

Module 10

Neuronal Signalling

Synopsis

The brain contains approximately 86 billion neurons that are located in different brain regions. Within each region, neurons are connected to each other to form neural circuits of bewildering complexity. To function in such circuits, each neuron must receive and process information entering from one set of neurons and then relay signals to other neurons in the circuit. The neuronal processes of signal reception and transmission are key elements of neuronal function and are located at opposite ends of the neuron. Specific attention will be focused on the operation of neural circuits in regions such as the hippocampus, cortex, cerebellum, basal ganglia and the respiratory centre in the brainstem. Neural circuits are established during development and remain relatively constant throughout adult life. The only exception in humans is the hippocampus, where the neuronal elements of the circuit are constantly being replaced by a process of neurogenesis.

In addition to the large number of neurons, the nervous system contains an equally large number of glial cells, such as astrocytes and the microglia, insinuate themselves between the neurons to provide a functional scaffold. This is not a passive scaffold, because the glial cells and neurons carry on a constant two-way dialogue that is essential for the computational operation of the neuronal circuits.

Neuronal morphology reveals that information is received at one end (the dendritic tree) and is transmitted at the synaptic endings at the opposite end. The key event is the process of synaptic transmission, during which presynaptic events release neurotransmitters from one neuron to induce localized excitatory or inhibitory postsynaptic events in the target neuron. The localized excitatory events are integrated to generate more global neural signals that trigger action potentials that initiate from the axon hillock. These action potentials then spread in two directions. They can flow in the forward direction down the axons to the synaptic endings. In addition, they can also flow backwards, and these back-propagating action potentials invade the dendritic tree to create global Ca^{2+} signals.

A very important aspect of neuronal signalling concerns the action potential frequency, which can vary over a wide range. At one extreme, there is the circadian clock, where the output of the suprachiasmatic clock neurons changes over 24 h. At the other end of the scale, we have the very rapid transfer of information that occurs between neurons in the 0.5–200 Hz frequency range that characterizes the brain rhythms detected by electroencephalograms (EEGs) during sleep and consciousness. Any consideration of synaptic transmission must take into account the fact that the rate of synaptic transmission is often extremely high. Another example of the significance of timing concerns neuronal coincident detection, during which a neuron integrates information coming in from more than one input, as occurs during the processes of learning and memory.

Modulation of neuronal activity can occur at many levels. Presynaptic events display a process of facilitation, whereby transmitter release is enhanced. Varying the permeability of K^+ channels can modulate membrane excitability, which determines whether or not a neuron will transmit information by firing an action potential. Information transfer across the synapse can also vary, and the mechanism of synaptic plasticity can persist for variable times. Enhancement of transmitter release through the process of facilitation is very brief, whereas the processes of long-term potentiation (LTP) and long-term depression (LTD), which are thought to be responsible for some memory processes, can last for hours. A process of memory consolidation functions to make these short-term changes more permanent through mechanisms that depend upon neuronal gene transcription and neuronal protein synthesis.

Ca^{2+} plays a key role in this modulation of neuronal activity in that it can act locally to influence excitability and synaptic strength, or it can have global effects on gene transcription, neuronal survival and axonal outgrowth. The neuron is not a static unit, but is highly plastic, and this underlies the process of learning and memory. The remarkable aspect of neuronal plasticity responsible for learning is that memory acquisition can occur within seconds, and can then be retained for a very

long time in long-term memory (months to years) through a process of memory consolidation. In considering neuronal signalling, we therefore have to understand how individual neurons carry out their day-by-day computational functions while retaining the ability to form memories by suddenly modifying this capacity to process information.

The section on hypothalamic pituitary regulation describes the interaction between the nervous and endocrine systems. The hypothalamus contains neurons that release a variety of hormones that regulate the endocrine cells located in the anterior pituitary. Some hypothalamic neurons, which send axons to the posterior pituitary, release hormones such as vasopressin and oxytocin.

Neurons in the respiratory centre located in the brainstem have the respiratory pacemaker mechanisms that control breathing.

Specialized neurons that function in the sensory systems are responsible for detecting information from both the internal and external environment. Photoreceptors in the eye are responsible for photoreception. Sensory neurons spread throughout the body function in nociception, temperature sensing and touch. Olfactory receptor cells in the nasal epithelium function in olfaction and taste receptors on the tongue function in taste. Hair cells in the ear are mechanoreceptors that function in hearing. Specialized cells and neurons function in the hypoxia-sensing mechanisms.

Brain regions

The brain is a highly complex organ that is divided into different functional regions (Module 10: Figure brain anatomy). Each region has a specific complement of neurons that are connected together into local circuits to generate characteristic output signals that are then sent either to other regions or to the periphery as is the case for the motor neurons. The high degree of brain connectivity between neurons is responsible for the multiple computational processes carried out in these different brain regions.

In the following description of neural cell signalling mechanisms, the primary emphasis will be on the way individual neurons interact with each other to form neural circuits. The organization and function of these integrated local circuits are described in the following brain regions:

- Cortex
- Limbic system
 - Amygdala
 - Hippocampus
 - Hypothalamus
 - Thalamus
- Basal ganglia
 - Striatum
 - Caudate nucleus
 - Putamen
 - Nucleus accumbens
 - Globus pallidus
 - Globus pallidus externa (GPe)
 - Globus pallidus interna (GPi)
 - Substantia nigra
 - Substantia nigra pars compacta (SNc)
 - Substantia nigra pars reticulata (SNr)
 - Subthalamic nucleus (STN)
 - Ventral tegmental area (VTA)
- Cerebellum
- Respiratory centre

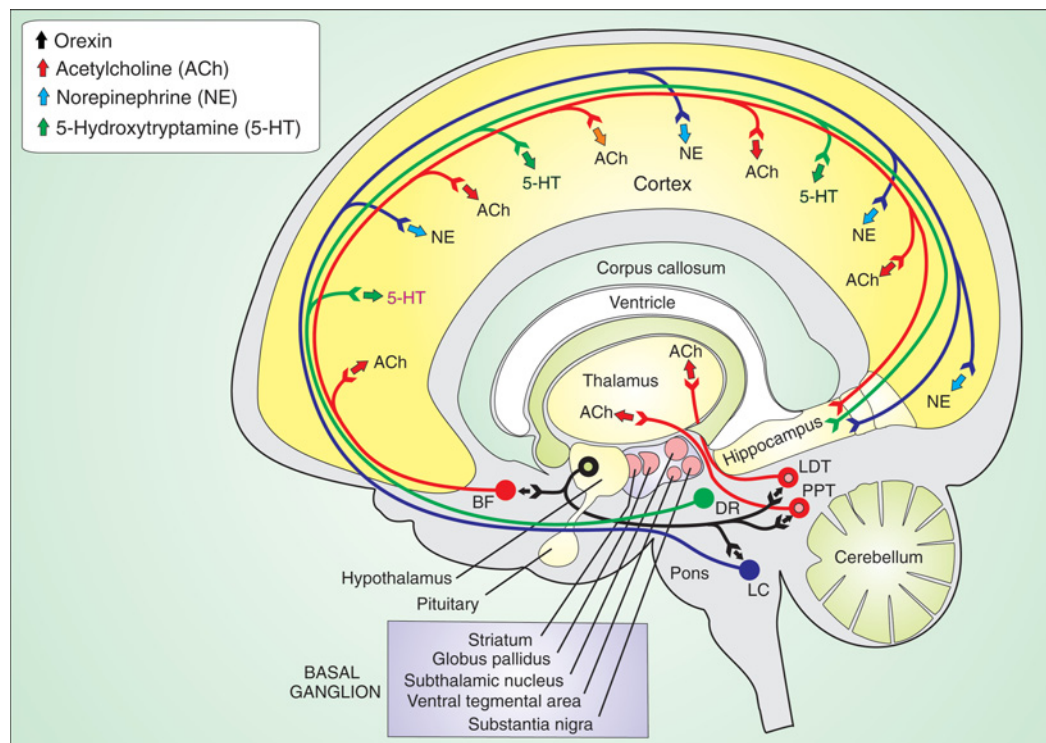
Brain connectivity and neural circuits

A brief description of some well-known neuronal circuits will highlight the typical architectural features that enable neurons to receive and transmit information.

To understand fully how neuronal function is controlled, it is necessary to consider neurons within their normal context where they are operating to receive and transmit information as part of a circuit. Each region of the brain has its characteristic set of neurons that are connected together to form circuits (Module 10: Figure brain circuitry and rhythms). The local circuits within many of these regions are based on complex interactions between excitatory (green) and inhibitory (red) neurons. Within different regions, the local circuits are often organized into highly integrated groups such as the cortical columns within the cortex.

The circuits that have been well-defined in the cortex and hippocampus are responsible for generating the brain rhythms that characterize brain function during sleep and consciousness. The delta and slow oscillations are a feature of sleep, whereas the faster theta and gamma oscillations operate during consciousness. These different rhythms can be detected either by an intracellular electrode that monitors the response of individual neurons or by an electrode in the extracellular space to record local field potentials (LFPs) that represent the integrated response of large neuronal assemblies (Module 10: Figure brain circuitry and rhythms). These LFPs result from the ion currents that are generated when neurons are responding to the excitatory or inhibitory neurotransmitters that drive the brain rhythms. The ability to detect these small extracellular potentials reflects the fact that large neuronal assemblies are firing synchronously with each other. This large-scale brain rhythm synchronization is essential for many brain functions such as working memory, attention selection and sensory stimulation.

An important feature of this neuronal synchronization within local circuits is that it often extends from one region to the next. For example, hippocampal theta oscillations occur in phase with those in the subiculum and

Module 10: | Figure brain anatomy**Anatomy of the human brain.**

The human brain has a number of discrete regions that carry out different functions. There is a large cortex that extends around towards the hippocampus. The middle of the brain has the corpus callosum, ventricles and the thalamus. Below the thalamus is the hypothalamus that extends down into the pituitary. The cerebellum at the back controls movement. The basal forebrain, midbrain and hindbrain contains the neurons that release transmitters, such as acetylcholine (ACh), 5-hydroxytryptamine (5-HT) and norepinephrine (NE) that constitute the ascending arousal system that controls the sleep/wake cycle.

neocortical theta oscillations (see vertical dashed line in Module 10: Figure brain circuitry and rhythms). Similarly, synchronous gamma oscillations occur throughout the cortex and entorhinal cortex. This high degree of connectivity co-ordinates the firing rate of neurons located in different brain regions. The vertical dashed line in Module 10: Figure brain circuitry and rhythms indicates the synchronous firing of an action potential in two neurons, one in the cortex and the other in the entorhinal cortex. Such connectivity is achieved by the excitatory and inhibitory neurons sending axons out to connect to circuits in other regions to integrate the activity of different brain regions. For example, the cortex receives a large direct afferent input from the hippocampus (Module 10: Figure brain circuitry and rhythms). Such interactions between different regions are often reciprocated. For example, the hippocampus sends an input to the deeper layers of the entorhinal cortex and also receives an input from neurons in the superficial layer of the latter (Module 10: Figure hippocampus).

The structure and function of individual neurons is well illustrated by considering specific regions such as the cerebellum, hippocampus and cortex. These regions of the brain have been studied extensively because they contain neurons that can easily be identified for physiological studies. Since brain function is so dependent on oscillatory activity, particular emphasis will be placed on the nature of the network oscillators and how the oscillatory activity

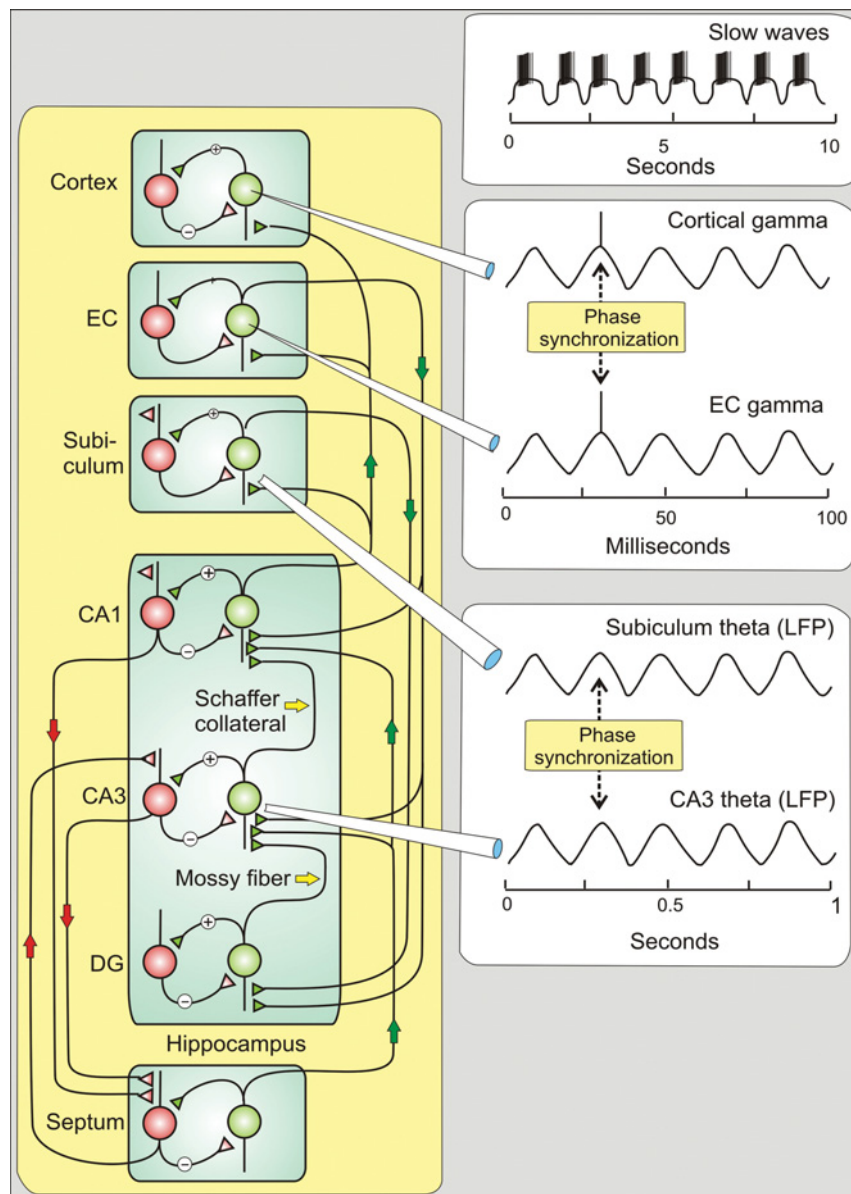
within local circuits are co-ordinated with those in other regions of the brain. Hippocampal oscillations provide a good example to illustrate how both theta and gamma oscillations are generated.

Another example of such neural oscillatory activity is found in the brainstem where the medullary respiratory centre contains a central pattern generator (CPG) that controls the pattern of breathing (Module 10: Figure pre-BötC respiratory circuit).

Cerebellum

The cerebellar cortex contains a number of cell types of which the Purkinje cells are the most obvious. They have large cell bodies (soma) that are lined up next to each other to form a palisade (Module 10: Figure cerebellum). It is the focal point of the cerebellar neural circuit. Information received from excitatory and inhibitory inputs are integrated at the level of the soma, and the final output, in the form of an action potential, is led down the axon, which is the sole output from the cerebellar cortex (Module 10: Figure cerebellar neural circuit).

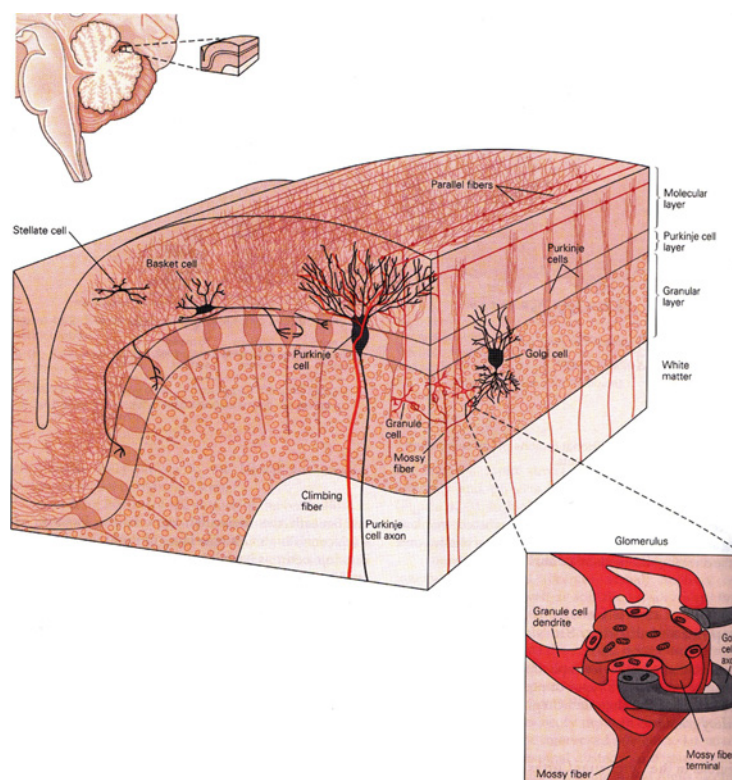
The complex organization of the Purkinje cell dendritic tree that emerges from the soma is nicely illustrated when it is filled with a Ca^{2+} -sensitive dye (Module 10: Figure Purkinje cell input-specific Ca^{2+} signals). The initial proximal dendritic segments are smooth, but the more distal branches are encrusted with myriads of small spines

Module 10: | Figure brain circuitry and rhythms**Brain circuitry and brain rhythms.**

An oversimplified view of the neural circuits located in some of the major regions of the brain. In many of these regions, such as the CA1, CA3 and cortex, the local circuits are based on complex interactions between excitatory (green) and inhibitory (red) neurons. These excitatory and inhibitory neurons can send out axons that connect to circuits in other regions to create the global connections that are so important for integrating the activity of different regions of the brain. The local circuits in each region generate the neural oscillations responsible for driving/integrating many brain functions. These oscillations can be detected by either intracellular electrodes that monitor the response of individual neurons or by electrodes in the extracellular space that record local field potentials (LFPs) that represent the integrated response of neuronal assemblies. The vertical dashed lines illustrate how gamma and theta oscillations can be synchronized in different brain regions. Details of the hippocampal circuitry are shown in Module 10: Figure hippocampus.

approximately 1 μm in diameter. Each Purkinje cell has approximately 200,000 spines, which are the sites that receive and process information coming in from the parallel fibres of the granule cells. The Purkinje cell has to integrate information received from three separate neurons: the parallel and climbing fibres that are excitatory, and the inhibitory input from the basket cells. Even though these three inputs innervate separate regions of the Purkinje cell, their positive or negative effects can be registered

and integrated immediately by electronic spread to induce either depolarization or hyperpolarization respectively. All of these excitatory and inhibitory inputs contribute to the function of the cerebellum in motor learning and control. This synapse has attracted considerable attention in that it displays long-term depression (LTD), a form of synaptic plasticity that has been implicated in motor learning.

Module 10: | Figure cerebellum**Organization of the cerebellum.**

The large Purkinje cells are organized in a distinct layer and are surrounded by other neurons such as the stellate cells, basket cells, granule cells and Golgi cells that make up the cerebellar neural circuit. Reproduced, with permission, from *Principles of Neural Science*, Kandel, E.R., Schwartz, J.H. and Jessell, T.M. Copyright (2000) The McGraw-Hill Companies, Inc.; see Kandel et al. 2000.

Purkinje cells thus display all the characteristics of a typical neuron. They have an elaborate dendritic tree encrusted with spines that receives information from other neurons.

Cortex

The cortex, which is also known as the cerebrum, is the largest part of the brain. The surface area of this outer cortical layer is highly wrinkled and the numerous indentations serve to enhance the surface area and the number of neurons. The cortex carries out many of the 'higher' brain functions that one associates with consciousness, such as perceptual awareness, thought, memory, spatial reasoning, language and the generation of motor commands. It is divided into two: the left and right hemispheres that are connected to each other by a bundle of nerve fibres known as the corpus callosum. Each hemisphere is divided into four lobes:

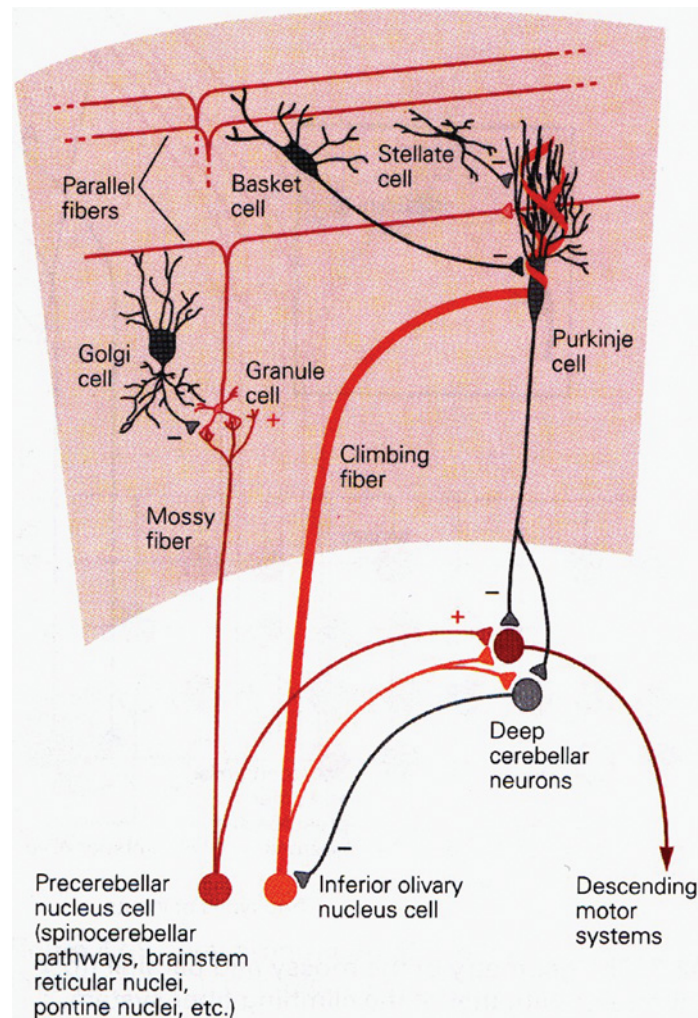
- Frontal lobe, which functions in reasoning, movement, emotions, planning, speech and problem solving.
- Parietal lobe, which functions in perception, orientation, recognition and movement.
- Occipital lobe, which functions in visual processing
- Temporal lobe, which functions in recognition of auditory stimuli, speech and memory.

Both hemispheres of the cortex contain motor areas that control voluntary movements. The premotor cortex selects out which movements to perform and these are then carried out by the primary motor cortex. The dorsolateral prefrontal cortex also plays a role in the decision-making process that controls voluntary movements.

The cortex consists of an extensive sheet of neural tissue that can be divided into six horizontal layers that contains different neuronal cell types and is often referred to as grey matter (Module 10: Figure dorsolateral prefrontal cortex). The white matter located beneath this region of grey matter contains the myelinated axons that provide the connections to other regions of the cortex and the brain. The typical organization of the cortical grey matter is illustrated by the dorsolateral prefrontal cortex, which is of particular interest, because changes in the operation of the local circuits in this area have been linked to schizophrenia.

Dorsolateral prefrontal cortex (DLPFC)

The dorsolateral prefrontal cortex (DLPFC) plays an important role in the executive control of the brain by participating in the process of working memory, which is responsible for carrying out thought processes and various behavioural sequences. During working memory, an initial input through either a sensory or thought process is maintained through a sustained and co-ordinated firing

Module 10: | Figure cerebellar neural circuit**Organization of the cerebellar neural circuit.**

A number of cell types regulate the activity of the Purkinje cells. The granule cells provide the parallel fibres that innervate the spines on the dendritic tree to provide an excitatory input that can induce localized Ca^{2+} signals in individual spines. The Golgi cells and the stellate/basket cells function as inhibitory interneurons. The Golgi cells inhibit the granule cells, whereas the stellate/basket cells inhibit the Purkinje cells. In addition to these four cell types, the cerebellum receives afferent input from mossy fibres, which innervate the granule cells, and climbing fibres, which wrap around the proximal dendrites of the Purkinje cells (see Module 10: Figure cerebellar climbing fibres for details of this climbing fibre innervation). Reproduced, with permission, from *Principles of Neural Science*, Kandel, E.R., Schwartz, J.H. and Jessell, T.M. Copyright (2000) The McGraw-Hill Companies, Inc.; see Kandel et al. 2000.

of groups of DLPFC pyramidal neurons that then enables the brain to decide upon the subsequent initiation of a behavioural response. This firing pattern is an emergent property of local circuits that depend upon interactions between the local fast-spiking inhibitory interneurons and the excitatory pyramidal neurons (Module 10: Figure dorsolateral prefrontal cortex). As for other cortical regions, these neurons are located within the six layers of the grey matter and are connected to each other both vertically and laterally to form discrete local circuits that are arranged in cortical columns, which are the functional units of the cortex. The neurons that operate within these columns are distributed between the six layers (Module 10: Figure dorsolateral prefrontal cortex):

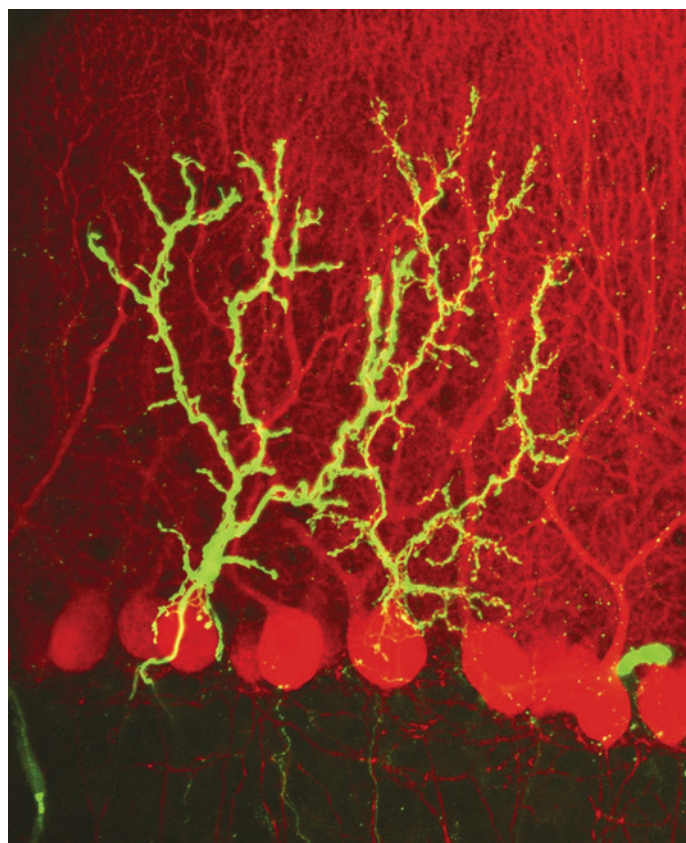
- Molecular layer 1: contains the Cajal–Retzius neurons, which express the Ca^{2+} -binding protein calretinin

(CR). In addition it has a dense network of axons in contact with the apical dendritic tufts of the pyramidal neurons.

- Molecular layers 2–4: contain the soma of the pyramidal neurons and numerous interneurons particularly the Double bouquet neurons, Chandelier neurons and wide arbor (basket) interneurons.

Double bouquet neurons, which express calbindin (CB) and calretinin (CR), have branched axons that innervate the distal dendrites of the pyramidal cells.

Chandelier neurons are also known as axo-axonic neurons because their axon terminals innervate the axon initial segment of the pyramidal neurons (red arrows in Module 10: Figure dorsolateral prefrontal cortex). This terminal region has vertical rows of boutons that resemble a chandelier neuron. These neurons express

Module 10: | Figure cerebellar climbing fibres**Innervation of cerebellar Purkinje neurons by climbing fibres.**

The palisade of Purkinje cells (stained red) has extensive dendritic trees. The climbing fibre (stained green) extends upwards to innervate the branched dendrites of the Purkinje cells. Reproduced, with permission, from Nishiyama, H. and Linden, D.J. (2004) Differential maturation of climbing fiber innervation in cerebellar vermis. *Journal of Neuroscience* 24:3926–3932. Copyright (2004) the Society for Neuroscience; see Nishiyama and Linden (2004).

parvalbumin (PV) and calbindin (CB) and are fast-spiking inhibitory neurons that regulate the output of the pyramidal neurons. In turn, the pyramidal neurons send out axonal collaterals that provide an excitatory feedback to the chandelier neurons (blue arrows in Module 10: Figure dorsolateral prefrontal cortex) thus completing a local circuit capable of generating brain rhythms.

Wide arbor (basket) interneurons form a similar local circuit with the pyramidal neurons. By innervating the pyramidal soma and its proximal dendrites, the wide arbor neurons can regulate the output of the local circuit.

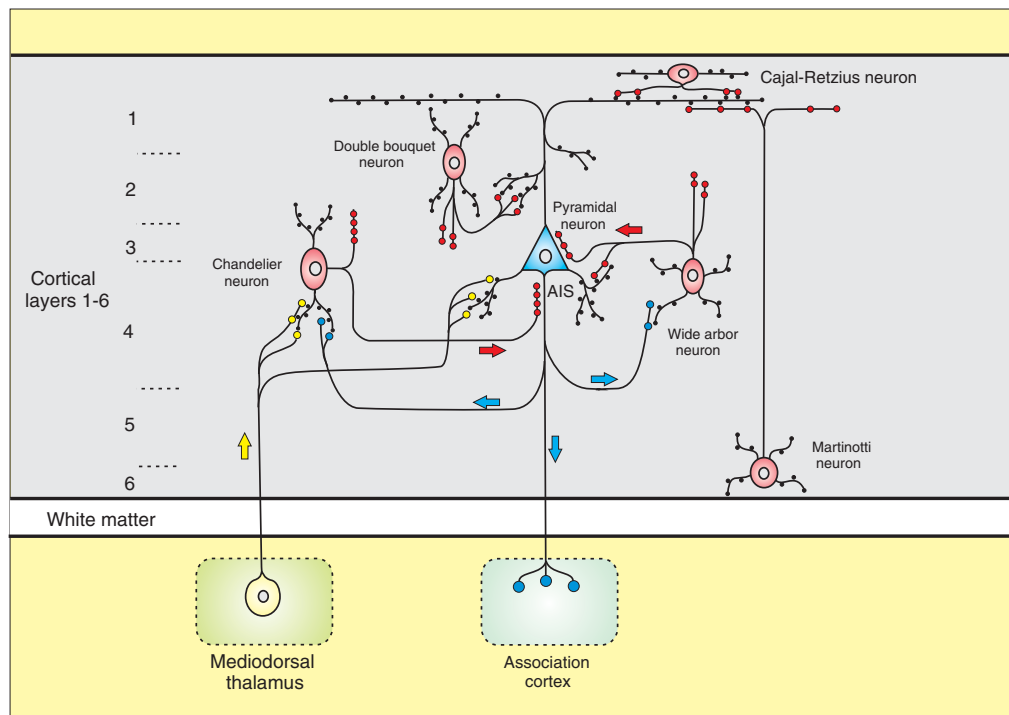
- Molecular layers 5 and 6: the two layers that face the white matter contain the Martinotti interneurons, which express calbindin and send their axons up to layer 1 where they innervate the distal dendrites of the pyramidal neurons.

The cerebral cortex is connected to various brain regions such as the hippocampus. It sends information out to these regions and it also receives information from the thalamus (yellow arrows in Module 10: Figure dorsolateral prefrontal cortex) and other regions of the brain.

The local circuits in the cortex are capable of generating brain rhythms that emerge from the feedback loops that operate between the inhibitory interneurons and the excitatory pyramidal neurons (Module 10: Figure brain circuitry and rhythms). These local circuits are remarkably similar to those located in the hippocampus that are capable of generating hippocampal gamma oscillations (Module 10: Figure gamma oscillatory mechanisms) and hippocampal theta oscillations (Module 10: Figure theta oscillatory mechanisms). Very similar mechanism may be responsible for setting up gamma and theta oscillations in the cortical regions.

Cortical columns

The cortex contains approximately 100,000 cortical columns, which are local assemblies of about 5000 interacting neurons. These neuronal assemblies are dedicated to a particular information processing task. The ability of each column to function independently of its neighbours is exemplified by the fact that they can enter into a sleep-like state during the local sleep processes when their neighbours remain in a wake state.

Module 10: | Figure dorsolateral prefrontal cortex**Dorsolateral prefrontal cortex neural circuitry.**

The neural circuitry contains populations of excitatory pyramidal neurons (blue) that have a main axon that innervates the association cortex and collateral axons that convey excitation (blue arrow) to a diverse population of inhibitory interneurons (red). The latter have branching axons that not only innervate other inhibitory interneurons, but also innervate the pyramidal neurons (red arrows) at the axon initial segment (AIS), the soma and the dendrites. These cortical local circuits also receive excitatory inputs (yellow arrow) from neurons located in the mediodorsal thalamus. This Figure is based on information obtained from Lewis et al. (2005) and Ross et al. (2006).

Limbic system

The limbic system, which is located below the cortex, consists of the amygdala, hippocampus, hypothalamus and thalamus.

Hippocampus

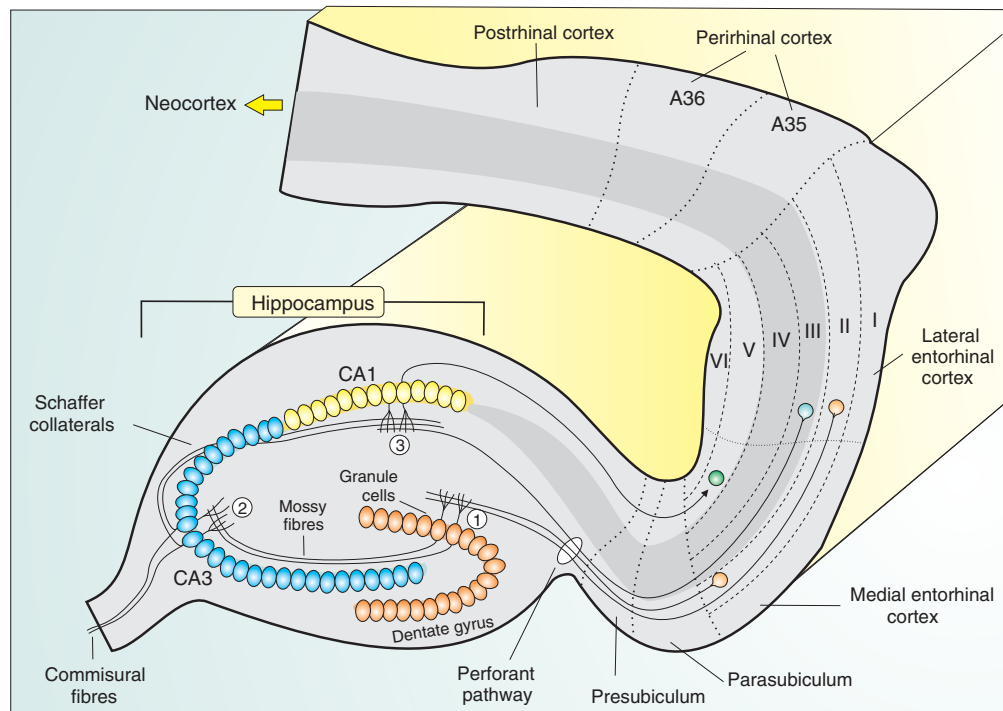
The hippocampus processes information from a number of sensory modalities (visual, auditory and somatic), and has been strongly implicated in the process of learning and memory. It is also able to generate rhythmical activity, and thus has clinical importance with regard to the generation of seizures. The organization of the hippocampus will be considered in relation to some of the neighbouring brain regions with which they share a two-way communication (Module 10: Figure brain circuitry and rhythms). At one end, the hippocampus communicates with the septum, whereas the other end is connected to various cortical regions. The cortical region closest to the hippocampus is the subiculum, which is followed by the parasubiculum, medial entorhinal cortex, lateral entorhinal cortex, regions A35 and A36 of the perirhinal cortex, postrhinal cortex and then to the neocortex (Module 10: Figure hippocampus). It is through these regions that the hippocampus extends its long-range influence over the oscillatory activity of the cortex.

There are two main neuronal cell types in the hippocampus: pyramidal neurons (granule cells, CA3 and CA1 neurons) and a large number of inhibitory hippocampal

interneurons (Module 10: Figure CA3 hippocampal neurons). The granule cells are located in a horseshoe-shaped area known as the dentate gyrus. Granule cells in the dentate gyrus region are of particular interest because they are constantly being replaced through a process of neurogenesis. The CA3 and CA1 neurons are also arranged in layers that are contiguous and form another horseshoe-shaped layer facing that of the dentate gyrus (Module 10: Figure hippocampus). The way in which the pyramidal and inhibitory neurons are organized will be described with reference to the CA3 region (Module 10: Figure CA3 hippocampal neurons). The cell bodies of the CA3 pyramidal cells are located in the stratum pyramidale (s. pyr). The axons extend out through the stratum oriens (s. ori), which also contains the proximal dendrites. The distal dendrites extend out through the stratum radiatum (s. rad) to terminate in the stratum lacunosum-moleculare (s. l-m). There are a large number of inhibitory hippocampal interneurons that interact in different ways with the pyramidal cells. The basket cells and the O-LM illustrate the different ways in which the axons of the inhibitory interneurons innervate the pyramidal cells.

The soma of the basket cells lie in the stratum pyramidale where they form a dense web of axons that interact with the axonal webs of neighbouring basket cells to form a highly connected layer of cells. This high degree of basket cell connectivity is enhanced further by the formation of numerous gap junctions between neighbouring cells. These

Module 10: | Figure hippocampus



Organization of the hippocampus and parahippocampal regions.

The hippocampus has a trisynaptic local circuit. The first synapses (1) are on the granule cells of the dentate gyrus (orange) that receives input from the perforant fibres. These granule cells send out axons called mossy fibres that extend into the CA3 region (blue), where they form the second group of synapses (2) by innervating the CA3 pyramidal cells. The axons from the CA3 neurons bifurcate: one part is directed down to the septum, whereas the other gives rise to Schaffer collaterals that complete the trisynaptic circuit by innervating the pyramidal neurons (3) in the CA1 region (yellow). The Schaffer collateral/hippocampal CA1 synapse (Module 10: Figure synaptic signalling mechanisms) has been studied extensively as a model for learning and memory.

basket cell axons innervate the soma region of the pyramidal cells to set up one of the hippocampal local circuits that generate gamma rhythms. The soma of the O-LM interneurons is located in the stratum oriens and the axons extend down through the layers to the stratum lacunosum-moleculare where they form an axonal web that innervates the distal dendrites of the pyramidal cells (Module 10: Figure CA3 hippocampal neurons). The basket cells and the O-LM interneurons thus interact with the pyramidal cells to set up two very different hippocampal local circuits that generate different neural rhythms (Module 10: Figure CA3 hippocampal local circuits).

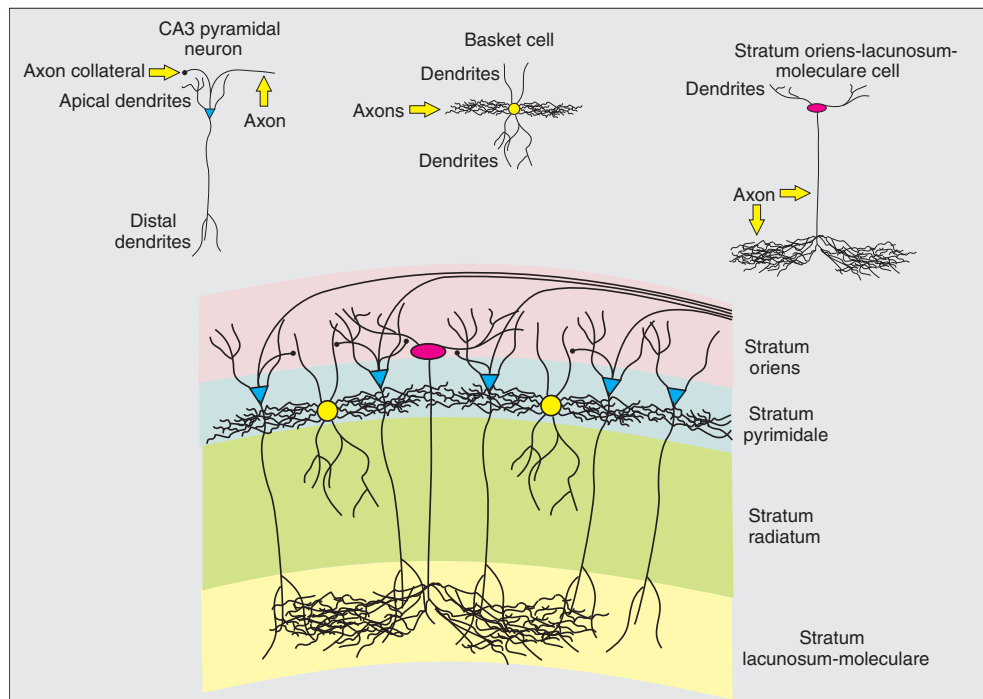
Hippocampal interneurons

There are a large number of hippocampal interneurons that can be distinguished from each other on the basis of their location, their protein expression patterns and their firing patterns in response to membrane depolarization (Module 10: Figure hippocampal interneurons):

- Axo-axonic cell, which express parvalbumin (PV) has its soma located in the stratum pyramidale and its axon innervates the axon hillock region of the pyramidal cell.
- Basket cell ($PV^+ CCK^-$), which expresses parvalbumin (PV) has its soma located in the stratum pyramidale and its axon innervates the soma region of the pyramidal cell. The extensive interconnected branching axons of

such basket cells are illustrated in Module 10: Figure CA3 hippocampal neurons. The basket interneurons are part of the basket cell/CA3 local circuit (Module 10: Figure CA3 hippocampal local circuits) that functions to generate hippocampal gamma oscillations.

- Basket cell ($PV^- CCK^+$), which expresses cholecystokinin (CCK) and vasoactive intestinal peptide (VIP), has its soma located in the stratum pyramidale and its axon innervates the soma region of the pyramidal cell.
- Basket cell ($PV^- CCK^+ VIP^+$), which expresses cholecystokinin (CCK) and vesicular glutamate transporter 3 (vGLUT3), has its soma located in the stratum pyramidale and its axon innervates the soma region of the pyramidal cell.
- Bis-stratified cell, which expresses parvalbumin (PV), neuropeptide Y (NPY) and somatostatin (Sst), has its soma located in the stratum pyramidale and its axon innervates the pyramidal cell at two regions: the soma and proximal dendrites, as well as the distal dendrites.
- Oriens-lacunosum moleculare (O-LM) cell, which express parvalbumin (PV), metabotropic glutamatergic receptor 1a (mGluR1a) and somatostatin (Sst), has its soma located in the stratum oriens and its long axon terminates in an extensive network that innervates the distal dendrites of the pyramidal cell as shown in more detail in Module 10: Figure CA3 hippocampal neurons.

Module 10: | Figure CA3 hippocampal neurons**Neuronal cell types in the CA3 region of the hippocampus.**

The CA3 region of the hippocampus has two main cell types: CA3 pyramidal neurons and a large number of interneurons. The drawing at the bottom illustrates how two of the main interneuronal cell types, the basket cells and the stratum oriens-lacunosum moleculare (O-LM) cells, interact with the layer of CA3 pyramidal neurons (blue). The local circuits that result from these interactions are illustrated in Module 10: Figure CA3 hippocampal local circuits.

The O-LM interneurons are part of the O-LM/CA3 local circuit (Module 10: Figure CA3 hippocampal local circuits) that functions to generate hippocampal theta oscillations.

- Schaffer-collateral-associated cell, which express calbindin (CB) and cholecystokinin (CCK), has its soma located in the stratum radiatum and its axon runs upwards with terminals in the stratum radiatum, pyramidale and oriens (Module 10: Figure hippocampal interneurons).
- Lacunosum-moleculare-radiatum-perforant path (LM-R-PP) cell, which express cholecystokinin (CCK), has its soma at the boundary between the stratum radiatum and molecular, and its axons terminate in both the stratum radiatum and stratum lacunosum-moleculare (s l-m). In addition the axons also extend out to the dentate gyrus (DG).
- Lacunosum-moleculare-perforant path (LM-PP) cell, which express cholecystokinin (CCK), has its soma at the boundary between the stratum radiatum and stratum lacunosum-moleculare (s l-m) and its axons terminate in the latter. In addition the axons also extend out to the dentate gyrus (DG).
- Trilaminar cell, which expresses the M2 muscarinic receptor, has its soma located in the stratum oriens and its axons terminate in three regions (stratum oriens, pyramidale and molecular). In addition, an axonal branch travels out of the hippocampus to innervate neurons in the subiculum.

- Back-projecting cell, which expresses somatostatin (Sst), has its cell body in the stratum oriens and sends its axons out widely throughout the hippocampus and dentate gyrus.
- Hippocampal septal cell, which expresses calbindin (CB) and somatostatin (Sst), has its cell body in the stratum oriens and sends its axons locally to the stratum radiatum, pyramidale and molecular and it also projects out to the septum.

These hippocampal interneurons are GABAergic neurons in that they synthesize and release the inhibitory neurotransmitter γ -aminobutyric acid (GABA). Their primary function is to provide an inhibitory input as part of the hippocampal neuronal circuits that characterize the multiple functions of the hippocampus.

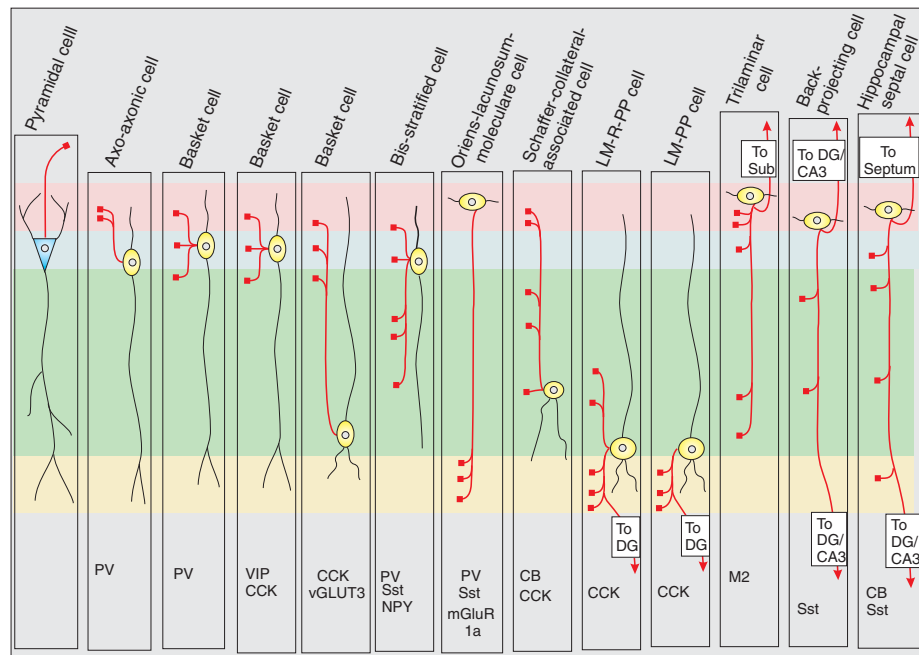
Hippocampal neuronal circuits

Hippocampal neuronal circuits can be divided into two main types: the hippocampal trisynaptic circuit that links the three main regions of the hippocampus (Module 10: Figure hippocampus) and the hippocampal local circuits that operate to generate rhythmical activity within each region (Module 10: Figure CA3 hippocampal local circuits).

Hippocampal trisynaptic circuit

The three main pyramidal neurons are joined together to form a trisynaptic circuit (synaptic connections 1–3 in Module 10: Figure hippocampus). The first synapses are

Module 10: | Figure hippocampal interneurons

**Hippocampal interneurons.**

The hippocampus has two main cell types: pyramidal cells (e.g. granule cells, CA1 and CA3 neurons) and a large number of inhibitory interneurons. These interneurons express different signalling components and can also be distinguished by the way their axons (shown in red) and dendrites (shown in black) terminate in different regions of the hippocampus. These four regions are colour-coded: stratum oriens (pink), stratum pyramidale (blue), stratum radiatum (green) and stratum lacunosum-moleculare (yellow). CB, calbindin; CCK, cholecystokinin; M2, muscarinic receptor type 2; mGluR1a, metabotropic glutamatergic receptor 1a; NPY, neuropeptide Y; PV, parvalbumin; Sst, somatostatin; VIP, vasoactive intestinal peptide; vGLUT3, vesicular glutamate transporter 3. Some of the interneurons send axons out to innervate neurons in other regions of the hippocampus such as the dentate gyrus (DG) or to other brain regions such as the subiculum (Sub) and septum. Information for this figure was taken from Figure 1 in Somogyi and Klausberger (2005).

on the granule cells of the dentate gyrus that receives input from the perforant fibres. These granule cells send out axons called mossy fibres that extend into the CA3 region, where they form the second group of synapses by innervating the characteristic pyramidal cells. These CA3 cells have both apical and basal dendritic trees. The axon from the CA3 neuron bifurcates: one part forms the commissural fibres that are directed down to the septum en route to the contralateral hippocampal region, whereas the other gives rise to Schaffer collaterals that complete the trisynaptic circuit by innervating the pyramidal neurons in the CA1 region. The axons emanating from the CA1 neurons are directed to various targets such as neurons located within layer V of the medial entorhinal cortex. In addition to sending information out, other regions of the brain provide an extensive input that regulates hippocampal activity. For example, axons from stellate cells in layer II of the entorhinal cortex enter the hippocampus through the perforant pathway to innervate the granule cells of the dentate gyrus. In addition, the hippocampus receives an extensive cholinergic input from the basal forebrain. Both cholinergic and GABAergic neurons located in the medial septum-diagonal band of Broca (MS-DSBB) provide an ascending input to both the pyramidal neurons and the interneurons.

As described for the Purkinje cells, the dendrites of the three cell types are encrusted with spines that receive the synaptic inputs, enabling them to communicate with each

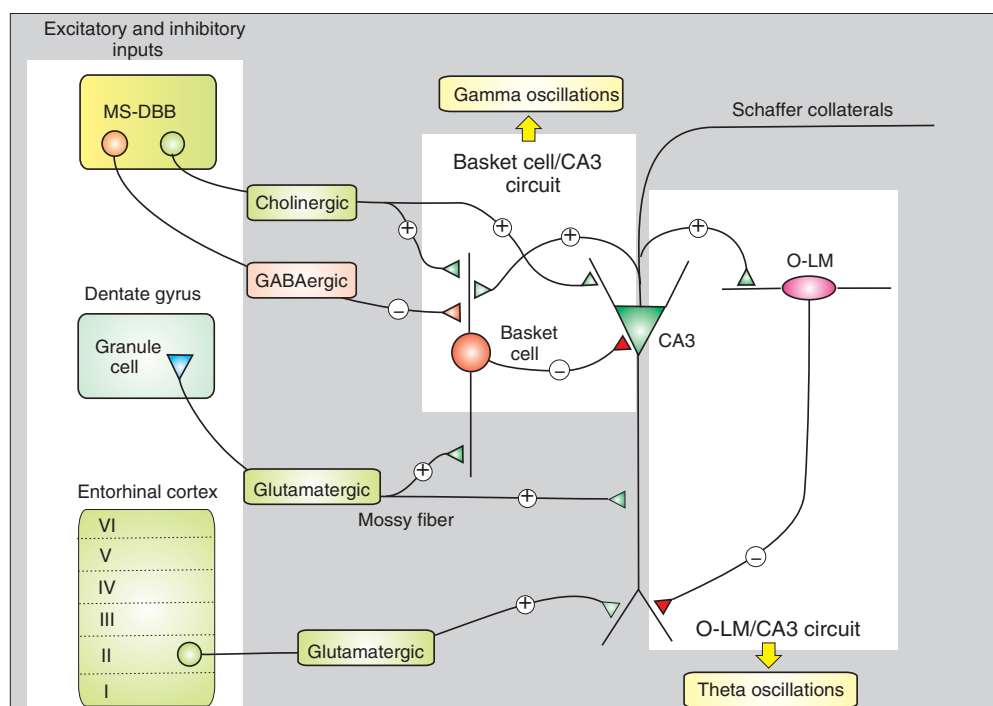
other. Details of the the Schaffer collateral/hippocampal CA1 synapse is shown in Module 10: Figure synaptic signalling mechanisms. All of these synaptic regions are highly plastic in that they reveal a process of long-term potentiation (LTP), which has been implicated in learning and memory.

Hippocampal local circuits

The circuits located within the CA3 region of the hippocampus illustrate how the excitatory pyramidal cells and the inhibitory hippocampal interneurons interact with each other to provide the local hippocampal circuits that generate hippocampal oscillations (Module 10: Figure CA3 hippocampal local circuits). The basic feature of these circuits is that the excitatory CA3 pyramidal neurons are connected to different inhibitory hippocampal interneurons. Two of these circuits are illustrated in Module 10: Figure CA3 hippocampal local circuits: the basket cell/CA3 local circuit and the oriens-lacunosum moleculare (O-LM)/CA3 local circuit.

Basket cell/CA3 local circuit

The dense web of axons, which characterize basket cell morphology, innervate the soma region of the CA3 neurons (Module 10: Figure CA3 hippocampal neurons) and are thus ideally located to inhibit the generation of action potentials at the axon hillock. The CA3 neurons send out axon collaterals that innervate the basket cell dendrites

Module 10: | Figure CA3 hippocampal local circuits**Hippocampal CA3 local neuronal circuit.**

The main components of the hippocampal CA3 local neuronal circuits are the CA3 pyramidal neurons and the inhibitory interneurons such as the basket cell and the oriens-lacunosum moleculare (O-LM) cell. The CA3 neurons provide an excitatory input to the inhibitory neurons, which, in turn, provide inhibitory inputs back to the CA3 neurons. The inhibitory interneurons interact with different regions of CA3: the axonal terminals of the basket cell (red triangles) innervate the soma whereas the long axons of O-LM innervate the distal dendrites. In addition, the components of these local circuits receive both excitatory and inhibitory inputs from other brain regions such as the medial septum-diagonal band of Broca (MS-DBB), the dentate gyrus and the entorhinal cortex. The structural organization of the CA3 pyramidal neurons and the basket cell and O-LM interneurons are shown in Module 10: Figure CA3 hippocampal neurons.

to provide the excitatory input to complete the circuit (Module 10: Figure CA3 hippocampal local circuits). An important feature of these basket cell neurons is their connectivity. The extensive interconnected branching axons of these basket cells are connected to each other through both chemical synapses and gap junctions. This connectivity plays an important role in enabling these inhibitory interneurons to operate in synchrony with each other during the generation of hippocampal gamma oscillations.

Oriens-lacunosum moleculare (O-LM)/CA3 local circuit

Like the basket cells, the O-LM interneurons receive an excitatory signal from the axonal collateral of the CA3 axons (Module 10: Figure 10 CA3 hippocampal neurons). Unlike the basket cells that innervate the CA3 soma, the O-LM axons travel down the different strata of the hippocampus to form terminal webs that innervate the distal regions of the CA3 neurons. This inhibitory input to the distal dendrites of the CA3 pyramidal neurons is part of the O-LM/CA3 circuit, which is completed by the collateral axons of the CA3 neurons providing an excitatory input to the O-LM interneurons (Module 10: Figure CA3 hippocampal neurons). The circuit formed between O-LM and CA3 operates to generate hippocampal theta oscillations.

Hippocampal oscillations

The hippocampal local circuits are typical network oscillators in that the positive and negative connections between the participating neurons set up oscillations with variable frequencies. Within each circuit, excitatory neurons feed positive signals through their axon collaterals to activate the interneurons, which then transmit an inhibitory signal back to the excitatory neurons to temporarily inhibit their stimulatory signal thereby setting up the oscillating neural rhythms that characterize many regions of the brain (Module 10: Figure CA3 hippocampal local circuits). Each circuit has a natural resonance, which depends on various parameters such as the rate of signal propagation across the participating synapses, which generate the excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs), and the electrical properties of the dendrites that transfer these EPSPs and IPSPs to the axon hillock where the action potentials are generated. The natural resonance of the basket cell/CA3 circuit generates hippocampal gamma oscillations whereas that of the O-LM/CA3 circuit produces the slower hippocampal theta oscillations.

Schizophrenia has been linked to alterations in the properties of these oscillations.

Hippocampal gamma oscillations

Hippocampal gamma oscillations are derived from the basket cell/CA3 local circuit, which is one of the hippocampal local circuits (Module 10: Figure CA3 hippocampal local circuits). Just how this circuit sets up the 20–40 Hz oscillations is still somewhat mysterious. There appear to be two main mechanisms. First, there are the excitatory and inhibitory synaptic mechanisms that enable the CA3 and basket cells to communicate with each other. Secondly, there are mechanisms that provide the tonic excitatory drive that can induce/modulate the gamma oscillations. The following model attempts to summarize some of the main signalling components that have been implicated in both driving and modulating these oscillations (Module 10: Figure gamma oscillatory mechanisms):

Excitatory and inhibitory hippocampal synaptic mechanisms

1. Since the CA3 neuron usually fires before the basket cell, it is appropriate to begin the sequence with the former firing action potentials (at the gamma oscillation frequency) that travel down both the Schaffer collateral towards the CA1 region as part of the hippocampal trisynaptic circuit (Module 10: Figure hippocampus) and the axonal collaterals that innervate the basket cells.
2. The basket cells also receive a glutamatergic innervations from the mossy fibres originating from the granule cells in the dentate gyrus (Module 10: Figure CA3 hippocampal local circuits). The glutamate released at the synapses on the dendrites of the basket cells acts on a number of glutamatergic synapses.
3. Glutamate activates α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors (AMPARs), which are composed mainly of the GluR4 subunit that enables them to activate fast excitatory postsynaptic potentials (EPSPs), which brings about the rapid depolarization necessary to initiate action potential firing.
4. Glutamate also activates NMDA receptors (NMDARs) that gate both Na^+ and Ca^{2+} and have been implicated in spike timing.
5. The action potentials induced in the basket cells travel down the branching web of axons that innervate multiple CA3 neurons where they form inhibitory synapses on the soma (Module 10: Figure gamma oscillatory mechanisms).
6. Depolarization of the terminals stimulates the opening of $\text{Ca}_v2.1$ P/Q-type channels to introduce the Ca^{2+} that triggers the release of GABA. These P/Q channels are tightly coupled to the Ca^{2+} sensors on the exocytotic machinery and this close proximity greatly reduces the synaptic delay thus enhancing both the speed and reliability of this inhibitory input on to the CA3 neurons.
7. The GABA released from the terminals act on the type A γ -aminobutyric acid (GABA) receptors (GABA_A Rs) that gate chloride and give rise to inhibitory postsynaptic potentials (IPSPs).

8. Summation of these GABA-induced IPSPs hyperpolarizes the membrane sufficiently to curtail the action potential firing of the CA3 neuron.
9. In addition to innervating the CA3 neurons, the basket cells also innervate other basket cells, which means that the firing of each cell is rapidly terminated by an inhibitory input from its neighbours.
10. The GABA released on to the basket cells activates GABA_A Rs to give IPSPs that will hyperpolarize the basket cell membrane to terminate its firing. This cessation of basket cell firing will then enable them to begin to depolarize to set the stage for the next period of burst firing. The rate at which the membrane depolarizes is determined by the tonic excitatory drive (see below), which represents the pacemaker mechanism responsible for determining the periodicity of this network oscillator.

Tonic excitatory drive

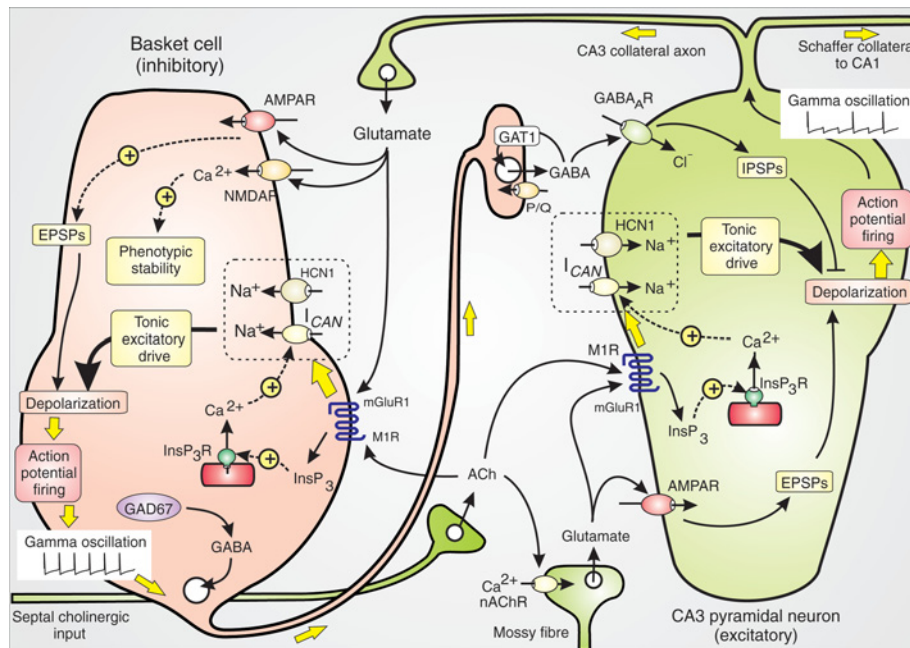
In order for the basket cell/CA3 local circuit to oscillate, it appears to require the input of a tonic excitatory drive. An important source of this drive are the neurons located within the septum and particularly within the medial septum-diagonal band of Broca (MS-DBB) which contains both GABAergic and cholinergic neurons that project to the hippocampus to innervate both the basket cells and the CA3 pyramidal cells (Module 10: Figure CA3 hippocampal local circuits). It seems that each neuronal cell type needs to be sufficiently excitable in order to participate in the reciprocal sequence of interactions necessary to drive the rapid gamma oscillatory cycle.

Particular attention will thus be focused on the septal cholinergic input, which has at least three possible modes of action (Module 10: Figure gamma oscillatory mechanisms):

- CA3 metabotropic responses
- Basket cell metabotropic responses
- Mossy fibre nicotinic responses

CA3 metabotropic responses

- a. Acetylcholine (ACh) acts through muscarinic 1 receptors (M1Rs) (Module 1: Table G protein-coupled receptors), which are known to be coupled through the heterotrimeric G protein G_q to phospholipase C β (PLC β) to activate the inositol 1,4,5-trisphosphate (InsP_3) Ca^{2+} signalling cassette (Module 2: Figure InsP_3 and DAG formation). The M1Rs may also act by inhibiting the SK Ca^{2+} -activated K^+ channels.
- b. The InsP_3 acts to release Ca^{2+} from the internal store, which might then stimulate a Ca^{2+} -dependent non-selective cationic current (I_{CAT}). The nature of the channel responsible for conducting I_{CAT} is not known, but likely candidates are TRPM4 and TRPM5 that perform a similar pacemaker function in the case of the respiratory pacemaker mechanism (Module 10: Figure respiratory pacemaker mechanism). The I_{CAT} provides the persistence inward current that contributes to the tonic excitatory drive necessary to maintain the gamma oscillation.

Module 10: | Figure gamma oscillatory mechanism**Hippocampal gamma oscillatory mechanism.**

The inhibitory basket cell (red) and the excitatory CA3 pyramidal neuron (green) interact with each other to form a network oscillator that is tuned to generate gamma oscillations. The collateral axons of the CA3 neuron release glutamate that depolarizes the basket cell whose axon terminals innervate the CA3 neurons to release GABA, which then acts on GABA_A receptors (GABA_AR) to induce the inhibitory postsynaptic potentials (IPSPs) which hyperpolarize the membrane to temporarily curtail the firing of the CA3 neurons. Operation of this self-contained network oscillator appears to be driven by a tonic excitatory drive that is activated by inputs from other brain regions such as the septal cholinergic input that releases acetylcholine (ACh), which acts through muscarinic 1 receptors (M1Rs). These metabotropic receptors appear to act by stimulating channels (dashed box) to provide the pacemaker depolarizations that drive oscillatory activity. See the text for information on the different signalling mechanisms that control this network oscillator.

- c. In addition to activating I_{CAT}, the M1Rs can also stimulate the hyperpolarizing-activated cyclic nucleotide-gated (HCN) channels, which are known to generate pacemaker activity in a number of excitable cells by maintaining a background influx of Na⁺ that will contribute to the tonic excitatory drive.
- d. When the basket cell has hyperpolarized the membrane (step 7), the next firing of an action potential by the CA3 neuron depends on the tonic excitatory drive to provide the inward currents for the pacemaker depolarization necessary to initiate the next gamma oscillatory cycle. Activation of the CA3 neuron gamma oscillation is thus determined by the fluctuating balance between the intermittent membrane hyperpolarization provided by the inhibitory GABAergic pathway (Steps 6–8) and the membrane depolarization activated by the tonic excitatory drive (Steps b–d).

Basket cell metabotropic responses

- e. Like the CA3 neurons described above, the basket cells also have a tonic excitatory drive activated by metabotropic receptors such as the M1Rs and the mGluR1s (Module 10: Figure gamma oscillatory mechanisms). These receptors stimulate the formation of InsP₃.
- f. The InsP₃ then acts on the InsP₃Rs to release Ca²⁺ that may function to stimulate I_{CAT} to contribute to the

tonic excitatory drive. This pacemaker activity may also be driven by HCN1 channels that are also activated by these metabotropic receptors.

Mossy fibre nicotinic responses

- g. A tonic excitatory drive may also be derived from the mossy fibre presynaptic Ca²⁺ release mechanism that depends on acetylcholine acting through ionotropic nicotinic acetylcholine receptors (nAChRs) to enhance the release of glutamate (Module 10: Figure mossy fibre presynaptic release).
- h. The increased glutamate could enhance oscillatory activity by increasing membrane depolarization by acting either on the AMPARs or on the metabotropic glutamatergic receptor 1 (mGluR1) as described earlier for the M1R.

Hippocampal theta oscillations

Hippocampal theta oscillations are derived from the oriens-lacunosum moleculare (O-LM)/CA3 local circuit, which is one of the hippocampal local circuits (Module 10: Figure CA3 hippocampal local circuits). Just how this circuit sets up the 4–10 Hz oscillations is still somewhat mysterious. There appear to be two main mechanisms. First, there are the excitatory and inhibitory synaptic mechanisms that enable the CA3 and O-LM interneurons to communicate with each other. Secondly, there are mechanisms that provide the tonic excitatory drive that can

induce/modulate the theta oscillations. The following model attempts to summarize some of the main signalling components that have been implicated in both driving and modulating these oscillations (Module 10: Figure theta oscillatory mechanisms):

Excitatory and inhibitory hippocampal synaptic mechanisms

1. The following sequence begins with the CA3 neuron firing action potentials that travel down both the Schaffer collateral towards the CA1 region as part of the hippocampal trisynaptic circuit (Module 10: Figure hippocampus) and the axonal collaterals that innervate the O-LM neurons as part of the local circuit.
2. The basket cells are also innervated by glutamatergic mossy fibres originating from the granule cells in the dentate gyrus (Module 10: Figure CA3 hippocampal local circuits). The glutamate released at the synapses on the dendrites of the basket cells acts on a number of glutamatergic synapses.
3. Glutamate activates AMPA receptors (AMPA), which are composed mainly of the GluR4 subunit that enables them to activate fast excitatory postsynaptic potentials (EPSPs) to bring about the rapid depolarization necessary to initiate action potential firing.
4. Glutamate also activates NMDA receptors (NMDARs) that gate both Na^+ and Ca^{2+} that will also contribute to the EPSP and they have also been implicated in spike timing.
5. When the depolarization reaches threshold, the action potentials induced in the O-LM neurons cells travel down to the web of axonal endings that innervate the distal dendrites of multiple CA3 neurons (Module 10: Figure theta oscillatory mechanisms).
6. Depolarization of the terminals stimulates the opening of $\text{Ca}_v2.1$ P/Q-type channels to introduce the Ca^{2+} that triggers the release of GABA. These P/Q channels are tightly coupled to the Ca^{2+} sensors on the exocytotic machinery and this close proximity greatly reduces the synaptic delay, which thus contributes to both the speed and reliability of this inhibitory input on to the CA3 neurons.
7. The GABA released from the terminals act on the type A γ -aminobutyric acid (GABA) receptors (GABA_A Rs) that gate chloride and give rise to inhibitory postsynaptic potentials (IPSPs).
8. Summation of these GABA-induced IPSPs hyperpolarizes the membrane sufficiently to curtail the action potential firing of the CA3 neuron thus completing the circuit that began with Step 1.
9. In addition to innervating the CA3 neurons, the O-LM interneuron cell also innervate other O-LM neurons, which means that the firing of each cell is rapidly terminated by an inhibitory input from its neighbours and this functions to synchronize the rhythm within the local population of interneurons.
10. The GABA released on to the basket cells activates GABA_A Rs to give IPSPs that will hyperpolarize the basket cell membrane to terminate its firing. This cessation of basket cell firing will then enable them to

begin to depolarize to set the stage for the next period of burst firing. The rate at which the membrane depolarizes is determined by the tonic excitatory drive (see below), which represents the pacemaker mechanism responsible for determining the periodicity of this network oscillator.

Tonic excitatory drive

In order for the O-LM/CA3 local circuit to oscillate it appears to require the input of a tonic excitatory drive. If the hippocampus is isolated from the other regions of the brain, it is incapable of generating theta oscillations. It requires a tonic excitatory drive that seems to be provided by neurons located within the septum and particularly within the medial septum-diagonal band of Broca (MS-DBB), which contains both GABAergic and cholinergic neurons that project to the hippocampus to innervate both the basket cells and the CA3 pyramidal cells (Module 10: Figure CA3 hippocampal local circuits).

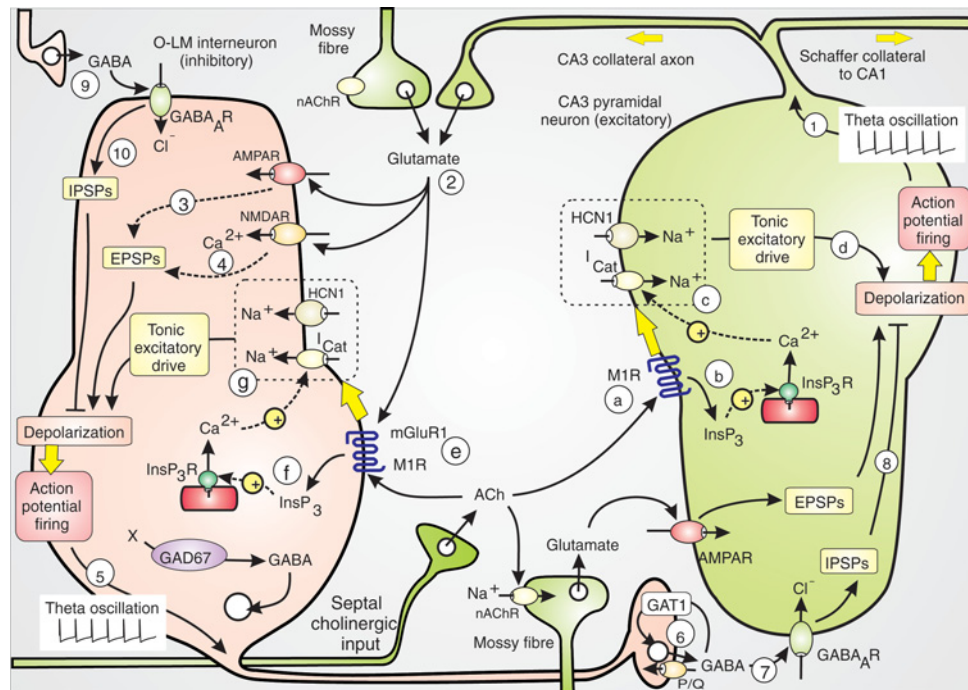
When rodents explore a new environment, 4–10 Hz theta oscillations, which are driven by cholinergic input from the medial septum, sweep through the hippocampus. This theta response can also be induced by injecting muscarinic agonists into the hippocampus. Lesions of the medial septal area, which disrupt the theta rhythm, interfere with spatial memory. The general idea is that the subcortical cholinergic input might control hippocampal memory processing by inducing fast network theta and gamma oscillations.

Particular attention is focused on their cholinergic input, which appears to act by stimulating metabotropic receptors located on both the CA3 and O-LM neurons (Module 10: Figure theta oscillatory mechanisms):

CA3 metabotropic responses

- a. Acetylcholine (ACh) acts through muscarinic 1 receptors (M1Rs) (Module 1: Table G protein-coupled receptors), which are known to be coupled through the heterotrimeric G protein G_q to phospholipase C_β (PLC_β) to activate the inositol 1,4,5-trisphosphate (InsP_3) Ca^{2+} signalling cassette (Module 2: Figure InsP_3 and DAG formation). The M1Rs may also act by inhibiting the SK Ca^{2+} -activated K^+ channels
- b. InsP_3 acts on the internal store to release Ca^{2+} , which might then stimulate a Ca^{2+} -dependent non-selective cationic current (I_{CAT}). The nature of the channel responsible for conducting I_{CAT} is not known, but likely candidates are TRPM4 and TRPM5 that perform a similar pacemaker function in the case of respiratory pacemaker mechanism (Module 10: Figure respiratory pacemaker mechanism). The I_{CAT} may provide the persistence inward current that contributes to the tonic excitatory drive necessary to maintain the theta oscillation.
- c. In addition to activating I_{CAT} , the M1Rs can also stimulate the hyperpolarizing-activated cyclic nucleotide-gated (HCN) channels, which are known to generate pacemaker activity in a number of excitable cells by maintaining a background influx of Na^+ that will contribute to the tonic excitatory drive.

Module 10: | Figure theta oscillatory mechanism



Hippocampal theta oscillatory mechanisms.

The inhibitory oriens-lacunosum moleculare (O-LM) cell (red) and the excitatory CA3 pyramidal neuron (green) interact with each other to form a network oscillator that is tuned to generate theta oscillations. The collateral axons of CA3 neurons release glutamate that depolarizes the O-LM cell whose axon terminals innervate the distal dendrites of the CA3 neurons to release GABA, which then acts on GABA_A receptors (GABA_AR) to provide the inhibitory postsynaptic potentials (IPSPs) that hyperpolarize the membrane to temporarily curtail the firing of the CA3 neurons. Operation of this self-contained network oscillator is regulated by a tonic excitatory drive provided by the septal cholinergic input that releases acetylcholine (ACh), which acts through muscarinic 1 receptors (M1Rs). These metabotropic receptors appear to act by stimulating channels (dashed box) to provide a pacemaker depolarization that drives oscillatory activity. See the text for information on the different signalling mechanisms that control this network oscillator.

- d. When the inhibitory influence of the basket cell (Steps 6–8) is temporarily withdrawn, the combination of inward currents that make up the tonic excitatory drive will begin to depolarize the membrane thus enabling the CA3 to set up the next theta oscillatory cycle. The firing of the CA3 neuron is thus determined by the fluctuating balance between the intermittent membrane hyperpolarization provided by the inhibitory GABAergic pathway (Steps 6–8) and the membrane depolarization activated by the tonic excitatory drive (Module 10: Figure theta oscillatory mechanisms).

Basket cell metabotropic responses

- e. Like the CA3 neurons described above, the basket cells appear to have a tonic excitatory drive activated by metabotropic receptors such as the M1Rs and the mGluR1s. There is a strong expression of mGluR1 receptors on the O-LM interneuron. These metabotropic receptors stimulate the formation of InsP₃ and release Ca²⁺ which may function to stimulate I_{CAT} that contributes to the tonic excitatory drive. This pacemaker activity may also be driven by HCN1 channels that are also activated by these metabotropic receptors.

This tonic excitatory drive that activates these theta oscillations seems to be responsible for metaplasticity, which is a key mechanism for enhancing learning and memory.

Thalamus

The thalamus is located in the forebrain where it has both sensory and motor functions. Most of the sensory information (except for olfaction) entering the brain is directed to the thalamus where it is processed before being directed to the overlying cortex.

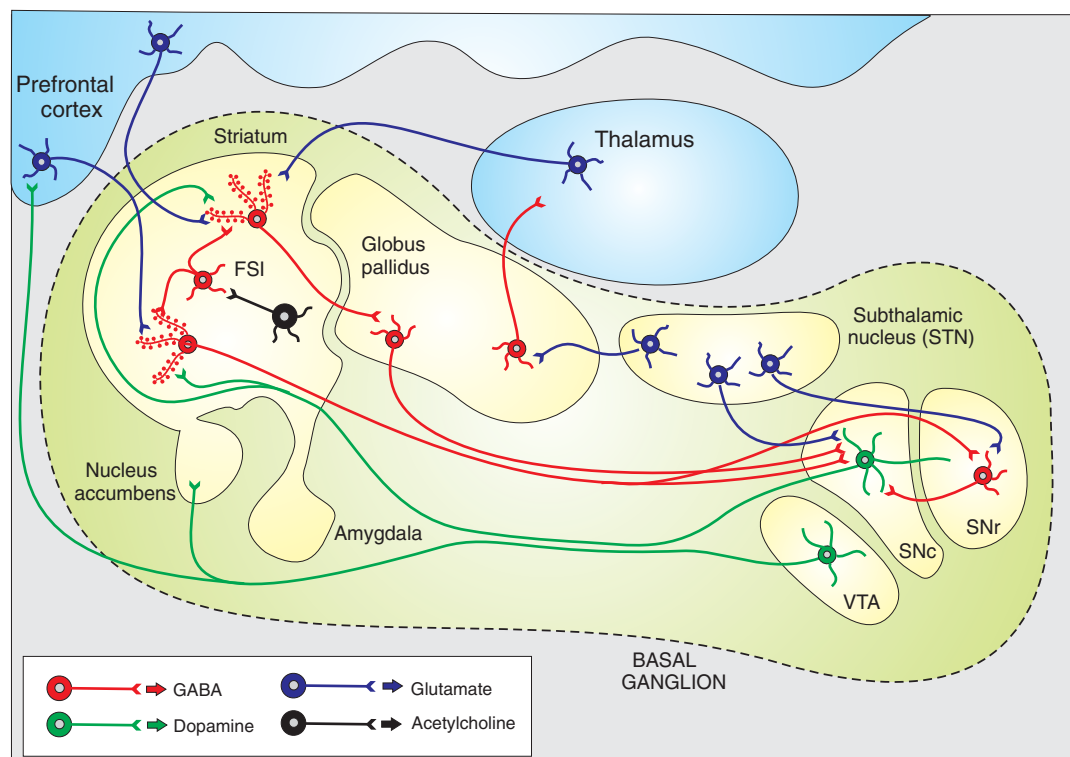
Basal ganglia

The two basal ganglia, which are located in each hemisphere at the base of the forebrain, are a self-contained functional group of neurons organized into a number of units known as nuclei (Module 10: Figure brain anatomy). The main nuclei are the striatum, globus pallidus, nucleus accumbens, substantia nigra, subthalamic nucleus (STN) and ventral tegmental area (VTA) (Module 10: Figure basal ganglia). The basal ganglia are responsible for regulating a number of different functions. One function is to control motor systems where it normally exerts an inhibitory function. When a particular motor function is required this inhibition is temporarily released following input from the prefrontal cortex that exerts an executive function.

Striatum

The striatum is one of the major nuclei of the basal ganglia (Module 10: Figure basal ganglia). It is the main input nucleus of the basal ganglia in that it receives information

Module 10: | Figure basal ganglia



Organization of the basal ganglia.

The basal ganglion is a self-contained functional unit containing a number of nuclei: striatum, nucleus accumbens, globus pallidus, subthalamic nucleus (STN), substantia nigra pars compacta (SNc), substantia nigra pars reticulata (SNr) and ventral tegmental area (VTA).

from many brain regions such as the cortex, hippocampus, thalamus and the neighbouring amygdala. It also receives an input for the substantia nigra pars compacta (SNc) that delivers dopamine, which is a major modulator of striatal function.

The main neuronal cell type in the striatum is the medium spiny neurons (MSNs) of which there are two main types: D1 dopamine receptor-expressing MSNs (D1+ MSNs) and the D2 dopamine receptor-expressing MSNs (D2+ MSNs). These MSNs are GABAergic neurons that provide inhibitory inputs that are restricted to the other nuclei within the basal ganglia such as the globus pallidus and the SNc.

The striatum also contains a small population of interneurons such as the GABAergic fast-spiking interneurons (FSIs) and the cholinergic interneurons (Module 10: Figure basal ganglia).

Medium spiny neuron (MSN)

Medium spiny neurons (MSNs) located in the striatum and nucleus accumbens have been implicated in drug addiction in that they undergo a process of long-term potentiation (LTP) as part of the change in the neural circuitry whereby individuals learn to anticipate a reward. There are two types of MSNs with very different electrophysiological properties. The D1 dopamine receptor-expressing MSNs (D1+ MSNs) are in a direct pathway that projects to the dopamine centre in the substantia nigra, whereas

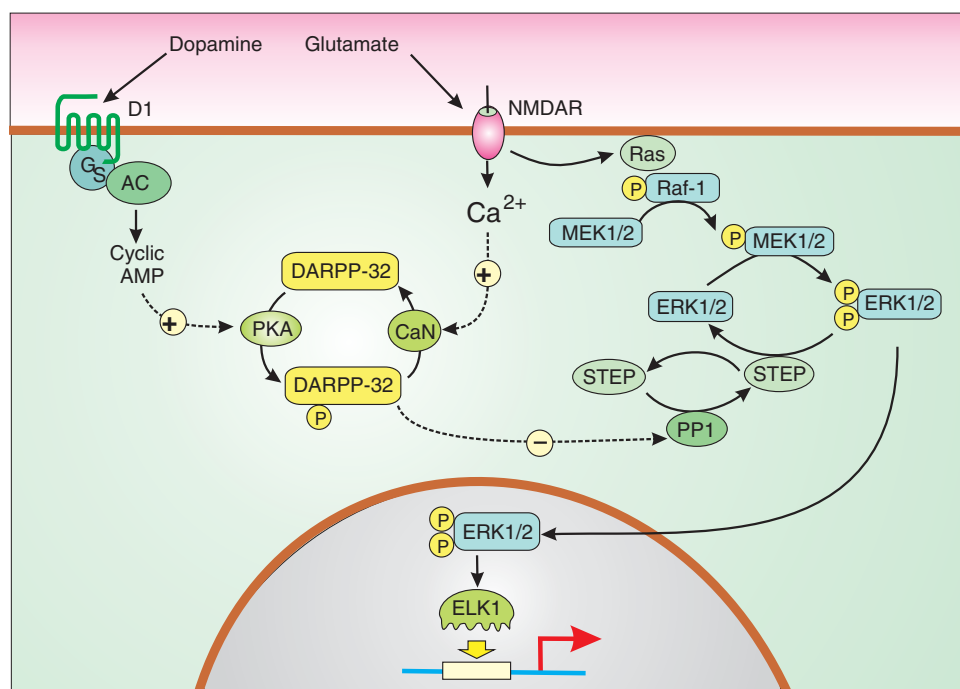
the D2 dopamine receptor-expressing MSNs (D2+ MSNs) send projections to the globus pallidus and thus influence the dopamine centres indirectly (Module 10: Figure basal ganglia).

A selective loss of these medium spiny neurons occurs during the development of Huntington's disease.

D1 dopamine receptor-expressing MSNs (D1 + MSNs)

The changes in neural plasticity associated with addiction in the D1 dopamine receptor-expressing MSNs (D1 + MSNs) seems to depend on the convergent action of signalling pathways induced by both dopamine and glutamate (Module 10: Figure medium spiny neuron signalling). Dopamine acts through the D1 receptor to activate the cyclic AMP signalling pathway, whereas glutamate acts through the *N*-methyl-d-aspartate (NMDA) receptor to activate both the entry of Ca^{2+} and the activation of the extracellular-signal-regulated kinase (ERK) pathway of the mitogen-activated protein kinase (MAPK) signalling system. Dopamine acting through cyclic AMP is able to alter the activity of protein phosphatase 1 (PP1), which normally acts through striatal-enriched phosphatase (STEP) to keep the ERK pathway in check.

One of the functions of the ERK pathway is to activate gene transcription, and this might be responsible for the neural plasticity underlying drug addiction. Activation of the NMDA receptor by itself usually has little effect on gene transcription, because the phosphorylation of ERK1/2 is kept in check by STEP, which is main-

Module 10: | Figure medium spiny neuron signalling**Convergence of the signalling pathways activated in medium spiny neurons by dopamine and glutamate.**

Medium spiny neurons have D₁ dopamine receptors that act through the cyclic AMP signalling pathway, and *N*-methyl-D-aspartate (NMDA) receptors that activate Ca²⁺ entry and can induce the extracellular-signal-regulated kinase (ERK) pathway of the mitogen-activated protein kinase (MAPK) signalling system (Module 2: Figure ERK signalling). There is evidence of cross-talk between these two pathways that converge on dopamine and cyclic AMP-regulated phosphoprotein of apparent molecular mass 32 kDa (DARPP-32), which is a potent inhibitor of protein phosphatase (PP1). (See the text for further details.)

tained in its active dephosphorylated state by protein phosphatase 1 (PP1). If glutamate receptors are activated concurrently with dopamine receptors, the latter can facilitate the ERK pathway by reducing the activity of PP1 by up-regulating its inhibitor dopamine and cyclic AMP-regulated phosphoprotein of apparent molecular mass 32 kDa (DARPP-32). When DARPP-32 is phosphorylated, it can begin to inhibit PP1 and this allows inactive phosphorylated STEP to accumulate. Once the STEP brake is removed, phospho-ERK accumulates, and will begin to drive the transcriptional events that will alter the phenotype of the neurons.

D2 dopamine receptor-expressing MSNs (D2 + MSNs)

The excitatory synapses on the D2 dopamine receptor-expressing MSNs (D2 + MSNs) display an unusual form of long-term depression (LTD). There is a strong relationship between Ca²⁺ and synaptic plasticity that applies to the mechanism of both long-term potentiation (LTP) and LTD. High concentrations of Ca²⁺ tend to trigger LTP, whereas lower concentrations activate LTD (Module 10: Figure Ca²⁺-induced synaptic plasticity). There are a number of proposals for the mechanisms that generate the Ca²⁺ that activates LTD and these mechanisms may vary between cell types. In the case of the D2 + MSNs and dentate gyrus neurons, anandamide (Module 1: Figure anandamide) activates TRPV1 channels to provide the Ca²⁺ signal that induces LTD by stimulating the endocyt-

osis of AMPARs (see the retrieval mechanism in Module 10: Figure Ca²⁺-induced synaptic plasticity).

Globus pallidus

The globus pallidus is one of the main output nuclei of the basal ganglia (Module 10: Figure basal ganglia). It receives its main input from the striatum and sends its inhibitory output mainly to the thalamus and to the brain stem.

Nucleus accumbens (NAc)

The nucleus accumbens (NAc), which is also known as the ventral striatum, is a key brain region responsible for reward, motivation and addiction. The NAc is an integral part of the basal ganglia and is closely linked to the ventral tegmental area (VTA) (Module 10: Figure basal ganglia). Both dopamine and 5-hydroxytryptamine are particularly active in the NAc. Many drugs act to increase the production of dopamine, which has been linked to compulsive drug use.

Substantia nigra

The substantia nigra, which is part of the basal ganglia (Module 10: Figure basal ganglia), consists of two nuclei: the substantia nigra pars compacta (SNc) and the substantia nigra pars reticulata (SNr).

Substantia nigra pars compacta (SNc)

The SNc is one of the substantia nigra nuclei that belong to the basal ganglia (Module 10: Figure basal ganglia). It is part

of the dopaminergic arousal system and is responsible for the dopaminergic input to the striatum. These SNc neurons have dopaminergic (DA) neuronal pacemaker mechanism that provides a tonic release of dopamine that modulates striatal activity. Alterations in the activity of these SNc neurons seem to be responsible for Parkinson's disease.

Substantia nigra pars reticulata (SNr)

The SNr is one of the substantia nigra nuclei that belong to the basal ganglia (Module 10: Figure basal ganglia). It receives its main input from the striatum and sends its inhibitory output mainly to the thalamus.

Subthalamic nucleus (STN)

The subthalamic nucleus (STN) is one of the nuclei belonging to the basal ganglia (Module 10: Figure basal ganglia). It receives inputs from the striatum and from the cortex. The main STN cell types are the glutamatergic neurons that project to the globus pallidus and the substantia nigra pars reticulata (SNr).

Ventral tegmental area (VTA)

The ventral tegmental area (VTA), which is a component of the limbic system, is an integral part of the basal ganglia (Module 10: Figure basal ganglia). It contains dopaminergic neurons that send inputs to both the nucleus accumbens (NAc) and to the cortex. The VTA plays a role in reward learning and has been implicated in the mode of action of addictive drugs (nicotine, amphetamines and cocaine) that may function by enhancing the efficacy of dopamine signalling in the NAc.

Release of dopamine from the VTA terminals in the cortex contributes to the tonic excitatory drive (Module 10: Figure tonic excitatory drive and neuronal rhythms). Dysfunction of this dopamine arousal system may be one of the contributory factors responsible for schizophrenia (Module 12: Figure schizophrenia).

Neurogenesis

One of the basic dogmas of neurobiology was that once development had ceased and the brain had formed, there was no further birth of neurons. While this is the case for much of the brain, it is now evident from recent studies on rodents that new neurons are being generated continuously in at least two regions of the brain: the subventricular zone and the dentate gyrus. Proliferation of the neuronal precursor cells seem to be regulated by ciliary neurotrophic factor (CNTF). In the case of the subventricular zone, the newly formed cells enter a rostral migratory stream that carries them to the olfactory bulb, where they become fully functional neurons. In the case of the dentate gyrus, which contains the granule cells that are a part of the trisynaptic circuit in the hippocampus (Module 10: Figure hippocampus), the processes of proliferation, migration and differentiation are much more localized. In the dentate gyrus of rodents, approximately 6% of the 2 million cells are replaced each month, which means that neurogenesis is a relatively slow process. Neurogenesis has also been described in humans, but appears to be restricted to

the dentate gyrus. The absence of neurogenesis in most of the human brain may be necessary in order for us to retain accumulated experiences as long-term memories. It is therefore significant that the circuitry of the brain region that is specialized for the storage of short-term memory is constantly being turned over. Perhaps the efficiency of this short-term memory store is maintained by constantly replacing new neurons.

As the name implies, neurogenesis is a process whereby new neurons are produced in the adult brain through a multistep process involving proliferation, survival, migration and differentiation that essentially recapitulates the events of neuron formation during development (Module 10: Figure granule cell neurogenesis). The new neurons are derived from neural stem cells located in a neurogenic niche. The canonical Wnt/ β -catenin pathway plays an important role in the maintenance and proliferation of the stem cell population within this niche. In the case of rodents, the new granular cells that develop from these stem cells become fully functional, indicating that this brain region has the capacity of self-renewal that may be essential for its role in short-term memory.

This process of neurogenesis is controlled by brain-derived neurotrophic factor (BDNF) acting through TrkB receptors. A major determinant of neurogenesis in the dentate gyrus is stress, which seems not to influence proliferation as much as the subsequent survival of the progenitor cells and their integration into the neural circuitry. Cell survival is an important factor, because approximately half of the new cells die 4 days after proliferation begins. An increase in the proportion of cells that die may account for the decline in the volume of this hippocampal region. Such a decline in hippocampal volume has been observed in patients suffering from temporal lobe epilepsy and manic-depressive illness. The neurogenesis hypothesis suggests that manic-depressive illness is caused by a stress-induced decline in the volume of the hippocampus.

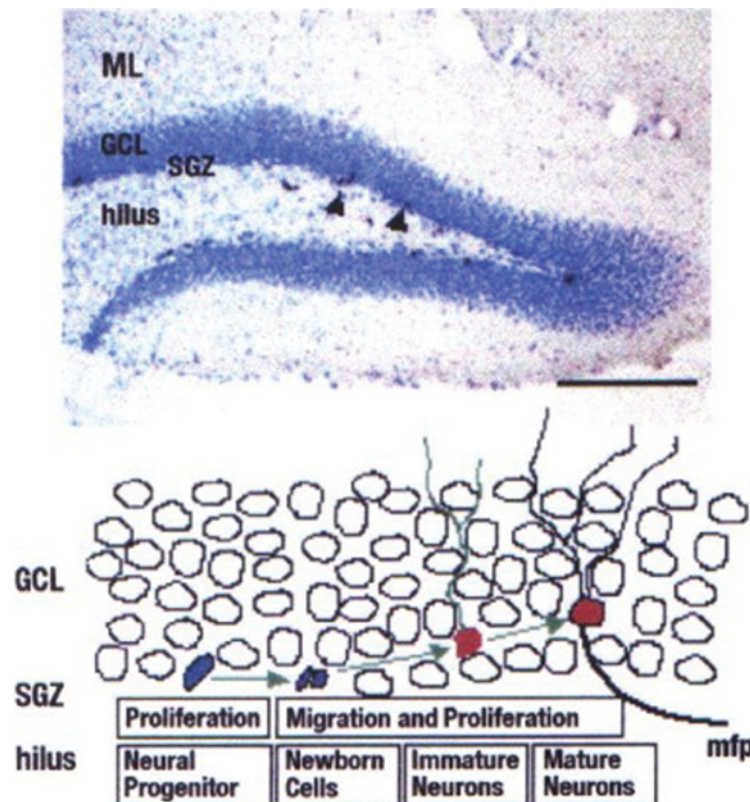
Neuronal morphology

Neurons are highly differentiated cells specialized to receive information at one end (the dendritic tree) and then to transmit it to other neurons from synaptic endings at the opposite side. Neurons can be divided into five main regions (Module 10: Figure neuronal morphology).

The neuronal endoplasmic reticulum is found in all of these regions (Module 10: Figure neuronal structure) in that it forms a continuous network that extends throughout the neuron and may function as a neuron-within-a-neuron.

Spines

The minute protuberances that encrust the surface of the dendrites are critical elements of synaptic transmission in that they receive information from other neurons. In general, each spine is connected to single synaptic endings to create the synapse, an autonomous unit that is the computational microprocessor used to store and transmit information in the brain (Module 10: Figure synaptic organization). Each neuron has thousands of these autonom-

Module 10: | Figure granule cell neurogenesis**Neurogenesis of granule cell neurons in the dentate gyrus region of the hippocampus.**

Putative stem cells in the subgranular zone (SGZ) proliferate continuously to provide a population of neuronal progenitor cells that have a neuronal lineage, but retain the capacity to proliferate. After several rounds of proliferation, these progenitors stop proliferating and then migrate into the granular cell layer (GCL), where they differentiate into fully functional granular cells. During this process of differentiation, the new neurons extend dendrites out into the molecular layer (ML) and an axon, which is the mossy fibre projection (mfp), grows out to innervate the hippocampal CA3 neurons, thus forming a part of the trisynaptic circuit (Module 10: Figure hippocampus). Reproduced from *Biol. Psychiatry*, Vol. 56, Duman, R.S., Depression: a case of neuronal life and death, pp. 140–145. Copyright (2004), with permission from the Society of Biological Psychiatry; see Duman 2004.

ous units, enabling it to receive many bits of information often relayed to it from different neurons.

Each spine is composed of a shaft of variable length and a bulbous head with a diameter of approximately 1 μm . Immediately below the spine surface that faces the synaptic cleft there is a postsynaptic density (PSD), where many of the signalling components responsible for synaptic transmission are located. A prominent feature of many spines is the spine apparatus, which is an extension of the endoplasmic reticulum (ER) that runs through the dendrites (Module 10: Figure dendritic endoplasmic reticulum). Both inositol 1,4,5-trisphosphate (InsP_3) receptors and ryanodine receptors (RYRs) have been located on the spine apparatus. In some cases, portions of this spine apparatus come to lie close to the PSD (Module 10: Figure hippocampal synapse). A protein called synaptopodin is necessary for the formation of the spine apparatus in some neurons (striatal and hippocampal neurons), but not in cerebellar neurons. The spine apparatus is absent from synaptopodin^{-/-} mice, which also show defects in hippocampal long-term potentiation (LTP) and spatial learning.

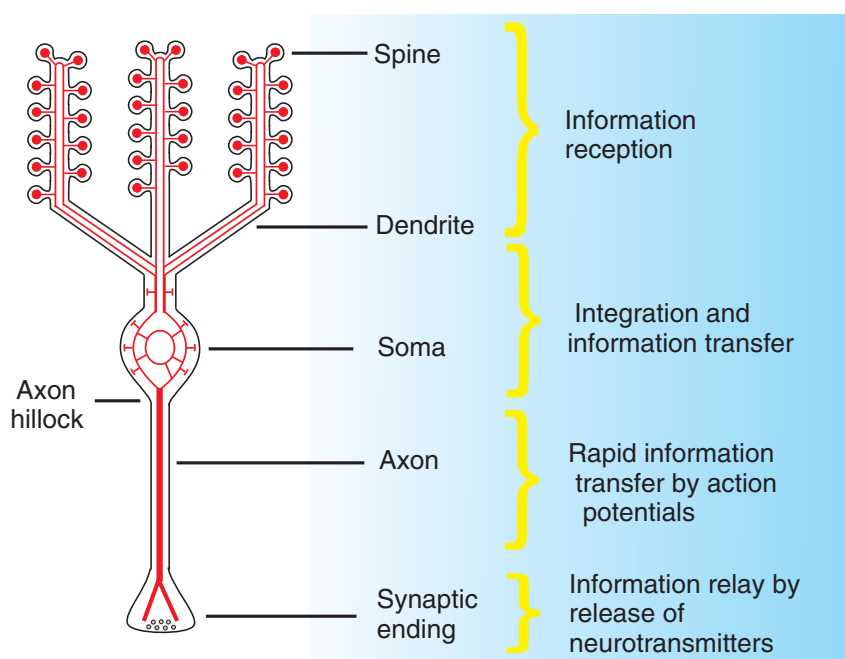
Postsynaptic density (PSD)

The postsynaptic density (PSD) is a specialized region associated with that part of the postsynaptic membrane that faces the presynaptic membrane (Module 10: Figure postsynaptic density). It shows up as a dense region in electron micrographs because of the large number of signalling components, cytoskeletal proteins and scaffolding elements that are crammed into this small region to organize the signalling events that occur during synaptic transmission.

The PSD can be divided into groups of functional elements such as the postsynaptic density (PSD) scaffolding and adaptor components, the postsynaptic density (PSD)-linked receptors, the postsynaptic density (PSD) cytoskeletal elements and the postsynaptic density (PSD) signalling elements.

Postsynaptic density (PSD) scaffolding and adaptor components

Postsynaptic density (PSD) scaffolding and adaptor components provide the framework that organizes many of signalling components, some of which face the synaptic cleft (neurotransmitter receptors and adhesion molecules), whereas others are directed towards the cytoplasm

Module 10: | Figure neuronal morphology**Neuronal morphology illustrating the main functional regions of a typical neuron.**

The spines and dendrites represent the receptive fields where information is received from other neurons. The soma, containing the nucleus, integrates all the information, and if the net result is membrane depolarization, then a special region known as the axon hillock (the region where the axon connects to the soma) generates an action potential responsible for rapid information transfer down the axon. When this action potential arrives at the synaptic endings, the membrane depolarization provides the signal to trigger release of the stored transmitters that carry information on to the next neuron in the circuit. Each region of the neuron has a specific function in the linear transfer of information from the dendritic region to the synaptic ending. The endoplasmic reticulum shown in red extends throughout the neuron and may be considered as a neuron-within-a-neuron (Module 10: Figure neuronal structure).

(signalling molecules and cytoskeletal modifying elements). A major component of this region is the PSD-95 family [PSD-95, PSD-93, and synapse-associated proteins 102 (SAP102) and 97 (SAP97)] of PDZ domain scaffolding proteins that are highly enriched in this synaptic region.

Neurologin

The neuroligin family (NL-1 to NL-4) are single transmembrane domain proteins located in the postsynaptic membranes of synaptic spines. There is a large extracellular acetylcholinesterase-like domain while the cytoplasmic tail has numerous interaction domains one of which is used to attach neuroligins to members of the postsynaptic density (PSD) scaffolding and adaptor components such as PSD-95 in the case of NL-1 (Module 10: Figure postsynaptic density). The NL-1 is found mainly in the excitatory synapses, whereas NL-2 is located in inhibitory synapses. This NL-2, which bridges the synaptic cleft at inhibitory neuronal junctions, associates with the adaptor protein gephrin. The NL-3 is more widely dispersed whereas NL-4 is strongly expressed in glycinergic neurons.

NL-1 seems to play a role in synaptic potentiation and this seems to be associated with an increase in its expression. The trafficking and synaptic localization of NL-1 seems to depend on synaptic activity. The increase in Ca^{2+} that occurs during synaptic activity stimulates Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), which then phosphorylates NL-1. This phosphorylation

of NL-1 contributes to its action in potentiation excitatory synapses.

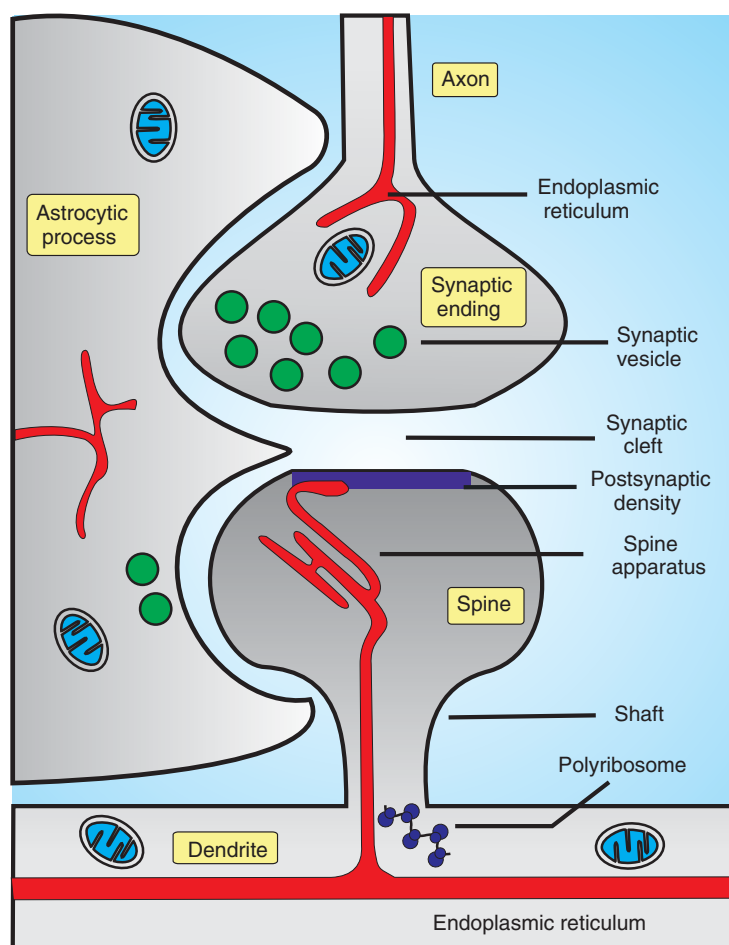
Given the importance of these neuroligins is synaptic function, it is clear that their expression needs to be carefully balanced to maintain a normal excitatory-to-inhibitory ratio. These neuroligins may thus make an important contribution to the excitation-inhibition (E-I) balance that ensures the brain rhythms operate correctly. Alterations in this E-I balance may occur in psychiatric diseases, such as bipolar disorder (BD). Mutations in the genes coding for NL-1, NL-3, NL-4 and neurexin-1 have also been associated with autism spectrum disorders (ASDs).

The effect of inflammation in Alzheimer's disease (AD) results in an increase in the activity of the gene repressor methyl-CpG-binding protein 2 (MeCP2) that reduces the expression of neuroligin 1 (NL-1) and this may play a significant role in enhancing the decline in cognition (Module 12: Figure Inflammation and Alzheimer's disease).

Postsynaptic density (PSD)-linked receptors

Most excitatory synapses use the neurotransmitter glutamate, which can act through three types of glutamate receptor: the α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, the *N*-methyl-D-aspartate (NMDA) receptors and the group I metabotropic glutamate receptors (mGluRs). The AMPA and NMDA receptors are located in the region of the postsynaptic

Module 10: | Figure synaptic organization

**Structural organization of a tripartite synaptic ending.**

The synapse, which is the microprocessor of the brain, connects one neuron (the synaptic ending) to the spine of another neuron. The presynaptic ending has the synaptic vesicles that are released into the synaptic cleft to activate receptors on the spine. The bulbous head of the latter is connected to the dendrite by a shaft. At the base of the shaft, where it connects to the dendrite, there usually is a single polyribosome, which may play a role in the neuronal protein synthesis necessary for memory acquisition and consolidation. Many spines, but by no means all, contain extensions of the dendritic endoplasmic reticulum (red). Such synaptic endings are usually enclosed within an astrocytic process, and the three components thus constitute a tripartite synapse (Module 7: Figure astrocyte tripartite synapse).

density (PSD), where they respond to glutamate released into the synaptic cleft. The mGluRs tend to lie outside the synaptic cleft, and thus respond to glutamate that spills out of the cleft (yellow arrows in Module 10: Figure postsynaptic density). The AMPA and NMDA receptor subunits have long C-terminal cytoplasmic tails that have peptide motifs such as ESEV and ESDV that enable them to bind to synapse-associated protein 97 (SAP97) and PSD-95 respectively.

Although the NMDA receptor is a fairly constant feature of the synapse, the AMPA receptor is much more variable with regard to its level of expression and insertion into the synapse. Its trafficking and translocation into the postsynaptic membrane (see step 3 in Module 10: Figure Ca^{2+} -induced synaptic plasticity) is a significant aspect of Ca^{2+} effects on synaptic plasticity, and is regulated by various proteins such as the glutamate-receptor-interacting protein (GRIP) and Stargazin. The latter is also connected to PSD-95.

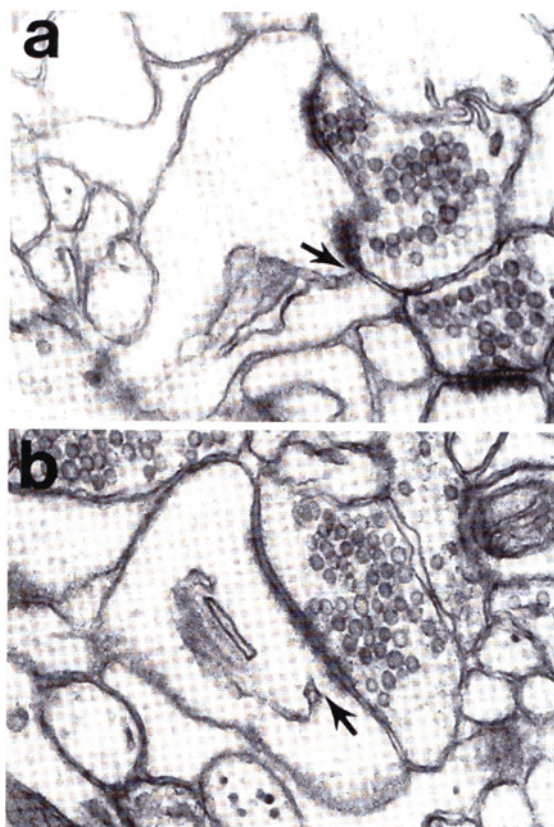
GRIP is also connected to the ephrin receptor (EphR), which makes contact with ephrin embedded in the presynaptic membrane.

Postsynaptic density (PSD) cytoskeletal elements

There are numerous actin filaments and actin-associated proteins within the postsynaptic density (PSD) (Module 10: Figure postsynaptic density). These actin filaments play an active role in changing the shape of the spines, particularly during learning and memory.

Postsynaptic density (PSD) signalling elements

The postsynaptic density (PSD) contains a large number of signalling components that regulate various signalling pathways during synaptic transmission. A prominent feature is the A-kinase-anchoring proteins (AKAPs) that are attached to the PSD scaffold. AKAP79, which binds protein kinase A (PKA) and protein phosphatase 2A (PP2A), is attached to synapse-associated protein 97

Module 10: | Figure hippocampal synapse

The structural organization of a typical synapse from the CA1 region of the hippocampus.

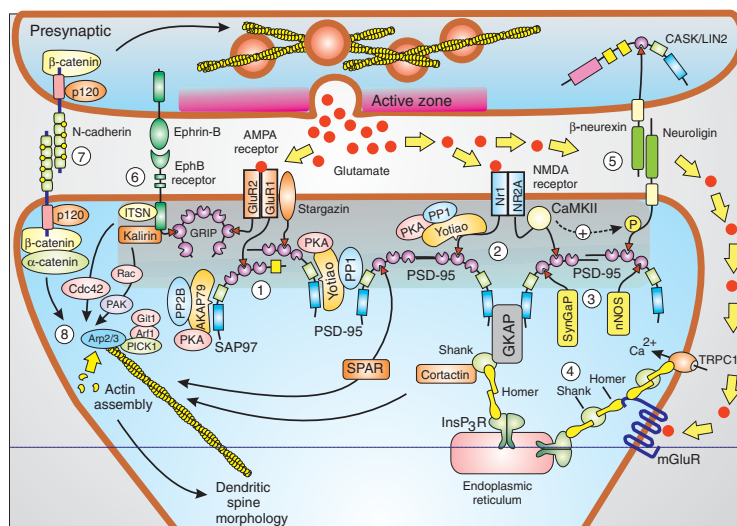
The presynaptic endings are loaded with synaptic vesicles, whereas the postsynaptic endings (i.e. the spines) are relatively empty, except for the spine apparatus. The arrows point to extensions of the spine apparatus that come into close contact with the postsynaptic density (PSD). The molecular organization of the PSD is illustrated in Module 10: Figure postsynaptic density). Reproduced, with permission, from Spacek, J. and Harris, K.M. (1997) Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. *J. Neurosci.* 17:190–203. Copyright (1997) by the Society for Neuroscience; see Spacek and Harris 1997.

(SAP97). Yotiao, which binds PKA and protein phosphatase 1 (PP1), is attached to both PSD-95 and to the NR1 subunit of the *N*-methyl-D-aspartate (NMDA) receptor.

All these different elements are connected to form a complex web of interacting signalling systems that can be grouped into the six functional zones shown in Module 10: Figure postsynaptic density:

1. The C-terminal cytoplasmic tails of the α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor subunits make contact with different scaffolds/adaptors. Glutamate receptor 2 (GluR2) interacts with the glutamate-receptor-interacting protein (GRIP), whereas GluR1 interacts with SAP97, which is also known as Disc, large homology 1 (DLG1). The latter also interacts with AKAP79, which associates with PKA and PP2B [calcineurin (CaN)]. The transmembrane Stargazin protein that interacts with the AMPA receptor binds to the abundant scaffold protein PSD-95, which associates with PKA and PP1. Through these interactions, the AMPA receptor associates with the kinase (PKA) and the phosphatases (PP2B and PP1) that determine its channel activity. For example, the PKA-dependent phosphorylation of Ser-845 contributes both to recycling and synaptic plasticity (Module 3: Figure AMPA receptor phosphorylation).
2. The scaffolding protein PSD-95, which is linked to subunits of the NMDA receptor, is also linked to a variety of proteins such as spine-associated RapGAP (GTPase-activating protein) (SPAR) and guanylate kinase-associated protein (GKAP) that links to Shank, which controls cortactin, another regulator of actin.
3. The PSD-95 scaffolding complex interacts with various signalling components such as SynGAP, which acts to reduce Ras activity (Module 10: Ca^{2+} -induced synaptic plasticity), and neuronal nitric oxide (nNOS).
4. The adaptor protein Homer provides a link between Shank and the inositol 1,4,5-trisphosphate receptor (InsP_3R), which may also be linked through Homer to the C-terminal region of the metabotropic glutamate receptor (mGluR). Homer may also link the mGluR to the canonical transient receptor potential 1 (TRPC1) (Module 10: Figure postsynaptic density).
5. The PSD-95 complex plays a role in synaptic adhesion in that it is linked to neuroligin-1 (NL-1), which binds to β -neurexin in the presynaptic membrane. This transsynaptic heterophilic connection plays a role in both synapse formation and maturation. The cytoplasmic tail of NL-1 has a number of protein–protein interaction domains and one of these anchors it to PSD-95. The trafficking and synaptic localization of NL-1 seems to depend on synaptic activity. The increase in Ca^{2+} that occurs during synaptic activity activates Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), which then phosphorylates NL-1. This phosphorylation of NL-1 contributes to its action in potentiation excitatory synapses.
6. An ephrin (Eph) receptor signalling pathway plays a critical role in regulating spine morphogenesis by controlling the remodelling of actin (Module 1: Figure Eph receptor signalling). Ephrin-B embedded in the presynaptic membrane activates the EphB receptor in the postsynaptic membrane (Module 10: Figure postsynaptic density). The EphB receptor has a C-terminal PDZ domain-binding motif that links into GRIP, which is also connected to the AMPA receptor. The EphB receptor also binds the guanine nucleotide exchange factors intersectin (ITSN) and kalirin-7. ITSN acts through Cdc42 to regulate actin assembly by activating the actin-related protein 2/3 (Arp2/3) complex (Module 2: Figure Cdc42 signalling). Kalirin-7, which is a guanine nucleotide exchange factor (GEF), acts through Rac and p21-activated kinase (PAK) to control actin assembly (Module 2: Figure Rac signalling).
7. One of the classical cadherin isoforms, N-cadherin, functions in both the formation and stabilization of synapses by binding together the pre- and postsynaptic membranes (Module 10: Figure postsynaptic density). The cadherin complexes are clustered on the edges of

Module 10: | Figure postsynaptic density



Molecular components of the postsynaptic density in an excitatory glutamatergic synapse.

The postsynaptic density (PSD) is an extensive macromolecular complex consisting of a wide range of signalling components organized on an elaborate framework of scaffolding proteins. The major scaffolding components belong to the PSD-95 family, represented here as PSD-95 and synapse-associated protein 97 (SAP97). They have three tandem PDZ domains (purple circles) that enable them to link the three types of glutamate receptor [α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), *N*-methyl-D-aspartate (NMDA) and metabotropic glutamate receptor (mGluR)] to various signalling components and adaptor molecules. The PSD is a very crowded region, and only a selected number of the full complement of molecules have been included, and these have been grouped into seven functional zones (see the text for further details).

the postsynaptic density and have both pre- and postsynaptic functions. The cadherin in the postsynaptic membrane is linked to actin through the typical dynamic β -catenin- α -catenin-Arp2/3 complex used by these cadherins (Module 6: Figure classical cadherin signalling). On the presynaptic side, β -catenin appears to have some role in organizing the actin-based molecular complexes that cluster the synaptic vesicles near the active zone.

8. A number of components function to remodel actin, which is particularly important for synaptic plasticity. The p120 catenin subunit attached to the cytoplasmic domain of N-cadherin contributes to actin assembly by activating Cdc42 (Module 2: Figure Cdc42 signalling) and Rac (Module 2: Figure Rac signalling) signalling mechanisms. In the latter case, p120 activates the p190RhoGAP that inhibits Rho, which will indirectly enhance signalling through Rac and Cdc42. The Arp2/3 complex that polymerizes actin is regulated by the Glt1-Arf1-PICK1 signalling cascade, which is explained in more detail in the synaptic plasticity section (see Step 7 in Module 10: Ca^{2+} -induced synaptic plasticity).

Neuronal endoplasmic reticulum

An important feature of neuronal structure is the endoplasmic reticulum that forms a continuous network that extends throughout the neuron (Module 10: Figure neuronal structure). In effect, this internal membrane system can be considered as a neuron-within-a-neuron.

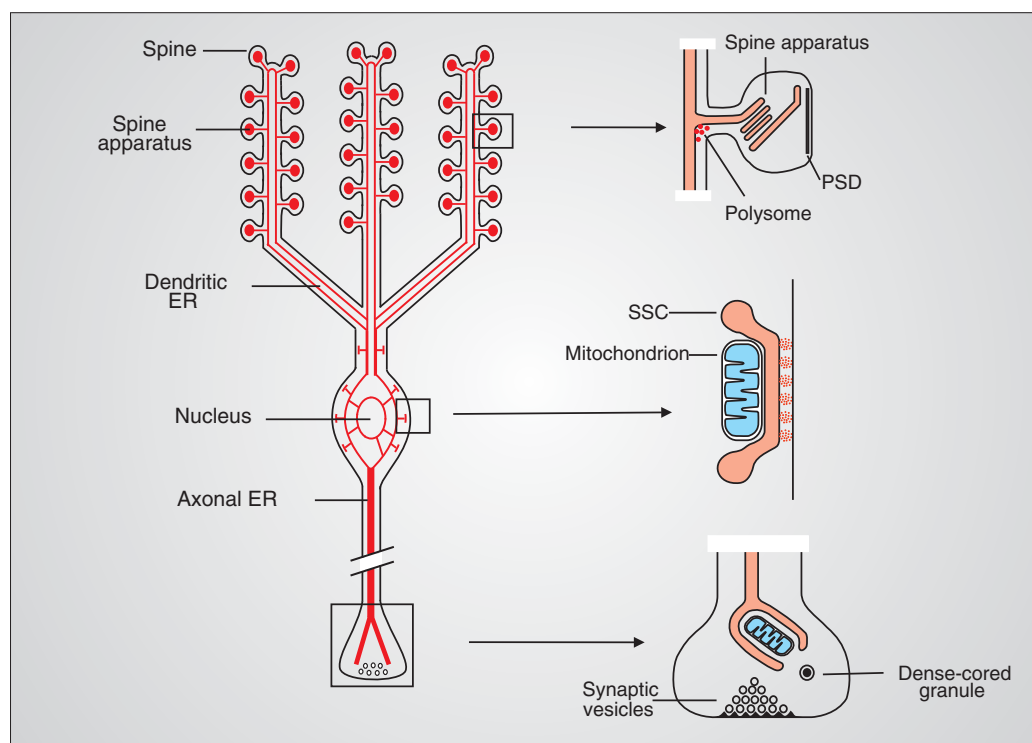
Dendrites

Dendrites are tubular structures emanating from the soma that bifurcate to form elaborate branched structures (Module 10: Figure neuronal morphology) with patterns that are characteristic for each type of neuron. The organization of this complex dendritic tree is nicely illustrated when it is filled with a Ca^{2+} -sensitive dye (Module 10: Figure Purkinje cell input-specific Ca^{2+} signal). Not only do they provide a framework to organize the spines, but also they can receive inputs, as occurs for the climbing fibres on the Purkinje cells (Module 10: Figure cerebellar climbing fibres). In some neurons, this input can induce dendritic transmitter release.

An important component of the dendrites is a meshwork of endoplasmic reticulum (ER), which sends connections into many of the spines (Module 10: Figure dendritic endoplasmic reticulum).

Soma

The soma contains the nucleus and much of the synthetic machinery required for maintaining the neuron. Proteins synthesized in the soma are packaged into vesicles to be dispersed throughout the neuron by an axonal transport system run by the microtubules. The soma also contains an elaborate endoplasmic reticulum (ER), portions of which come into close contact with the plasma membrane to form subsurface cisternae (SSC) (Module 10: Figure neuronal structure) that may play a role in regulating neuronal excitability. Junctophilin 3 (JP3) may play a critical role in maintaining the structural organization of these subsurface cisternae (SSCs) where the voltage-operated Ca^{2+}

Module 10: | Figure neuronal structure**Organization of the endoplasmic reticulum (ER) in a typical neuron.**

Beginning with the soma, the endoplasmic reticulum (ER) (shown in red on the left and coloured pink on the right) is attached to the nuclear membrane and extends outwards to invade all parts of the neuron. Within the soma and proximal dendrites, it comes into close contact with the plasma membrane to form specialized structures known as subsurface cisternae (SSC). The ER extends further up into the dendrites and sends small protuberances into many of the spines. The ER lying within the spine is often referred to as the spine apparatus. On the opposite side of the cell, the ER extends down the axon and into the synaptic ending.

channels, ryanodine receptors and the Ca^{2+} -sensitive K^{+} channels can interact with each other.

Axons

Rapid information transfer is carried out by the axons, which are long thin tubular extensions that emanate from the soma. They contain mitochondria, endoplasmic reticulum and the microtubular tracks that carry vesicles to and from the synaptic endings. The assembly and maintenance of the microtubular tracks is maintained by the neuron-specific microtubule protein tau.

Tau

Tau is a neuron-specific microtubule-associated protein that functions in the assembly and maintenance of neuronal microtubules. Its activity is regulated by phosphorylation by glycogen synthase kinase 3 (GSK-3), and protein phosphatase 2A (PP2A) functions to reverse the phosphorylation.

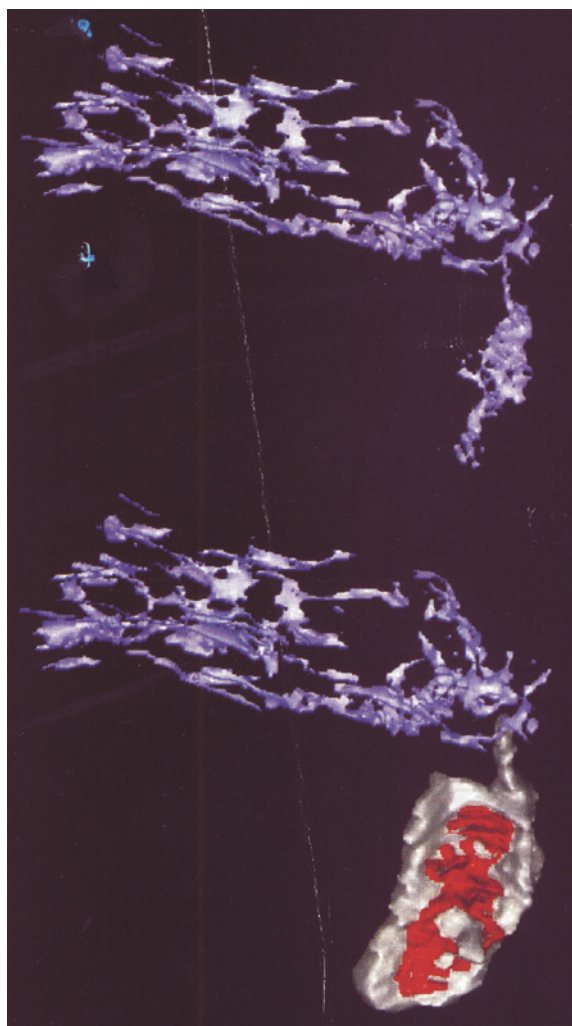
Hyperphosphorylation of tau results in a change in its shape that not only disrupts the microtubules, but also results in the altered tau molecules polymerizing to form the neurofibrillar tangles that are one of the hallmarks of Alzheimer's disease (Module 12: Figure amyloid plaques and tangles). Mutations in tau are responsible for frontotemporal dementia with Parkinsonism linked to

chromosome 17 (FTDP-17), which has many of the hallmarks of Alzheimer's disease.

Synaptic endings

Axons have synaptic endings specialized to release the neurotransmitters stored up in the synaptic vesicles (Module 10: Figure synaptic organization). Many synapses contain several thousand small synaptic vesicles with only a few of these docked to the membrane and ready to be released when an action potential arrives. Indeed, action potentials often invade nerve ending without releasing a vesicle. The probability that a spike will release a vesicle (P_{REL}) lies in the range of 0.1 to 0.3. However, this probability can increase through a process of facilitation. In the case of the hypothalamic and vasopressin neurons, the probability of release of the large dense-core vesicles from their nerve endings in the posterior pituitary declines to 0.0025.

In addition to the synaptic vesicles, the endings also have extensions of the endoplasmic reticulum (ER), often closely associated with a mitochondrion. Synaptic endings have the fusion machinery responsible for the Ca^{2+} -dependent process of exocytosis. There is increasing evidence that the Ca^{2+} responsible for triggering exocytosis during presynaptic events can be derived from both external and internal sources (Module 4: Figure Ca^{2+} -dependent exocytosis).

Module 10: | Figure dendritic endoplasmic reticulum

Organization of the endoplasmic reticulum (ER) in a dendrite and spine. A three-dimensional reconstruction of the endoplasmic reticulum (ER) is shown, taken from a segment of a dendrite with a small connection (bottom right) that extends into a spine, where it forms the spine apparatus. Note the narrow connection that would pass through the stalk region of the spine. In the lower part of the figure, the plasma membrane of the spine has been included. Reproduced, with permission, from Spacek, J. and Harris, K.M. (1997) Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. *J. Neurosci.* 17:190–203. Copyright (1997) by the Society for Neuroscience; see Spacek and Harris 1997.

Synaptic vesicle

Synaptic vesicles contain the neurotransmitters that are released from synaptic endings (Module 10: Figure synaptic organization). In electron micrographs, these individual packets of transmitter appear as small (42nm in diameter) smooth-surfaced vesicles that are crowded into the pre-synaptic endings (see Module 10: Figure hippocampal synapse). However, a proteomic analysis of purified vesicles has revealed that these minute organelles are associated by a large number of proteins that cover a large part of the membrane surface (Module 10: Figure synaptic vesicle). Approximately 80 proteins were identified, many of which are integral vesicle proteins but some of the others might be loosely associated with the vesicle or are con-

taminants. An important aspect of this study is that it has provided an estimate of just how many protein molecules there are on each vesicle and it is this analysis that has led to the molecular model shown in Module 10: Figure synaptic vesicle. For example, for each vesicle there are 70 synaptobrevins, 32 synaptophysins, 10 Rab3As, 8 synapsins and 15 synaptotagmins. In addition, there are approximately 12 of the neurotransmitter transporters. Many of these molecules are part of the exocytotic machinery used during the process of transmitter release (Module 4: Figure Ca^{2+} -induced membrane fusion).

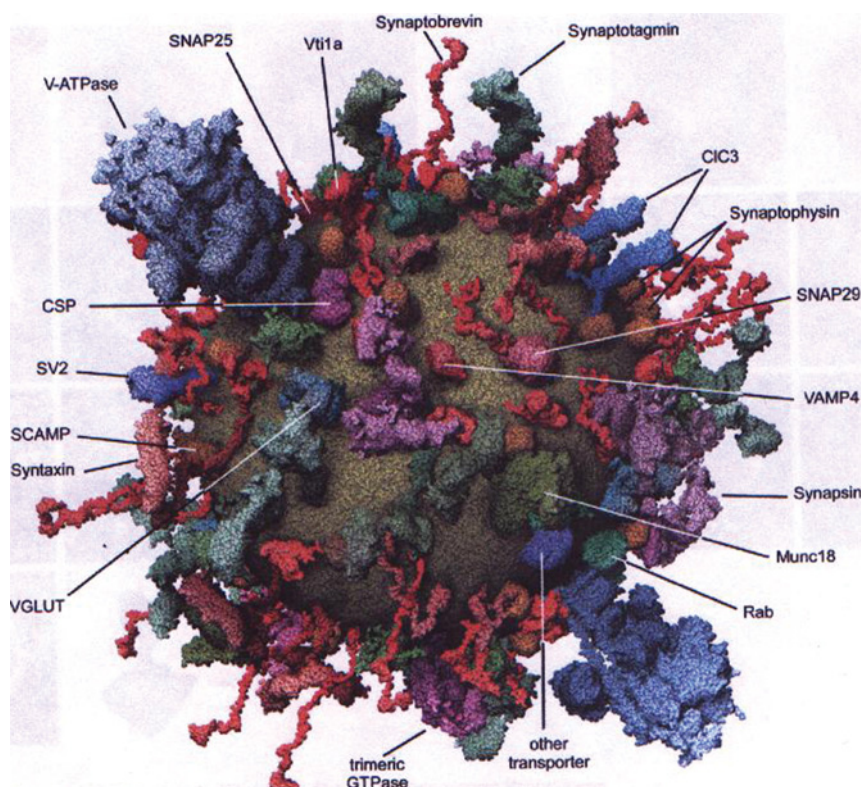
Neuron-within-a-neuron

The neuron-within-a-neuron concept attempts to explain how the plasma membrane and the endoplasmic reticulum (ER) form a binary membrane system to control many neuronal processes, such as membrane excitability, synaptic plasticity and gene transcription. The neuron-within-a-neuron concept was developed to emphasize the fact that the ER shares many of the properties usually associated with the plasma membrane. For example, the ER membrane can integrate information (such as second messengers), it is excitable in that it can conduct Ca^{2+} waves, and it has a memory system in that its degree of excitability can vary depending on its previous activity. Most of these properties depend upon priming the inositol 1,4,5-trisphosphate receptors (InsP_3Rs) and ryanodine receptors (RYRs) located on the ER. In order to understand neuronal function, therefore, it is necessary to consider the ER and the plasma membrane as a highly integrated binary membrane system. The plasma membrane integrates excitatory and inhibitory inputs to generate action potentials, whereas the ER has a more modulatory role, functioning to regulate excitability and contributing to the mechanisms of synaptic plasticity.

The neuron has a complex morphology that is adapted for the rapid process of synaptic transmission, i.e. how information is transmitted from one neuron to the next.

Synaptic transmission

The synapse is the microprocessor responsible for transmitting information from one neuron to the next. It is composed of a presynaptic part, the synaptic ending of one neuron that is closely applied to a postsynaptic component, usually a spine or part of a dendrite of the target neuron. During synaptic transmission, information is transferred from neuron to neuron across the synapse. What is remarkable about this neuronal information transfer process is the speed of transmission: information is passed from one neuron to the next in less than 2 ms. Such rapid transmission of information is consistent with the high-frequency brain rhythms that occur in different regions of the brain. A variety of neuronal Ca^{2+} entry and release channels are used for this transfer of information. A process of priming the inositol 1,4,5-trisphosphate receptors (InsP_3Rs) and ryanodine receptors (RYRs) is of particular importance in modulating the sensitivity of these release channels. These channels may also function in neuronal coincident detection to generate the Ca^{2+} signals used during

Module 10: | Figure synaptic vesicle

Molecular model of a synaptic vesicle.

This model represents some of the most abundant proteins that were extracted from synaptic vesicles purified from rat brain. Reproduced from Takamori et al (2006).

learning and memory. Despite the fact that neurons can receive a large number of individual inputs, it is clear that these can be kept apart, and this depends upon a process of input-specific signalling whereby each synapse can transfer information as an autonomous unit independently of its neighbours.

Neuronal information transfer

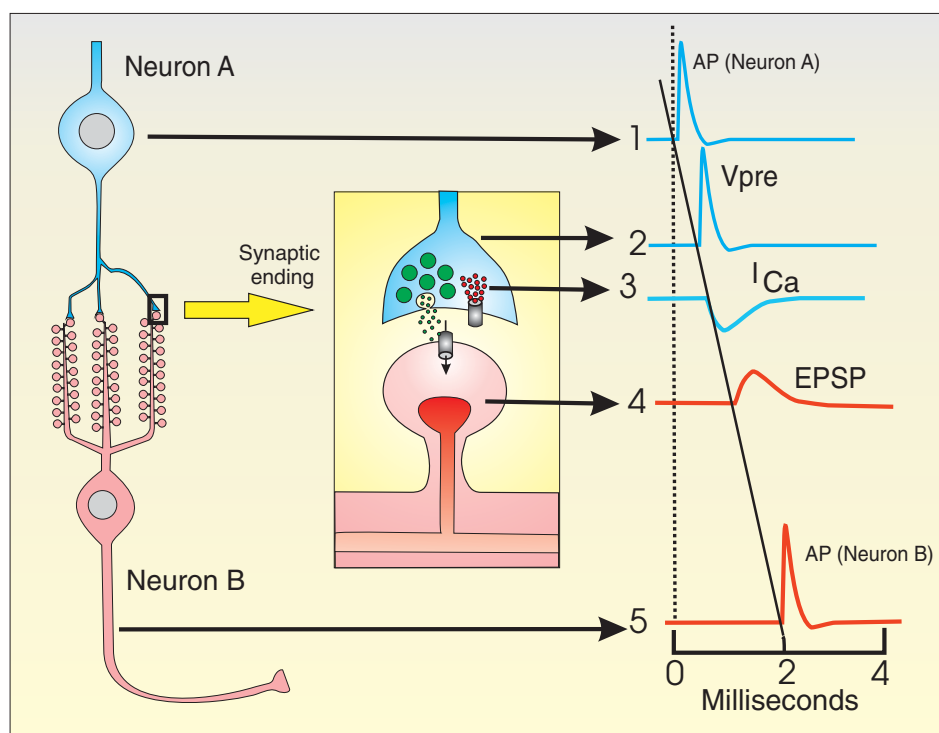
The flow of information through the synaptic microprocessor will be considered in an example where neuron A is an excitatory neuron functioning to excite neuron B. The two main events in synaptic transmission are the presynaptic events (Events 1–3) that result in the release of neurotransmitters from the synaptic ending, which are followed by the postsynaptic events (Events 4 and 5) (Module 10: Figure kinetics of neurotransmission):

1. Neuron A fires its action potential (AP) that initiates at the soma and then propagates down the axon at approximately 100 m/s, which means that it arrives at the synaptic ending (shown in blue in the inset) following a delay of about 0.2 ms, depending on the length of the axon.
2. When the action potential invades the synaptic endings, it provides the depolarizing signal (V_{pre}) responsible for triggering the release of neurotransmitters.
3. The depolarization activates voltage-operated channels (VOCs) to produce an inward current (I_{Ca}) due to the

entry of the Ca^{2+} responsible for triggering exocytosis (Module 4: Figure Ca^{2+} -induced membrane fusion). In effect, the electrical signal (the action potential) has been transduced into a chemical signal (the neurotransmitter) that then diffuses across the narrow synaptic cleft to activate the postsynaptic events.

4. Neurotransmitters such as glutamate act to open excitatory receptors, such as the α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor to produce the inward flow of Na^+ and Ca^{2+} that results in the excitatory postsynaptic potential (EPSP).
5. The individual EPSPs generated at each spine are summed to produce the global depolarization that then excites an action potential in neuron B, thus completing the flow of information from neuron A to neuron B.

There are two major signalling events during this information transfer across the synapse. At the presynaptic ending, the key event is the depolarization-dependent influx of Ca^{2+} that triggers exocytosis. The transmitters that are released then act on the postsynaptic endings to bring about both the postsynaptic changes in membrane potential and the postsynaptic generation of Ca^{2+} signals. Ca^{2+} signalling is thus a critical feature for both the pre- and post-synaptic events. Before considering these events in more detail, it is appropriate to describe the operation of the neuronal Ca^{2+} entry and release channels in more detail.

Module 10: | Figure kinetics of neurotransmission

A typical example of the time course of neuronal information transfer during synaptic transmission.

The flow of information between an excitatory neuron A and neuron B can be separated into five sequential events that are described in the text. One of the synapses is expanded in the middle of the figure to illustrate the way information is transferred from neuron A to neuron B. The transfer of information takes about 2 ms, during which an action potential (AP) in A excites an AP in B.

Neuronal Ca^{2+} entry and release channels

Neurons generate Ca^{2+} signals during both the presynaptic and postsynaptic events. During the former, an increase in Ca^{2+} is responsible for releasing transmitter. When the transmitter acts on postsynaptic receptors, there often is an increase in Ca^{2+} that can be derived from activation of both neuronal Ca^{2+} entry mechanisms and neuronal Ca^{2+} release mechanisms. Although the expression and distribution of the different Ca^{2+} signalling components vary from neuron to neuron, there are some general features that apply to most neurons.

Neuronal Ca^{2+} entry mechanisms

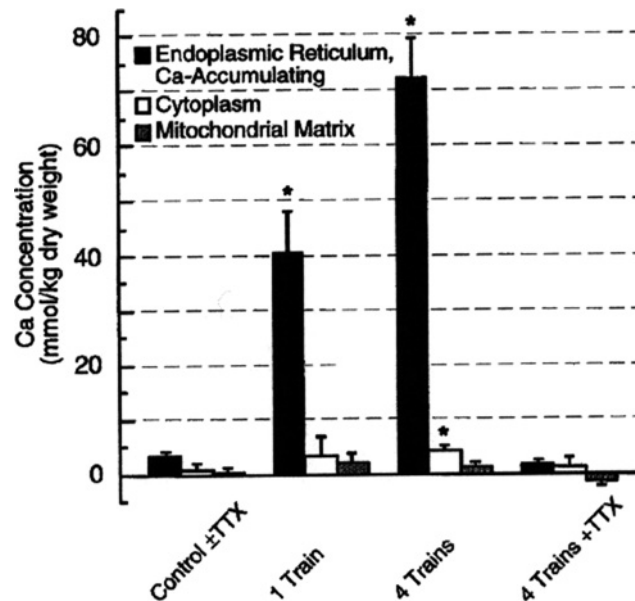
One of the primary Ca^{2+} entry mechanisms in neurons is through various members of the voltage-operated channels (VOCs) that have variable distributions over the neuron (Module 3: Table VOC classification). The presynaptic endings have members of the Ca_v2 family of N-type, P/Q-type and R-type channels. Other parts of the neuron, such as the dendrites and soma, have members of the Ca_v1 family of L-type channels. It is these channels that provide the Ca^{2+} signals in the soma that are responsible for activating neuronal gene transcription (Module 10: Figure neuronal gene transcription). In addition, Ca^{2+} enters through the receptor-operated channels (ROCs) such as the glutamate-sensitive *N*-methyl-D-aspartate (NMDA) receptors and

α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors that tend to be clustered at the synaptic postsynaptic densities (Module 10: Figure postsynaptic density).

Neuronal Ca^{2+} release mechanisms

Neurons express both inositol 1,4,5-trisphosphate receptors (InsP_3Rs) and ryanodine receptors (RYRs) located on the endoplasmic reticulum (ER), which is dispersed throughout the neuron. As described for the neuron-within-a-neuron concept, this extended ER network has a significant role to play in neuronal signalling, both as a source and as a sink for Ca^{2+} . The reciprocal interaction that occurs between Ca^{2+} entry and release channels is illustrated in Module 2: Figure Ca^{2+} -induced Ca^{2+} release. Following a burst in neuronal activity that brings Ca^{2+} in from the outside [e.g. as a result of activating voltage-operated channels (VOCs) or receptor-operated channels (ROCs)], the ER contributes to the recovery process by functioning as a sink to soak up the Ca^{2+} . The accumulation of Ca^{2+} in the lumen of the ER then has an important role in priming the inositol 1,4,5-trisphosphate receptors (InsP_3Rs) and ryanodine receptors (RYRs), during which their sensitivity to release messengers is markedly enhanced.

Conventional mechanisms are used to release this stored Ca^{2+} . Of particular significance are the VOCs and ROCs

Module 10: | Figure ER Ca^{2+} loading

Activity-dependent loading of the endoplasmic reticulum (ER) of a hippocampal neuron.

Following stimulation of a hippocampal neuron, there was a marked increase in the Ca^{2+} content of the endoplasmic reticulum (ER) (solid bars), with little or no change in the cytoplasm (open bars) or mitochondria (dotted bars). The uptake of Ca^{2+} was graded in that it was larger after four trains compared with one train of action potentials. Reproduced, with permission, from Pozzo-Miller, L.D., Pivovarova, N.B., Leapman, R.D., Buchanan, R.A., Reese, T.R. and Andrews, S.B. (1997) Activity-dependent calcium sequestration in dendrites of hippocampal neurons in brain slices. *J. Neurosci.* 17:8729–8738. Copyright (1997) by the Society of Neuroscience; see Pozzo-Miller et al. 1997.

that generate the trigger Ca^{2+} that can activate the RYRs, as occurs in the hippocampal neuron (Module 10: Figure synaptic signalling mechanisms). In the case of the Purkinje cell, metabotropic receptors coupled to phospholipase C (PLC) activate the inositol 1,4,5-trisphosphate (InsP_3)/ Ca^{2+} signalling cassette, which plays a major role in regulating Ca^{2+} release by the InsP_3 Rs. Since the latter are dependent on both InsP_3 and Ca^{2+} , this dual regulation has important implications in that the two messengers can act synergistically. For the channel to open, both messengers need to be present, and this means that the InsP_3 Rs could function in neuronal coincident detection. Such coincident detection has been implicated in the mechanism of synaptic plasticity that underlies learning and memory. The neuronal Ca^{2+} entry and release channels are thus a highly versatile system responsible for either localized input-specific Ca^{2+} signalling in spines or more global Ca^{2+} signals that spread more widely throughout the neuron.

Priming the inositol 1,4,5-trisphosphate receptors (InsP_3 Rs) and ryanodine receptors (RYRs)

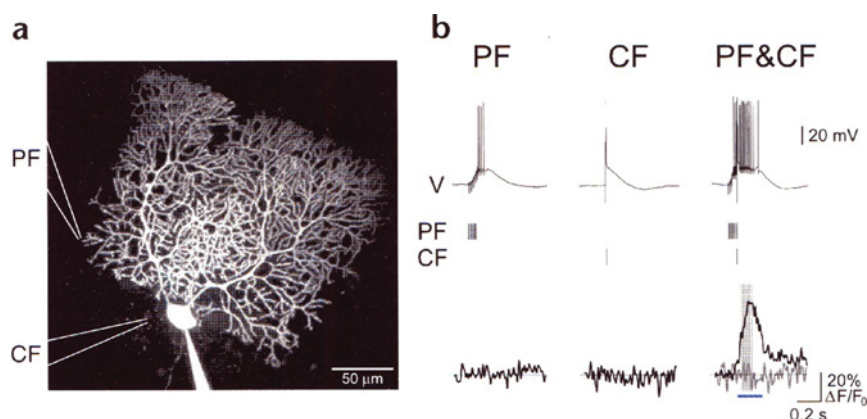
The sensitivity of the inositol 1,4,5-trisphosphate receptors (InsP_3 Rs) and ryanodine receptors (RYRs) can be primed by different mechanisms. An increase in Ca^{2+} -mobilizing messengers, such as InsP_3 or cyclic ADP-ribose (cADPR), is important, and so is the Ca^{2+} loading of the internal stores. For example, the latter can be primed by repetitive bouts of membrane depolarization that allow external Ca^{2+} to enter the neuron and be taken up by the internal stores, as occurs during membrane depolarization and

back-propagating action potentials. Once primed, these stores will release large amounts of Ca^{2+} following synaptic stimulation. The ability of the endoplasmic reticulum (ER) to load up with Ca^{2+} is phenomenal. For example, a 1 s stimulation at 50 Hz caused the ER Ca^{2+} content to increase from 3.5 to 40.0 mmol/kg of dry weight (Module 10: Figure ER Ca^{2+} loading). If this 1 s priming burst was repeated four times at 30 s intervals, the ER content of Ca^{2+} increased further to 72 mmol/kg of dry weight. The critical point is that the accumulation of Ca^{2+} was dependent on the degree of neuronal activity. As a consequence of this store loading, the luminal concentration of Ca^{2+} will increase the sensitivity of the InsP_3 Rs and the RYRs, thus enhancing the probability of triggering a global Ca^{2+} signal. Once primed, the internal ER network is capable of producing a regenerative Ca^{2+} wave, as has been described in CA1 neurons (Module 10: Figure dendritic Ca^{2+} wave).

Neuronal coincident detection

An important aspect of the neural plasticity responsible for learning and memory is an ability to detect and associate the arrival of two separate neural events. Neurons have developed molecular targets capable of such neuronal coincident detection:

- *N*-methyl-D-aspartate (NMDA) receptors. The NMDA receptors respond to the postsynaptic depolarization, and the glutamate coming from the presynaptic ending (Module 10: Figure synaptic signalling mechanisms).

Module 10: | Figure coincident detection**Coincident detection at the parallel fibre/Purkinje cell synapse.**

A rat Purkinje neuron was loaded with the low-affinity Ca^{2+} indicator Magnesium Green to measure Ca^{2+} . The climbing fibre (CF) or the parallel fibres (PF) could be activated using glass electrodes positioned as shown in a. The changes in Ca^{2+} were recorded in one of the spiny branches. No Ca^{2+} signal was recorded following eight stimuli given at 100 Hz to the PF (bottom trace in b). Likewise, a single stimulus to the CF also gave no response. However, when the CF was stimulated together with the PF, there was a large Ca^{2+} response that greatly exceeded the sum of the two separate responses. This neuronal coincident detection depends critically on the order and timing of the two stimuli. The synergistic response was greatest when the PF stimulation preceded the CF stimulation by 50–200 ms. Reproduced by permission from Macmillan Publishers Ltd: *Nat. Neurosci.*, Wang, S.S.-H., Denk, W. and Häusser, M. (2000) Coincident detection in single dendritic spines mediated by calcium release, 3:1266–1273. Copyright (2000); <http://www.nature.com/neuro>; see Wang et al. 2000.

- **Inositol 1,4,5-trisphosphate receptors (InsP_3Rs).** The InsP_3Rs , which are sensitive to both Ca^{2+} and InsP_3 , can detect the Ca^{2+} generated as a result of postsynaptic depolarization and the InsP_3 generated by the activation of the metabotropic receptors. An example of such coincident detection has been described in Purkinje cells, which are innervated by parallel fibres (PFs) and by climbing fibres (CFs). When activated separately, there is no Ca^{2+} signal, but this increases enormously when these two inputs are stimulated together (Module 10: Figure coincident detection). In the latter case, the InsP_3R is the coincident detector, which may explain long-term depression (LTD) at the parallel fibre/Purkinje cell synapse (Module 10: Figure long-term depression)
- **Dopamine and cyclic AMP-regulated phosphoprotein of apparent molecular mass 32 kDa (DARPP-32).** The DARPP-32 protein, which is an inhibitor of the protein phosphatase 1 (PP1), can integrate information coming from dopamine and glutamate in medium spiny neurons (Module 10: Figure medium spiny neuron signalling), and this process has been implicated in drug addiction.

Input-specific signalling

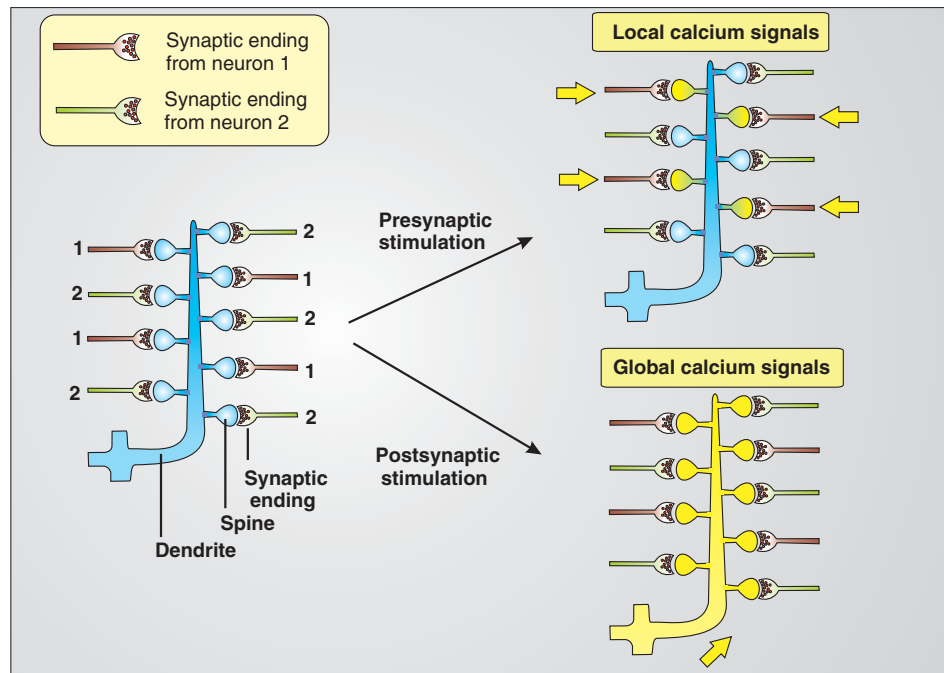
Each neuron has a large number of synapses receiving inputs from many neurons. Learning studies have revealed that individual synapses can be selectively modified, leaving unchanged all the other neighbouring synapses. It is therefore evident that individual synaptic connections are capable of input-specific signalling. What this means in the case of spines is that they can function autonomously with regard to the generation of the second messengers that are used for learning and memory. Particularly good evidence

for such input-specific signalling has emerged from Ca^{2+} signalling studies where presynaptic signalling gives rise to spine-specific signals, whereas postsynaptic stimulation, as occurs during back-propagating action potentials, gives rise to a global Ca^{2+} signal that spreads throughout the dendrites and spines (Module 10: Figure input-specific signalling).

The high concentration of Ca^{2+} buffers in neurons plays a major role in restricting Ca^{2+} signals to localized regions. Another way of ensuring the spatial restriction of internal messengers is to enhance the OFF mechanisms responsible for their removal. For example, the inositol 1,4,5-trisphosphate (InsP_3) 3-kinase responsible for metabolizing InsP_3 is strongly expressed in spines, where it is localized by being attached to actin in the region of the postsynaptic density (PSD), and this may ensure that this Ca^{2+} -mobilizing messenger is confined to the spine. This miniaturization of neuronal signalling systems means that each neuron can process and store an enormous amount of information.

Since Ca^{2+} is one of the major messengers responsible for modifying synaptic transmission, much effort has been concentrated on the process of input-specific Ca^{2+} signalling in spines. In contrast with the electrical signals [excitatory postsynaptic potential (EPSPs) and inhibitory postsynaptic potentials (IPSPs)] that smear out over the dendrite through electrotonic spread, the Ca^{2+} signal and associated biochemical changes remain localized within each activated spine, and this is the basis of the input specificity that underlies the processes of learning and memory. Both the memory acquisition and memory consolidation phases are input-specific in that individual synapses can be changed independently of neighbouring synapses. This input specificity is necessary, because most neurons receive inputs from multiple partners. For

Module 10: | Figure input-specific signalling

Input-specific localized Ca^{2+} signals and global Ca^{2+} signals in the dendritic tree of neurons.

The panel on the left shows a portion of a typical dendritic tree receiving multiple inputs from two neurons. The synaptic endings from neuron 1 are shown in brown, whereas those from neuron 2 are in green. During presynaptic stimulation of the endings from neuron 1 (yellow arrows in the top panel on the right), only those spines that are activated display Ca^{2+} signals. By contrast, postsynaptic stimulation via a back-propagating action potential (single arrow in the lower right-hand panel) results in a Ca^{2+} signal developing uniformly throughout the dendrites and into all of the spines. These more global events, where Ca^{2+} increases over larger regions of the neuron, can also invade the nucleus, with important implications for gene activation.

example, neuron B (Module 10: Figure summation of excitatory inputs) receives inputs from neurons A and C. During learning, neuron B can alter the strength of its synaptic connections to A (e.g. synapses 1, 2 and 3), while leaving unchanged those from neuron C (e.g. synapses i, ii and iii). In some cases, these synapses might lie close together, and such input specificity can only occur because each spine is an autonomous unit that functions independently of its neighbours.

Presynaptic events

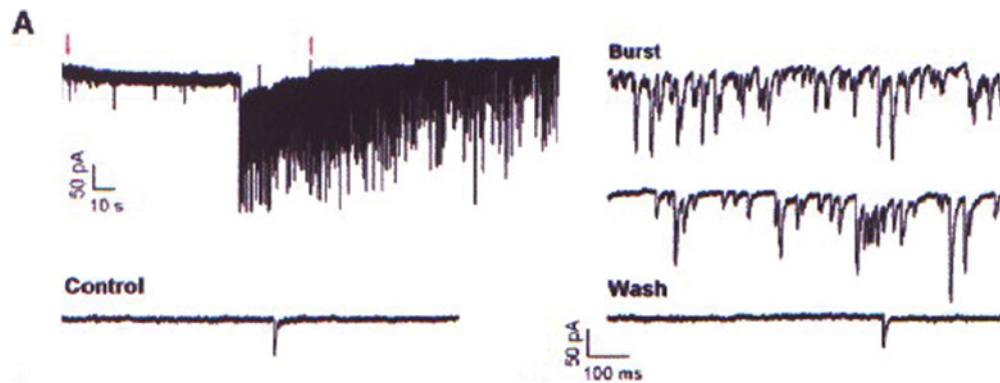
Presynaptic events refer to the processes that occur in synaptic endings to control the Ca^{2+} -dependent release of transmitter. There are two main mechanisms for controlling transmitter release (Module 4: Figure Ca^{2+} -dependent exocytosis). Transmitter release can be triggered either by entry of external Ca^{2+} or by the release of Ca^{2+} from the internal stores. Such transmitter release usually occurs at synaptic endings, but there also is a mechanism of dendritic transmitter release, which will be considered separately. The main mechanism, which is controlled by entry, is described in the section on exocytosis triggered by Ca^{2+} entry through voltage-operated channels (VOCs). In this case, entry through a VOC associated with the exocytotic machinery provides the localized pulse of Ca^{2+} that triggers exocytosis (Module 4: Figure Ca^{2+} -induced membrane fusion).

The other mechanism is exocytosis triggered by Ca^{2+} release from internal stores. In this case, exocytosis is triggered by Ca^{2+} released from the endoplasmic reticulum (ER) Ca^{2+} store found in the synaptic endings (see panel B in Module 4: Figure Ca^{2+} -dependent exocytosis). Release of Ca^{2+} from inositol 1,4,5-trisphosphate receptors (InsP_3Rs) and the ryanodine receptors (RYRs) has been implicated in the control of exocytosis both in neuronal presynaptic terminals and in other cell types:

- Mossy fibre presynaptic Ca^{2+} release.
- Cerebellar basket cell presynaptic Ca^{2+} release.
- Hypothalamic neuronal presynaptic Ca^{2+} release.
- Neocortical glutamatergic presynaptic Ca^{2+} release.
- Astrocytes use InsP_3 -induced release of Ca^{2+} to trigger the exocytosis of vesicles containing glutamate as part of the astrocyte-neuronal communication system (Module 7: Figure astrocyte tripartite synapse).

Mossy fibre presynaptic Ca^{2+} release

The axons of the hippocampal granule cells are known as mossy fibres, which are part of the hippocampal trisynaptic circuit (Module 10: Figure hippocampus). What this simple diagram does not show is that the mossy fibre axon divides repeatedly, resulting in about 50 boutons, where transmitter is released on to each CA3 hippocampal neuron. If an electrode is placed in the CA3 neuron, spontaneous miniature excitatory postsynaptic currents (mEPSCs) are

Module 10: | Figure mossy fibre presynaptic release**Spontaneous release of transmitter from mossy fibre boutons.**

The trace (top left) shows the frequency of miniature excitatory postsynaptic currents (mEPSCs) in response to the addition and removal of 20 μ M nicotine (first and second arrows respectively). The other traces are expanded versions of segments of control, the high-frequency large amplitude mEPSCs induced by nicotine and the recovery after washing out the nicotine. Reproduced from *Neuron*, Vol. 38, Sharma, G. and Vijayaraghavan, S., Modulation of presynaptic store calcium induces release of glutamate and postsynaptic firing, pp. 929–939. Copyright (2003), with permission from Elsevier; see Sharma and Vijayaraghavan 2003.

recorded that reflect the spontaneous release of transmitter from these boutons (Module 10: Figure mossy fibre presynaptic release). The frequency of these mEPSCs is very low under resting conditions, but increases dramatically following addition of nicotine that acts on nicotinic acetylcholine receptors (nAChRs), which are ROCs (receptor-operated channels) that introduce Ca^{2+} into the cell. This introduction of Ca^{2+} appears not to be responsible for triggering exocytosis, but seems to act indirectly by enhancing the release of internal Ca^{2+} . The following hypothesis has been developed to explain this priming role of the nAChRs (upper panel in Module 10: Figure mossy fibre presynaptic Ca^{2+} release). The idea is that the opening of the nAChRs allows Ca^{2+} to enter the bouton to charge up the internal store, which results in sensitization of the ryanodine receptors (RYRs). Once these RYRs are sensitized, they begin to generate spontaneous Ca^{2+} sparks that then trigger the release of transmitter. What is surprising about the action of nicotine is the very long latency between the time it is added (first arrow in Module 10: Figure mossy fibre presynaptic release) and the time when the large increase in the mEPSC frequency occurs. This latency can be explained by the time it takes for the nAChRs to charge up the internal store. Likewise, the recovery after removing nicotine (shown by the second arrow) takes a long time. Again, this can be explained by the time it takes for the charged store to return to its resting level. This rundown may be slowed if much of the Ca^{2+} that is released during the spark is taken up by mitochondria and cycled back into the endoplasmic reticulum (ER), as shown in the lower panel of Module 10: Figure mossy fibre presynaptic Ca^{2+} release.

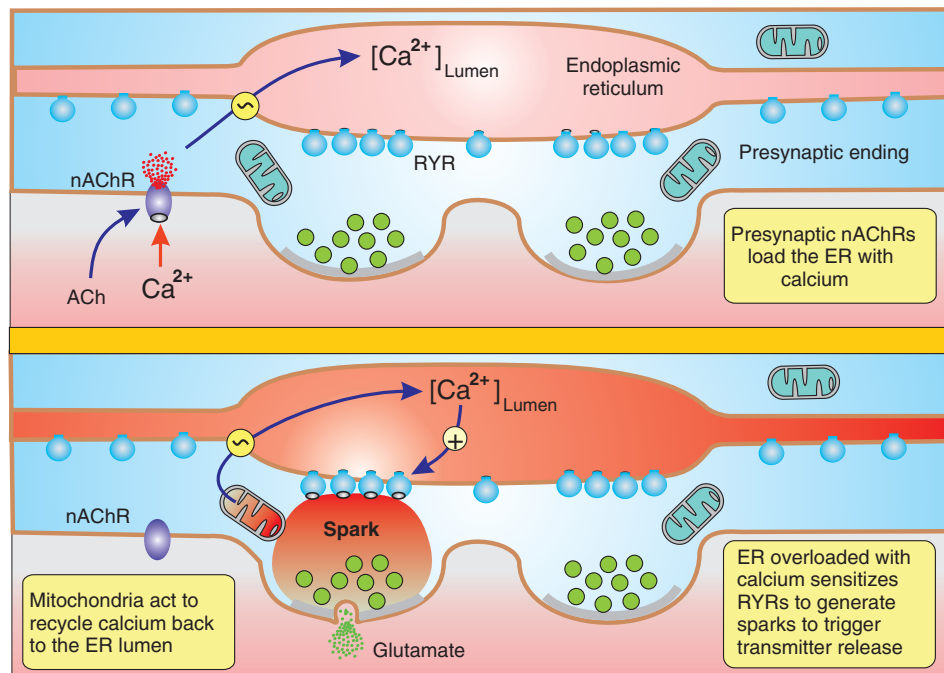
Cerebellar basket cell presynaptic Ca^{2+} release

The cerebellar basket cells send out their axons, which form a basket-shaped synaptic ending that ramifies over the surface of the soma of the Purkinje cell (Module 10:

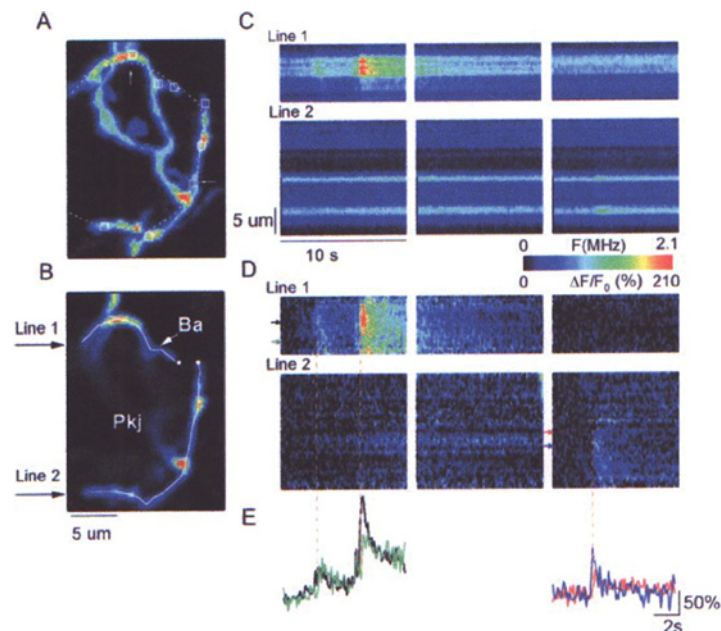
Figure cerebellum). This is an inhibitory synapse that releases the transmitter γ -aminobutyric acid (GABA), and this can account for the large-amplitude miniature inhibitory postsynaptic potentials (mIPSPs) that can be recorded from the Purkinje cells. Ryanodine receptors (RYRs) appear to contribute to the release of GABA from these basket cell synaptic endings. In addition, there are indications that release from stores within the nerve ending can give rise to spontaneous Ca^{2+} transients (SCaTs) (Module 10: Figure basket cell Ca^{2+} transients). The frequency of these SCaTs was greatly enhanced by ryanodine, and they were not prevented by blocking the VOCs (voltage-operated channels), suggesting that they depend upon release from internal stores. Just how they are triggered has not been determined, but they may also depend on store loading, as described above for the mossy fibre boutons.

Hypothalamic neuronal presynaptic Ca^{2+} release

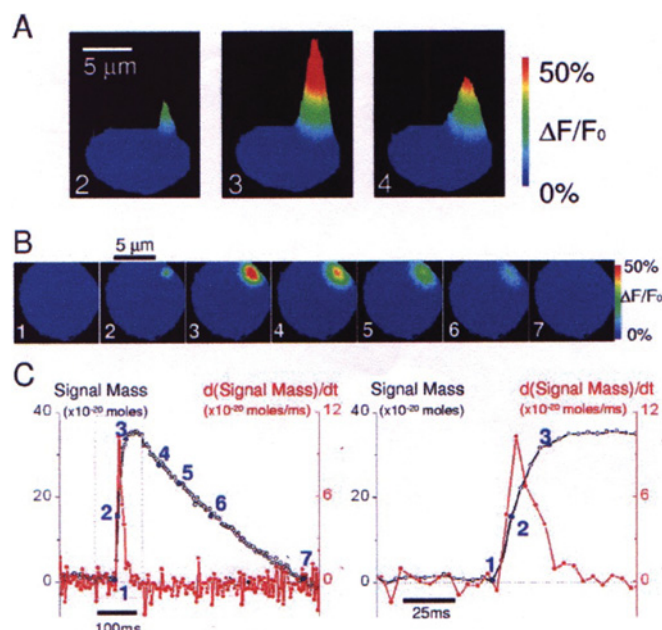
Hypothalamic neurons send their axons down to the posterior pituitary, where they release peptides into the blood from large nerve terminals. These terminals have an unusual release mechanism that depends on the release of Ca^{2+} from an internal store by ryanodine receptors (RYRs). Isolated nerve terminals display localized Ca^{2+} transients that have been called syntillas (derived from the Latin word *scintilla*, meaning a spark) (Module 10: Figure hypothalamic Ca^{2+} syntilla). These syntillas occur in the absence of external Ca^{2+} . What is unusual about these syntillas is that their frequency is enhanced by depolarizing the membrane. It is suggested that these syntillas might be activated by a conformational coupling mechanism similar to that found in skeletal muscle. A voltage-operated L-type Ca^{2+} channel in the plasma membrane might be directly coupled to a RYR located in the endoplasmic reticulum (ER) within the nerve terminal.

Module 10: | Figure mossy fibre presynaptic Ca^{2+} release**Spontaneous transmitter release from a mossy fibre bouton triggered by a Ca^{2+} spark.**

The working hypothesis shown in this figure suggests that activation of the nicotinic acetylcholine receptor (nAChR) introduces Ca^{2+} into the bouton that begins to load the endoplasmic reticulum (ER). When the ER becomes overloaded, the ryanodine receptors (RYRs) become sensitized and begin to release spontaneous Ca^{2+} sparks that trigger transmitter release. (The information and ideas for this figure were taken from Sharma and Vijayaraghavan 2003.)

Module 10: | Figure basket cell Ca^{2+} transients**Spontaneous Ca^{2+} transients recorded from the synaptic endings of cerebellar basket cells.**

A and B. A three-dimensional reconstruction of the basket-shaped terminal of a basket cell (Ba) on the surface of a Purkinje cell (Pkj). The two lines that were used for the scans shown on the right are indicated in B. C. Spatiotemporal profiles of the Ca^{2+} -dependent fluorescence. D. The corresponding $\Delta F/F_0$ signals. E. Time course of the Ca^{2+} transients recorded at the black and green arrows in line scan 1 (see panel D) and the red and blue arrows in line scan 2. Reproduced, with permission, from Conti, R., Tan, Y.P. and Llano, I. (2004) Action potential-evoked and ryanodine-sensitive spontaneous Ca^{2+} transients at the presynaptic terminal of a developing CNS inhibitory synapse. *J. Neurosci.* 24:6946–6957. Copyright (2004) by the Society for Neuroscience; see Conti et al. 2004.

Module 10: | Figure hypothalamic Ca^{2+} syntilla

Ca^{2+} syntillas in a hypothalamic nerve terminal.

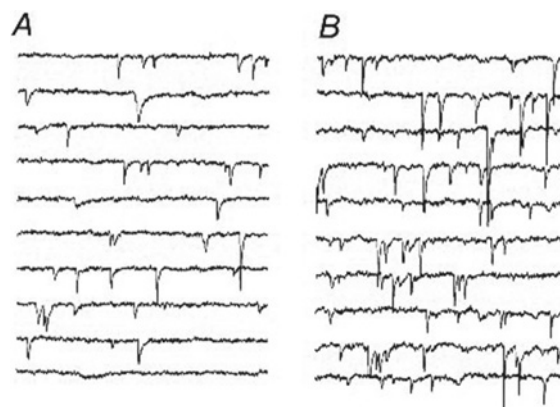
A and B. Images of a spontaneous Ca^{2+} syntilla in a hypothalamic nerve terminal loaded with Fluo3 and incubated in a Ca^{2+} -free solution and held at -80 mV. C. The time course of the syntilla shown in black, with the first derivative shown in red. An expanded time scale on the right shows that these syntilla develop very fast. Reproduced, with permission, from De Crescenzo, V., ZhuGe, R., Velázquez-Marrero, C., Lifshitz, L.M., Custer, E., Carmichael, J., Lai, F.A., Tuft, R.A., Fogarty, K.E., Lemos, J.R. and Walsh, J.V. (2004) Ca^{2+} syntillas, miniature Ca^{2+} release events in terminals of hypothalamic neurons, are increased in frequency by depolarization in the absence of Ca^{2+} influx. *J. Neurosci.* 24:1226–1235. Copyright (2004) by the Society for Neuroscience; see De Crescenzo et al. 2004.

Neocortical glutamatergic presynaptic Ca^{2+} release

Barrel cortex neurons were used to monitor miniature excitatory postsynaptic currents (mEPSCs) from the nerve terminals of neocortical neurons (Module 10: Figure miniature EPSCs). Under control conditions, the mEPSC frequency was low, but was markedly increased following activation of the metabotropic glutamatergic receptors that are coupled to the formation of inositol 1,4,5-trisphosphate (InsP_3). The interpretation of these results is that the formation of InsP_3 triggers the release of Ca^{2+} from the internal store through a mechanism that may be amplified by ryanodine receptors (Module 10: Figure neocortical Ca^{2+} release).

Modulation of transmitter release

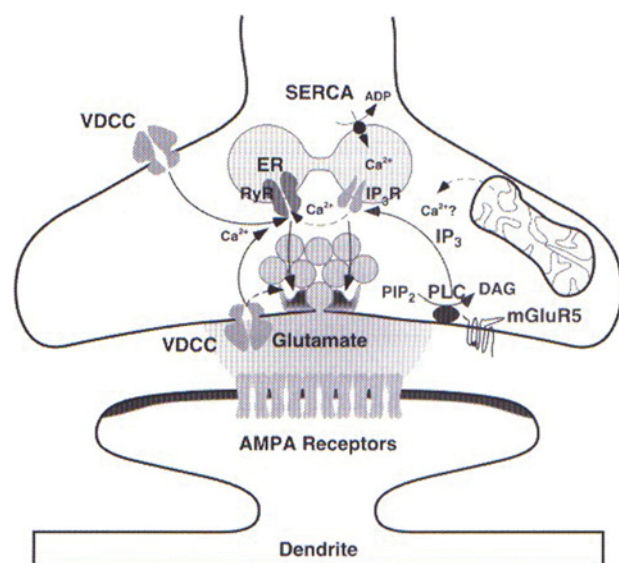
Presynaptic autoreceptors (i.e. receptors that respond to the transmitter that is being released) and heteroreceptors (i.e. receptors that respond to transmitters other than those being released) can regulate the amount of transmitter that is released during exocytosis both positively and negatively. They do this primarily by acting through G proteins to modulate both K^+ and Ca^{2+} channels and the exocytotic machinery itself. An important component of this regulation is described in the section on the modulation of the Ca_v2 family of N-type and P/Q-type channels, which are responsible for triggering the Ca^{2+} entry signal that induces exocytosis (Module 3: Figure Ca_v2 channel family).

Module 10: | Figure miniature EPSCs

Activation of presynaptic transmitter release by metabotropic receptor activation.

The spontaneous miniature excitatory postsynaptic currents (mEPSCs) from layer II neurons of the barrel cortex were used to monitor transmitter release. The traces in A show the control rate. Following activation of the metabotropic receptors, there was a marked increase in frequency, as shown in B. It is proposed that this increase in frequency is due to the release of Ca^{2+} from the internal store within the presynaptic ending (Module 10: Figure neocortical Ca^{2+} release). Reproduced from Simkus, C.R.L. and Stricker, C. (2002) The contribution of intracellular calcium stores to mEPSCs recorded in layer II neurones of rat barrel cortex. *J. Physiol.* 545:521–535, with permission from Blackwell Publishing; see Simkus and Stricker 2002.

An important form of presynaptic regulation depends on the retrograde signalling mediated by ligands formed in the postsynaptic ending. A particularly prominent form

Module 10: | Figure neocortical Ca^{2+} release

Role of inositol 1,4,5-trisphosphate receptors (InsP₃Rs) and the ryanodine receptors (RYRs) in presynaptic transmitter release.

Metabotropic glutamate receptors produce inositol 1,4,5-trisphosphate (InsP₃), which releases Ca^{2+} from a presynaptic store to trigger neurotransmitter release. Ryanodine receptors (RYRs) may amplify this signal, since they can also be induced to release Ca^{2+} through a process of Ca^{2+} -induced Ca^{2+} release (CICR). The Ca^{2+} that activates these RYRs can come either from the InsP₃Rs or from Ca^{2+} entering through the voltage-dependent Ca^{2+} channels (VDCCs). Reproduced from Simkus, C.R.L. and Stricker, C. (2002) The contribution of intracellular calcium stores to mEPSCs recorded in layer II neurones of rat barrel cortex. *J. Physiol.* 545:521–535, with permission from Blackwell Publishing; see Simkus and Stricker 2002.

of such regulation depends on the endocannabinoid retrograde signalling mechanism that operates in many different regions of the brain.

Endocannabinoid retrograde signalling mechanism

The endocannabinoids anandamide and 2-arachidonylglycerol (2-AG) are particularly active in the brain where they function as retrograde messengers relaying information from postsynaptic sites back to the presynaptic terminals (Module 10: Figure endocannabinoid retrograde signalling). This retrograde signalling system operates at both excitatory and inhibitory synapses.

At a typical glutamatergic synapse, glutamate can have a number of actions. It can activate AMPA receptors (AMPA-Rs) to provide the excitatory postsynaptic excitatory potential (EPSP) responsible for synaptic transmission. In addition, glutamate can elevate the intracellular level of Ca^{2+} by promoting entry through NMDA receptors (NMDARs) or be acting on metabotropic glutamatergic receptors (mGluRs) to generate InsP₃ and the release of stored Ca^{2+} . There is a tight relationship between this elevation of Ca^{2+} and synaptic plasticity (Module 10: Figure Ca^{2+} -induced synaptic plasticity). In addition to activating these memory processes, the elevation in Ca^{2+} can also stimulate the formation of both anandamide (Module 1: Figure anandamide) and 2-AG (Module 2: Fig-

ure InsP₃/DAG recycling), which have multiple messenger functions.

The anandamide and 2-AG diffuse out from the postsynaptic region and make contact with the cannabinoid receptor CB1 on the presynaptic ending to trigger two signalling events that can markedly influence transmitter release. First, the $\beta\gamma$ dimer can modulate the Cav2 family of N-type and P/Q-type channels, which are responsible for triggering the Ca^{2+} entry signal that induces exocytosis (Module 3: Figure Cav2 channel family). Secondly, the $\beta\gamma$ dimer can also activate the GIRK K^+ channel and the resulting hyperpolarization will reduce the depolarization-dependent release of transmitter.

A similar endocannabinoid retrograde signalling mechanism operates at GABAergic inhibitory synaptic endings (Module 10: Figure endocannabinoid retrograde signalling). The GABAergic endings normally make synapses on dendrites or perisomatic regions as occurs for the fast-spiking inhibitory basket cells that innervate the soma region of CA3 hippocampal neurons (Module 10: Figure gamma oscillatory mechanisms). In such cases, the Ca^{2+} signal responsible for activating endocannabinoid formation seems to depend on the opening of voltage-operated Ca^{2+} channels (VOCs). The anandamide (ANA) and 2-AG then activate CB1 receptors on the presynaptic terminals to inhibit release through mechanisms similar to those just described for the excitatory synaptic ending.

This retrograde signalling by the endocannabinoid operates in a variety of neuronal assemblies:

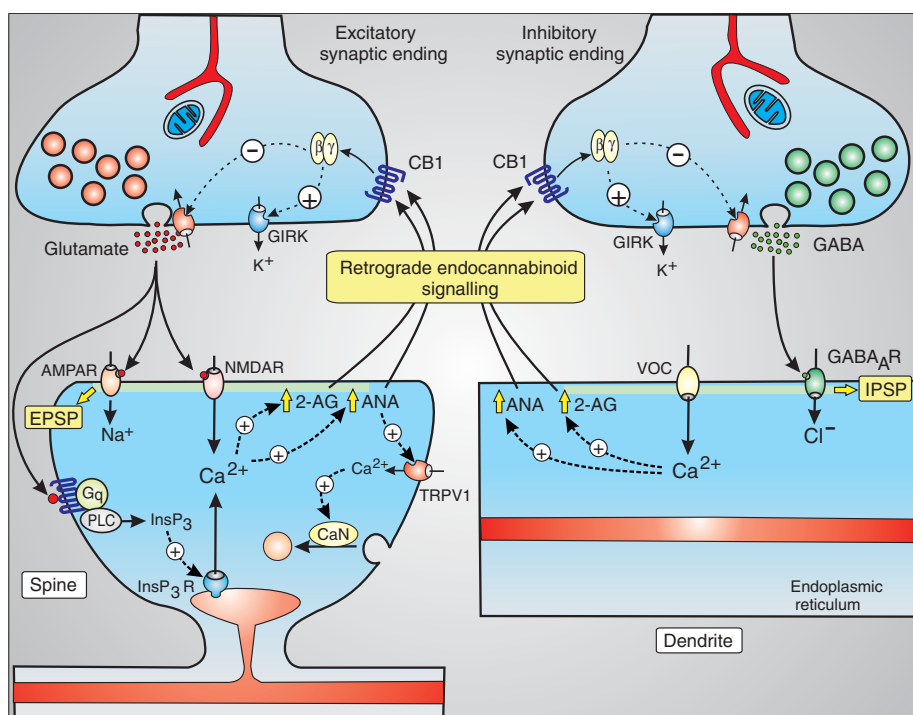
- In oxytocin neurons, termination of the burst of action potentials depends on the Ca^{2+} -dependent formation of endocannabinoids that feedback by binding to presynaptic CB1 receptors to inhibit the release of the glutamate that contributes to the depolarizing drive that maintains bursting activity (See step 9 in Module 10: Figure oxytocin neurons).
- The terminals of NPY/AgRP neuronal collaterals, which operate in the control of food intake and body weight, express CB1 receptors that switch off the inhibitory inputs to the POMC/CART neurons (See step 6 in Module 7: Figure control of food intake)
- Anandamide may have an analgesic effect by inhibiting transmitter release at the terminals of peripheral sensory neurons (Module 10: Figure endocannabinoid retrograde signalling).

Miniature excitatory postsynaptic currents (mEPSCs)

The existence of miniature excitatory postsynaptic currents (mEPSCs) recorded from postsynaptic neurons reveal that some presynaptic neurons are spontaneously releasing small packets of neurotransmitter that are detected as mEPSCs. The frequency of these mEPSCs in certain presynaptic endings is accelerated by the release of Ca^{2+} from internal stores (Module 10: Figure miniature EPSCs).

Dendritic transmitter release

Although synaptic endings are the classical sites of transmitter release, transmitters can also be released from

Module 10: | Figure endocannabinoid retrograde signalling**Endocannabinoid retrograde signalling mechanisms.**

Endocannabinoids formed at postsynaptic sites can act in a retrograde manner to modulate presynaptic transmitter release at both glutamatergic excitatory and GABAergic inhibitory synaptic endings. See the text for further details.

the dendrites. Such dendritic transmitter release of γ -aminobutyric acid (GABA) occurs in thalamic interneurons (Module 10: Figure dendritic GABA release). It has also been described in the olfactory bulb and substantia nigra. Another example is found in hypothalamic oxytocin neurons, which can release oxytocin from both the conventional axonal terminal site and the dendrites (Module 10: Figure magnocellular neurons). In the case of the latter, intracellular Ca^{2+} stores play a role in this dendritic release mechanism. The dendritic release of oxytocin is thought to operate a feedback mechanism whereby oxytocin increases the bursting activity of the neuron, thus potentiating the axonal release mechanism (see step 5 in Module 10: Figure oxytocin neuron).

Thalamic interneurons

The thalamus has an elegant system for controlling sensory signal processing. Release of γ -aminobutyric acid (GABA) from dendrites of thalamic interneurons is modulated by extrathalamic inputs, such as the serotonergic neurons coming from the dorsal raphe nucleus. Firing of these neurons varies during the sleep/wake cycle. The fastest rates occur during wakefulness, and function to increase visual contrast sensitivity of the thalamic neurons by inducing a local release of GABA. An unusual feature of the release mechanism is that dendritic transmitter release is controlled by canonical transient receptor potential 4 (TRPC4) channels (Module 10: Figure dendritic GABA release). 5-Hydroxytryptamine (5-HT) stimulates the release of GABA by activating TRPC4 channels, which

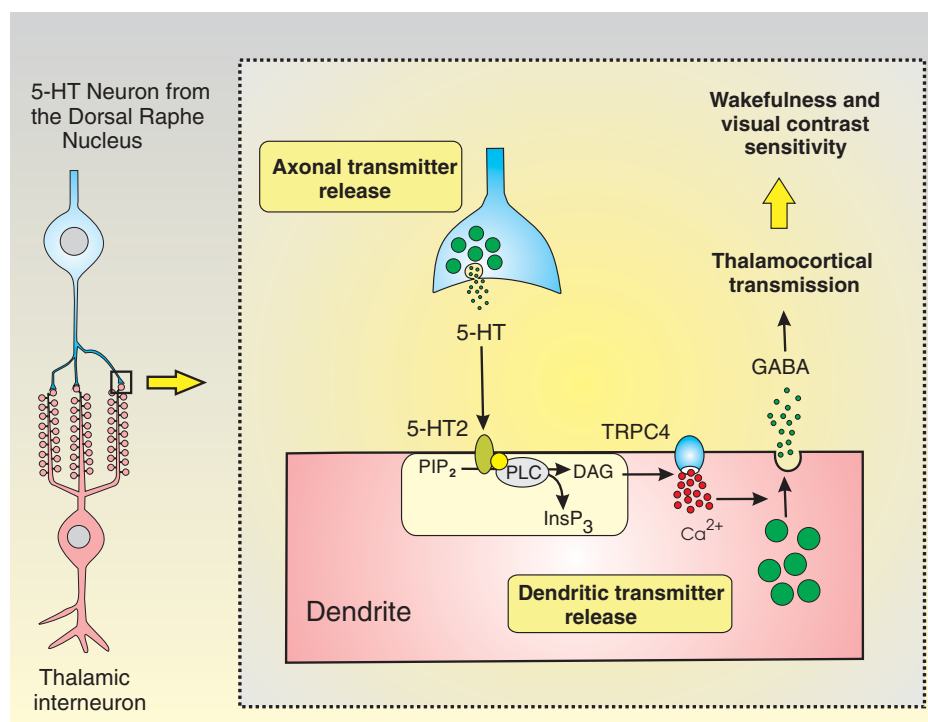
promote the entry of Ca^{2+} that triggers the release of GABA from the dendrites of the thalamic interneurons. The GABA then acts locally to enhance contrast processing.

Postsynaptic events

Neurotransmitters diffusing across the synaptic cleft interact with receptors on the surface of the spines or dendrites to initiate the postsynaptic events. These appear either as rapid postsynaptic changes in membrane potential (either excitatory or inhibitory) or as the somewhat slower generation of second messengers, such as the postsynaptic generation of Ca^{2+} signals. Initially, these postsynaptic events are highly localized to the individual synapses, but, upon repetitive stimulation, they can spread outwards to create more global signals. For example, the minute electrical signals at individual synapses can sum to create larger depolarizations capable of invading the soma to trigger action potentials. Likewise, the recruitment of second messengers such as Ca^{2+} can also invade the nucleus to activate the nuclear processes necessary for remodelling, as occurs during learning and memory.

It is important to emphasize the difference between the postsynaptic changes in membrane potential and the parallel postsynaptic generation of Ca^{2+} signals. The former are the basic computational elements or bits of information that are used to transfer information between neurons, whereas the latter function in synaptic plasticity. As such, this postsynaptic generation of Ca^{2+} signals is responsible

Module 10: | Figure dendritic GABA release

**Dendritic γ -aminobutyric acid (GABA) release from thalamic interneurons.**

5-Hydroxytryptamine (5-HT) neurons from the dorsal raphe nucleus innervate the dorsal lateral geniculate nucleus in the thalamus, where they act on the dendrites of the thalamic neurons to stimulate the release of γ -aminobutyric acid (GABA). This synaptic region thus displays the two main forms of transmitter release. Firstly, there is the classical form of axonal transmitter release responsible for releasing 5-HT. Secondly, 5-HT acts on metabotropic 5-HT₂ receptors coupled to the inositol lipid-signalling pathway. Some messenger, as yet unidentified, activates the canonical transient receptor potential 4 (TRPC4) channels, and the entry of Ca²⁺ triggers GABA release. (Information taken from Munsch et al. 2003.)

for the temporary (short-term memory) and permanent (long-term memory) modifications of synaptic transmission that occur during learning and memory. There are two major types of postsynaptic Ca²⁺ signals. Firstly, there is the highly localized input-specific Ca²⁺ signalling in spines. Secondly, there is more global Ca²⁺ signalling, where Ca²⁺ is elevated in large areas of the neuron. These localized and global Ca²⁺ signals are controlled by the interplay between the neuronal Ca²⁺ entry and release channels. A characteristic of this modification of synaptic plasticity is that it depends upon input-specific signalling.

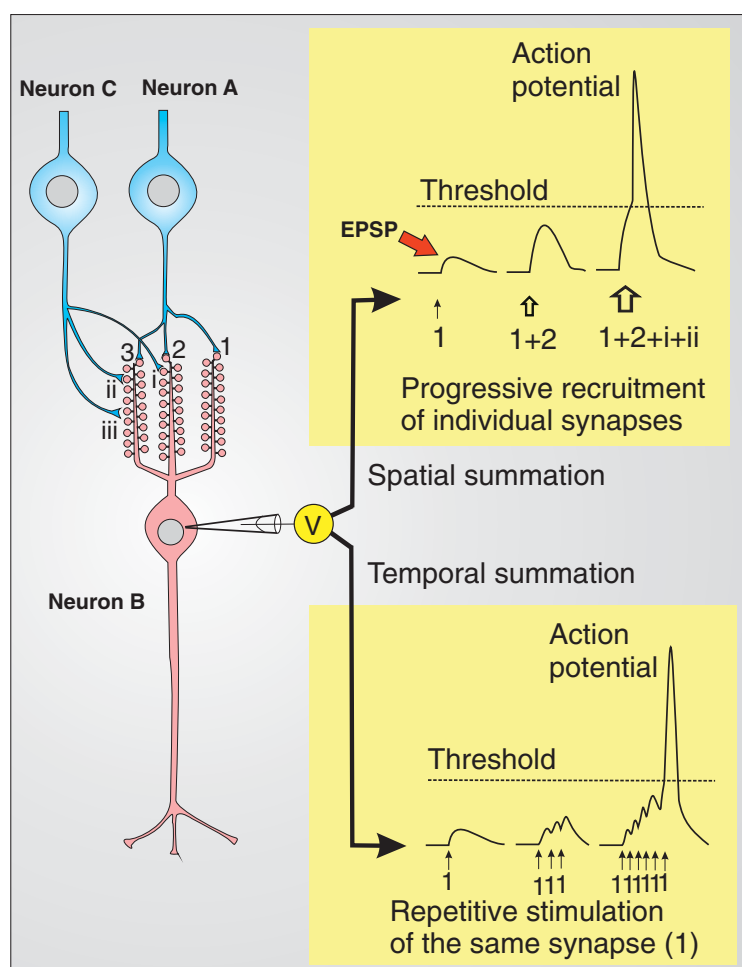
Postsynaptic changes in membrane potential

At excitatory synapses, such as the parallel fibre/Purkinje cell synapse, neurotransmitters such as glutamate bind to receptor-operated Na⁺ channels such as the AMPA receptors to produce a small depolarization, the excitatory postsynaptic potential (EPSP), which is one of the output signals of this neuronal microprocessor (Module 10: Figure synaptic signalling mechanisms). In the Schaffer collateral/hippocampal CA1 synapse, the EPSP induced by the AMPA receptor facilitates the opening of N-methyl-D-aspartate (NMDA) receptors that enhance the depolarization by allowing Ca²⁺ to enter the spine. Different events occur at inhibitory synaptic endings, where the membrane is hyperpolarized rather than depolarized. For example, γ -aminobutyric acid (GABA) receptors respond to GABA by opening Cl[−] channels, resulting

in an inhibitory postsynaptic potential (IPSP) causing the membrane to hyperpolarize and to depress synaptic transmission.

The EPSPs and IPSPs described above are all fast responses resulting from the activation of receptor-operated channels (ROCs), such as the AMPA and NMDA receptors. However, metabotropic receptors, such as the group I metabotropic receptors (e.g. mGluR1), can also induce EPSPs that develop more slowly. Since these mGluR1s are usually located in a perisynaptic region, they are only activated during intense stimulation when glutamate spills out of the synaptic cleft (Module 10: Figure postsynaptic density). There are indications that these slow EPSPs might depend upon an mGluR1 activation of TRPC1, which is a member of the canonical transient receptor potential (TRPC) family. Indeed, the mGluR1 and TRPC1 might be linked together through the protein Homer to produce a signalling complex. When glutamate activates mGluR1, it liberates G α_q , which then has two functions: it can stimulate phospholipase C (PLC) to produce inositol 1,4,5-trisphosphate (InsP₃), which releases Ca²⁺ from the internal store, or it can activate TRPC1 to promote the entry of external Ca²⁺.

The EPSP generated at individual synapses is not large enough to excite the postsynaptic neuron. In order for neuron A to excite neuron B sufficiently to induce an action potential, the individual EPSPs must sum together, either by combining with those from other spines (spatial

Module 10: | Figure summation of excitatory inputs

Spatial and temporal summation of excitatory inputs is responsible for triggering action potentials.

This simple circuit contains two excitatory neurons (A and C) that each makes three separate synaptic contacts on the dendritic tree of neuron B. The insets on the right represent the voltage (V) recorded from neuron B following different types of spatial and temporal inputs, as described in the text.

summation) or by activating a single or a group of synapses repeatedly (temporal summation) (Module 10: Figure summation of excitatory inputs). In order for neuron B to fire an action potential, its resting membrane potential must depolarize towards a threshold value. Activation of a single synapse (first arrows in the two insets of Module 10: Figure summation of excitatory inputs) causes a small EPSP that fails to reach threshold. During spatial summation, inputs from other synaptic regions of neuron A (e.g. synapses 1 and 2) or from the synapses of other neurons (e.g. synapses i and ii from neuron C) combine to induce a sufficiently large EPSP to trigger an action potential. During temporal summation, large EPSPs arise through repetitive firing of a single synapse (e.g. synapse 1 from neuron A). Spatial and temporal summation is used to integrate inputs from different neurons. If such summation depolarizes the soma sufficiently to reach threshold, it will induce the neuron to fire an action potential. This action potential has an interesting property in that it not only propagates in the forward direction, i.e. down the axon towards the synaptic endings, but also spreads

as a back-propagating action potential that invades the dendrites.

In summary, the EPSPs and the IPSPs represent the bits of electrical information that are used to process and transmit information through neural circuits. These electrical signals spread electronically throughout the dendritic tree, where they sum with those from other spines to produce the integrated electrical drive to either excite or inhibit neurons from firing action potentials. The minute contributions from each synapse are integrated electronically to produce the global signal necessary to excite the postsynaptic cell. As such, these electronic signals are not input (spine)-specific because they rapidly spread away from each synapse. Such input-specific signalling is carried out by the second messengers and associated biochemical changes that are confined within the synaptic endings. A typical example is the postsynaptic generation of Ca^{2+} signals that are localized to the synaptic regions. It is these input-specific local biochemical signals that are used to bring about the synaptic modifications responsible for learning and memory.

Postsynaptic generation of Ca^{2+} signals

Neurons can generate Ca^{2+} signals with widely different spatial and temporal characteristics. Input-specific Ca^{2+} signalling in spines is the most precise form of spatial signalling, and is an example of elementary Ca^{2+} events. Under certain circumstances, particularly during intense stimulation, these input-specific signals confined to the spines can initiate global Ca^{2+} signalling by spreading out to other regions of the neuron. This synaptic stimulation of global Ca^{2+} signals can appear as a regenerative Ca^{2+} wave that may travel down the dendrites to invade the soma and nucleus.

Input-specific Ca^{2+} signalling in spines

Input-specific Ca^{2+} signals, which are confined to individual spines, are the smallest units of neuronal integration. These highly localized signals are derived from both neuronal Ca^{2+} entry and release channels. The way in which these two sources are used varies considerably between neurons, as shown for the Schaffer collateral/hippocampal CA1 synapse and the parallel fibre/Purkinje cell synapse (Module 10: Figure synaptic signalling mechanisms).

Schaffer collateral/hippocampal CA1 synapse

Much is known about the Schaffer collateral/hippocampal CA1 synapse that forms between the CA3 and CA1 neurons as part of the hippocampal trisynaptic circuit (Module 10: Figure hippocampus). The spines on the CA1 neuron have three glutamate-sensitive receptors capable of responding to the glutamate released from the synaptic endings of the Schaffer collaterals coming from the CA3 neurons (Module 10: Figure synaptic signalling mechanisms). The α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor gates Na^+ to give the excitatory postsynaptic potential (EPSP), and this local depolarization has two important consequences. Firstly, it removes the Mg^{2+} block of the *N*-methyl-D-aspartate (NMDA) receptor, allowing it to open in response to glutamate to allow Ca^{2+} to enter the spine. Secondly, the depolarization can activate voltage-operated channels (VOCs) to provide another source of Ca^{2+} entry. Under certain circumstances, this influx of external Ca^{2+} through the NMDA receptors and the VOCs can be amplified further by release of internal Ca^{2+} by activating the type 2 ryanodine receptor (RyR2) through the process of Ca^{2+} -induced Ca^{2+} release (CICR).

The spine also has metabotropic type 1 glutamate receptors (mGluR1s), which are usually located outside the postsynaptic density and are coupled to the formation of inositol 1,4,5-trisphosphate (InsP_3) (Module 10: Figure postsynaptic density). There do not appear to be any InsP_3 receptors on the spine apparatus in hippocampal CA1 neurons, but they are located on the dendrites (Module 10: Figure synaptic signalling mechanisms). There is evidence that, when stimulation is excessive, InsP_3 spills out of the spine to set up a dendritic Ca^{2+} wave by activating these receptors. This synaptic stimulation of global Ca^{2+} signals can create waves that travel down the dendrite and

into the soma to engulf the nucleus (Module 10: Figure somatic and dendritic Ca^{2+} waves).

All these sources of Ca^{2+} have been implicated in long-term potentiation (LTP) at the Schaffer collateral/hippocampal CA1 synapse. These Schaffer collateral/hippocampal CA1 synapses display input-specific signalling in that the Ca^{2+} signals that develop following stimulation of the Schaffer collaterals are largely confined to individual spines (Module 10: Figure hippocampal input-specific Ca^{2+} signals).

Parallel fibre/Purkinje cell synapse

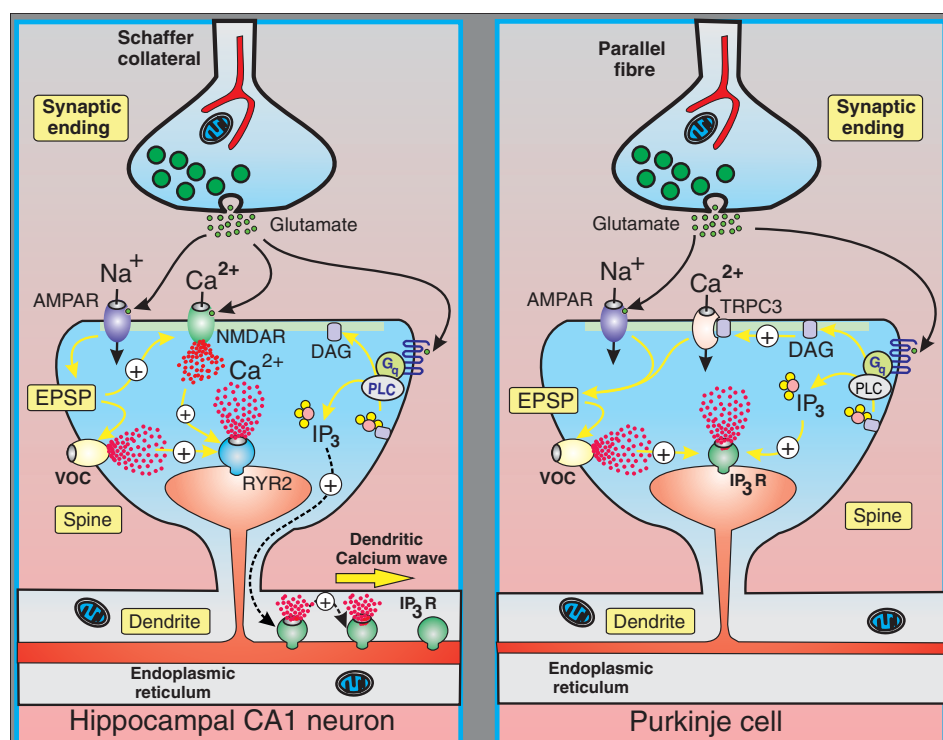
The parallel fibre/Purkinje cell synapse forms between the granule cells, which send out long parallel fibres that make contact with the spines on the Purkinje cells (Module 10: Figure cerebellar neural circuit). The presynaptic ending of the parallel fibre releases glutamate that then activates different glutamate receptors on the spines to induce both an elevation of Ca^{2+} and membrane depolarization (Module 10: Figure synaptic signalling mechanisms). This excitatory postsynaptic potential (EPSP) has two components. There is a fast depolarization that results from the activation of AMPA receptors that gate Na^+ . The other action of glutamate is to stimulate the metabotropic type 1 glutamate receptors (mGluR1s) to form both inositol 1,4,5-trisphosphate (InsP_3) and diacylglycerol (DAG). The functional organization of these two receptor responses to glutamate is shown in Module 10: Figure postsynaptic density. The InsP_3 releases Ca^{2+} from the endoplasmic reticulum (ER) whereas DAG seems to function by stimulating TRPC3 that results in a slow EPSP. There also are voltage-operated channels (VOCs) that introduce Ca^{2+} that acts synergistically with InsP_3 to release Ca^{2+} . This integration of Ca^{2+} and InsP_3 derived from different sources may play an important role in the process of long-term depression (LTD) (Module 10: Figure long-term depression).

Input-specific Ca^{2+} signals have been observed in the spines of Purkinje cells (Module 10: Figure Purkinje cell input-specific Ca^{2+} signal) and dendrites (Module 10: Figure Ca^{2+} signals in spines and dendrites). In some cases, it is possible to recognize the fast contribution made by Ca^{2+} entry and the slower release signal derived from the InsP_3 receptors (Module 10: Figure stimulus intensity and spine Ca^{2+} responses). The fact that the spatial and temporal characteristics of the Ca^{2+} signal in spines can vary, depending on stimulus intensity, has important implications for understanding the Ca^{2+} -dependent changes in synaptic plasticity described later.

Global Ca^{2+} signalling

Neurons display a variety of Ca^{2+} signals ranging from input-specific Ca^{2+} signalling in spines to more global signals that develop either within certain regions or sometimes can engulf large areas of the neuron. Generation of such global signals often depends upon a functional interaction between the neuronal Ca^{2+} entry and release channels. These global signals can be induced through

Module 10: | Figure synaptic signalling mechanisms



The spines on hippocampal CA1 neurons and Purkinje cells have a distinctive set of signalling components responsible for generating neuron-specific postsynaptic responses.

The hippocampal CA1 neuron is activated by glutamate released from the Schaffer collateral. Similarly, Purkinje cells are activated by glutamate released from the synaptic endings of parallel fibres. However, there are marked differences in the way glutamate acts in these two examples, as described in the text.

membrane depolarization and back-propagating action potentials or through synaptic stimulation of global Ca^{2+} signals, or a combination of the two.

Back-propagating action potentials

One of the most reliable ways of inducing a Ca^{2+} signal in neurons is to depolarize the membrane to open the voltage-operated channels (VOCs) distributed over the plasma membrane. This can be done experimentally by immersing neurons in a high K^+ solution. When functioning *in vivo*, such a depolarization occurs each time that the neuron fires an action potential. This action potential travels down the axon and is responsible for triggering the brief pulse of Ca^{2+} in the synaptic terminal. In addition, the action potential can travel in the opposite direction to invade the dendritic tree (Module 10: Figure input-specific signalling), and is referred to as a back-propagating action potential. The ability of the action potential to invade the dendrites is regulated by $\text{K}_{\text{v}}4.2$ channels. As this back-propagating action potential invades the dendritic tree, it generates a uniform elevation of Ca^{2+} that spreads along the dendrites and invades all the spines. In this case, there is no input specificity because Ca^{2+} flooding in through the VOCs is distributed throughout the dendrites and spines and thus creates a global signal. An important feature of this dendritic Ca^{2+} signal is that, during the recovery period, much of it is sequestered in the endoplasmic re-

ticulum (ER), where it can function in priming the inositol 1,4,5-trisphosphate receptors (InsP_3Rs) and ryanodine receptors (RYRs).

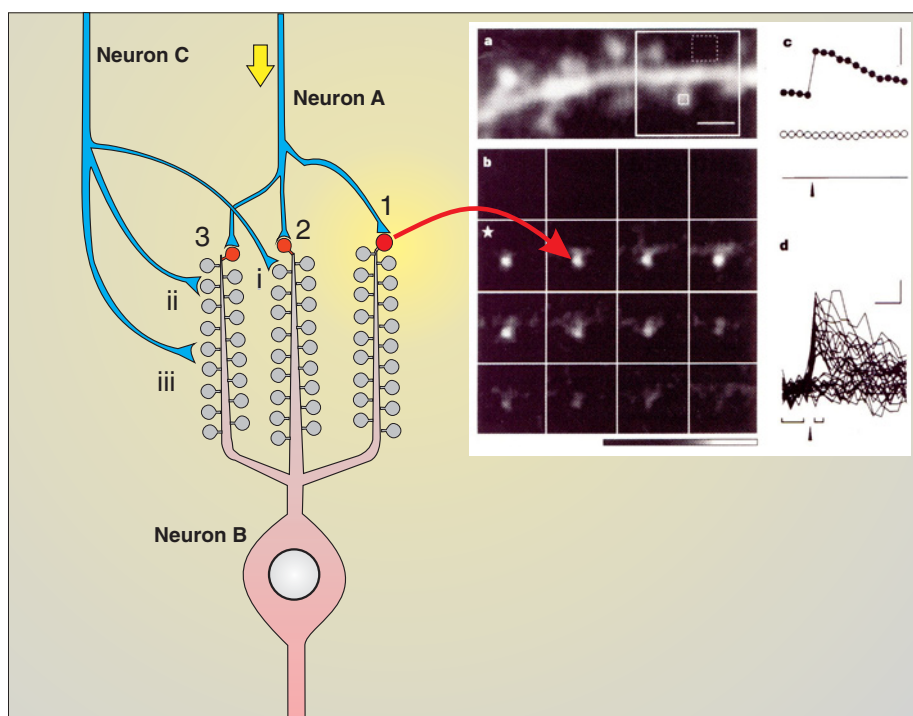
Synaptic stimulation of global Ca^{2+} signals

A good example of a global Ca^{2+} signal triggered by synaptic stimulation is the dendritic response that occurs in Purkinje cells following activation of the climbing fibre (Module 10: Figure Purkinje cell global Ca^{2+} signals). The striking observation is that the global Ca^{2+} signal was very much larger in the dendrites than it was in the soma. Confocal imaging reveals that this small signal in the soma was localized to a 2–3- μm -thick plasmalemmal ring, with very little spread towards the inside of the cell (Module 10: Figure Ca^{2+} signals in Purkinje cell soma). Such global signals often appear as dendritic Ca^{2+} waves.

Dendritic Ca^{2+} wave

Dendritic Ca^{2+} waves are global Ca^{2+} signals, which appear as regenerative intracellular Ca^{2+} waves, and are of particular interest in that they begin at synaptic sites and travel down the dendrites to invade the nucleus (Module 10: Figure somatic and dendritic Ca^{2+} waves). Such dendritic waves have been recorded in a number of neurons:

- For hippocampal CA1 neurons, dendritic waves occur following either synaptic stimulation

Module 10: | Figure hippocampal input-specific Ca^{2+} signals**Input-specific Ca^{2+} signals localized to a single spine.**

The diagram on the left illustrates how neuron B can receive different inputs coming from neurons A and C to form input-specific synaptic endings on different spines. When neuron A generates an action potential, it produces localized Ca^{2+} signals in spines 1, 2 and 3 without any effect on neighbouring spines. The inset illustrates such a localized Ca^{2+} signal in a single spine (red arrow) in the dendritic region of a CA1 hippocampal neuron (large box in panel a). The panels in b represent 64 ms recordings of Ca^{2+} ; note the rapid increase that occurs in a single spine upon a brief stimulation applied at the time of the white asterisk. The average measurements in the spine (small solid box in a) and in a background area (dashed box in a) are shown in c. Summaries of 34 responses are shown in d, which illustrates that not all the stimuli evoked a response, thus illustrating the quantal nature of transmitter release. Reproduced by permission from Macmillan Publishers Ltd: *Nature*, Mainen, Z.F., Malinow, R. and Svoboda, K. (1999) Synaptic calcium transients in single spines indicate that NMDA receptors are not saturated. 399:151–155. Copyright (1999); <http://www.nature.com>; see Mainen et al. 1999.

(Module 10: Figure dendritic Ca^{2+} wave) or cholinergic stimulation (Module 10: Figure neuronal Ca^{2+} wave).

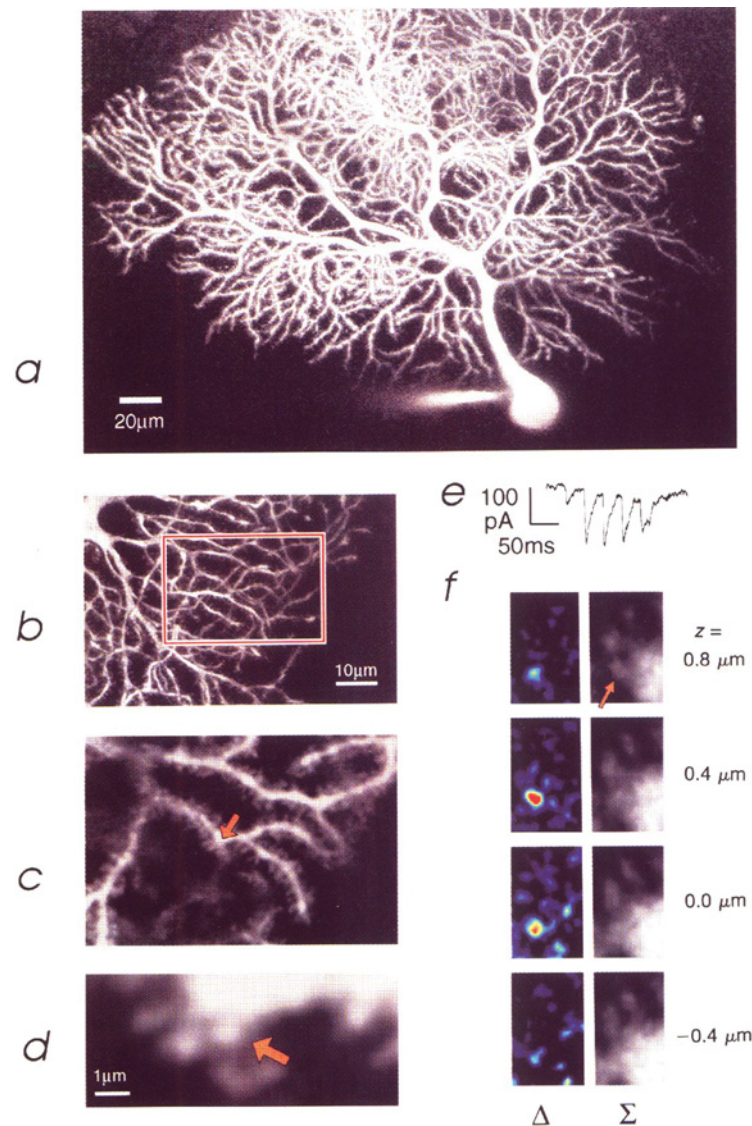
- Basolateral amygdala (BLA) neurons generate dendritic waves very similar to those described above for the CA1 neurons.
- The waves in pre-Bötzinger pacemaker neurons depend on the activation of mGluR5 receptors (Module 10: Figure respiratory pacemaker mechanism).

For all these examples, the wave seems to be triggered by inositol 1,4,5-trisphosphate (InsP_3) formed in the spine which diffuses into the dendrites to trigger a dendritic Ca^{2+} wave (Module 10: Figure synaptic signalling mechanisms). Since these InsP_3 receptors are sensitive to both InsP_3 and Ca^{2+} , wave initiation can be influenced both by the supply of InsP_3 from the synapses and by Ca^{2+} entering through voltage-operated channels (VOCs) during depolarization. Such a summation of these two internal messengers may explain the synergistic interaction observed between synaptic inputs and back-propagating action potentials in neocortical pyramidal neurons (Module 10: Figure neocortical Ca^{2+} wave).

If conditions are favourable, such dendritic Ca^{2+} waves can travel down to the soma and enter the nucleus (Module 10: Figure somatic and dendritic Ca^{2+} waves). The main factor that determines whether such dendritic waves can reach the nucleus seems to depend on the activation of metabotropic glutamatergic or cholinergic inputs, which create the global elevation of InsP_3 that sensitizes the InsP_3 receptors in the dendrites and soma to set the stage for wave transmission from the spines all the way to the nucleus.

In addition to the dendritic waves, it is also possible that there are somatic Ca^{2+} waves that are ignited by Ca^{2+} entering the soma during depolarization (Module 10: Figure somatic and dendritic Ca^{2+} waves). The generation of such dendritic and somatic Ca^{2+} waves capable of invading the nucleus may be particularly important with regard to the activation of neuronal gene transcription responsible for learning and memory.

A similar global Ca^{2+} signal has been recorded in CA3 neurons following intense stimulation. The signal begins in the dendritic region, but soon engulfs the whole cell, including the soma. Much of the Ca^{2+} comes from the outside, but there is a component of internal release

Module 10: | Figure Purkinje cell input-specific Ca^{2+} signals**Localized Ca^{2+} signalling in single spines of a Purkinje neuron.**

a. A Purkinje neuron filled with a Ca^{2+} indicator (Calcium Green) was stimulated through the parallel fibre input (five pulses at 50 Hz). Panels b–d illustrate the regions under investigation, with the arrows pointing to the active spine that gave the Ca^{2+} signal shown in panel f. Σ is the resting fluorescence level taken at four different depths, while Δ represents the difference images (stimulated compared with resting), which show that the Ca^{2+} response occurs in a single spine with little activation of the dendrite. Reproduced, with permission, from Denk, W., Sugimori, M. and Llinas, R. (1995) Two types of calcium response limited to single spines in cerebellar Purkinje cells. *Proc. Natl. Acad. Sci. U.S.A.* 92:8279–8282. Copyright (1995) National Academy of Sciences, U.S.A.; see Denk et al. 1995.

that depends upon priming the inositol 1,4,5-trisphosphate receptors (InsP_3Rs) and ryanodine receptors (RYRs) through variations in the Ca^{2+} content of the internal store.

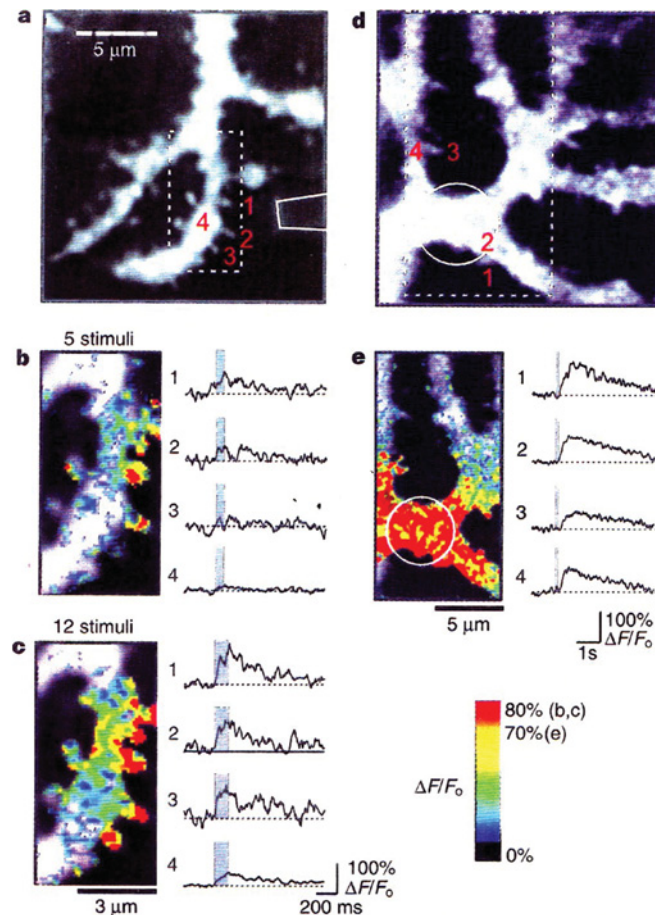
Modulation of neuronal activity

Neural activity can be modulated both in the short term (changes that last for a few seconds) and for much longer periods (years), as occurs in learning and memory. An example of the former is the change in neuronal excitability, which regulates whether or not a neuron will fire an action potential. Another example is the process of facilitation, whereby the amount of neurotransmitter being released

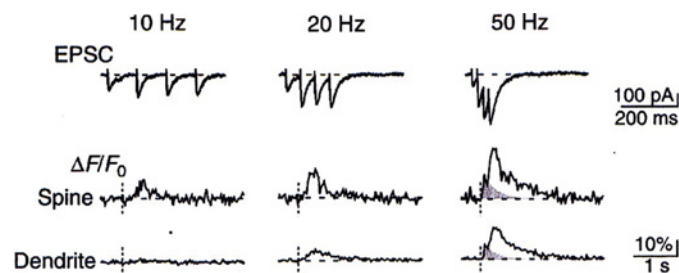
from the synaptic ending can be altered. Longer-term modulation of neuronal activity occurs during learning and memory.

Neuronal excitability

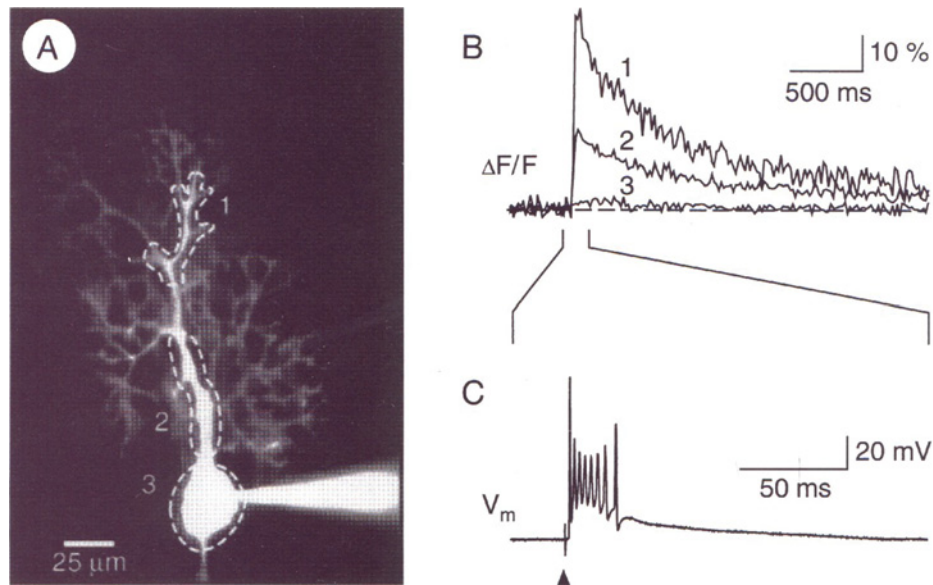
The decision about whether or not to fire an action potential depends upon the balance between the excitatory and inhibitory inputs arriving on the dendritic tree. The major inhibitory inputs on neurons use neurotransmitters such as γ -aminobutyric acid (GABA) receptors and glycine receptors that are Cl^- channels. When these channels open, the resulting change in potential depends on the electrochemical gradient, which is determined by the internal concentration of Cl^- . The neuronal transport of

Module 10: | Figure Ca^{2+} signals in spines and dendrites**Localized Ca^{2+} signalling in the spines and dendrites of a Purkinje cell.**

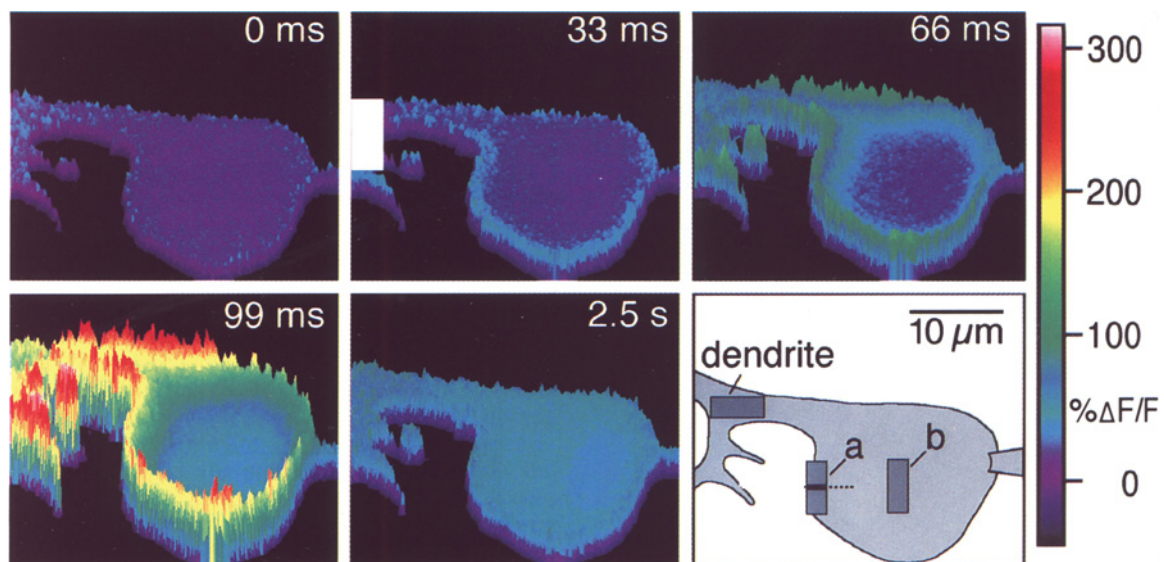
Panel a represents a region of the dendritic tree of a rat Purkinje cell, with the stimulus electrode on the right-hand side. When the parallel fibres were stimulated, Ca^{2+} signals were recorded either in the spines (1, 2 and 3) or in the dendrite (4). At low stimulus intensities (five stimuli; panel b), Ca^{2+} signals were restricted to the spines, whereas at higher stimulus intensity (12 stimuli; panel c), the Ca^{2+} spread into the dendrite. When caged inositol 1,4,5-trisphosphate (InsP_3) was photolysed in a local region (the circle in panel d), there was a large elevation of Ca^{2+} that spread into neighbouring spines and dendrites (panel e). Reproduced by permission from Macmillan Publishers Ltd: *Nature*, Finch, E.A. and Augustine, G.J. (1998) Local calcium signalling by inositol 1,4,5-trisphosphate in Purkinje cell dendrites. 396:753–756. Copyright (1998); <http://www.nature.com>; see Finch and Augustine 1998.

Module 10: | Figure stimulus intensity and spine Ca^{2+} responses**Effect of varying stimulus intensity on Ca^{2+} responses in single spines or neighbouring dendrites.**

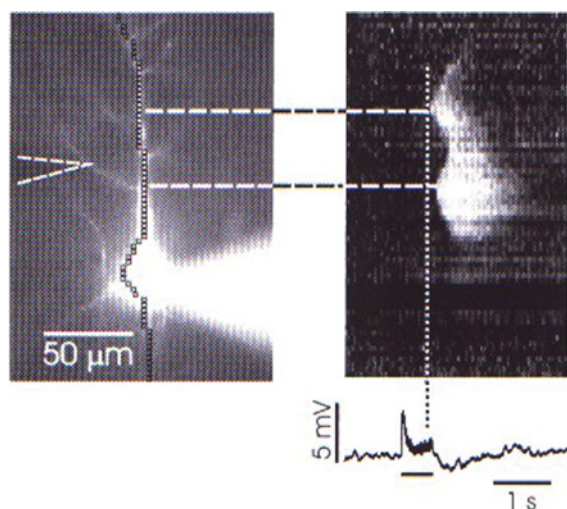
If the parallel fibres are stimulated at low frequencies (10 Hz), a Ca^{2+} signal appears in the spine after a latency of 200 ms; it reaches a peak after a further 260 ms, but there is no response in the dendrites. The slow response is caused by the time taken for glutamate to act on the metabotropic glutamate 1 receptors (mGluR1) to generate inositol 1,4,5-trisphosphate (InsP_3), which then releases Ca^{2+} from the internal store. At higher stimulation intensities (20 Hz), the spine response was larger, and some Ca^{2+} appeared in the dendrite. When stimulated at 50 Hz, glutamate activated the α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors sufficiently to induce an excitatory postsynaptic current (EPSC) that depolarizes the membrane sufficiently to open voltage-operated channels (VOCs) to give an early Ca^{2+} spike that develops with a short delay of 7 milliseconds. This early signal due to Ca^{2+} entry (the grey shaded area) was then followed by a much larger signal as the slower developing InsP_3 -dependent release of Ca^{2+} began to appear. Reproduced by permission from Macmillan Publishers Ltd: *Nature*, Takechi, H., Eilers, J. and Konnerth, A. (1998) A new class of synaptic response involving calcium release in dendritic spines. 396:757–760. Copyright (1998); <http://www.nature.com>; see Takechi et al. 1998.

Module 10: | Figure Purkinje cell global Ca^{2+} signals**Activation of a global Ca^{2+} signal in the dendritic tree of a Purkinje cell following parallel fibre stimulation.**

The climbing fibres that innervate the proximal regions of the dendritic tree release glutamate that activates α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors on the Purkinje cell, resulting in membrane depolarization, which sets off a complex spike that lasts for about 25 ms. As this spike invades the dendritic tree, it activates the voltage-operated channels (VOCs), resulting in a global Ca^{2+} signal throughout the dendritic tree. In addition, the action potential invades the soma, but the resulting Ca^{2+} signal is relatively small and confined to the outer region because of the large surface-to-volume ratio (Module 10: Figure Ca^{2+} signals in Purkinje cell soma). Reproduced, with permission, from Eilers, J., Callewaert, G., Armstrong, C. and Konnerth, A. (1995) Calcium signaling in a marrow somatic submembrane shell during synaptic activity in cerebellar Purkinje neurons. *Proc. Natl. Acad. Sci. U.S.A.* 92:10272–10276. Copyright (1995) National Academy of Sciences, U.S.A.; see Eilers et al. 1995.

Module 10: | Figure Ca^{2+} signals in Purkinje cell soma**Depolarization-induced Ca^{2+} signals in a Purkinje cell.**

Ca^{2+} was recorded using the Ca^{2+} indicator Calcium Green-5N. When the neuron was depolarized for 100 ms from -60 mV to 0 mV at the time of the second image (white flag), there was a rapid increase in Ca^{2+} that was mainly confined to a narrow ring of cytoplasm immediately below the plasma membrane of the soma. Reproduced, with permission, from Eilers, J., Callewaert, G., Armstrong, C. and Konnerth, A. (1995) Calcium signaling in a marrow somatic submembrane shell during synaptic activity in cerebellar Purkinje neurons. *Proc. Natl. Acad. Sci. U.S.A.* 92:10272–10276. Copyright (1995) National Academy of Sciences, U.S.A.; see Eilers et al. 1995.

Module 10: | Figure dendritic Ca^{2+} wave

Dendritic Ca^{2+} waves evoked by localized synaptic stimulation of hippocampal CA1 neurons.

The patch electrode on the bottom right was used to introduce the Ca^{2+} indicator (Fura-2/AM), which has spread throughout the neuron. The dashed arrowhead on the left indicates the position of the electrode used to stimulate some of the local branches of the dendritic tree with a train of 50 pulses at 100 Hz. The electrical trace at the bottom records the change in the membrane potential at the soma. The Ca^{2+} response was measured in a series of small squares (superpixels) running down the length of the neuron to produce a series of line scans that were then stacked up to produce the spatiotemporal plot shown on the right. Note that the Ca^{2+} signal initiated at two branch points where the side branches joined the main dendritic shaft. Two waves then spread down the shaft. The dotted vertical line indicates that these two waves began about 500 ms after the beginning of the stimulation, and at different times from each other. Reproduced from Nakamura, T., Lasser-Ross, N., Nakamura, K. and Ross, W.N. (2002) Spatial segregation and interaction of calcium signalling mechanisms in rat hippocampal CA1 pyramidal neurons. *J. Physiol.* 543:465–480, with permission from Blackwell Publishing; see Nakamura et al. 2002.

Cl^- depends on members of the cation-chloride cotransporters responsible for either the influx or efflux of Cl^- . In embryonic neurons, the $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter 1 (NKCC1) dominates resulting in high intracellular $[\text{Cl}^-]$, which means that the membrane depolarizes in response to neurotransmitters such as GABA. When neurons mature, the level of NKCC1 declines and there is an increased expression of the K^+/Cl^- cotransporter 2 (KCC2), which results in the characteristic low internal levels of Cl^- that gives rise to the hyperpolarizing action of GABA and glycine in adult neurons.

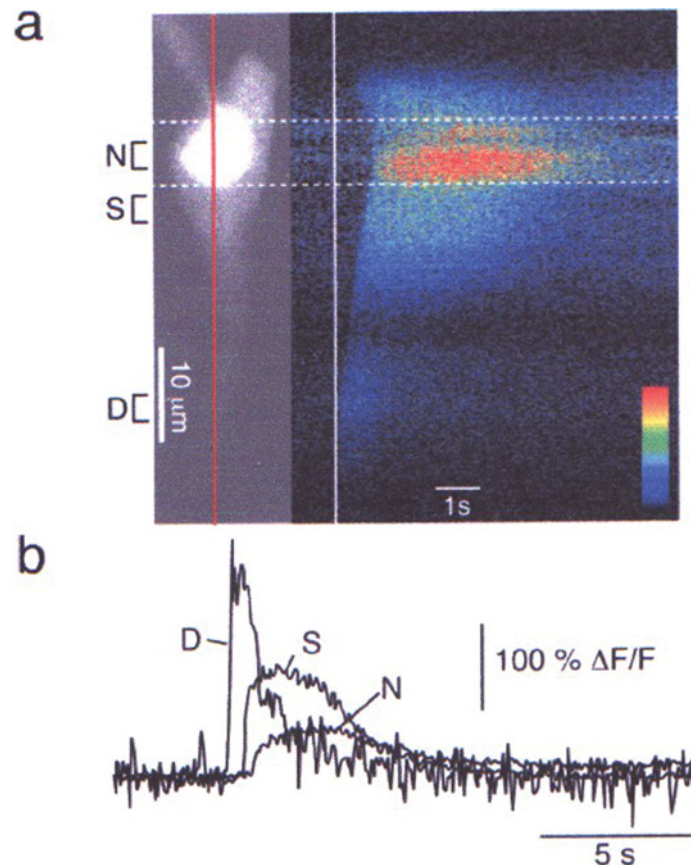
The excitatory inputs on neurons depend on the actions of neurotransmitters such as acetylcholine, ATP and glutamate that open channels that conduct inward current (Na^+ or Ca^{2+}). If the neuron is sufficiently depolarized, it will reach the threshold where it can begin to fire action potentials (Module 10: Figure summation of excitatory inputs). The subsequent pattern of firing will then depend upon other factors, not least of which is the activation of Ca^{2+} -sensitive K^+ channels, which regulate neuronal excitability and thus influence the way information is coded by neurons. These K^+ channels can be activated by Ca^{2+} entering through the voltage-operated Ca^{2+} channels in the plasma membrane or by the release channels in the

endoplasmic reticulum. Junctophilin 3 (JP3) may play a critical role in maintaining the structural organization of the subsurface cisternae (SSCs) (Module 10: Figure neuronal structure) where the voltage-operated Ca^{2+} channels, ryanodine receptors and the Ca^{2+} -sensitive K^+ channels can interact with each other.

The two main channels for regulating excitability are the large-conductance (BK) channels and the small-conductance (SK) channels. The former have a role in spike repolarization. As the BK channels are voltage- and Ca^{2+} -dependent, they activate quickly and their large conductance ensures that the membrane capacitance is quickly recharged. As the potential returns towards its resting value, the BK channels close and any subsequent after-hyperpolarization depends on the existence of other channels such as the SK channels. Modification of these SK channels by signalling systems such as the cyclic AMP pathway can markedly influence the firing frequency of neurons (Module 10: Figure neuronal SK channel function). In addition to the SK channels, there are apamin-insensitive channels that show much slower kinetics in that it takes 0.5–1 s to reach the peak and remain open often for many seconds, and are responsible for the slow after-hyperpolarizing potential (sAHP). This channel, which appears to require the neuronal calcium sensor (NCS) protein hippocalcin, seems to be activated by release of Ca^{2+} from internal stores and is particularly sensitive to neuromodulators in that the sAHP is suppressed by activation of metabotropic, muscarinic, serotonin, histamine and dopamine receptors.

Facilitation

The probability of release from synaptic endings is relatively low for small synaptic vesicles (0.1–0.3) and even lower for large dense-core vesicles (LDCVs). However, this probability is not fixed and the amount of transmitter released from the presynaptic terminal can either increase (facilitation) or decrease (depression) during repetitive neuronal activity. The phenomenon of facilitation is usually studied by applying two pulses in rapid succession. This ‘paired pulse facilitation’ (PPF) protocol unveils whether events that occur during the first stimulus can alter the amplitude of the subsequent postsynaptic potential. An increase in amplitude (facilitation) is evident in fast synaptic transmission at both excitatory and inhibitory connections. This short-term synaptic enhancement operates over a time scale of milliseconds to several seconds, and seems to depend on Ca^{2+} . One of the explanations for this phenomenon is the ‘residual Ca^{2+} hypothesis’ originally proposed by Katz and Miledi. The idea is that a residue of Ca^{2+} remains after the first impulse, which can facilitate release by adding to the Ca^{2+} signal of the next response. The operation of this system is thus very dependent on the OFF mechanism, particularly the $\text{Na}^+/\text{Ca}^{2+}$ exchanger and the mitochondria, which are the low-affinity high-capacity Ca^{2+} removal mechanisms responsible for dissipating Ca^{2+} signals (Module 5: Figure Ca^{2+} uptake and extrusion).

Module 10: | Figure neuronal Ca^{2+} wave

A Ca^{2+} wave originating in the dendrites travels down to the soma and invades the nucleus.

a. The fluorescent image of the neuron on the left illustrates the position of the nucleus (N), soma (S) and dendrite (D). The neuron was imaged along the red line and the individual line scans were stacked up to create the spatiotemporal plot shown on the right. The white line illustrates when the carbachol was added. Note that there was an immediate elevation in Ca^{2+} in the dendrite, which then spread towards the soma before invading the nucleus. The time course of this Ca^{2+} wave is shown in panel b. Reproduced, with permission, from Power, J.M. and Sah, P. (2002) Nuclear calcium signaling evoked by cholinergic stimulation in hippocampal CA1 pyramidal neurons. *J. Neurosci.* 22:3454–3462. Copyright (2002) by the Society for Neuroscience; see Power and Sah 2002.

Learning and memory

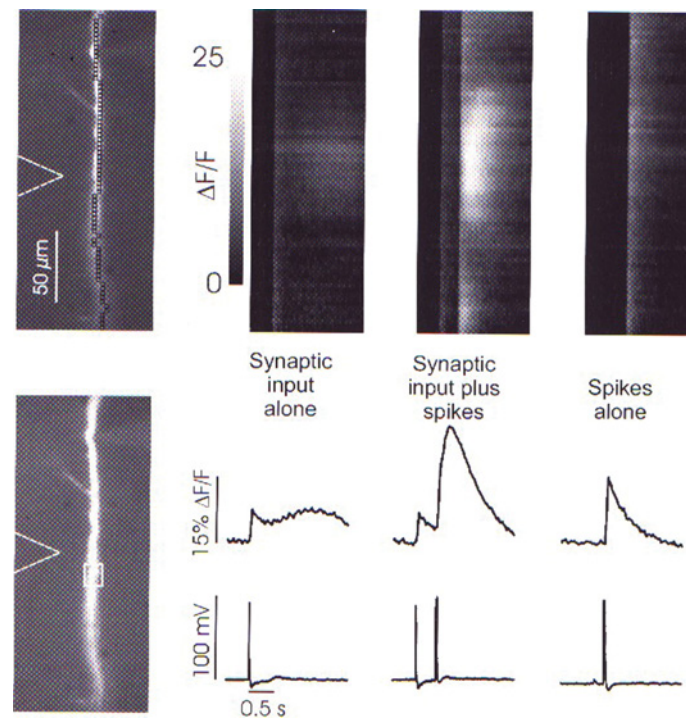
Longer-term modulatory events are mainly concerned with the process of learning and memory. The synapse is often considered to be a fixed computational unit used to transmit information from one neuron to the next. In reality, this junction is highly 'plastic' in that its ability to transmit bits of information can be rapidly adjusted either up or down. Such modifications, which can be long-lasting, are known as long-term potentiation (LTP) or long-term depression (LTD), and are thought to be responsible for the early acquisition phases of learning and memory. For both of these examples, there is a close relationship between Ca^{2+} and synaptic plasticity. The remarkable aspect of both LTP and LTD is that the modifications can occur within seconds and can then persist for long periods (hours). These temporary modifications can be made more permanent (lasting for years) if they are consolidated by the synthesis of new proteins directed by information coming from the nucleus. Synaptic plasticity can thus be divided into two processes: an early memory acquisition phase, when specific synapses are modified by input-specific signalling mechanisms, which is followed

by a later phase of memory consolidation when neuronal gene transcription and protein synthesis function to make these early modifications more permanent.

Neural plasticity has also been implicated in the modification of those neurons that are altered during drug addiction. The relationship between neural plasticity and drug addiction has been studied in medium spiny neurons.

Memory acquisition

While carrying out their daily computations, each synapse can undergo sudden modifications of synaptic efficiency through either long-term potentiation (LTP) or long-term depression (LTD) when subjected to either intense stimulation (as is duplicated experimentally) or a unique coincidence of stimuli (see later). Why is it that the normal stimuli used for information processing pass through the synapse without leaving a memory trace, whereas other stimuli induce longer-lasting changes such as LTP and LTD? In considering this problem, the Canadian neuroscientist David Hebb proposed that such permanent modifications occur when two neurons fire together. As described earlier,

Module 10: | Figure neocortical Ca^{2+} wave**Dendritic Ca^{2+} signals in layer 5 neocortical pyramidal neurons.**

In the panels at the top, Ca^{2+} responses were measured in a series of small squares (superpixels) running down a length of the long dendritic region of a neocortical neuron to produce a series of line scans that were then stacked up to produce the spatiotemporal plots shown on the right. The dashed arrowhead on the left marks the position of the stimulating electrode used to stimulate one of the dendritic branches at 100 Hz for 0.5 s. A patch electrode on the soma (not shown) was used to activate two back-propagating action potentials (spikes) that occurred over 30 ms. The traces on the bottom illustrate the changes in Ca^{2+} concentration that occurred in a small dendritic region (white box). Synaptic input alone induced a brief opening of voltage-operated channels (VOCs), resulting in a small but rapid increase in Ca^{2+} , followed by a slower secondary phase that may have resulted in some release from internal stores. Back-propagating spikes alone caused a somewhat larger early increase in Ca^{2+} concentration, which decayed back to the resting level. However, when the synaptic response was followed 0.5 s later by the spikes, there was a very much larger secondary increase in Ca^{2+} concentration. It seems that the inositol 1,4,5-trisphosphate (InsP_3) produced by the synaptic activity acted synergistically with the Ca^{2+} generated by the spikes to produce a very large dendritic Ca^{2+} signal. Reproduced from Larkum, M.E., Watanabe, S., Nakamura, T., Lasser-Ross, N. and Ross, W.N. (2003) Synaptically activated Ca^{2+} waves in layer 2/3 and layer 5 rat neocortical pyramidal neurons. *J. Physiol.* 549:471–488, with permission from Blackwell Publishing; see Larkum et al. 2003.

neurons normally fire sequentially, with one neuron activating the next neuron and so on through the circuit. However, if the two neurons fire together, then there is a strengthening of their synaptic contacts. This has led to the well-known mnemonic ‘neurons that fire together wire together’. In order to translate this simultaneous activity into a change in synaptic efficiency, there has to be a mechanism of coincident detection, i.e. an ability to associate two events in both time and space. The idea of associativity implies that somewhere within the synapse there have to be biochemical entities that function as neuronal coincident detectors to integrate information coming from two separate sources.

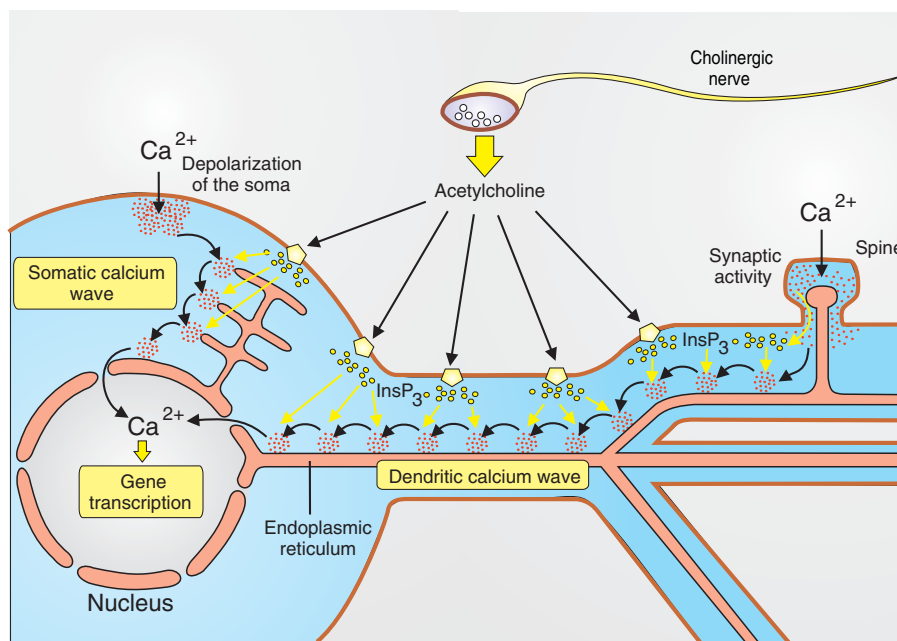
The synaptic plasticity that occurs during LTP is thought to be restricted to individual synapses mainly based on a mechanism of input-specific Ca^{2+} signalling in spines. However, such spine-specific signalling may not be absolutely precise in that neighbouring spines can be sensitized by signals spreading from the active spine through a mechanism known as heterosynaptic metaplasticity. There also is a process of homosynaptic metaplasticity whereby activation of mGluR or cholinergic receptors can provide a

priming mechanism to enhance subsequent LTP especially to weak stimuli.

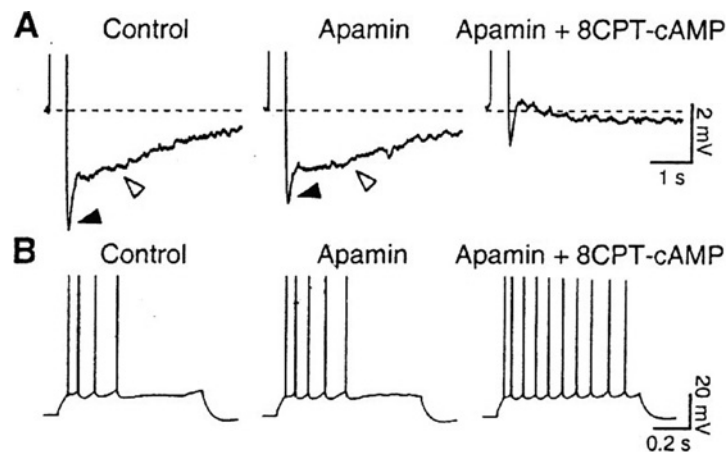
Those synapses that have experienced such a coincidence during the brief period of memory acquisition are modified in some way during this acquisition phase. They are marked out for the later processes of memory consolidation. Some of the early modifications are the phosphorylation of the transmitter receptors, the exocytosis of vesicles containing AMPA receptors and the polymerization of actin and there also is a localized increase in neuronal protein synthesis (Module 10: Figure Ca^{2+} -induced synaptic plasticity).

Neuronal coincident detectors

Associative learning depends upon the ability to associate two events in both time and space. Such associativity implies that somewhere within the synapse there have to be biochemical coincident detectors that integrate information coming from two separate neuronal inputs (Module 10: Figure coincidence detectors). Some of the proposed coincidence detectors [e.g. N-methyl-D-aspartate (NMDA) receptors and inositol

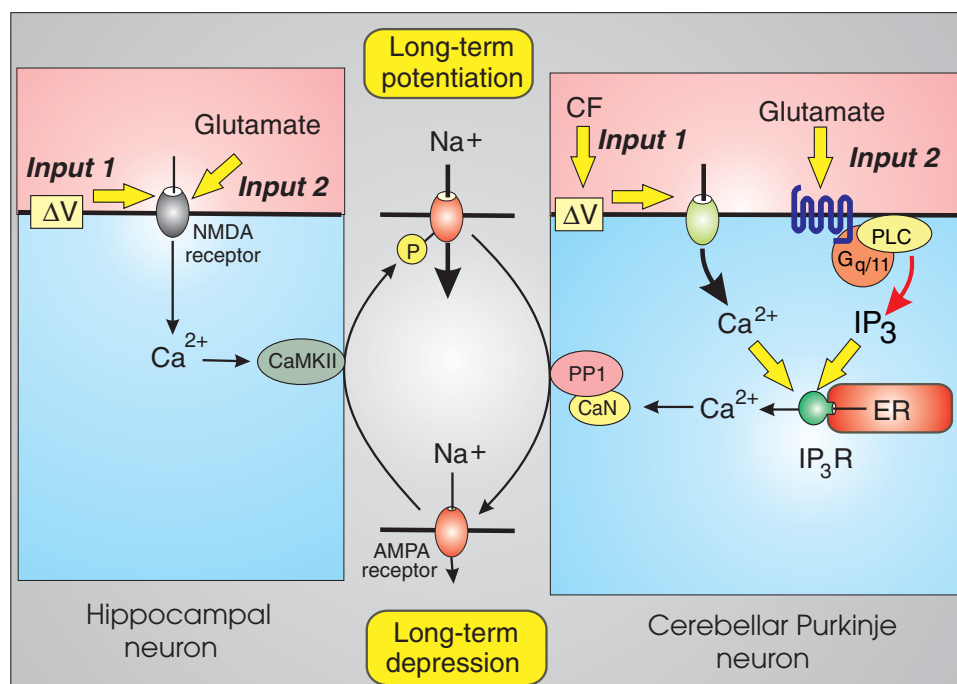
Module 10: | Figure somatic and dendritic Ca^{2+} waves**Neuronal somatic and dendritic Ca^{2+} waves.**

The Ca^{2+} that controls neuronal gene transcription may reach the nucleus by Ca^{2+} waves that initiate at the synapses and propagate down the spines and in to the nucleus. The entry of Ca^{2+} at synapses may ignite dendritic Ca^{2+} waves that propagate by means of inositol 1,4,5-trisphosphate (InsP_3) receptors, which are located in the endoplasmic reticulum, that produce the regenerative Ca^{2+} signal through a process of Ca^{2+} -induced Ca^{2+} release (CICR). Acetylcholine released from cholinergic neurons acts on muscarinic receptors to produce the InsP_3 (yellow dots) that sensitizes the InsP_3 receptors in the dendrites and soma that is necessary for wave propagation. A similar process could account for somatic waves spreading in from the surface of the soma.

Module 10: | Figure neuronal SK channel function**The role of small-conductance (SK) channels in modulating the firing frequency of CA1 hippocampal neurons.**

The activation of the apamin-sensitive small-conductance (SK) channels and apamin-insensitive channels regulates the firing pattern of CA1 hippocampal neurons. **A.** Following a 400 ms depolarizing current pulse, the control displays a fast after-hyperpolarizing potential (fAHP) (closed arrowhead) followed by a slow after-hyperpolarizing potential (sAHP) (open arrowhead). The fast early response was reduced by apamin, whereas the slower response was completely suppressed by an analogue of cyclic AMP. Under control conditions, an 800 ms depolarizing pulse induces a brief burst of four action potentials. Addition of apamin to reduce the fast AHP resulted in a small increase in firing frequency, whereas inhibition of the slow AHP with 8CPT-cyclic AMP abolished the adaptation process, resulting in a prolonged burst of firing. Reproduced, with permission, from Stocker, M., Krause, M. and Pedarzani, P. (1999) An apamin-sensitive Ca^{2+} -activated K^+ current in hippocampal pyramidal neurons. *Proc. Natl. Acad. Sci. U.S.A.* 96:4662–4667. Copyright (1999) National Academy of Sciences, U.S.A.; see Stocker et al. 1999.

Module 10: | Figure coincidence detectors



The function of neuronal coincident detectors in hippocampal neurons and cerebellar Purkinje cells.

In the case of the hippocampal neuron on the left, the coincident detector is the *N*-methyl-D-aspartate (NMDA) receptor that responds to membrane depolarization (ΔV) of the postsynaptic membrane (input 1) and glutamate from the presynaptic ending (input 2). The Ca^{2+} that enters then activates Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), which phosphorylates the α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor, resulting in long-term potentiation. In the Purkinje neuron on the right, the coincident detector is the inositol 1,4,5-trisphosphate receptor (IP_3R), which responds to Ca^{2+} derived from the depolarization (ΔV) induced by the climbing fibre (CF; input 1) and IP_3 generated in response to glutamate stimulation from the parallel fibres (input 2). The Ca^{2+} then activates calcineurin (CaN) and protein phosphatase 1 (PP1), which dephosphorylates the AMPA receptor, resulting in long-term depression (LTD).

1,4,5-trisphosphate (InsP_3) receptors] are responsible for generating Ca^{2+} signals, which play a central role in modifying synaptic plasticity. For both LTP and LTD, there is a clear relationship between Ca^{2+} and synaptic plasticity. Ca^{2+} is derived from both Ca^{2+} entry and release channels, which interact with each other to create the unique Ca^{2+} signalling patterns that bring about either LTP or LTD. Since these channels are the primary targets of the different neuronal inputs, they have been considered to be coincident detectors (Module 10: Figure coincidence detectors).

When considering the central question of how these coincident detectors function, it is necessary to remember that the end result is a burst of Ca^{2+} within the synaptic spine. Some of the best examples of synaptic plasticity are long-term depression (LTD) at the parallel fibre/Purkinje cell synapse and long-term potentiation (LTP) at the Schaffer collateral/CA1 hippocampal cell synapse.

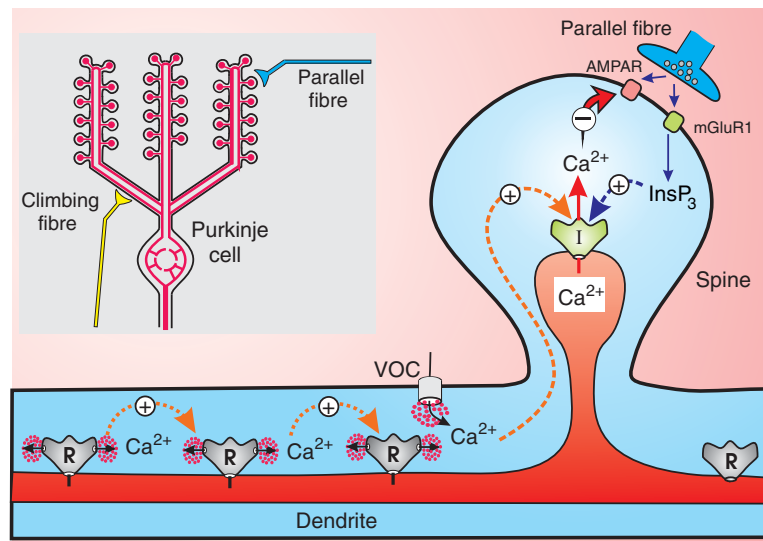
Long-term depression (LTD)

Long-term depression (LTD), which is the process that brings about a reduction in the efficacy of neuronal synapses, occurs in many areas of the CNS. It has been studied in most detail in the hippocampus and cerebellum where there is a strong relationship between Ca^{2+} and synaptic plasticity (Module 10: Figure Ca^{2+} -induced synaptic plasticity).

LTD at the Purkinje cell synapse plays an important role in cerebellar motor learning. The Purkinje cell receives an input from the granule cell in the form of the parallel fibres that innervate the individual spines to form the parallel fibre/Purkinje cell synapse (Module 10: Figure synaptic signalling mechanisms). During motor learning, these synapses undergo long-term depression (LTD). In keeping with the Hebb rule, this LTD occurs when the Purkinje cell is active (depolarized) at the time the relevant synapses receive their input from the parallel fibres. This coincidence is achieved through the activation of two separate inputs: the climbing fibres and the parallel fibres (Module 10: Figure long-term depression). Even though these two inputs innervate separate regions of the Purkinje cell, they are able to interact at the level of the synapse. A major problem concerns the identification of the molecular coincident detector that co-ordinates the information from these two inputs within the synaptic spines where LTD occurs.

The climbing fibre, which innervates the proximal dendrites, acts to depolarize the entire dendritic tree to activate voltage-operated channels (VOCs), resulting in an elevation of intracellular Ca^{2+} within the dendrites and spines. The current debate centres on the nature of the signal that is provided by the parallel fibres. One suggestion is that the inositol 1,4,5-trisphosphate (InsP_3)/ Ca^{2+} signalling cassette might provide the signal coming from

Module 10: | Figure long-term depression



The proposed role of the inositol 1,4,5-trisphosphate (InsP_3)/ Ca^{2+} signalling cassette in coincident detection cerebellar long-term depression (LTD).

The climbing fibre, which innervates the proximal dendrites (grey inset), depolarizes the entire dendritic tree to activate voltage-operated channels (VOCs), resulting in an elevation of intracellular Ca^{2+} that spreads through the dendrites and into the spines (dashed yellow arrows). The parallel fibres that directly innervate the spines release glutamate, which acts on both the α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors and metabotropic type 1 glutamate receptors (mGluR1s). The latter produce inositol 1,4,5-trisphosphate (InsP_3) that acts on the InsP_3 receptor (I), which functions as a coincident detector by responding to the simultaneous presentation of Ca^{2+} and InsP_3 -released Ca^{2+} from the internal store into the spine, where it acts to inhibit the AMPA receptor.

the metabotropic type 1 glutamate receptors (mGluR1s) that are activated by the parallel fibres during coincident detection. In this case, it is the inositol 1,4,5-trisphosphate (InsP_3) receptor that functions as the coincident detector to integrate the signals coming from the two inputs (Module 10: Figure coincidence detectors).

Timing plays a critical aspect in the integration of the two inputs during coincident detection. It turns out that the most effective timing is for the parallel fibre (input 2) to fire 50–200 ms before the climbing fibres (input 1). This timing constraint can be explained on the basis of the fact that the InsP_3 has a longer half-life than Ca^{2+} , and can thus provide a reasonably long window during which the subsequent firing of the climbing fibre can be detected, as shown in the following sequence of events illustrated in Module 10: Figure LTD timing:

- One of the parallel fibres fires to induce an input-specific elevation of InsP_3 in a selected spine (marked in red).
- After the parallel fibre has fired, the level of InsP_3 in this spine remains elevated for a 50–200 ms window.
- Activation of the climbing fibre during this window induces a global elevation of Ca^{2+} throughout the dendritic tree (yellow). When this Ca^{2+} enters those spines that have an elevated level of InsP_3 (the spine marked in green), these two messengers act synergistically to release Ca^{2+} from the internal store.
- The large increase in Ca^{2+} that occurs in such selected spines is then responsible for a spine-specific induction of LTD that is restricted in an input-specific way to

those spines that ‘saw’ the simultaneous elevation in both Ca^{2+} and InsP_3 (spine marked in white).

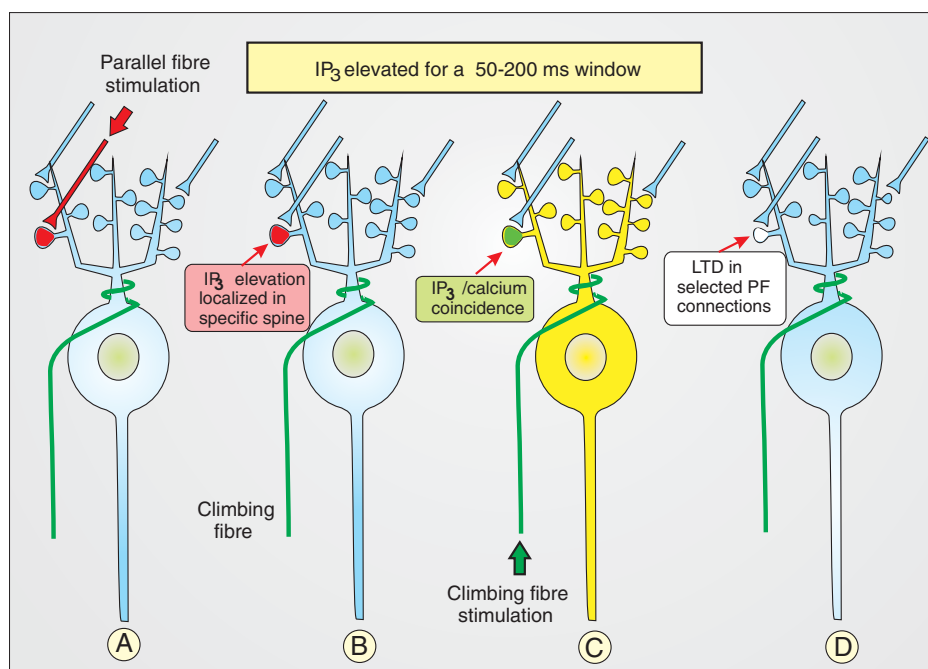
An alternative explanation is that the nitric oxide (NO)/cyclic GMP signalling pathway might provide the signal from the parallel fibres that is responsible for coincidence detection. The idea is that when the parallel fibre is activated, the presynaptic increase in Ca^{2+} activates neuronal NO synthase (nNOS) to form NO, which then diffuses across to the Purkinje cell, where it activates the cyclic GMP signalling cascade and somehow interacts with the Ca^{2+} signal to bring about LTD.

Long-term potentiation (LTP)

Hippocampal LTP

The Schaffer collateral/CA1 hippocampal neuron synapse undergoes either long-term potentiation (LTP) or long-term depression (LTD), depending on the frequency of stimulation. A 100 Hz train of action potentials lasting for just 1 s can induce LTP, whereas a much longer period of stimulation (10 min) at 1–3 Hz produces LTD. In these experiments, the stimuli were applied to a single synaptic input (the Schaffer collateral) that then undergoes either LTP or LTD, depending on stimulus intensity. As such, this would appear to contradict the Hebb rule (see the section on memory acquisition) in that there is no apparent requirement for input from another neuron. However, there is a way whereby the Hebb rule might apply, at least for the induction of LTP in this experimental paradigm. The brief period of intense stimulation may release enough glutamate to activate

Module 10: | Figure LTD timing



The role of inositol 1,4,5-trisphosphate (IP₃) in timing the coincidence window between parallel fibre and climbing fibre firing during the induction of long-term depression (LTD).

This is an example of input-specific signalling, because only those spines that receive a parallel input are susceptible to the subsequent signal from the climbing fibre to bring about long-term depression (LTD). A–D illustrate the proposed sequence of events that occur during LTD at the parallel fibre/Purkinje cell synapse, as described in the text.

the α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors to depolarize the membrane sufficiently to remove the Mg^{2+} block on the *N*-methyl-D-aspartate (NMDA) receptors, so that they will open in response to glutamate. In this case, the intense stimulation is duplicating what happens normally, where the NMDA receptor functions as the coincident detector that integrates the two components of the Hebbian response, i.e. the depolarization of the CA1 cell (the postsynaptic response) and the glutamate coming from the Schaffer collateral (the presynaptic event) (Module 10: Figure synaptic signalling mechanisms). Opening of the NMDA receptor allows Ca^{2+} to enter the spine, and this contributes to the induction of LTP. However, LTP may be facilitated by Ca^{2+} derived from the opening of ryanodine receptors (RYRs), inositol 1,4,5-trisphosphate receptors (InsP₃Rs) and the voltage-operated channels (VOCs). These different sources appear to be tuned to different levels of stimulation. Lower stimulation levels recruit the RYRs, but as the stimulus intensity increases, there is progressive recruitment of the InsP₃Rs, and then finally the VOCs. In the case of the RYRs, the Ca^{2+} that enters through the NMDA receptor may be amplified further by stimulating the RYRs to release more Ca^{2+} from the internal store through a process of Ca^{2+} -induced Ca^{2+} release (CICR). There is some indication that this release might be mediated by the RYR3, because some forms of synaptic plasticity and spatial learning were found to be impaired in RYR3^{-/-} mice.

So far, we have just considered the Schaffer collateral input on to the CA1 neuron and how the Hebb rule can apply if the synapse is activated at high frequency. In reality, however, the synaptic input on to the CA1 neurons is considerably more complicated. Additional neural inputs provide signalling pathways that can modulate the ability of spines to undergo LTP through a process known as metaplasticity. For example, prior activation of mGluR or cholinergic receptors can provide a priming effect to enhance subsequent LTP especially to weak stimuli.

In summary, hippocampal LTP depends upon an increase in the concentration of Ca^{2+} within the spine that can be derived from Ca^{2+} entry or release from internal stores. Under normal circumstances, entry through the NMDA receptors seems to be the primary mechanism to drive the process of Ca^{2+} and synaptic plasticity (Module 10: Figure Ca^{2+} -induced synaptic plasticity).

Metaplasticity

Metaplasticity is a key element in learning and memory because it determines the sensitivity of spines to respond to signals that initiate the plasticity changes associated with LTP or LTD. Prior history is all important in setting spine sensitivity and this depends on the activation of the other neural pathways that innervate the hippocampus. Such metaplasticity can last for tens of minutes and is homosynaptic. This hippocampal region receives an ascending input from the cholinergic system originating in the medial septum and diagonal band of Broca

(Module 10: Figure CA3 hippocampal local circuits). Activating this cholinergic input induces hippocampal oscillations (Module 10: Figure theta oscillatory mechanisms). The resulting 4–10 Hz theta rhythm (θ) dramatically sensitizes synaptic plasticity. This cholinergic-induced theta rhythm sweeps through the hippocampus whenever rodents explore a new environment. Lesions to the medial septal area that disrupt this rhythm impair spatial memory. By setting up this network oscillator, the cholinergic stimulation induces periodic phases of membrane depolarization, during which the postsynaptic spine is sensitive to the presynaptic pulse of glutamate, exactly as predicted by the Hebbian rule.

The group I metabotropic receptors that signal through the G_q family may also contribute to LTP under certain conditions. In particular, this signalling pathway seems to enhance LTP to weak stimuli. Prior activation of the metabotropic glutamate receptor (mGluR) can provide a priming effect to enhance subsequent LTP that can last for minutes.

Synapses can also display a process of heterosynaptic metaplasticity whereby the induction of input-specific LTP at an individual neuron can reduce the threshold of neighbouring synapses to undergo LTP to subthreshold stimuli. This localized potentiation, which spreads about 10 μm from the activated synapse, can last for about 10 minutes. The nature of the signal that spreads from the activated synapse to the neighbouring synapses has not been determined.

Cerebellar cell long-term potentiation (LTP)

The parallel fibre/Purkinje cell synapse, which shows long-term depression (LTD), also displays long-term potentiation (LTP) that functions to reset the system by reversing the effects of LTD. The activation of LTP is induced by repetitive stimulation of the parallel fibres at 1 or 4 Hz, and these two frequencies appear to be operating through different mechanisms. The 1 Hz stimulation seems to require nitric oxide (NO), but is independent of cyclic GMP, whereas the 4 Hz stimulation may operate presynaptically and depends upon cyclic AMP. More information is needed about how these messengers act to bring about LTP at this synaptic junction.

Ca^{2+} and synaptic plasticity

There is a central role for Ca^{2+} in orchestrating the multiple processes responsible for learning and memory in the mammalian brain. It functions during the early phase of memory acquisition when memories are initially formed and it also activates the regulatory mechanisms responsible for the later phases of memory consolidation when processes that depend on neuronal gene transcription and protein synthesis consolidate the early changes in the strength of synaptic transmission. The action of Ca^{2+} in memory acquisition is complicated by the fact it can either increase or decrease the strength of central glutamatergic synapses. This bimodal action is revealed physiologically as the long-term potentiation (LTP) or long-term depression (LTD) of the AMPA receptors responsible for fast

excitatory neurotransmission. The remarkable aspect of LTP and LTD is that both modifications are controlled by Ca^{2+} . This dual action of Ca^{2+} causing us either to remember (LTP) or to forget (LTD) depend on the generation of high or low levels of Ca^{2+} respectively (Module 10: Figure Ca^{2+} -induced synaptic plasticity). To carry out these sophisticated alterations in synaptic plasticity, neurons recruit many components of the calcium signalling toolkit that are tightly packed together within the dendritic spines.

Each spine is an autonomous unit capable of input-specific signalling, which means that the Ca^{2+} signals used for learning and memory can modify synapses on individual spines selectively leaving neighbouring synapses unchanged.

The next question to consider is how this brief pulse of Ca^{2+} activates the panoply of downstream responses with their very different time courses. Some of the responses, such as AMPA receptor phosphorylation by CaM kinases (CaMKK and CaMKII), spine growth through actin remodelling and the insertion of AMPA receptors, occur quickly, whereas the activation of protein synthesis develops slowly and goes on for much longer. The initial burst of Ca^{2+} may recruit other signalling pathways to complete these later events associated with memory consolidation. For example, Ca^{2+} activates the Ras signalling pathway by stimulating RasGRF and inhibiting SynGAP. The Ca^{2+} /CaM complex interacts with RasGRF1, which is one of the Ras guanine nucleotide release-inducing factors (RasGRFs) (Module 2: Table monomeric G protein toolkit), responsible for activating Ras (Module 2: Figure Ras signalling). The complexity of Ca^{2+} signalling is evident by the fact that, in addition to stimulating Ras through RasGRF1, Ca^{2+} also inhibits SynGAP by acting through CaMKII to increase Ras activity. This activation of Ras is a 'supersensitive threshold detector' in that the interaction between Ca and the Ras pathway is highly cooperative.

The release of brain-derived neurotrophic factor (BDNF) is activated by Ca^{2+} . The dense-core vesicles containing BDNF are associated with calcium-dependent activator protein for secretion 2 (CAPS2) that responds to Ca^{2+} to trigger the release of BDNF in the immediate vicinity of the spine. This spine-specific mechanism ensures that spine growth is restricted to those spines receiving glutamate stimulation. BDNF thus acts in an autocrine manner through TrkB receptors to continue the early Ca^{2+} -dependent stimulation of Ras. Ras occupies a pivotal signalling node because it activates both the PtdIns 3-kinase and MAPK signalling pathways.

An increase in intracellular Ca^{2+} is thus a major signal responsible for bringing about synaptic plasticity. The following sequence provides more details as to how Ca^{2+} acts to modify the properties of the spine such that its sensitivity to glutamate is either enhanced (LTP) or reduced (LTD) (Module 10: Figure Ca^{2+} -induced synaptic plasticity):

1. Each spine has access to a number of Ca^{2+} sources: Ca^{2+} can enter through NMDA receptors (NMDAR) or $\text{Ca}_v1.2$ L-type channels, which are voltage-operated Ca^{2+} channels, and it can also be released from the

endoplasmic reticulum (ER) by ryanodine receptors (RYRs) and inositol 1,4,5-trisphosphate (InsP₃) receptors. In some neurons, such as the medium spiny neurons (MSNs), LTD is induced by Ca²⁺ entering through TRPV1 channels in response to anandamide. The Ca²⁺ signal generated by the NMDARs, which induces LTP, tends to be large whereas the other sources produce the smaller signals responsible for LTD. In response to the different stimulus regimes, the high concentration pulse of Ca²⁺ within the spine recruits the Ca²⁺ sensor calmodulin (CaM) to initiate the processes responsible for LTP such as receptor sensitization through phosphorylation, receptor insertion and spine actin formation. On the other hand, these different processes are reversed by lower concentrations of Ca²⁺ that promote LTD by dephosphorylating these receptors, retrieving cell-surface receptors and depolymerizing the actin filaments.

Receptor sensitization

2. One of the earliest actions of Ca²⁺/CaM is to activate Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), which is a highly versatile enzyme that can phosphorylate a range of substrates. One of these substrates is the excitatory AMPA receptor, which is phosphorylated on Ser-831 to increase the unitary conductance of the channel (Module 3: Figure AMPA receptor phosphorylation). In addition, proteins associated with the AMPA receptor, such as C kinase 1 (PICK1), glutamate receptor interacting protein (GRIP) and transmembrane AMPA receptor regulatory proteins (TARP), are also phosphorylated by CaMKII when Ca²⁺ levels are high to cause the receptor sensitization associated with LTP. On the other hand, low levels of Ca²⁺ activate calcineurin (CaN), which dephosphorylates the protein phosphatase 1 (PP1) responsible for reversing these phosphorylation reactions to cause LTD (Module 10: Figure Ca²⁺ control of LTD and LTP). The CaN is anchored to AKAP79 and is located close to the AMPA receptors in the postsynaptic density (PSD) (Module 10: Figure postsynaptic density).

Receptor insertion

3. Another way in which the synapse is potentiated is through an increase in the insertion of new AMPA receptors, which is activated by CaMKII (Module 10: Figure Ca²⁺-induced synaptic plasticity). AMPA receptors located in recycling endosomes at the base of the spine are constantly being recycled in and out of the membrane. During insertion, vesicles are carried on actin filaments to special exocytotic sites on the side of the spine. The myristoylated alanine-rich C-kinase substrate (MARCKS) protein may play a role in attaching actin to the membrane. A process of endocytosis at a neighbouring site retrieves receptors and returns them to the recycling endosome. During Ca²⁺-induced LTP, there is an increase in the insertion of AMPARs resulting in an enhanced sensitivity to glutamate. During LTP, one of the actions of Ca²⁺ is to stimulate myosin

Vb (MyoVb) to increase vesicle trafficking to the membrane. At resting levels of Ca²⁺, MyoVb is in an inactive folded conformation, but this opens up in response to Ca²⁺ to enable one end to bind to the scaffolding protein Rab11-family interacting protein 2 (FIP2) that interacts with Rab11 on the endosome whereas the myosin heads at the opposite end bind to actin to propel the cargo towards the membrane (see panel C in Module 4: Figure myosin motor). Once this attachment is triggered, there is no further requirement for Ca²⁺ and this means that the myosin can move its cargo towards the membrane after the level of Ca²⁺ has returned to its low resting level.

4. When vesicles are associated with the surface, an increase in Ca²⁺ triggers exocytosis allowing the AMPARs to pass into the plasma membrane and they then diffuse into the synaptic region to enhance synaptic sensitivity. The exocytotic machinery resembles that used for the fusion of synaptic vesicles at the presynaptic ending (Module 4: Figure Ca²⁺-induced membrane fusion). However, the SNARE complex responsible for driving fusion in the spine is very different. The syntaxin-3 (Stx-3) and SNAP-47 in the plasma membrane interacts with synaptobrevin-2 (Syb-2) in the vesicle to form a fusion complex that associates with complexin (Cmx). Just how Ca²⁺ triggers this fusion mechanism remains to be determined.
5. The decrease in the number of receptors that occurs during LTD is caused by an increase in the rate of receptor retrieval through the process of endocytosis, which is induced by lower levels of Ca²⁺ that acts through the Ca²⁺/calmodulin-dependent protein phosphatase calcineurin (CaN). The CaN acts to stimulate some of the key components responsible for the control of endocytosis (Module 4: Figure scission of endocytic vesicles). The activity-regulated cytoskeletal-associated protein (Arc) plays a critical role in facilitating this process of endocytosis.

Another putative control mechanism depends on the metabotropic mGluR1 receptors activating a protein tyrosine phosphatase (PTP) to remove a phosphate from Tyr-876 from the Glu2A subunit on the AMPA receptor, which then enables it to interact with BRAG2. The latter is an Arf guanine nucleotide-exchange factor (GEF) for Arf6, which then recruits AP2 to the membrane to initiate the process of clathrin-mediated endocytosis (Module 4: Figure endocytosis).

Spine actin remodelling

6. Actin remodelling is an important aspect of the change in spine morphology associated with the changes in synaptic strength. The dynamic equilibrium of actin is altered by Ca²⁺ with high levels promoting polymerization and LTP, whereas depolymerization is caused by the low levels that induce LTD (Module 10: Figure Ca²⁺-induced synaptic plasticity). Monomeric G proteins feature very significantly in this remodelling process by activating the Cdc42 signalling mechanism (Module 2: Figure Cdc42 signalling) and the Rac signalling mechanism (Module 2: Figure Rac signalling),

which are two of the main mechanisms for controlling actin assembly. High levels stimulate the Rac guanine nucleotide-exchange factors α -Pix, TIAM and Kalirin to increase the conversion of Rac.GDP into Rac.GTP, which then acts through p21-activated kinase (PAK) and LIMK-1 to phosphorylate cofilin to inhibit its ability to de-polymerize actin. In addition, the phosphorylation of gelsolin by CaMKII acts to stabilize the growing actin filament. The increase in actin functions to increase the length of the spine, it stabilizes AMPA receptors within the post-synaptic density and provides the filaments used for the trafficking and insertion of new AMPA receptors. The activity-regulated cytoskeletal-associated protein (Arc) plays an important role in stabilizing the actin filaments responsible for changing spine morphology.

7. During LTD, there is a reduction in actin polymerization that depends on two mechanisms. Firstly, the low concentrations of Ca^{2+} , which activate calcineurin (CaN), dephosphorylate cofilin leading to actin depolymerization. Secondly, the Arp2/3 complex that drives actin polymerization is switched off by the scaffolding protein interacting with C α -kinase 1 (PICK1). This inhibitory activity of PICK1 is regulated by the Arf signalling mechanism (Module 2: Figure Arf signalling). When Arf1 is bound to GTP, it inhibits PICK1 thus enabling Arp2/3 to polymerize actin as occurs during LTP (as described in 6 above). When LTD is induced, the Arf1-GTP complex is inactivated by G protein-coupled receptor kinase interactor 1 (Git1), which is a guanine nucleotide-exchange factor (GEF). When the inhibitory action of Arf1 is removed, PICK1 inhibits further actin polymerization and the actin filaments depolymerize to bring about the shrinkage of the spines as part of the changes that occur during LTD. It is not clear whether the lower levels of Ca^{2+} that induce LTD play any role in regulation of this Git1-Arf1-PICK1-Arp2/3 signalling pathway.

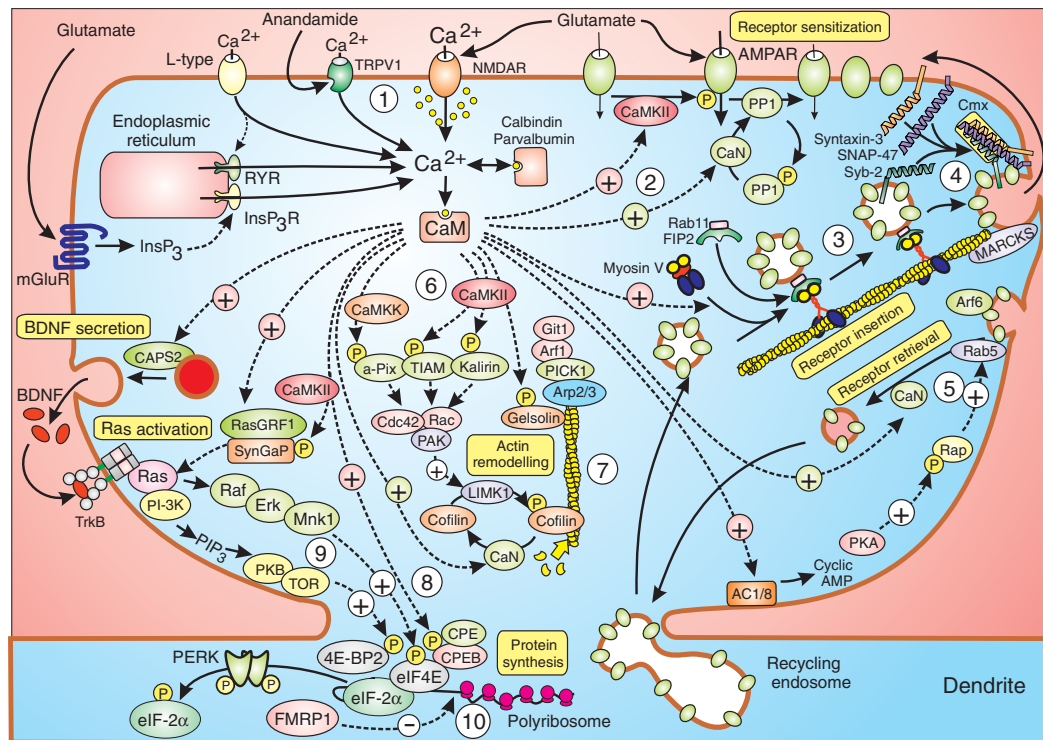
Protein synthesis

8. A local increase in neuronal protein synthesis may play an important role during memory acquisition by 'marking' those spines that are undergoing input-specific modifications during learning and memory. Somehow, the brief electrical events and Ca^{2+} signals must leave behind a tag to mark out those spines that have to undergo the memory consolidation processes necessary to form more permanent memories. These tags are thought to be proteins that are formed during the acquisition phase. But how are the early events linked to a local activation of neuronal protein synthesis in each spine? One mechanism seems to depend on the BDNF, which is released from the spine, acting in an autocrine manner through TrkB receptors to stimulate both the PtdIns 3-kinase and MAPK signalling pathways. There also are indications that the brief pulse of Ca^{2+} within the spine may diffuse down to the polysome located in the dendrite at the base of the spine (Module 10: Figure Ca^{2+} -induced synaptic plasticity). In addition, the long-term changes in syn-

aptic plasticity require new protein synthesis that it induced in a spine-specific manner. Each spine has a polyribosome that contains mRNAs that code for many of the signalling and structural components responsible for the changes in synaptic strength such as Arc, CaMKII, LIMK1 and the GluR1 subunit of the AMPA receptor. The initiation of protein synthesis depends on the eukaryotic initiation factor 2 (eIF-2) (Module 9: Figure regulation of eIF-2 α cycling). Phosphorylation of eIF-2 by PERK, which occurs during endoplasmic reticulum (ER) stress signalling (Module 2: Figure ER stress signalling) inhibits its initiation activity and this could account for a decrease in protein synthesis that would contribute to the calcium-induced memory loss in Alzheimer's disease (AD). This highly localized increase in translation is activated by Ca^{2+} and its associated Ras signalling pathway. One of the actions of Ca^{2+} is to stimulate CaMKII to phosphorylate the cytoplasmic polyadenylation element binding protein 1 (CPEB1) to alleviate its repression of mRNA translation.

9. The PtdIns 3 kinase signalling pathway acts through PKB to stimulate the target of rapamycin (TOR) (Module 9: Figure target of rapamycin signalling) to phosphorylate the cap-binding eukaryote initiation factor 4E (eIF4E), which is a rate-limiting step for the translation of most mRNAs. The MAPK pathway uses MAPK integrating kinase 1 (Mnk1) to phosphorylate the eIF4E-binding protein (4E-BP) that normally prevents eIF4E from binding mRNA. The PHLPP phosphatase that acts through Ras results in a decrease in the MAP kinase signalling pathway and this mechanism reduces neuronal protein synthesis required for long-term memory formation.
10. The fragile X mental retardation protein 1 (FMRP1) binds to mRNA and can inhibit protein synthesis. It can also reduce actin polymerization by inhibiting the p21-activated kinase (PAK) (Module 10: Figure Ca^{2+} -induced synaptic plasticity). Mutation of the *FMRP1* gene, which reduces the expression of FMRP1, is the cause of fragile X syndrome (FXS).

The dual role of Ca^{2+} in controlling both long-term potentiation (LTP) and long-term depression (LTD) (Module 10: Figure coincidence detectors) seems to depend on the way Ca^{2+} is delivered. When different patterns of Ca^{2+} elevation were delivered by the photolysis of caged Ca^{2+} , it was found that a brief high-amplitude pulse induced LTP, whereas a prolonged low-amplitude signal gave LTD. Such variations in the level of Ca^{2+} may then bring about either LTP or LTD by inducing either positive or negative effects on AMPA receptor phosphorylation, receptor insertion and actin polymerization. The main enzymes involved in the phosphorylation/dephosphorylation reactions are the kinases Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) and protein kinase A (PKA), which are opposed by the phosphatases Ca^{2+} /calmodulin-dependent protein phosphatase calcineurin (CaN) [also known as protein phosphatase 2B (PP2B)] and protein phosphatase 1 (PP1). Further details of the function of these different enzymes

Module 10: | Figure Ca^{2+} -induced synaptic plasticityMechanisms of Ca^{2+} -induced synaptic plasticity.

A local increase in Ca^{2+} within the spine triggers a number of events that culminate in the processes of synaptic plasticity. High levels activate long-term potentiation (LTP) that depends on events such as AMPA receptor (AMPA) phosphorylation by CaMKII, which also activates the process of receptor insertion that increases the number of receptors in the postsynaptic membrane. Ca^{2+} also triggers a process of actin remodelling that is part of the morphological change responsible for an increase in the length and volume of the spine. Lower levels of Ca^{2+} that activate calcineurin (CaN) reverse these processes resulting in long-term depression (LTD). An increase in Ca^{2+} also activates protein synthesis by polysomes located in the dendrites at the base of the spine. See text for further details.

with regard to the control of AMPA receptor sensitivity are provided in Module 3: Figure AMPA receptor phosphorylation.

The CaMKII has autocatalytic properties, which ideally suits it for a memory function. High levels of Ca^{2+} promote LTP by stimulating CaMKII and by recruiting the cyclic AMP signalling pathway to promote dephosphorylation of the AMPA receptor through the co-operation between calcineurin and PP1. Activity of the latter is regulated by its associated inhibitor 1 (I1), which inhibits PP1 when it is phosphorylated. By dephosphorylating I1, calcineurin removes this inhibition, thus enabling PP1 to dephosphorylate the AMPA receptor. Through its ability to oppose the phosphorylation events responsible for memory, PP1 is a mechanism for forgetfulness. There also is evidence that this enzyme may play a role in the decline of cognition during ageing.

Cyclic AMP plays a role in LTP by stimulating PKA to phosphorylate I1, thus inactivating PP1 and helping to promote LTP (Module 10: Figure Ca^{2+} control of LTD and LTP). High levels of Ca^{2+} are known to activate adenylyl cyclase to produce the cyclic AMP that will help to promote LTP by turning off the dephosphorylation of the AMPA receptor. Such a mechanism is supported by the observation that CaN and PKA are held together by AKAP79, which is one of the A-kinase-anchoring proteins

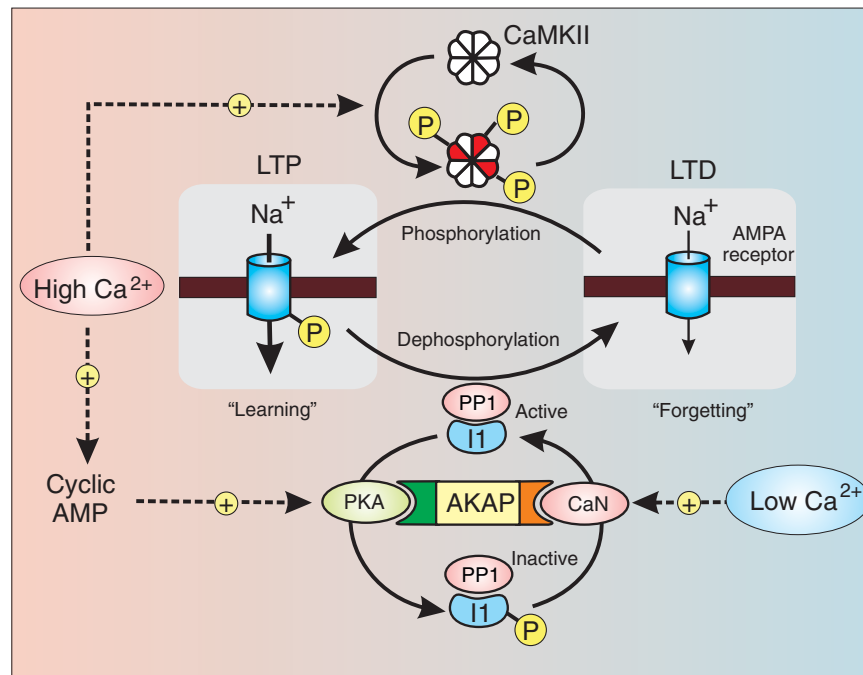
(AKAPs), located within the postsynaptic density (PSD) (Module 10: Figure postsynaptic density).

Low levels of Ca^{2+} , which are unable to activate CaMKII, induce LTD by stimulating the pathways that reverse the processes that are activated during LTP. It has been suggested that Alzheimer's disease (AD) might be caused by an up-regulation of Ca^{2+} signalling that constantly erases the memories formed by the process of LTP.

Some neuropsychiatric diseases such as depression might be caused by a decrease in dietary omega-3 fatty acids that result in a decline in the ability of anandamide to activate LTD.

Activity-regulated cytoskeletal-associated protein (Arc)

The activity-regulated cytoskeletal-associated protein/activity-regulated gene 3.1 (Arc/Arg3.1), which is usually referred to as Arc, is a typical immediate early gene product that has an important role in synaptic plasticity. The structure of the Arc protein indicates that it has an adaptor function in that it contains a number of protein-protein interaction domains. At the N-terminal end there is a coiled-coil (CC) domain. This is followed by an endophilin 3-binding region and a dynamin 2-binding domain that are used for its role in facilitating the process of endocytosis (see below).

Module 10: | Figure Ca^{2+} control of LTD and LTP

Dual action of Ca^{2+} in controlling either long-term depression (LTD) or long-term potentiation (LTP).

Long-term potentiation (LTP) occurs when high levels of Ca^{2+} activate Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), resulting in phosphorylation of the α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors. Conversely, low levels of Ca^{2+} activate calcineurin (CaN), leading to AMPA receptor dephosphorylation and long-term depression (LTD). Further details of how different levels of Ca^{2+} can alter the balance between phosphorylation/dephosphorylation are provided in Module 3: Figure AMPA receptor phosphorylation.

Following the neural activity that leads to memory formation, there is an increase in Arc transcription and Arc translation. In the case of the former, the mRNA formed by neuronal gene transcription (Module 10: Figure neuronal gene transcription) associates with the ribonucleoprotein (RNP) particle that is distributed out into the dendrites by the neuronal mRNA transport system (Module 4: Figure kinesin cargo transport in neurons). In addition to this increase in Arc transcription, there also is a rapid increase in Arc translation from the mRNA transcripts that are located at the base of those spines that are activated following neuronal activity. This local formation of Arc, which appears within minutes and continues to be produced for 2–4 h, functions as a 'master regulator' of synaptic plasticity. Its precise function is somewhat mysterious in that it seems to play a role in both long-term potentiation (LTP) and long-term depolarization (LTD). It is not clear how Arc can function in these diametrically opposite effects. One possibility is that Arc may act on LTP and LTD at different times. During the early process of memory acquisition, Arc may function to maintain LTP by stabilizing the actin-dependent changes in spine morphology. On the other hand, Arc is also used later if these memories are erased by the process of LTD. In this case, Arc is known to facilitate the process of endocytosis that removes AMPA receptors from the spine surface (see step 4 in Module 10: Figure Ca^{2+} -dependent synaptic plasticity). Arc may also act by interfering with the role of BDNF that functions in activating the protein synthesis required for memory con-

solidation (see step 7 in Module 10: Figure Ca^{2+} -dependent synaptic plasticity).

Fragile X mental retardation protein 1 (FMRP1)

The fragile X mental retardation protein 1 (FMRP1) is an mRNA-binding protein that co-operates with microRNAs (miRNAs) to inhibit protein synthesis. One of the functions of FMRP1 is to inhibit protein synthesis in synaptic spines (Module 10: Figure Ca^{2+} -dependent synaptic plasticity). Another function of FMRP1 is to act as an adaptor protein to attach the ribonucleoprotein (RNP) particles to the KIF5 motor for transport out into the dendrites of neurons (Module 4: Figure kinesin cargo transport in neurons).

The FMRP1 gene is mutated in fragile X syndrome (FXS).

Memory consolidation

The rapid Ca^{2+} -dependent changes in synaptic plasticity that occur during the memory acquisition phase are not particularly permanent and begin to fade unless they are consolidated by additional processes that depend upon both RNA and protein synthesis. Synaptic plasticity can thus be divided into an early acquisition phase induced by mechanisms that are independent of protein synthesis, and a late phase that requires the synthesis of macromolecules. The problem to understand is how the small number of synapses on each neuron that have undergone the early

phase are selected to be consolidated by a mechanism that requires information emanating from the nucleus.

A possible mechanism, which is gaining in popularity, is that of synaptic tagging. The idea is that, during the early phase, the synapses that have undergone a temporary synaptic modification are given a biochemical tag, which enables them to sequester the mRNA and/or proteins coming from the nucleus that are then responsible for stabilizing the synaptic change. The formation of the putative flag, which occurs very quickly during the early acquisition phase, provides a biochemical address to which the somewhat slower biosynthetic events (gene transcription and protein synthesis) can be directed for consolidation to occur later on a much lower time scale. This flag may depend on the local activation of protein synthesis that is triggered by the spine-specific Ca^{2+} signal (step 8 in Module 10: Figure Ca^{2+} -induced synaptic plasticity).

Such a tagging mechanism may explain a major problem in neurobiology concerning the way in which electrical activity confined to specific synapses located on the dendritic tree can send signals into the nucleus to activate neuronal gene transcription and neuronal protein synthesis. However, this problem may have been somewhat exaggerated, because the need for specificity may be solved by placing a tag on the synapses that have to be modified. Then all that is required is for there to be a general up-regulation of nuclear events to produce the precursors that are then dispersed throughout the dendrites, where the tagged synapses can deploy them to strengthen their connections. Such a scenario could explain the significance of slow-wave sleep (SWS) in memory consolidation. The specific suggestion is that the slow oscillations (1 Hz) and delta (1–4 Hz) (δ) that sweep through the brain as propagating waves during non-rapid eye movement sleep (NREM) serve to stimulate the nuclear events responsible for producing the mRNA and proteins used to stabilize synaptic connections. The $\text{Cav}1.2$ L-type channels may play an important role in consolidation by providing the Ca^{2+} signals that promote the signalling pathways that regulate both the local protein synthesis at the spines (see Step 8 in Module 10: Figure Ca^{2+} -induced synaptic plasticity) and the signalling pathways responsible for neuronal gene transcription (Module 10: Figure neuronal gene transcription). Whatever the mechanism turns out to be, it is clear that the neural signals that are associated either with the early acquisition phase or some later stage, such as during sleep, are required to activate gene transcription in the nucleus.

Mutations in *CACNA1C* and *CACNB2*, which code for the $\alpha 1c$ and β subunits of the $\text{Cav}1.2$ L-type channels (Module 3: Figure $\text{Cav}1.2$ L-type channel), are a significant risk factor for a number of psychiatric disorders.

Neural plasticity and drug addiction

Addiction to drugs seems to depend on the modification of a brain reward circuitry. This is an example of phenotypic remodelling of the signalsome, because the signalling mechanisms that induce addiction act by altering gene transcription. There is increasing evidence that neural plasticity depends upon an interaction between dopamine

and glutamate signalling pathways, as has been studied in medium spiny neurons.

Neuronal gene transcription

An increase in intracellular Ca^{2+} plays a critical role in controlling neuronal gene transcription. In addition to carrying out the rapid but short-lasting events linking changes in Ca^{2+} to synaptic plasticity within the spines, Ca^{2+} also plays a dominant role in activating the genetic processes that serve to consolidate the early memory events. What is fascinating about this role of Ca^{2+} in memory consolidation is that it employs many of the same signalling pathways used by growth factors to regulate cell proliferation. An excellent example of the conservation of signalling pathways is that the same mechanism is adapted to perform many different control functions. In the case of neurons, the main adaptation is to couple neurotransmitter action or electrical activity in the plasma membrane to gene activation in the nucleus. This coupling is achieved primarily by using Ca^{2+} , which enters through $\text{Cav}1.2$ L-type channels (Module 3: Figure $\text{Cav}1.2$ L-type channel) that are voltage-operated channels (VOCs), as the primary messenger to initiate the plasma membrane-to-nucleus signalling cascades (Module 10: Figure neuronal gene transcription). Despite the fact that other entry pathways, such as the *N*-methyl-D-aspartate (NMDA) receptors, can give rise to Ca^{2+} signals similar to those induced by the VOCs, they are much less effective in activating gene transcription. The significance of the VOCs is that they are closely coupled to other signalling pathways, such as the mitogen-activated protein kinase (MAPK) and cyclic AMP signalling cascades through Ca^{2+} . Most of these Ca^{2+} actions are carried out by calmodulin (CaM), which is released from its association with neurogranin and is then free to act on various signalling pathways. Ca^{2+} mediates its effects either through direct Ca^{2+} -mediated gene transcription by activating Ca^{2+} /calmodulin-dependent protein kinase IV (CaMKIV), or by using indirect Ca^{2+} -mediated gene transcription mechanisms, where Ca^{2+} acts by recruiting other pathways such as those regulated through MAPK or cyclic AMP.

One of the major targets for these signalling cascades is the transcription factor cyclic AMP response element-binding protein (CREB) and its associated CREB-binding protein (CBP), which functions as a transcriptional co-activator to link CREB to the basal transcriptional complex (Module 4: Figure CREB activation). The CREB/CBP complex has emerged as the prototypical transcriptional factor that is activated during the consolidation of memory. Other transcription factors that are activated include serum response factor (SRF), ETS-like transcription factor-1 (Elk-1) and the nuclear factor of activated T cells (NFAT).

A large number of genes are activated during this process of Ca^{2+} -dependent memory consolidation and many of these code for the postsynaptic density (PSD) signalling elements that function in Ca^{2+} and synaptic plasticity (Module 10: Figure postsynaptic density):

- Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII)
- Activity-regulated cytoskeletal-associated protein (Arc)
- Inositol 1,4,5-trisphosphate receptor (InsP_3R)
- Homer, which binds to the InsP_3R
- GluR1 and GluR2 (subunits of the AMPA receptor)
- NR2A and NR2B (subunits of the NMDA receptor)

The activity of the transcription factors, such as CREB and NFAT, that are responsible for activating these neuronal genes is also tightly regulated by protein acetylation of both histones and various transcription factors. With regard to the latter, the activity of peroxisome-proliferator-activated receptor γ (PPAR γ) coactivator-1 α (PGC-1 α) and p53 in neurons is activated by SIRT1. The change in p53 acetylation induced by SIRT1 may reduce its ability to induce apoptosis. One of the important functions of PGC-1 α is to activate the genes responsible for energy metabolism and antioxidant defences and a defect in this pathway contributes to Parkinson's disease (PD) (Module 12: Figure signalling pathways in Parkinson's disease). In this respect, the level of SIRT1 is elevated in mouse models of other neurodegenerative diseases such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) and this might be an adaptive response to reduce the stress of these diseases particularly by up-regulating antioxidant defences. The relationship between acetylation and AD includes a role for both HDAC2 and HDAC4 as modulators of gene transcription (Module 12: Figure amyloids and Ca^{2+} signalling).

The acetylation of histones by histone acetyltransferases (HATs) such as p300 and CBP serves to remodel chromatin such that it becomes easier to access by the transcription factors and cofactors (Module 10: Figure neuronal gene transcription). This open accessible chromatin is reversed by two of the histone deacetylases (HDACs), HDAC2 and HDAC4, which are regulated in different ways.

HDAC2 is a resident nuclear protein that suppresses the nuclear gene transcription responsible for memory consolidation by deacetylating histones leading to chromatin condensation. The level of HDAC2 is increased in Alzheimer's disease (AD) and this may account for the phenotypic changes that appear to underlie the neurodegenerative changes associated with this disease.

HDAC4 is also a repressor of nuclear gene transcription. The same Ca^{2+} signal that activates gene transcription also phosphorylates HDAC resulting in this repressor being exported from the nucleus.

In a mouse model of AD, the decline in synapses and memory formation lost during neurodegeneration were ameliorated using HDAC inhibitors such as sodium butyrate and suberoylanilide hydroxamic acid (SAHA), which has raised the possibility that it might be possible to recover some long-term memories in patients suffering from dementia.

Neuronal mRNA transport

Many of the mRNA transcripts that are formed during neuronal gene transcription, particularly those involved in memory formation, leave the nucleus and are transported

out into the dendrites. Transcripts of signalling components, such as Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) and activity-regulated cytoskeletal-associated protein (Arc), are packaged together on messenger ribonucleoprotein (RNP) particles made up of proteins such as fragile X mental retardation protein 1 (FMRP1), Pur α and mStaufen. The FMRP1 acts as an adaptor that attaches this RNP particle to the KIF5 motor that transports it along the microtubules out into the dendrites (Module 4: Figure kinesin cargo transport in neurons).

Direct Ca^{2+} -mediated gene transcription

Ca^{2+} stimulates gene transcription using different mechanisms that are initiated either in the cytoplasm or in the nucleus (Module 10: Figure neuronal gene transcription):

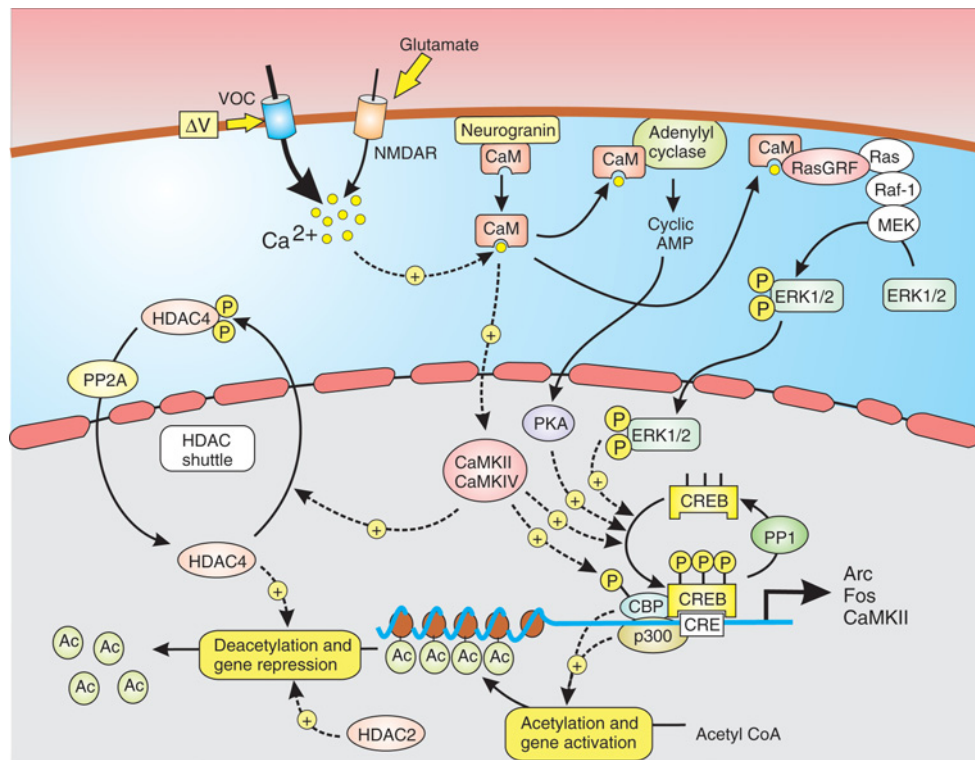
- Ca^{2+} acts through calmodulin (CaM) to stimulate calcineurin (CaN) to dephosphorylate nuclear factor of activated T cells (NFAT), resulting in its translocation into the nucleus to induce transcription of genes such as that for the inositol 1,4,5-trisphosphate (InsP_3) type 1 receptor ($\text{InsP}_3\text{R1}$).
- The Ca^{2+} /CaM complex formed in the cytoplasm translocates into the nucleus to activate Ca^{2+} /CaM-dependent protein kinase II (CaMKII) and IV (CaMKIV) to phosphorylate cyclic AMP response element-binding protein (CREB). This translocation of CaM into the nucleus functions as a memory of prior neuronal activity in that it can prime the responsiveness of CREB to Ca^{2+} -dependent phosphorylation. It seems that the nuclear level of CaM is limiting for this phosphorylation process. Translocation of CaM could also serve to integrate signals from different inputs.
- Ca^{2+} can act within the nucleus by stimulating the CREB-binding protein (CBP). Phosphorylation of CREB by CaMKII, extracellular-signal-regulated kinase 1/2 (ERK1/2) or protein kinase A (PKA) is not, in itself, sufficient for CREB activation, which also requires a Ca^{2+} -dependent activation of its associated CBP. Since CBP is a coactivator of many other genes, it is easy to see why Ca^{2+} is so significant in regulating gene transcription.

Indirect Ca^{2+} -mediated gene transcription

In addition to the direct Ca^{2+} -mediated pathways described above, Ca^{2+} can also transmit information into the nucleus by recruiting other signalling pathways. Both the Ras/mitogen-activated protein kinase (MAPK) and cyclic AMP signalling pathways have Ca^{2+} -sensitive components enabling them to sense the Ca^{2+} entering across the plasma membrane (Module 10: Figure neuronal gene transcription):

- Many neurons express isoforms of the Ca^{2+} -sensitive adenylyl cyclases (Module 2: Table adenylyl cyclases). The localization of types 1 and 8 in the hippocampus and cerebellum indicates a possible role in synaptic plasticity. In response to Ca^{2+} entry, during synaptic transmission, therefore, there will be an accompanying burst

Module 10: | Figure neuronal gene transcription

**Ca²⁺-mediated neuronal gene transcription.**

Elevation of Ca²⁺ through depolarization of the neuronal membrane (ΔV) to stimulate voltage-operated channels (VOCs) or through glutamate-dependent NMDA receptor (NMDAR) stimulation acts to release calmodulin (CaM) bound to neurogranin. Ca²⁺/CaM then induces gene transcription either directly through nuclear factor of activated T cells (NFAT) or Ca²⁺/CaM-dependent protein kinase II (CaMKII), or indirectly by recruiting the Ras/mitogen-activated protein kinase (MAPK) or cyclic AMP-dependent signalling pathways.

of cyclic AMP, which could have several functions. It could act locally to mediate the early synaptic modifications, such as long-term potentiation (LTP) (Module 10: Figure Ca²⁺ control of LTD and LTP), or it could have more global effects by inducing the translocation of PKA into the nucleus (Module 10: Figure neuronal gene transcription).

- The other major signalling pathway recruited by Ca²⁺ is the Ras/MAPK pathway. The entry of Ca²⁺ through the L-type channel acts locally to stimulate the Ras guanine nucleotide release-inducing factors (RasGRFs) that activates Ras and the MAPK signalling pathway (Module 2: Figure Ras signalling). The activated extracellular-signal-regulated kinase 1/2 (ERK1/2) then translocates into the nucleus to phosphorylate cyclic AMP response element-binding protein (CREB) (Module 10: Figure neuronal gene transcription).

Neuronal protein synthesis

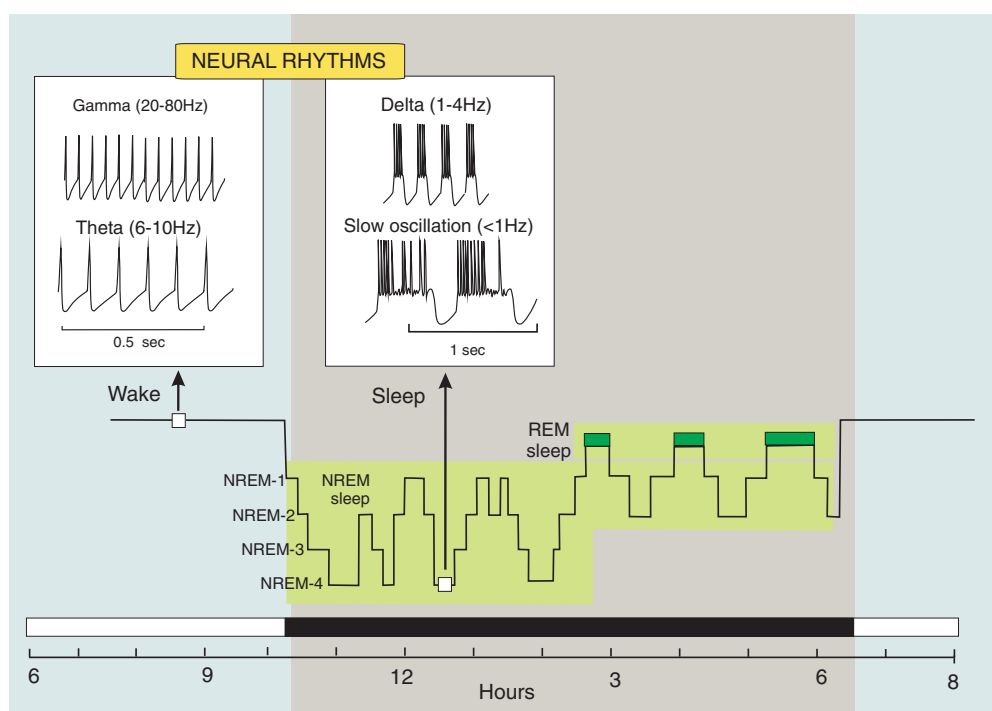
The protein synthesis that plays a critical part in both memory acquisition and memory consolidation has to be highly localized to those synapses that are being modified, and this is achieved by having a polyribosome associated with each spine (Module 10: Figure synaptic organization). Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) mRNA is one of the few dendritic mRNA molecules that

have been identified. The 3'-untranslated region (UTR) of CaMKII mRNA has elements that target it to the dendrites where it is used to increase the synthesis of this enzyme during synaptic plasticity.

This increase in protein synthesis that occurs during memory acquisition is facilitated by the Ca²⁺/CaMKII-dependent phosphorylation of the cytoplasmic polyadenylation element binding protein (CPEB) that initiates mRNA polyadenylation and protein synthesis (see step 8 in Module 10: Figure Ca²⁺-induced synaptic plasticity).

Sleep and consciousness

The neural activity of the brain during wakefulness when one is conscious and aware of ones surroundings is very different to that during sleep. Electroencephalogram (EEG) recordings indicate a rapid alteration in neural activity at the onset of sleep as the brain switches from consciousness to a period of unconsciousness. There are two types of sleep. Firstly, there is a global sleep process during which the entire brain essentially goes off line as a result of a marked reduction in its responsiveness to sensory stimulation. This prolonged period of sleep consists of two main phases: rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep phases that cycle with an ultradian frequency of approximately 90 min (Module 10: Figure sleep phases). Secondly, there are

Module 10: | Figure sleep phases**Sleep phases.**

Sleep consists of two main phases: non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. The NREM sleep has four different stages. During the wake state, neurons in the brain fire at the fast theta and gamma neural rhythms, which then switch rapidly to the delta and slow oscillations that characterize sleep.

local sleep processes where isolated regions of the brain go to sleep.

A number of sleep and wake regulatory mechanisms are responsible for switching the brain between states of sleep and wakefulness (Module 10: Figure sleep/wake cycle regulation). One of the master regulators of the global sleep/wake cycle is the ascending arousal system, which wakes up the sleeping brain and is responsible for maintaining the period of wakefulness. This arousal system consists of a heterogeneous population of neurons located within the brainstem, midbrain, basal forebrain and hypothalamus that produce sleep/wake regulatory molecules that are released throughout the major regions of the brain such as the cortex and hippocampus (Module 10: Figure brain anatomy). For sleep to occur, this arousal system has to be switched off by the sleep-inducing systems. The sleep/wake cycle is also controlled by the circadian control of sleep and by the endogenous sleep-regulatory molecules, such as adenosine and $\text{TNF}\alpha$, which are produced locally and may be particularly important in regulating the local sleep processes.

The ascending arousal system controls the sleep/wake cycle by modulating the tonic excitatory drive that controls the neuronal circuits responsible for the different brain rhythms that occur during both wakefulness and the NREM and REM sleep phases. Such oscillations in the EEG recordings reflect the synchronous responses of large assemblies of neurons that oscillate in phase with each other. Such brain rhythm synchronization mechanisms

are particularly important in information processing throughout both the wake and sleep cycle.

Considerable progress has been made in recording the activity of individual neurons in order to understand how specific neuronal cell types contribute to sleep and the various activities associated with sleep such as memory consolidation.

Global sleep

The global sleep process is not homogeneous but consists of two main phases of sleep: rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep phases that cycle with an ultradian frequency of approximately 90 min (Module 10: Figure sleep phases).

Most of the sleep period is NREM (80%) with the remaining being REM sleep that increases in frequency prior to waking.

Non-rapid eye movement (NREM) sleep

Non-rapid eye movement (NREM) sleep can be divided into four distinct phases (NREM-1 to NREM-4) (Module 10: Figure sleep phases). Stages 1 and 2 occur at the beginning and are associated with lighter sleep, whereas Stages 3 and 4 occur during the deeper slow wave sleep (SWS) periods. The defining characteristics of NREM sleep are two oscillatory brain rhythms: delta (1-4 Hz) oscillations and the slow oscillations (<1 Hz) that are particularly evident during deeper periods of sleep. These two slow rhythms are characterized by the membrane potential alternating

between Down states (hyperpolarized) and Up states (depolarized). During the Up state, there often are high frequency oscillations resembling those seen in the awake brain.

The onset of these slow oscillations occurs when the tonic excitatory drive normally activated by the ascending arousal system is reduced at the onset of sleep. Following this decrease in the external drive, the neurons descend into the slow oscillatory states that may depend more on an endogenous slow oscillation mechanism rather than the network oscillatory mechanisms that drive the much faster gamma and theta oscillations. An important aspect of the slow wave oscillation in each neuron is that it occurs in near synchrony with its neighbours that appear to be orchestrated through a slow oscillation synchronization and wave propagation mechanism.

Rapid eye movement (REM) sleep

Rapid eye movement (REM) sleep is characterized by rapid and random movements of the eyes and are the periods when dreaming takes place. The REM epochs occur towards the end of the sleep period and tend to be short at the beginning but then become longer before awakening (Module 10: Figure sleep phases). The precise function of REM sleep has not been fully determined but it may play a role in memory organization and retention.

Local sleep processes

Parts of the brain can be in a sleep state while the rest of the brain is awake. This phenomenon is particularly evident during sleep walking, which is an example of a parasomnia where individuals can be both awake and asleep. These epochs of sleep-like states, which are characterized by typical slow wave oscillations, seem to be localized to individual cortical columns. The question arises as to which of the sleep regulatory mechanisms determine which of the individual columns enter into a period of sleep. These columns may be switched off as part of the generalized homeostatic mechanism of sleep regulation whereby the probability of falling asleep is advanced if the assemblies of neurons within a column have been particularly active in information processing. The build-up of endogenous sleep-regulatory molecules are probably responsible for the switch from wake to sleep-like states in highly localized regions of the brain.

Sleep and wake regulatory mechanisms

There are a number of systems responsible for regulating the sleep/wake cycle (Module 10: Figure sleep/wake cycle regulation). The brain can be rapidly switched between sleep and consciousness by the activation of an ascending arousal system that consists of groups of neurons that project their axons throughout the brain where transmitters such as orexin, acetylcholine (ACh), norepinephrine (NE), 5-hydroxytryptamine (5-HT), histamine and dopamine (DA) are released on to the excitatory and inhibitory neurons that constitute the functional neural circuits. These transmitters then act on receptors that are coupled to signalling pathways that induce the tonic excitatory drive (Module 10: Figure tonic excitatory drive and neuronal

rhythms) that enables these neurons to process information during the wake state.

The orexinergic neurons that release orexin are master regulators in that they also act by stimulating the other arousal neurons (Module 10: Figure sleep/wake cycle regulation). The orexinergic neurons may also integrate the action of a number of other regulators of the sleep/wake cycle such as the circadian control of sleep and factors such as ghrelin, leptin and glucose.

The onset of sleep is initiated by various sleep-inducing systems that act to switch off the ascending arousal system. Sleep can be induced by the endogenous sleep-regulatory molecules or are a result of various neuronal systems such as the circadian control of sleep or the neurons located in the preoptic area of the brain such as the ventrolateral preoptic (VLPO) neurons.

Ascending arousal system

The ascending arousal system consists of a number of different neurons located mainly in the brainstem, midbrain, basal forebrain and hypothalamus. The term ascending arousal system is somewhat of a misnomer because it does not adequately describe all its functions. In addition to arousing the brain from sleep, it also is responsible for maintaining the wake state and can also adjust the arousal state of neural circuits participating in a particular piece of behaviour. The following arousal systems, which are located in different regions of the brain, use the following neuronal cell types (Module 10: Figure sleep/wake cycle regulation):

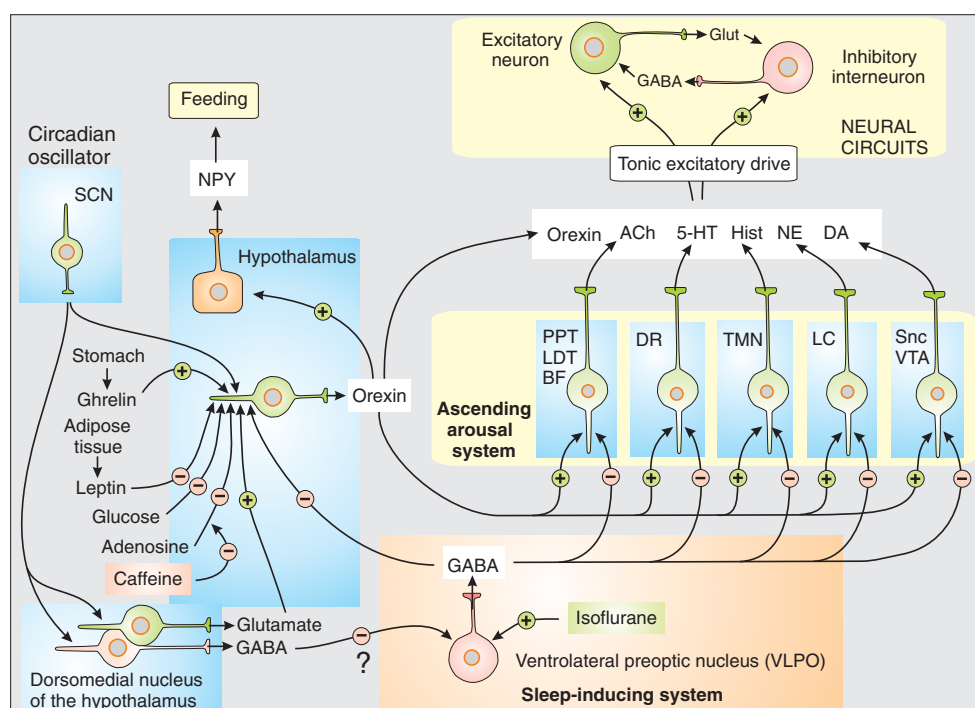
- Cholinergic arousal system
- Dopaminergic arousal system
- Histaminergic arousal system
- Noradrenergic arousal system
- Orexinergic arousal system
- Serotonergic arousal system

Some of the components of this system are also responsible for regulating the patterns of activity during REM sleep and are often referred to as REM-on or REM-off neurons. The activity of these two neuronal types, which have opposing actions, oscillate in a rhythmical manner and are responsible for the 90 min cycles that characterizes the period of REM sleep (Module 10: Figure sleep phases). The neurotransmitters released from the neuronal components of this ascending arousal system regulate neuronal activity by adjusting the level of the tonic excitatory drive.

Cholinergic arousal system

The cholinergic neurons that function in the ascending arousal system are located in two main regions (Module 10: Figure brain anatomy). Firstly, the pedunculopontine tegmental (PPT) nucleus and the laterodorsal tegmental (LDT) nucleus of the pontomesencephalic tegmentum contains cholinergic neurons that project primarily to the thalamus, whereas the cholinergic neurons in the medial septum and the diagonal band of Broca, located in the basal forebrain (BF), project primarily to the hippocampus and cortex.

Module 10: | Figure sleep/wake cycle regulation



Regulation of the sleep/wake cycle.

The ascending arousal system consists of neurons distributed in different brain regions such as the orexinergic neurons in the lateral hypothalamus; cholinergic neurons in the basal forebrain (BF), pedunculopontine tegmental (PPT) nucleus and the laterodorsal tegmental (LDT) nucleus; serotonergic neurons in the dorsal raphe (DR); histaminergic neurons in the tuberomammillary nucleus (TMN); noradrenergic neurons in the locus coeruleus (LC); dopaminergic neurons in the substantia nigra pars compacta (Snc) and ventral tegmental area (VTA). The neurotransmitters released by this arousal system act on neural circuits to regulate the sleep/wake cycle. The orexinergic neurons that release orexin are master regulators in that they also act by stimulating the other arousal neurons. The orexinergic neurons may also integrate the action of a number of other regulators of the sleep/wake cycle. Sleep-inducing systems such as the VLPO neurons have the opposite effect in that they release GABA to inhibit both the orexinergic and the other arousal neurons.

The LDT and PPN neurons project to many regions of the brain where they release acetylcholine and contribute to the regulation of the sleep/wake cycle (Module 10: Figure sleep/wake cycle regulation). The activity of these neurons increases on waking and arousal when they act to inhibit the REM-off neurons. One of their functions is to activate cortical gamma oscillations in the awake brain. However, they may also play a role in inducing the active oscillations that occur during the Up state of slow oscillations during sleep.

Dopaminergic arousal system

The midbrain dopaminergic (DA) neurons, which are located in the substantia nigra pars compacta (SNc) and in the ventral tegmental area (VTA), function in the voluntary control of movement and reward planning. These DA neurons have autonomous pacemaker mechanisms capable of generating two types of rhythms. Firstly, there are slow (1 Hz) tonic spiking oscillations that control motor, cognitive and motivational mechanisms. Secondly, there are regular bursting patterns that seem to provide a reward signal that controls reinforcement learning and may also contribute to various addictions. There is considerable interest in the dopaminergic (DA) neuronal pacemaker mechanisms

because alterations in the handling of Ca^{2+} during the course of each oscillatory cycle seem to be responsible for Parkinson's disease.

Dopaminergic (DA) neuronal pacemaker mechanisms

The dopaminergic (DA) substantia nigra pars compacta (SNc) neurons have two basic oscillatory modes. There are slow (1 Hz) tonic oscillations where single action potentials fire at a frequency of about 1 Hz. Following either glutamatergic or cholinergic stimulation, this tonic firing of the dopaminergic neurons is switched to an oscillatory mode with action potentials firing rapidly on the crest of each burst.

Generation of the slow tonic firing pattern is determined by an interplay between plasma membrane channels and an intracellular Ca^{2+} oscillation as outlined in the following sequence of events (Module 10: Figure tonic oscillation in DA neurons):

1. The rising phase of each spike depends on an inward Na^+ current (I_{Na}) resulting from the opening of a tetrodotoxin-sensitive Na^+ channel.
2. This rapid depolarization of the plasma membrane activates a $\text{Cav}1.3$ channel, which belongs to the $\text{Cav}1$ family of L-type channels, to initiate an influx of

external Ca^{2+} that contributes to the intracellular Ca^{2+} transient that occurs during each oscillatory cycle.

3. The entry of Ca^{2+} through the Cav1.3 channel stimulates ryanodine receptors (RYRs) through Ca^{2+} -induced Ca^{2+} release (CICR) to enhance the amplitude of each Ca^{2+} transient.
4. The rapid global elevation of Ca^{2+} during each transient activates the small-conductance (SK) potassium channels and contributes to the large membrane hyperpolarization that terminates each action potential.
5. This large membrane hyperpolarization activates a hyperpolarizing-activated cyclic nucleotide-gated (HCN) channel and the opening of this HCN channel may contribute some of the inward Na^{+} current responsible for the slow pacemaker depolarization that sets the stage for the next action potential.
6. Various mechanisms come into play during the falling phase of the transient to reduce the level of the Ca^{2+} back towards the resting level. Initially, some of the Ca^{2+} is bound to cytosolic Ca^{2+} buffers, such as calbindin D-28k (CB), but this has a small effect because these SCn neurons have a low buffer capacity.
7. Some Ca^{2+} will also enter the mitochondria through the uniporter located on the inner mitochondrial membrane (Module 10: Figure tonic oscillation in DA neurons). This Ca^{2+} is returned to the cytoplasm by the mitochondrial $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger (NCX) in order to avoid overloading the mitochondrion with Ca^{2+} .
8. It is not clear what the mitochondrial matrix Ca^{2+} levels are during the course of the tonic oscillations. It is clear that some Ca^{2+} enters the mitochondrion during each transient, but what remains to be established is whether all of this Ca^{2+} is returned to the cytoplasm during the recovery period following each transient. Are the mitochondrial Ca^{2+} oscillations precisely mirrored by those in the cytoplasm? Does the Ca^{2+} rise and fall back to a basal level during each transient or is the concentration held at some tonic level that is constantly being topped up by influx during each transient? Some support for the latter scenario has come from measurements of the mitochondrial membrane potential that was found to flicker at a frequency of about 50–60 s (see lower panel in Module 10: Figure tonic oscillation in DA neurons). This flickering between a depolarized and hyperpolarized state may reflect an underlying oscillation in the level of Ca^{2+} within the mitochondrial matrix. When the membrane is hyperpolarized, the uptake of Ca^{2+} may exceed that of the extrusion mechanism resulting in a gradual build up in the level of Ca^{2+} . However, as soon as the membrane depolarizes, the entry of Ca^{2+} will decline sufficiently to enable the NCX extrusion mechanism to reduce the level of Ca^{2+} resulting in an oscillation in the mitochondrial level of Ca^{2+} occurring in phase with the flickering membrane potential.

The tonic elevation of mitochondrial Ca^{2+} may be necessary to stimulate the respiratory pathway to produce the ATP to maintain whole-cell Ca^{2+} homeostasis. Such high respiration rates come with the risk of pro-

ducing increased levels of reactive oxygen species (ROS). However, the mitochondrion appears to have a negative-feedback mechanism based on the ability of ROS to stimulate the opening of the uncoupling protein 4 (UCP4) and uncoupling protein 5 (UCP5) channels to leak H^{+} that will depolarize the inner mitochondrial membrane potential thus reducing respiration and the entry of Ca^{2+} . This negative-feedback loop, which depends on the periodic elevation of ROS, may explain the flickering of the mitochondrial membrane potential.

Whatever the dynamics of mitochondrial Ca^{2+} turn out to be, a breakdown in the operation of the protective feedback mechanisms necessary to manage the persistent elevation in Ca^{2+} and the ensuing oxidant stress may well explain why the SNc neurons are so vulnerable to oxidative damage.

9. In order to maintain whole-cell Ca^{2+} homeostasis, there must also be a close matching between the amount of Ca^{2+} entering and leaving the cell. The amount of Ca^{2+} entering the cell via the Cav1.3 channels is removed by plasma membrane extrusion processes such as the plasma membrane Ca^{2+} -ATPase (PMCA) and $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger (NCX).
10. The sarco-/endo-plasmic Ca^{2+} -ATPase (SERCA) pump replenishes the internal store by pumping Ca^{2+} back into the ER.

A remarkable feature of each Ca^{2+} transient is that Ca^{2+} remains elevated for much of the interspike interval. In other words, Ca^{2+} is constantly 'in play' and a number of mechanisms are used to ensure that the Ca^{2+} ON reactions are precisely balanced by the Ca^{2+} OFF reactions in order to maintain Ca^{2+} homeostasis of these SNc neurons. There is a sense that these SNc neurons are constantly on a 'knife edge' with regard to Ca^{2+} and ROS regulation and this makes them particularly vulnerable to oxidative damage and is the basis of the calcium and ROS hypothesis of Parkinson's disease.

Histaminergic arousal system

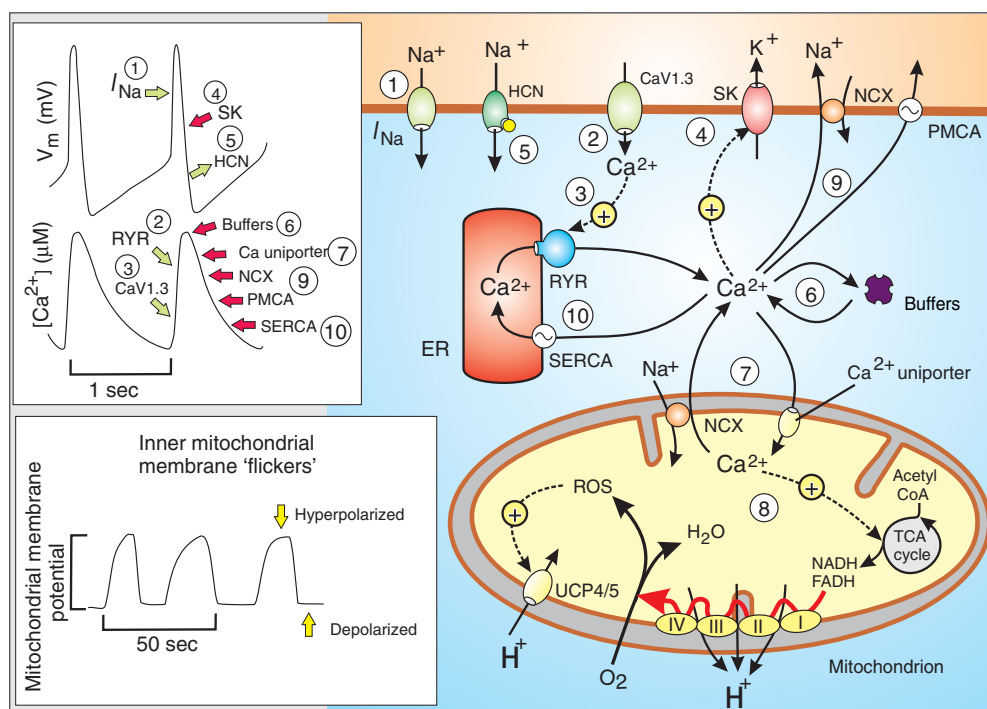
The histaminergic neurons that function in the ascending arousal system are located in the posterior hypothalamus (Module 10: Figure brain anatomy).

Noradrenergic arousal system

The locus coeruleus (LC), which is located in the junction between the pons and midbrain (Module 10: Figure brain anatomy), contains the noradrenergic neurons that are highly active during wakefulness, but begin to switch off during NREM sleep and become very quiescent during REM sleep.

Orexinergic arousal system

The orexinergic neurons, which are located in the lateral hypothalamus (Module 10: Figure brain anatomy), are responsible for releasing orexin that plays a prominent role in wakefulness by exciting multiple neurons of the ascending arousal system (Module 10: Figure sleep/wake cycle regulation). There are approximately 7,000 orexin neurons that project widely throughout the brain. They have

Module 10: | Figure tonic oscillations in DA neurons**Tonic oscillations in dopaminergic (DA) neurons.**

Dopaminergic (DA) neurons have an autonomous oscillatory mechanism that generates a regular tonic firing pattern. The membrane potential (V_m) is unstable in that there is a slow pacemaker potential and this depolarization triggers an action potential at intervals of approximately 1 per second (see inset on the left). Operating in phase with this membrane oscillation, there are repetitive Ca^{2+} transients with each rising phase coinciding with the action potential while the slow falling phase occurs during the pacemaker period. The repetitive Ca^{2+} transients load up the mitochondria to enhance oxidative respiration and a resulting increase in the formation of reactive oxygen species (ROS). Inner mitochondrial membrane potential flickers, which occur at a much lower frequency (see lower inset on the left), may reflect the existence of a feedback mechanism to curb excess Ca^{2+} levels and ROS formation. See text for further details of the interplay between the membrane and intracellular Ca^{2+} oscillations. Information for this Figure was taken from the work of Jim Surmeier and his colleagues (Guzman et al., 2010; Surmeier et al., 2011).

particularly dense projections to the paraventricular nucleus of the thalamus, arcuate nucleus, locus coeruleus, dorsal raphe and the tuberomammillary nucleus (TMN).

These orexin neurons are master regulators in that they integrate information from many different sources to regulate multiple processes. In addition to having an important role in regulating sleep and wakefulness, they also play a major role in the control of food intake and body weight.

A decrease in the function of this orexinergic arousal system has been linked to narcolepsy.

Serotonergic arousal system

The dorsal raphe nucleus (DRN) contains the serotonergic neurons that release the 5-hydroxytryptamine (5-HT) as part of the ascending arousal system (Module 10: Figure sleep/wake cycle regulation).

Sleep-inducing systems

The action of the multiple arousal systems that induce and maintain wakefulness are counteracted by the sleep-inducing systems such as the endogenous sleep-regulatory molecules, circadian control of sleep and the ventrolateral preoptic (VLPO) neurons that release GABA to inhibit both the orexinergic and the other arousal neurons (Module 10: Figure sleep/wake cycle regulation).

Endogenous sleep-regulatory molecules

There are a number of endogenous sleep-regulatory molecules such as adenosine, nitric oxide (NO), tumour necrosis factor α (TNF α), interleukin-1 (IL-1) and growth hormone-releasing factor (GHRH) that build up during neuronal activity and may contribute to the switch from wakefulness to sleep. Some of these sleep-regulatory molecules seem to be linked together in that IL-1 and TNF α act through the transcription factor NF- κ B to increase the formation of adenosine and NO.

The endogenous sleep-regulatory molecules are produced during neural activity and their accumulation thus reflects the intensity of neural activity, which is often restricted to individual neural columns. Such a build-up of these regulators may explain how such neuronal assemblies can enter into a local sleep state independently of neighbouring cortical columns during the wake state. In addition to controlling local sleep, these regulatory molecules can diffuse away to influence the activity of neurons of the ascending arousal system, which will contribute to the wake/sleep switch at the onset of global sleep.

Circadian control of sleep

The way in which the circadian clock controls the sleep/wake cycle is still not fully understood. Clock

neurons from the suprachiasmatic nuclei (SCN) have modest projections to the orexin neurons and may also innervate the sleep-inducing VLPO neurons. However, the main SCN output is directed towards the dorsomedial nucleus of the hypothalamus (DMH), which contains neurons that project both to the orexin neurons and the VLPO neurons (Module 10: Figure sleep/wake cycle regulation). There are indications that the neurons that project to the lateral hypothalamus are excitatory neurons that release glutamate and may thus induce arousal. The other neurons that project to the VLPO neurons appear to be inhibitory neurons that release GABA and may thus also contribute to arousal by inhibiting the sleep-inducing system.

Ventrolateral preoptic (VLPO) neurons

The induction of sleep is strongly influenced by neurons located in the preoptic area of the brain. The ventrolateral preoptic (VLPO) neurons are particularly important in initiating and maintaining NREM sleep. The VLPO neurons contribute to the onset of sleep by firing rapidly to release GABA and galanin that inhibits many members of the ascending arousal system (Module 10: Figure sleep/wake cycle regulation). The activity of these VLPO neurons may be regulated by the circadian control of sleep.

The volatile anaesthetic isoflurane acts by stimulating the subpopulation of VLPO neurons that normally release GABA to promote sleep. The isoflurane seems to act by closing a background K^+ channel to depolarize the VLPO neurons.

Tonic excitatory drive

The ascending arousal system is responsible for regulating the sleep/wake cycle by adjusting the level of the tonic excitatory drive of both the excitatory neurons and the inhibitory interneurons. An important feature of this tonic excitatory drive is that it is applied equally to both the excitatory and inhibitory neurons as it is essential for proper brain function for this stimulation to be finely balanced. There are indications that alterations in this excitation-inhibition (E-I) balance may occur in psychiatric diseases such as bipolar disorder (BD) where excessive excitation may be responsible for the manic phase whereas depression may result from excessive inhibition.

The activity of the arousal system is lowest during NREM sleep, but increases during REM sleep and is most active during the wake period. Even within the wake period, the activity of the arousal system can vary in that it can be markedly increased in those parts of the brain that are carrying out a particular piece of behaviour. Variations in the activity of the arousal system is translated into variations in the tonic excitatory drive that determines the hierarchy of the neural rhythms with the lowest frequencies occurring during sleep (i.e. the slow oscillations and delta rhythms) that then switch to the higher frequency theta and gamma rhythms of the wake state (Module 10: Figure tonic excitatory drive and neuronal rhythms). In effect, the variable tonic excitatory drive induced by the ascending arousal system functions as a rhythm rheostat

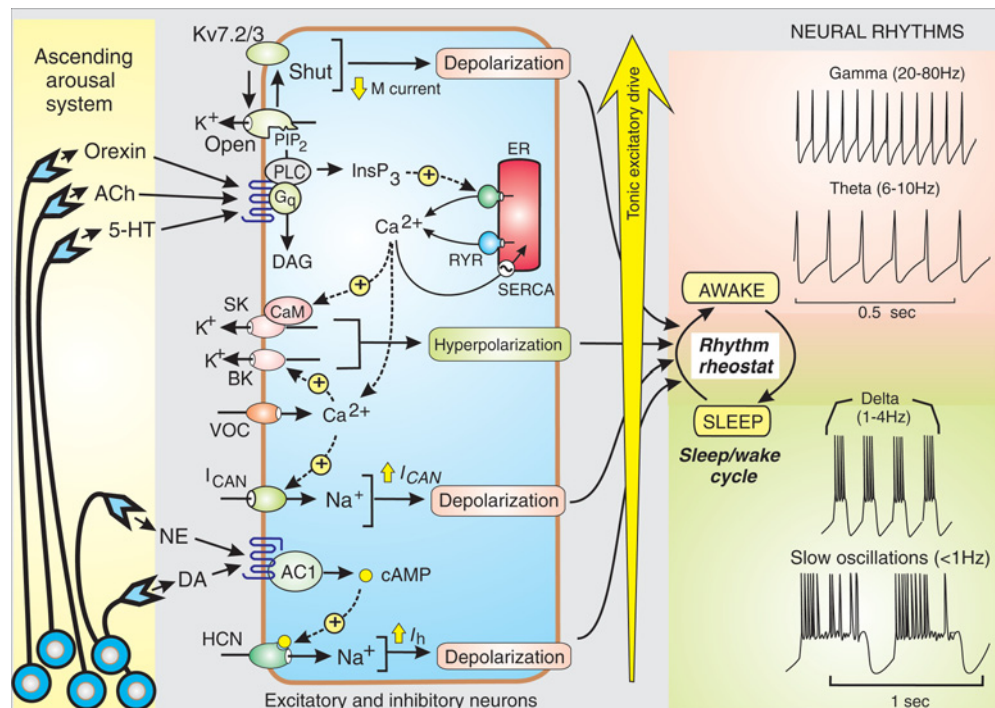
capable of generating the panoply of neural rhythms that occur during the course of the sleep/wake cycle.

A variety of signalling mechanisms are used to control the ion channels responsible for the tonic excitatory drive that depolarizes the target neurons to regulate their oscillatory outputs. Transmitters, such as orexin, acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) act on receptors that are coupled to the phosphoinositide signalling pathway that has multiple outputs (Module 2: Figure PtdIns4,5P₂ signalling). A particularly important mechanism depends on the PtdIns4,5P₂ regulation of ion channels and exchangers to regulate the M current by closing the K_v7.2 and K_v7.3 potassium channels. The PtdIns4,5P₂ keeps the channel open, but when PtdIns4,5P₂ is hydrolysed by the transmitters released by the ascending arousal system, the channel closes. Switching off this M current depolarizes the membrane to increase neuronal activity.

The norepinephrine released from neurons originating in the locus coeruleus (LC) (Module 10: Figure sleep/wake cycle regulation) acts through the cyclic AMP signalling pathway to enhance the activity of the hyperpolarizing-activated cyclic nucleotide-gated (HCN) channel (Module 3: Figure HCN channels) responsible for the I_h current.

The characteristics of neuronal rhythms are also modulated by a large variety of potassium channels. Voltage-dependent K_v3 channels, which open and close very quickly, play an important role in spike repolarization. The Ca²⁺-sensitive K⁺ channels, such as the large conductance (BK) channels and the small conductance (SK) channels, have an important role in determining rhythm frequency by regulating the interval between the spikes. The contribution of these two channels to neuronal rhythms is determined by the nature of the activating Ca²⁺ signal. The BK channels are often closely associated with the voltage-operated Ca²⁺ channels (VOCs), which enables their low-affinity Ca²⁺-binding sites to respond to the high levels of Ca²⁺ found near the opening of these Ca²⁺ entry channels. In this way, the BK channels function primarily to facilitate the repolarization of the individual action potentials. By contrast, the SK channels, which have calmodulin (CaM) as a Ca²⁺ sensor (Module 10: Figure tonic excitatory drive and neuronal rhythms), respond to more global elevations such as those that occur when Ca²⁺ is released from internal stores by InsP₃ receptors or ryanodine receptors (RYRs).

The next aspect to consider is how variations in the activity of these tonic excitatory drive components contribute to the different oscillatory rhythms that occur during the course of the sleep/wake cycle. When brain slices are studied *in vitro*, neuronal activity is often absent, but can be induced by the addition of agonists such as ACh that begin by inducing the slow oscillations and delta rhythms. As the intensity of the ascending arousal stimulation increases, the characteristic theta and gamma rhythms of the awake state begin to appear (Module 10: Figure tonic excitatory drive and neuronal rhythms). The mechanisms responsible for setting up these rhythms will be described by considering the two extremes: the slow oscillation mechanism and the gamma oscillation mechanism

Module 10: | Figure tonic excitatory drive and neuronal rhythms**Tonic excitatory drive and the control of neuronal rhythms.**

The ascending arousal system releases transmitters such as orexin, acetylcholine (ACh), 5-hydroxytryptamine (5-HT) and norepinephrine (NE) that activate signalling systems that control neural rhythms which occur during the sleep/wake cycle. The tonic excitatory drive mechanism depends on membrane depolarization that results from closing the Kv7.2/Kv7.3 channels responsible for the M current and the opening of the Ca²⁺-activated non-specific cation channel (I_{CAN}) and the hyperpolarizing-activated cyclic nucleotide-gated (HCN) channel responsible for the I_h current.

Slow oscillation mechanism

The slow oscillation (<1 Hz), which is a characteristic feature of non-rapid eye movement (NREM) sleep (Module 10: Figure sleep phases), has typical Up states when the membrane potential (V_m) is depolarized to about -65 mV that alternates with a Down state where the membrane is hyperpolarized by 10–15 mV (Module 10: Figure slow oscillation mechanism). The mechanism responsible for generating these oscillations is still not clear. The first aspect to consider is what drives these oscillations. Two main mechanisms seem to be driving these oscillations. The first is a tonic excitatory drive exerted by the ascending arousal system, which sets the stage for oscillations to occur. The second mechanism seems to depend on the spontaneous synaptic input from excitatory neurons that contribute to the initiation of each oscillatory cycle. The much reduced activity of the ascending arousal system combined with the spontaneous input from glutamatergic neurons may provide a sufficient level of excitation to drive the slow oscillation. The way in which these two mechanisms interact with each other may explain how these slow oscillations are synchronized throughout the brain.

When brain slices are studied *in vitro*, neuronal activity is often absent, but can be activated by adding agonists such as acetylcholine (ACh) normally associated with the tonic excitatory drive exerted by the ascending arousal system (Module 10: Figure tonic excitatory drive and neuronal rhythms). This observation suggests that, during NREM

sleep, neurons may receive sufficient excitation through these tonic excitatory drive mechanisms to enable them to generate slow oscillations. During the conditions that prevail in NREM sleep, therefore, the brain may be considered to be an excitable medium in that most of the neurons are in an excitable state capable of beginning a slow oscillation. When a neuron or a group of neurons initiate a slow oscillation, the excitation then entrains neighbouring neurons to set up a slow oscillation synchronization and wave propagation that spreads throughout the brain travelling at approximately 3 m/s in an anterior/posterior direction.

The next question to consider is what initiates the Up state in the pacemaker region, which is usually located in the frontal areas of the brain where neurons are known to receive a higher degree of synaptic input. The glutamate released spontaneously from these synaptic endings then acts on AMPA receptors (AMPA) to trigger the excitatory postsynaptic potentials (EPSPs) that provide the depolarization to switch the membrane potential from the Down to the Up state to initiate the slow oscillation (Module 10: Figure slow oscillation mechanism). The action potentials that occur during this Up state will then spread excitation through the axon collaterals to activate neighbouring neurons thus initiating the slow oscillation synchronization and wave propagation mechanism. What initiates the Down state remains uncertain, but there are two possible mechanisms. Firstly, since inhibitory neurons also display slow oscillations, it is feasible that when they

are sufficiently activated they fire action potentials that releases GABA, which will then act on GABA receptors to open chloride channels to provide the inhibitory postsynaptic potentials (IPSPs) to repolarize the membrane. This may be an important mechanism to control the slow oscillation synchronization and wave propagation mechanism. Secondly, the hyperpolarization may be driven by an endogenous mechanism based on the generation of a Ca^{2+} transient that activates the small conductance (SK) channels to generate the I_{SK} current as described below (Module 10: Figure slow oscillation mechanism).

The following endogenous ion channels and signalling pathways have been implicated in the slow oscillation mechanism (Module 10: Figure slow oscillation mechanism):

Ca_v3 family of T-type channels

The Ca_v3 family of T-type channels are switched on at the beginning of an Up state to provide the low-threshold Ca^{2+} potential (LTCP). This initial LTCP rapidly inactivates to become a persistent inward current, also known as the T window current, which provides a tonic depolarization that helps to maintain the Up state. These T channels will also introduce a small but persistent influx of Ca^{2+} that may load up the internal stores to sensitize and then to activate the InsP_3 receptors or ryanodine receptors (RYRs) to generate a Ca^{2+} transient that could curtail the Up state by inducing membrane hyperpolarization. To understand how this Ca^{2+} transient is generated, it is necessary to consider the phosphoinositide signalling pathway that is activated by many of the transmitters that operate the ascending arousal system.

Phosphoinositide signalling pathway

A major target for many of the transmitters of the ascending arousal system is the phosphoinositide signalling pathway that has different outputs that may play a major role in generating the slow oscillation (Module 10: Figure slow oscillation mechanism). At the low agonist concentrations mentioned earlier, the generation of InsP_3 will be small and insufficient to induce much depletion of the internal store. However, through the mechanism of priming the inositol 1,4,5-trisphosphate receptors (InsP_3Rs) and ryanodine receptors (RYRs), the small but tonic elevation of InsP_3 may be sufficient to enable the store to periodically release its Ca^{2+} . This release will only occur when the stores are sufficiently loaded with the Ca^{2+} that enters the cell through the T type channels during the course of the Up state. As shown in Module 10: Figure slow oscillation mechanism, it is proposed that this release of Ca^{2+} occurs at the end of each Up state and may be responsible for inducing the rapid hyperpolarization of the Down state by opening the small conductance (SK) channels to generate the I_{SK} current. As the Ca^{2+} released from the internal store is pumped back into the ER by the SERCA pump or extruded from the cell, the level of Ca^{2+} will fall progressively during the Down state and the corresponding decline in I_{SK} will contribute to the pacemaker depolarization that leads up to the onset of the next Up state.

The pacemaker depolarization may also depend on activation of the hyperpolarizing-activated cyclic nucleotide-gated (HCN) channel.

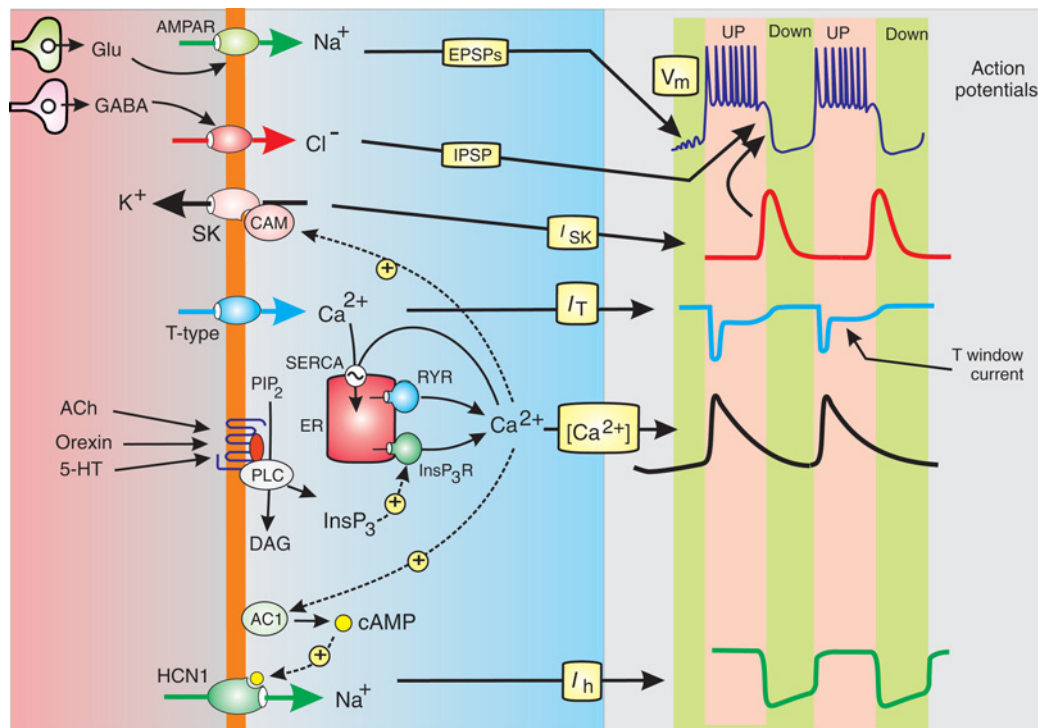
Hyperpolarizing-activated cyclic nucleotide-gated (HCN) channel

The hyperpolarizing-activated cyclic nucleotide-gated (HCN) channel will be switched on by the hyperpolarization that terminates the Up state (Module 10: Figure slow oscillation mechanism). Opening of the HCN1 channels provides the inward current that will contribute to the pacemaker depolarization that sets the stage for the next Up state.

Network gamma oscillation mechanism

The fast gamma oscillations (20–80 Hz) are generated through the operation of a typical network oscillator (Module 10: Figure network gamma oscillations). The main elements of these oscillators are the feedback interactions between the inhibitory interneurons and the excitatory neurons. Examples of such networks are found in the hippocampal local circuits where the excitatory CA3 pyramidal neurons interact with the basket cell and the oriens-lacunosum moleculare (O-LM) inhibitory interneurons (Module 10: Figure CA3 hippocampal local circuits). A unique feature of many of these circuits is that each interneuron sends out a network of axons that make contact with and thus controls the activity of many excitatory neurons (Module 10: Figure CA3 hippocampal neurons). This anatomical arrangement is a central feature of the mechanism responsible for setting up gamma oscillations (Module 10: Figure network gamma oscillations). A large number of the excitatory neurons receive an input from a single inhibitory interneuron (red arrow in Module 10: Figure network gamma oscillations) that innervates their axon initial segments (AIS) and is thus ideally positioned to influence the generation of action potentials. To complete the network, each pyramidal neuron sends an axon collateral back to innervate the inhibitory interneuron (green arrow).

The inhibitory interneuron has a primary role in setting up the gamma rhythm in that it fires an action potential at the crest of each gamma cycle (Arrow 1) and this induces the hyperpolarization that occurs synchronously in all the excitatory neurons. While all the excitatory neurons participate in each gamma cycle by responding to GABA to produce a hyperpolarization response, they fire much less frequently and the resulting action potentials occur within a narrow time window towards the end of the pacemaker depolarization (Arrows 2 and 3). The inhibitory interneuron registers each of these action potentials as a small excitatory postsynaptic potential (EPSP). These postsynaptic changes in membrane potential then sum (as illustrated in Module 10: Figure summation of excitatory inputs) to activate the interneuron to fire an action potential to initiate another gamma oscillatory cycle. In the case of the hippocampus, the ionic mechanisms involved in such a feedback mechanism between excitatory and inhibitory neurons are illustrated in Module 10: Figure gamma oscillatory mechanisms.

Module 10: | Figure slow oscillation mechanism**Slow oscillation mechanisms.**

This hypothetical scheme describes the main mechanisms that have been implicated in the neuronal slow membrane potential (V_m) oscillations that are characterized by periodic Up (red panels) and Down (green panels) states. See text for a description of the major ion channels and the role of Ca^{2+} in generating these oscillations. The information used to construct this Figure was obtained from Crunelli et al., 2005; Crunelli et al., 2006; Errington et al., 2012.

Brain rhythm synchronization

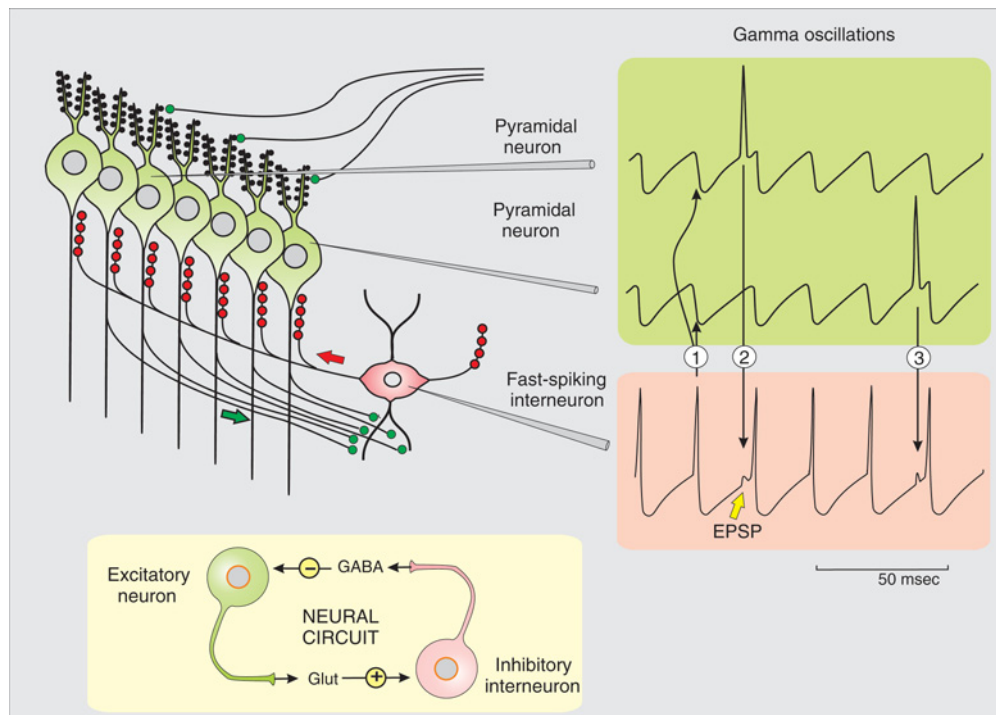
A remarkable feature of neuronal rhythms is that they are highly synchronized within local regions and often over much longer distances between different brain regions (Module 10: Figure brain circuitry and rhythms). Different synchronization mechanisms are used depending on the type of oscillation. A slow oscillation synchronization and wave propagation mechanism is used to spread slow oscillations throughout the brain. A more precise mechanism of phase synchronization of gamma oscillations is used to provide the precise timing required for information processing and memory formation.

Slow oscillation synchronization and wave propagation

The slow oscillations that occur during non-rapid eye movement (NREM) sleep (Module 10: Figure sleep phases) are highly synchronized within brain regions and this functions to tune a number of neural processes particularly those that function in memory consolidation. Synchronization of the slow oscillation mechanism depends on the generation of a propagating wave that serves to synchronize the activity of neurons in different regions of the brain. This slow wave originates in the frontal areas of the brain and then spreads at approximately 3 m/s in an anterior/posterior direction. In some cases, the propagating wave can form spirals consistent with the concept that

the participating neurons are all in an excitable state as a consequence of the slow oscillation mechanism (Module 10: Figure slow oscillation mechanism).

When the neurons in the frontal region depolarize sufficiently to initiate the slow oscillation Up state, there is a burst of action potentials that spread excitation through their axon collaterals to neighbouring neurons to set up the propagating wave. However, such a mechanism is likely to be somewhat haphazard and may not explain the high degree of synchrony that occurs as the wave spreads through the brain. The onset of the hyperpolarization that terminates the Up state may also provide a robust synchronization mechanism. There are two mechanisms to explain the timing of this repolarization event. Firstly, the onset of a Ca^{2+} transient towards the end of the slow oscillation mechanism may activate small conductance (SK) channels to generate the I_{SK} current that repolarizes the membrane. Secondly, activation of the inhibitory neurons during the course of the wave releases GABA to open chloride channels to hyperpolarize the membrane to initiate the Down state (Module 10: Figure slow oscillation mechanism). It is possible that the first mechanism may serve to regulate the timing of the pacemaker neurons that initiate the wave, whereas the inhibitory neurons, which are known to regulate multiple excitatory neurons, may provide the synchronization signal during the course of the propagating wave.

Module 10: | Figure network gamma oscillations**Neuronal network gamma oscillations.**

Numerous neural circuits (see inset at the bottom) consist of fast spiking inhibitory interneurons (red) and excitatory neurons (green) interacting with each other through a positive/negative-feedback loop. A unique feature of many circuits is that each interneuron controls the activity of many excitatory neurons all of which send axon collaterals back to the inhibitory interneuron. The interneuron fires an action potential on each gamma cycle (Arrow 1) and this serves to induce a hyperpolarization that occurs synchronously in all the excitatory neurons. While all the excitatory neurons participate in each gamma cycle, they fire much less frequently towards the end of the pacemaker depolarization (Arrows 2 and 3). The inhibitory interneuron registers each of these action potentials as a small excitatory postsynaptic potential (EPSP) that sum to activate the interneuron to fire an action potential to initiate another gamma oscillatory cycle.

Phase synchronization of gamma oscillations

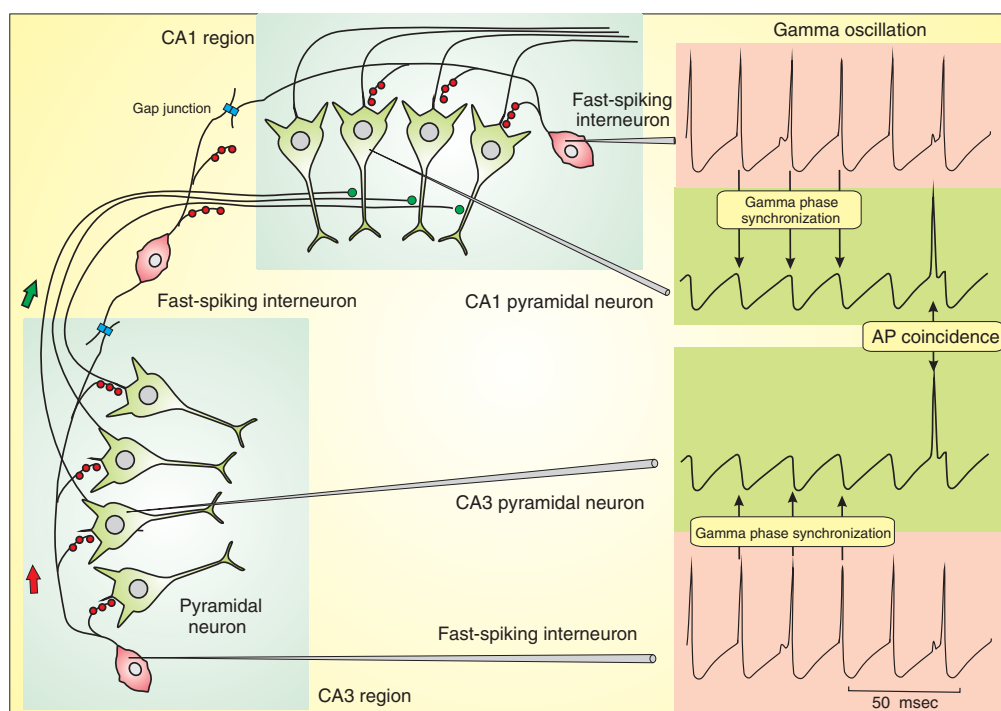
Synchronization of gamma oscillations depends on a unique feature of the local neural circuitry where an individual inhibitory interneuron can innervate a large number of pyramidal neurons (Module 10: Figure network gamma oscillations). In this way, a single interneuron can entrain the inherent rhythmical activity, which depends on the tonic excitatory drive, of a large population of pyramidal neurons. This control of synchronization by the inhibitory neurons may extend over longer distances by virtue of the fact that these inhibitory neurons are connected to each other through both synaptic connections and gap junctions (Module 10: Figure gamma oscillation phase synchrony). Examples of synchronization over more distant regions such as between the hippocampus and the subiculum and cortex (see phase synchronization of theta oscillations in Module 10: Figure brain circuitry and rhythms), are established more by the theta than gamma oscillations.

During development, there are changes in synchrony, which is a feature of the restructuring of the neural circuitry that occurs during childhood through to adolescence and adulthood. At the end of adolescence, the mature brain is characterized by a marked increase in the synchronization of both the theta and gamma rhythms that is essential for the computational precision of the adult brain. Such neural synchrony is impaired in patients with

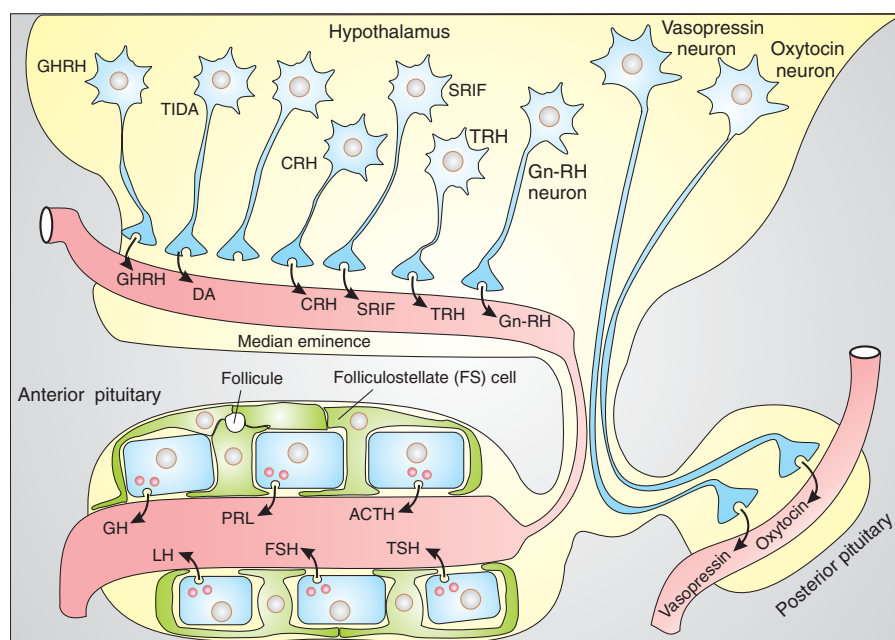
schizophrenia and this may explain why this disorder appears during the transition from adolescence to adulthood, which corresponds to the time when such adult theta and gamma oscillation synchronized patterns begin to emerge.

Hypothalamic pituitary regulation

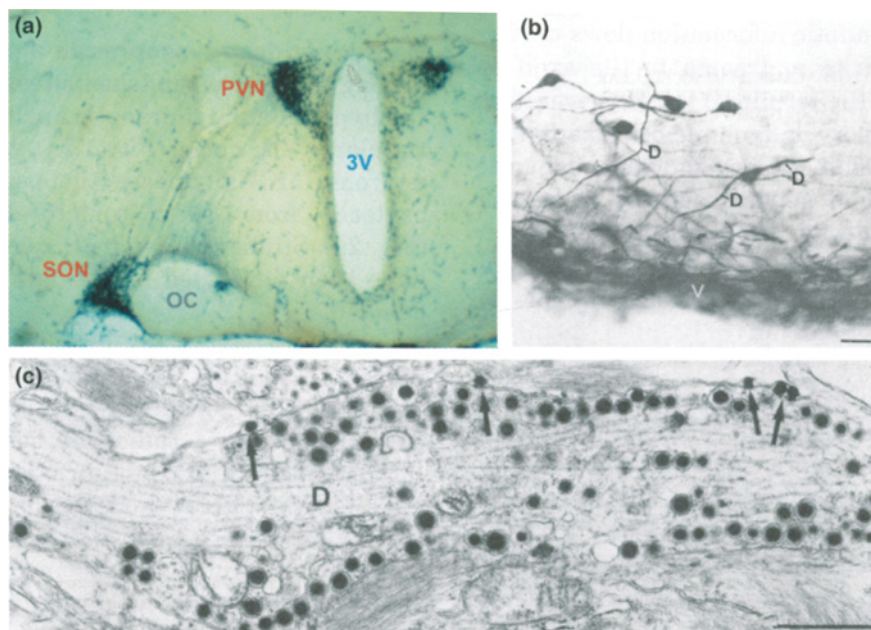
The hypothalamus and the pituitary form a tightly integrated neuroendocrine unit that regulates many body functions such as reproduction, growth, metabolism, adaptation to environmental changes and particularly those dealing with stress. The neural components of this regulatory system are located in the hypothalamus. The various neurohormones that are synthesized by these hypothalamic neurons are released from two distinct sites. Many of these neurons send out axons that make close contact with a nexus of blood vessels within the median eminence where they release their factors into the hypothyseal artery (Module 10: Figure hypothalamic pituitary regulation). These neurohormones then travel down to the anterior pituitary to control the release of a range of hormones by the endocrine cells. A separate group of magnocellular neurons (MCNs) pass their axons down to the posterior pituitary where they release neurohormones such as vasopressin or oxytocin directly into the circulation.

Module 10: | Figure gamma oscillation phase synchrony**Hippocampal gamma oscillation phase synchrony.**

Neurons within the CA3 and CA1 regions of the hippocampus have synchronous gamma oscillations orchestrated by a network of inhibitory interneurons connected through synapses and gap junctions. Action potential (AP) coincidence occurs when individual pyramidal neurons in the regions fire together on the peaks of the gamma oscillations.

Module 10: | Figure hypothalamic pituitary regulation**The hypothalamic pituitary regulatory system.**

Neurons located within the hypothalamus synthesize a number of factors, which are released into the hypophyseal portal circulation at the median eminence and travel down to the anterior pituitary. The latter has endocrine cells, which respond to the factors by releasing a range of hormones, and folliculostellate (FS) cells that form a network that surrounds the endocrine cells. Further details of anterior pituitary structure are shown in Module 10: Figure anterior pituitary structure. A separate group of neurons synthesize vasopressin or oxytocin that is then released into the circulation at the posterior pituitary.

Module 10: | Figure location of magnocellular neurons**Location and structure of magnocellular neurons.**

a. The cell bodies of the two types of magnocellular neurons (MCNs) (oxytocin and vasopressin neurons) are located in the supraoptic nucleus (SON), which lies close to the optic chiasm (OC), and the paraventricular nucleus (PVN) located next to the third cerebral ventricle (3V). b. Each MCN has 1–3 dendrites (D) that extend down to form an interconnected web within the ventral glial lamina. c. A typical dendrite (D) showing the numerous large dense-core vesicles (LDCVs) that contain either oxytocin or vasopressin. The arrows indicate examples of the exocytotic events responsible for the dendritic release of oxytocin and vasopressin. Reproduced from Ludwig, M. and Pittman, Q.J. (2003) Talking back: dendritic neurotransmitter release. *Trends Neurosci.* 26:255–261. Copyright (2003), with permission from Elsevier; see Ludwig and Pittman 2003.

Hypothalamus

The hypothalamus is located in the diencephalon region of the brain. It has a special role as an interface between the nervous and endocrine systems located in the pituitary. The organization of this hypothalamic pituitary regulatory system is illustrated in Module 10: Figure hypothalamic pituitary regulation. The hypothalamus contains a large number of neurosecretory neurons that tend to be grouped together into so-called nuclei such as the supraoptic nucleus (SON), the paraventricular nucleus (PVN) and the arcuate nucleus (AN). For example, the magnocellular neurons (MCNs), which release oxytocin and vasopressin, are located in the SON and PVN (Module 10: Figure location of magnocellular neurons), whereas the gonadotropin-releasing hormone (GnRH) neurons are found in the medial preoptic area of most species. These hypothalamic neurons synthesize a large number of factors and hormones, some of these, such as gonadotropin-releasing hormone (GnRH), are released into the median eminence and travel to the anterior pituitary to control the endocrine cells that release a variety of hormones. Some of the other neurons, such as the MCNs that consist of the oxytocin neurons and vasopressin neurons, send their axons to the posterior pituitary where they release hormones such as oxytocin (OT) and vasopressin (VP).

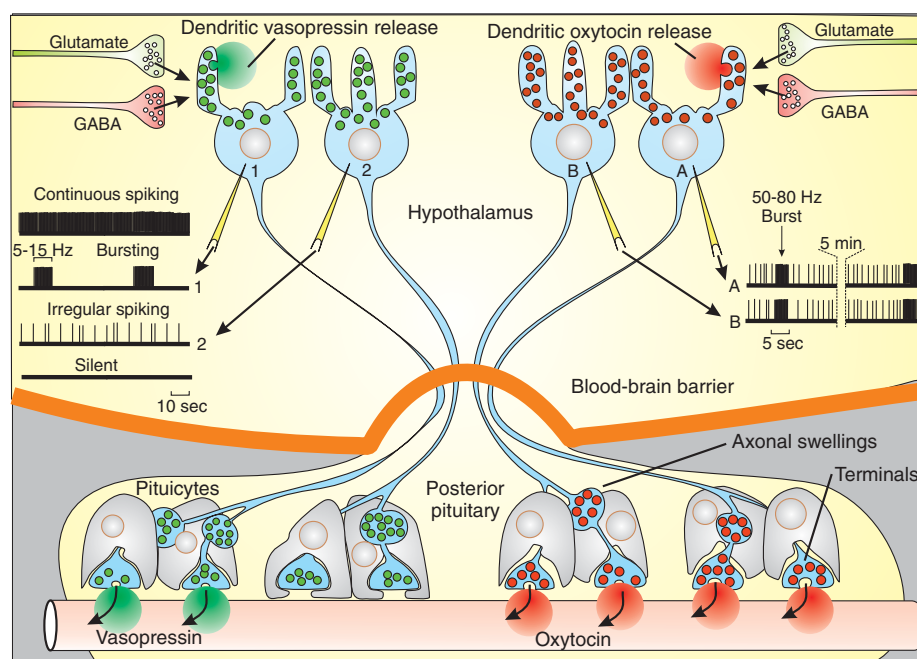
Magnocellular neurons (MCNs)

There are two types of magnocellular neurons (MCNs): the oxytocin neurons and the vasopressin neurons. The

cell bodies of these two neuronal cell types are located in both the supraoptic nucleus (SON) and the paraventricular nucleus (PVN) (Module 10: Figure location of magnocellular neurons). Each MCN has 1–3 dendrites and, in the case of those in the SON, they extend down into the ventral glial lamina where they ramify to form a dendritic meshwork (see panel b in Module 10: Figure location of magnocellular neurons). These dendrites contain numerous large dense-core vesicles (LDCVs) that participate in a process of dendritic transmitter release, which means that these MCNs release oxytocin and vasopressin from two sites located at either ends of the cell (Module 10: Figure magnocellular neurons). The long axons, which extend down into the posterior pituitary, are extensively branched to form approximately 1840 nerve endings for each of the MCNs. Each ending has approximately 258 LDCVs containing either oxytocin or vasopressin and this enormous increase in the number of release sites means that large amounts of hormone can be released into the blood within a short period of time.

The bursts of action potentials, which trigger the release of hormone from the nerve endings, have different patterns in the two types of MCNs as illustrated by the electrical recordings shown in Module 10: Figure magnocellular neurons. At rest, the vasopressin neurons are either silent or they fire spikes that have three distinct patterns: irregular spikes at a low frequency (neuron 2), bursts of spikes that last for 5–10 s at 5–15 Hz that occur every 20–40 s (neuron 1) or continuous spiking. The last may be unphysiological as it only occurs under extreme

Module 10: | Figure magnocellular neurons

**The magnocellular neurons of the hypothalamus.**

The magnocellular neurons, which are located within the ON and PVN regions of the hypothalamus, are typical neurosecretory cells. Each MCN has 2–4 dendrites and this somatic/dendritic region contain 15000 vesicles of oxytocin (OT) or 60000 vesicles of vasopressin (VP). The long axons of these two neurons extend down into the posterior pituitary where they divide into a large number of terminals (2000/cell) each of which have approximately 250 vesicles that contain 85000 molecules of oxytocin or vasopressin that are released into the blood during activation. Pituicytes wrap around these endings reducing their access to the blood vessel, but this restriction is removed during secretion. Information for this figure was taken from Leng and Ludwig 2008.

conditions. The bursting pattern is most effective in inducing the release of vasopressin. These bursts are autonomous events and there is no evidence of the synchronized bursting found in the oxytocin neurons. Release of oxytocin also depends on short 50–80 Hz bursts of action potentials that last for approximately 5 s (neurons A and B). These bursts have a much lower frequency compared with those seen in the vasopressin neurons in that they occur every 5–10 min. What is remarkable about these bursts is that despite their long periodicity they occur synchronously throughout the entire population of approximately 3000 oxytocin neurons. There are different mechanisms for controlling these oxytocin neurons and vasopressin neurons.

Oxytocin neurons

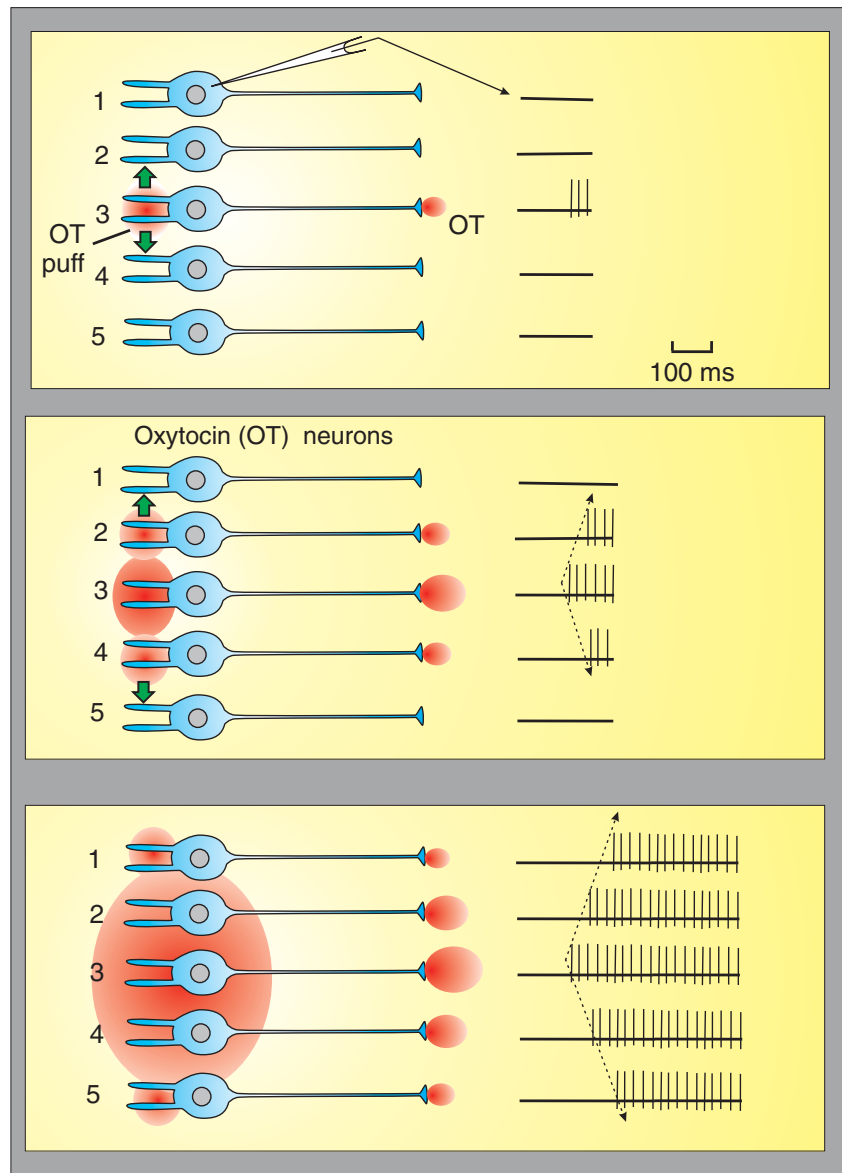
Oxytocin neurons are one of the magnocellular neurons (MCNs) located in the supraoptic nuclei (SON) and paraventricular nuclei (PVN) of the hypothalamus (Module 10: Figure location of magnocellular neurons). These neurosecretory cells synthesize oxytocin, which is then released from two different sites. It is released from nerve terminals into blood vessels in the posterior pituitary (Module 10: Figure magnocellular neurons). They also display dendritic transmitter release whereby oxytocin is released from the dendrites directly into the brain. Oxytocin has multiple functions depending on its site of release. The oxytocin released from the dendrites diffuses into the brain

where it functions as a global messenger to act either locally as an autocrine/paracrine signal for the synchronization of oxytocin neurons or it diffuses further afield to influence neurons that function in social recognition and in processes of learning and memory. On the other hand, the oxytocin released into the blood from the nerve terminals modulates the uterus smooth muscle cell membrane oscillator that drives contractions during labour (Module 7: Figure uterus activation) and it also controls the milk-ejection reflex during suckling.

Much of our information on the control of the oxytocin neurons has been derived from studies on the milk-ejection reflex. In response to suckling, oxytocin neurons begin to fire action potentials at a slow frequency, but at periodic intervals of 5–10 min there is a rapid 50–80 Hz burst of action potentials that last for about 5 s (see neurons A and B in Module 10: Figure magnocellular neurons). What is remarkable about these short bursts is that they occur synchronously in all of the oxytocin neurons in both the SON and PVN. Such synchronization of oxytocin neurons ensures that oxytocin is released from all of the nerve endings over a short period of time resulting in the large elevation in the blood levels of this hormone that is necessary to drive the milk-ejection reflex.

Synchronization of oxytocin neurons

The synchronization mechanism seems to depend on the dendritic release of oxytocin that functions as a regenerative paracrine messenger that spreads out to trigger all the

Module 10: | Figure oxytocin neuron synchronization**Synchronization of action potential bursts in oxytocin neurons.**

The ability of the oxytocin neurons to generate bursts of action potential depends on the dendritic release of oxytocin that then sets up a dendrodendritic communication system. The upper panel shows five oxytocin neurons lined up next to each other. Neuron 3 functions as a pacemaker in that it spontaneously releases oxytocin (OT) from its dendrites and it also begins to initiate a burst of action potentials that trigger release of OT from the nerve endings. The middle panel shows how the OT from the dendrites of neuron 3 diffuses across to the neighbouring cells (neurons 2 and 4) to trigger a similar series of events (green arrows): dendritic release of OT, burst initiation and OT release from the nerve endings. This positive-feedback mechanism then sets up a regenerative mechanism that enables all the neurons to fire in close synchrony with each other. The dashed arrows show how the wave of excitation, which is reflected in the initiation of the bursts, spreads from the pacemaker neuron 3 to the neighbouring neurons.

neurons to burst in unison with each other (Module 10: Figure oxytocin neuron synchronization). The idea is that a stochastic event in a pacemaker neuron initiates a burst of action potentials through a process that coincides with the release of oxytocin from the dendrites (see neuron 3 in the top panel of Module 10: Figure oxytocin neuron synchronization). The oxytocin diffuses rapidly to the dendrites of neighbouring neurons that are then activated to initiate the bursts that release oxytocin (cells 2 and 4 in the middle panel). This paracrine stimulation process represents the regenerative component of the oxytocin wave (see the dashed arrows on the middle and lower panels) that

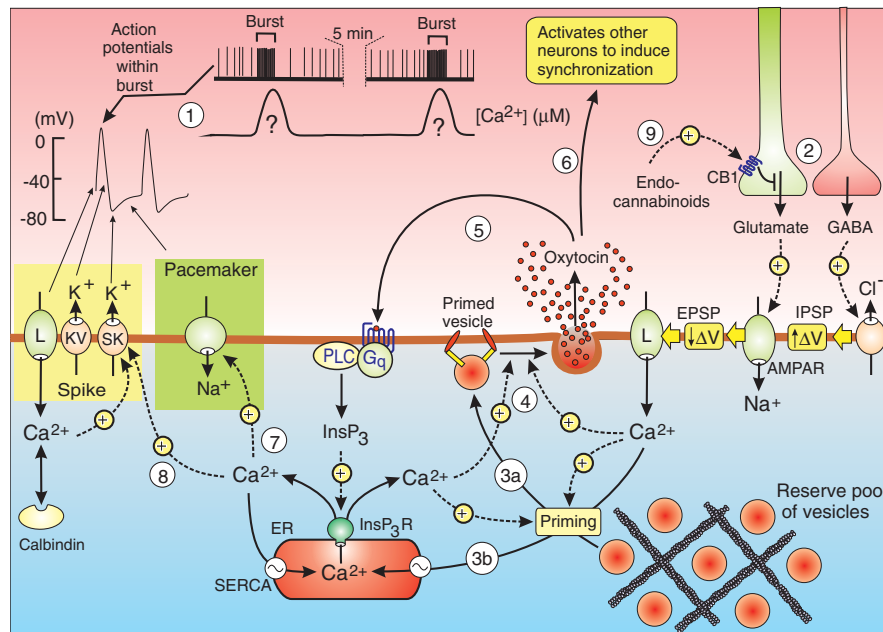
initiates bursting in all of the oxytocin neurons within a very short period.

This synchronized bursting activity depends on a number of interconnected control mechanisms. The ability to generate bursts is controlled by the balance between the excitatory (glutamate) and inhibitory (GABA) neural inputs derived from neurons located in other regions of the brain. An increase in the excitatory drive induces the depolarization necessary to initiate both the bursting pattern and the closely related process of dendritic oxytocin release. The latter provides the critical positive-feedback component that not only drives/maintains the burst through an

autocrine mechanism but it also generates the paracrine synchronization messenger to initiate bursting in neighbouring cells. The following hypothesis illustrates some of the proposed control systems that may co-operate with each other to initiate an action potential burst in oxytocin neurons (Module 10: Figure oxytocin neuron):

1. During a suckling response, bursts of action potentials occur every 5–10 min. The interburst interval is characterized by random action potentials that occur at a much lower frequency. The ion channels responsible for the spike of each action potential are still being established. The upstroke depends on inward currents derived from both Na^+ and Ca^{2+} channels. The latter are likely to be L-type Ca^{2+} channels. The downstroke is initiated by one of the voltage-dependent K^+ (Kv) channels and this is then followed by the opening of the small-conductance (SK) Ca^{2+} -activated K^+ channels, which may open briefly in response to the brief pulse of Ca^{2+} that enters through the L-type channels to give rise to the Ca^{2+} -dependent afterhyperpolarization (AHP). The AHP then recovers quickly and another action potential will occur if the membrane depolarizes sufficiently to reach the threshold responsible for activating the inward currents. During the prolonged interburst period, this pacemaker depolarization develops slowly, which explains the low frequency of action potentials, but the depolarizing drive becomes stronger during the burst to account for the much higher frequency of bursting. Since this bursting behaviour is driven by the suckling response, the initiation of a burst must depend on information reaching the oxytocin neurons from the various neuronal inputs.
2. The oxytocin neurons receive both excitatory (glutamate) and inhibitory (GABA) neural inputs. The latter acts through GABA receptors to induce inhibitory postsynaptic potentials (IPSPs) and the resulting hyperpolarization will reduce neuronal activity. On the other hand, the excitatory neurons release glutamate that act through AMPA receptors to induce the excitatory postsynaptic potentials (EPSPs), which are responsible for initiating random low-frequency action potentials during the interspike interval. It is argued that these action potentials may promote a priming process that gradually increases the readiness of the neurons to initiate a burst.
3. The big question concerns the nature of the priming process that is responsible for increasing the sensitivity of the neuronal population such that some of the cells will function as pacemakers to release the oxytocin that then rapidly recruits the remaining follower cells as described earlier (Module 10: Figure oxytocin neuron synchronization). In effect, the time it takes for this priming process to occur determines the length of the interspike interval and hence the bursting frequency. There are at least two ways in which this priming might occur.
 - 3a. The large dense-core vesicles (LDCVs) containing oxytocin are stored in a reserve pool of vesicles and before they can be released they have to be translocated to the plasma membrane where they form a readily releasable pool of primed vesicles capable of responding to the Ca^{2+} signals that stimulate release (Module 10: Figure oxytocin neuron). Since priming is a Ca^{2+} -sensitive process, it is possible that the small Ca^{2+} signals associated with the inter-burst action potentials may be responsible for transferring vesicles to the plasma membrane. These primed vesicles may then respond to some initiation event in the pacemaker neurons to initiate the release of oxytocin, which will then activate a positive-feedback processes as described below.
 - 3b. Another possible priming mechanism may depend on loading the endoplasmic reticulum (ER) with Ca^{2+} , which is known to enhance the sensitivity of the release channels, such as the ryanodine receptors (RYRs) and InsP_3 receptors (InsP_3Rs), that are responsible for releasing Ca^{2+} . Such store loading has been incorporated into a model to explain the mechanism of Ca^{2+} oscillations (Module 6: Figure Ca^{2+} oscillation model). In this case, the action potentials that occur during the inter-burst period would provide the entry of Ca^{2+} , which gradually loads up the internal store to sensitize the cells. This enhanced sensitivity finally results in the pacemaker neurons spontaneously releasing the Ca^{2+} that then triggers the release of oxytocin.
4. These two putative priming mechanisms are not mutually exclusive and could act together to set up the intermittent bursting pattern. Also, they have one aspect in common; they both propose that the onset of the burst depends on the release of oxytocin that then initiates the oxytocin-dependent positive-feedback mechanism that has implications for both the synchronization mechanism and perhaps also the onset and termination of the burst.
5. Once oxytocin is released, it feeds back in an autocrine manner to stimulate oxytocin receptors to increase the formation of InsP_3 that then releases more Ca^{2+} . An interesting aspect of this mechanism is that once Ca^{2+} release begins in one dendritic region, it will spread through to the other dendrites and soma in the form of a regenerative Ca^{2+} wave that would maximize both the amplitude and spatial spread of the Ca^{2+} signal and the release of oxytocin. A dendritic Ca^{2+} wave has been described in respiratory neurons where it functions in the respiratory pacemaker mechanism to synchronize the activity of the neurons during the respiratory burst (Module 10: Figure respiratory pacemaker mechanism). Unfortunately, there are no *in vivo* measurements of Ca^{2+} in oxytocin neurons, but the prediction of the current hypothesis is that the increase in $[\text{Ca}^{2+}]$ should begin either just before or at the beginning of the burst and to continue rising rapidly during the course of the burst as shown in Module 10: Figure oxytocin neuron.
6. One of the functions of the oxytocin released from the dendrites is to function as a paracrine messenger to activate other neurons as part of the

Module 10: | Figure oxytocin neuron



Control of bursting in oxytocin neurons.

This figure illustrates a hypothesis concerning the mechanisms used by oxytocin neurons to generate periodic bursts of action potentials that occur every 5–10 min. See text for further details of the proposed sequence of events. Much of the information shown in this slide is based on the hypothesis proposed by Rossoni et al 2008.

synchronization mechanism discussed earlier (Module 10: Figure oxytocin neuron synchronization).

7. The proposed build-up of Ca^{2+} during the course of the burst may play a role in both the maintenance and then the inhibition of the burst. It is known that oxytocin can increase the frequency of action potentials and this is associated with a prolonged depolarization. This depolarization may depend on the activation of a Ca^{2+} -sensitive inward current or a hyperpolarization-activated inward current.
8. As the concentration of Ca^{2+} builds up during the course of the burst, the persistent activation of the SK channels will finally cause the burst to terminate.
9. Termination of the burst may also depend on the Ca^{2+} -dependent formation of endocannabinoids that feedback by binding to presynaptic CB1 receptors to inhibit the release of the glutamate that contributes to the depolarizing drive that maintains bursting activity. The CB1 receptors may act either by reducing the activity of Ca^{2+} channels or by activating GIRK channels (Module 1: Figure anandamide).

Oxytocin

Oxytocin is a nine-amino-acid peptide that is synthesized and released by oxytocin neurons (Module 10: Figure oxytocin neuron). These neurons have two separate release sites: oxytocin can be released into the blood through the nerve terminals or it is released from the dendrites directly into the brain (Module 10: Figure magnocellular neurons). As a result of these different release sites, oxytocin has a number of different functions:

- Oxytocin released into the blood modulates the uterus smooth muscle cell membrane oscillator that drives contractions during labour (Module 7: Figure uterus activation).
- Oxytocin controls the milk-ejection reflex during suckling.
- When released from the dendrites, oxytocin functions in the synchronization of the oxytocin neurons (Module 10: Figure oxytocin neuron synchronization).
- There is a relationship between oxytocin and CNS function that may help to explain a number of behavioural responses and particularly those concerned with social interactions.

Oxytocin and CNS function

The oxytocin neurons located in the hypothalamus have a number of functions. They have a conventional neurosecretory function in that they synthesize and release oxytocin into the blood to carry out a variety of hormonal effects. In addition, they can also act within the CNS to modulate a large number of functions. The oxytocin neurons send projections to the limbic areas where they target specific neurons located in the septum and hippocampus. Oxytocin also has a more global action that depends on the dendritic release of large amounts of oxytocin that diffuses out into the brain to influence many different behavioural responses. Many of these functions seem to be related to social interactions such as maternal care, pair-bonding, sexual behaviour and social memory. Oxytocin is also released during physical and emotional stress.

Alterations in the operation of this global neuromodulatory role of oxytocin have been linked to a variety

of mental disorders including anxiety-related syndromes, postpartum depression, social phobias and autism. With regard to autism, an alteration on the global action of oxytocin may interfere with the ability to process and remember the social stimuli necessary for successful social interactions. A possible link to autism has been strengthened by the observations that polymorphisms in the gene that codes for the oxytocin receptor have been found in various autism spectrum disorders.

Vasopressin neurons

Vasopressin neurons, which are one of the magnocellular neurons (MCNs), are located in the supraoptic nuclei (SON) and paraventricular nuclei (PVN) of the hypothalamus (Module 10: Figure hypothalamic pituitary regulation). These neurons synthesize vasopressin that is released from both ends of the neuron (Module 10: Figure magnocellular neurons). Some of the vasopressin is released from the soma and dendrites whereas the remainder travels down the axon to the posterior pituitary where it is released into the circulation. The primary function of the vasopressin neurons is to release vasopressin, which is also known as antidiuretic hormone (ADH). As the latter name implies, one of the functions of vasopressin is to conserve water. During dehydration, vasopressin-induced antidiuresis conserves water by increasing the permeability of the apical membrane of the collecting duct to extract water from the final urine (Module 7: Figure collecting duct function). However, vasopressin has many other functions including liver cell signalling mechanisms (Module 7: Figure liver cell signalling) and vasoconstriction. All of these functions require that blood levels of vasopressin be maintained over prolonged periods of time. This modus operandi is very different to that found in the oxytocin neurons that are designed to generate periodic bursts of oxytocin. Although the control systems in the two cell types share many common components, closer inspection reveals that they are set up in different ways to cope with their two very different functions.

One obvious similarity between the oxytocin and vasopressin neurons is that they both use bursts of action potentials that are generated at the soma and travel down the axon to provide the depolarizing signals to stimulate hormone release (Module 10: Figure magnocellular neurons). However, the patterning of these bursts and the way they are controlled is very different for the two cell types. The oxytocin neurons are tied together by a wave of oxytocin released from the dendrites that enables them to burst in synchrony with each other (Module 10: Figure oxytocin neuron synchronization). By contrast, the bursting in vasopressin neurons is asynchronous. The vasopressin neurons display four different spiking patterns: silent, irregular spiking, bursting and continuous spiking (Module 10: Figure magnocellular neurons). These bursting patterns are fairly labile in that each neuron can switch between these different patterns depending on the stimulus conditions. It is thought that at any one time about 50% of the neurons are in a bursting mode, which is the most efficient pattern for stimulating the release of vasopressin, whereas the others are in the irregular or silent mode. In

time, these less active neurons may switch into the bursting mode while the previously bursting neurons decrease their activity perhaps to enable them to recover.

The control systems that regulate vasopressin release must not only generate the periodic bursts that drive secretion but they must also operate the self-regulatory process that switches the responsibility for secreting vasopressin such that only a proportion of the population are operating on-line (bursting) at any given moment. Control of these two processes depends on the generation and function of the periodic bursts of action potentials as described in the following sequence of events (Module 10: Figure vasopressin neuron):

1. The vasopressin neurons receive both excitatory (glutamate) and inhibitory (GABA) neural inputs from the osmosensory neurons that function in osmoreception. The inhibitory neurons act through GABA receptors to induce inhibitory postsynaptic potentials (IPSPs) and the resulting hyperpolarization will reduce neuronal activity. On the other hand, the excitatory neurons release glutamate that act through AMPA receptors to induce the excitatory postsynaptic potentials (EPSPs), which are responsible for initiating the bursts.
2. Summation of the EPSPs gives rise to the depolarization that initiates the burst. The two insets illustrate the main feature of these bursts. The inset at the bottom shows two bursts and the corresponding changes in Ca^{2+} concentration. Each burst is often preceded by a pacemaker depolarization, which persists as a plateau potential (white arrow) during the course of the burst. At the end of the burst, the potential increases and often overshoots to form the slow after-hyperpolarization (sAHP, red arrow), which gradually recovers during the inter-burst interval. The concentration of Ca^{2+} increases rapidly during the beginning of the burst and then remains elevated until it recovers at the end of the burst. The upper panel illustrates the nature of the first two action potentials of a typical burst and the corresponding changes in Ca^{2+} concentration. The EPSPs are responsible for depolarizing the membrane sufficiently to activate the L-type Ca^{2+} channels that open briefly to initiate the spike, during which there is an entry of external Ca^{2+} that then plays an important role in orchestrating the rest of the pulse.
3. The brief pulse of external Ca^{2+} activates ryanodine receptors (RYRs) to trigger release of Ca^{2+} from the ER through a process of Ca^{2+} -induced Ca^{2+} release (CICR) (Module 2: Figure Ca^{2+} -induced Ca^{2+} release). The Ca^{2+} entering the cytoplasm from both the external and internal sources accounts for the brief increase in Ca^{2+} associated with the first spike. The same sequence is repeated during the subsequent spikes and these small pulses sum to account for the rapid elevation in Ca^{2+} that occurs early during the burst (see lower inset Module 10: Figure vasopressin neurons).
4. One of the functions of this elevation of Ca^{2+} is to activate the Ca^{2+} -sensitive K^{+} channels that seem to be responsible for the recovery phase. The opening of both

the small-conductance (SK) Ca^{2+} -activated K^+ channels and the large-conductance (BK) channels seem to contribute to the Ca^{2+} -dependent after-hyperpolarization (AHP). The AHP then recovers quickly and the membrane depolarizes sufficiently to trigger another action potential. This depolarization is facilitated by the Ca^{2+} -dependent depolarizing after-potential (DAP), which depends on the opening of an inward current that remains to be identified. It is this activation of the DAP that is responsible for the plateau potential that maintains the persistent spiking pattern during the course of the burst. The primary function of this burst is to provide the train of action potentials that travel down the axon to the terminals in the posterior pituitary where they trigger the release of vasopressin (Module 10: Figure magnocellular neurons).

5. The increase in Ca^{2+} that occurs in the soma and dendrites during the course of the burst not only controls the ionic channels that contribute to the action potentials but it also acts to trigger the dendritic release of the large dense-core vesicles (LDCVs) that contain both vasopressin and dynorphin (Module 10: Figure vasopressin neuron). These two neuropeptides are released from the same vesicle, but in very different proportions. The very small amounts of dynorphin have a restricted autocrine mode of action by feeding back to terminate the burst. The larger amounts of vasopressin have both autocrine and paracrine actions. They can feedback to influence burst duration and they are able to diffuse out into the brain where they have a paracrine action, one of which is to co-ordinate the activity of other vasopressin neurons as part of the population feedback mechanism described earlier.
6. The dynorphin that gradually builds up during the course of the burst acts on κ opioid receptors to terminate the burst. The inhibitory signal seems to switch off the ion channels responsible for the DAPs that maintain the plateau potential (Module 10: Figure vasopressin neuron).
7. Vasopressin also functions to terminate the burst by inhibiting the release of glutamate by the excitatory neural inputs responsible for the EPSPs that initiate the spikes. Vasopressin may also act to inhibit the L-type channels responsible for the influx of Ca^{2+} ions.
8. In addition to having these direct effects on the components responsible for bursting, vasopressin can also act on V1b receptors that are coupled to the formation of InsP_3 and the release of internal Ca^{2+} . Just how this putative InsP_3 -dependent release of Ca^{2+} influences bursting patterns is not clear but it could contribute to the role of vasopressin as a 'population feedback signal' to co-ordinate the activity of the vasopressin neuronal population as described below.
9. During a prolonged period of stimulation, as might occur during several days of dehydration, the responsibility for secreting vasopressin seems to alternate within the vasopressin neuronal population. The vasopressin released from the dendrites may diffuse out to provide a population feedback signal to ensure that the vasopressin neurons adjust their firing patterns to maxim-

ize the output of the population as a whole. Such a regulatory role is consistent with the observation that vasopressin can be both stimulatory and inhibitory depending on the functional state of the neuron. For those neurons that are bursting, a gradual accumulation of vasopressin will switch them into a lower activity mode (see point 7 above), whereas the vasopressin diffusing away to less-active neurons will excite them into a bursting mode to take over the duties of maintaining a constant supply of vasopressin. The InsP_3 -dependent release of Ca^{2+} may be capable of switching neurons into a bursting mode by raising the resting level of Ca^{2+} sufficiently to activate the inward channels that depolarize the membrane. Clearly, there is a need for more information on how vasopressin acts to modulate the activity of these vasopressin neurons.

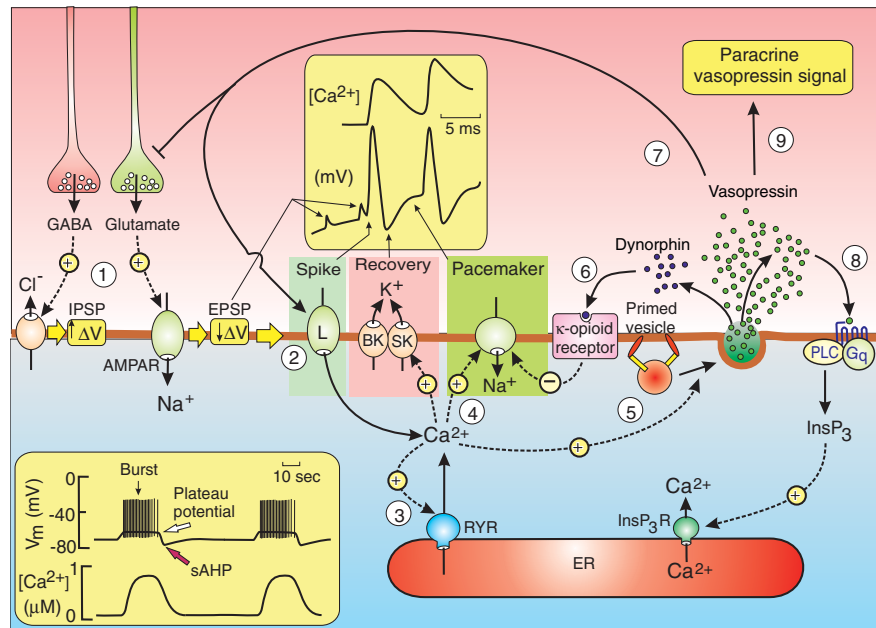
The regular bursts of action potentials that are generated at the soma travel down the axons to the multiple nerve terminals in the posterior pituitary where vasopressin is released (Module 10: Figure magnocellular neurons).

Kisspeptin neurons

Hypothalamic kisspeptin neurons located in the anteroventral periventricular (AVPV) and arcuate nuclei function to regulate the activity of the gonadotrophin-releasing hormone (GnRH) neurons (Module 10: Figure kisspeptin neuronal circuit). There is a regulatory circuit comprising the hypothalamic neurons (kisspeptin and GnRH neurons), the gonadotrophs in the anterior pituitary that release gonadotropins and the gonads that release the sex steroids. Although GnRH neurons are known to be regulated by many different neurotransmitters (e.g. GABA and glutamate), recent studies have shown that hypothalamic kisspeptin neurons may function as 'gatekeepers' to control both the onset of puberty and to maintain adult reproductive activity. Such a central role in the regulation of reproduction is supported by the fact that they integrate many of the stimuli that control reproductive behaviour. If conditions are favourable, the kisspeptin neurons release kisspeptin that is a powerful stimulus for the GnRH neurons. The latter have the G protein-coupled receptor (GPCR) GPR54 that acts through the formation of the second messengers inositol 1,4,5-trisphosphate (InsP_3) and diacylglycerol (DAG) that induce the bursts of action potentials that trigger the release of GnRH (Module 10: Figure GnRH neuron). The GnRH then acts through the GnRH receptor (GnRHR) on gonadotrophs in the anterior pituitary to release luteinising hormone (LH) and follicle-stimulating hormone (FSH) (Module 10: Figure gonadotroph regulation).

These gonadotropins then act on the gonads to increase the formation of the sex steroids that can feedback to regulate the kisspeptin neurons (Module 10: Figure kisspeptin neuronal circuit). In females, estradiol increases the synthesis of kisspeptin in the AVPV neurons, but inhibits that in the arcuate neurons. Testosterone inhibits the synthesis of kisspeptin in the arcuate neurons. The gonads also synthesize and release the inhibins that feedback to inhibit

Module 10: | Figure vasopressin neuron



Control of burst patterning in vasopressin neurons.

This figure illustrates the proposed mechanisms responsible for generating periodic bursts of action potentials that occur every 30–40 s (see bottom inset). The action potentials are located on an elevated plateau potential (white arrow). At the end of the burst, the potential suddenly increases to form the slow after-hyperpolarization (sAHP, red arrow), which then recovers during the interspike interval before the next burst. The inset at the top shows the time course of the first two action potentials of the burst and the corresponding changes in $[Ca^{2+}]$. See text for further details of the numbered sequence of events. Much of the information shown in this slide was taken from Brown 2004 and Brown and Bourque 2006.

the expression of FSH by the gonadotrophs (Module 10: Figure gonadotroph regulation).

Kisspeptin

The kisspeptin gene (*Kiss1*) was first identified in tumour cells where it appeared to function as a cancer metastasis suppressor. The peptide was named both kisspeptin and metastin but, as used here, the former is now used more widely. Subsequently, it was found to have an important role as a neuropeptide and was called kisspeptin, which is the name that will be used here. The *Kiss1* gene encodes a precursor protein (145 amino acid), which is then cleaved into a 54 amino acid protein (kisspeptin). There are also shortened C-terminal peptides of 14, 13 and 10 amino acids that have full biological activity. This neuropeptide acts through GPR54, which is a typical G protein-coupled receptor (GPCR) that is coupled through $G_{q/11}$ and phospholipase C to generate the second messengers inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DAG). Following the discovery of human families with GPR54 mutations, which suffered from infertility (hypogonadotropic hypogonadism), the kisspeptin neurons were found to regulate the activity of the gonadotrophin-releasing hormone (GnRH) neurons (Module 10: Figure kisspeptin neuronal circuit).

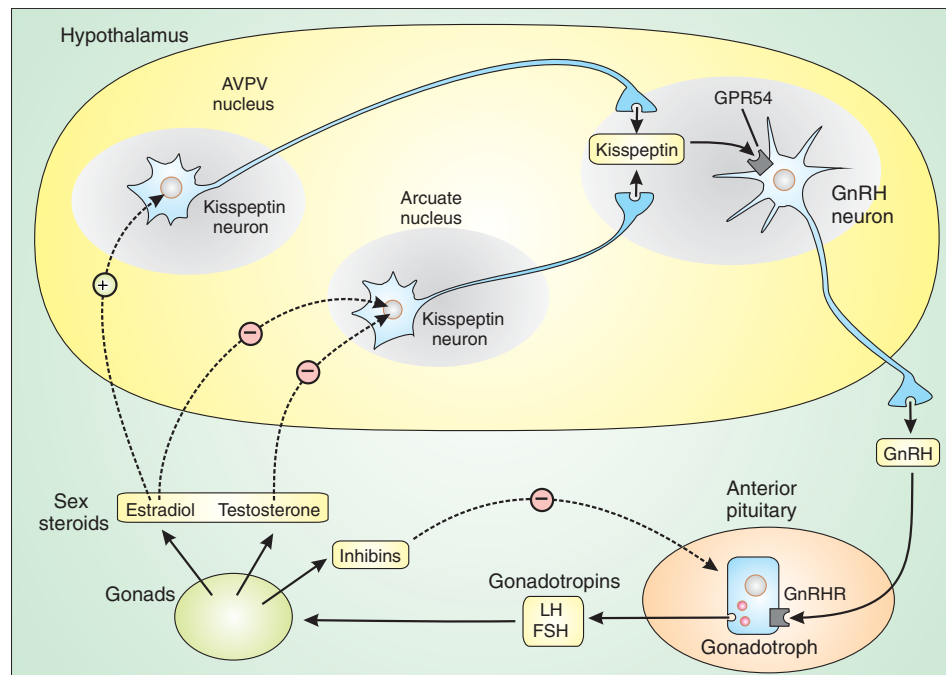
Gonadotropin-releasing hormone (GnRH) neurons

The gonadotropin-releasing hormone (GnRH) neurons located within the medial preoptic area of the hypothalamus are responsible for releasing pulses of GnRH that

passes down to the anterior pituitary (Module 10: Figure hypothalamic pituitary regulation). The GnRH then activates the gonadotrophs to release the two gonadotropins luteinising hormone (LH) and follicle-stimulating hormone (FSH) (Module 10: Figure gonadotroph regulation). The GnRH neurons release pulses of GnRH every 30–60 min and the secretion of each pulse is activated by a burst of action potentials that last for 4–5 min (See large panel at the bottom of Module 10: Figure GnRH neuron). The nature of the oscillatory mechanism responsible for generating these bursts of neural activity every 30–60 min is still not clear. It is possible that these neurons are driven by other oscillatory neurons, such as the kisspeptin neurons that play such an important role in regulating the activity of the GnRH neurons (Module 10: Figure kisspeptin neuronal circuit). Until this is established, it will be assumed that the oscillator resides in the GnRH neuron. The primary function of this oscillator is to generate periodic bursts of action potentials as outlined in the following hypothesis (Module 10: Figure GnRH neuron):

1. A spontaneous release of Ca^{2+} from the internal stores seems to be responsible for initiating each burst of action potentials. The release of Ca^{2+} depends on both the inositol 1,4,5-trisphosphate ($InsP_3$) receptors and ryanodine receptors (RYRs). The nature of the oscillator responsible for timing the long 30–60 min intervals between the bursts has not been established, but must depend upon some desensitization/resensitization mechanism. The mechanism of Ca^{2+} oscillations

Module 10: | Figure kisspeptin neuronal circuit

**Kisspeptin neuronal circuit.**

Kisspeptin neurons located in the AVPV (anteroventral periventricular) and arcuate nuclei within the hypothalamus control the activity of the gonadotropin-releasing hormone (GnRH) neurons. They release kisspeptin that acts on GPR54 receptors on the GnRH neurons to release GnRH, which then acts on GnRHR receptors on gonadotrophs in the anterior pituitary to release the gonadotropins LH (luteinising hormone) and FSH (follicle-stimulating hormone). These gonadotropins then act on the gonads to release the sex steroids that can feedback to regulate the kisspeptin neurons. In females, oestradiol increases the synthesis of kisspeptin in the AVPV neurons, but inhibits that in the arcuate neurons. Testosterone inhibits the synthesis of kisspeptin in the arcuate neurons.

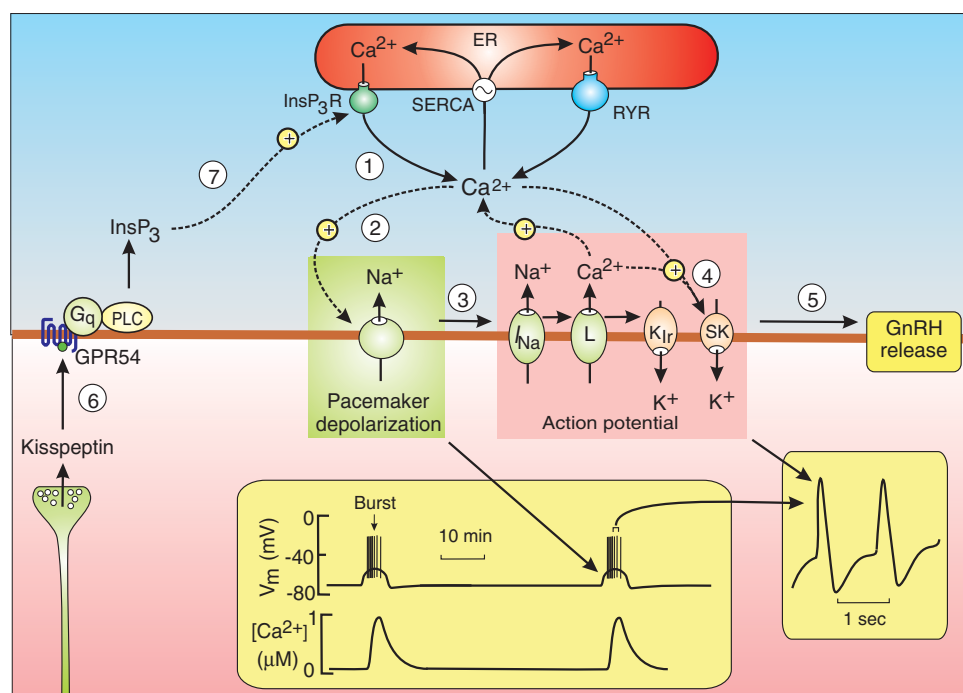
responsible for many other cytosolic Ca^{2+} oscillators, which have much shorter periodicities ranging from a few seconds to 3–4 min (Module 6: Figure cellular oscillators), can be explained by a store-loading mechanism (Module 6: Figure Ca^{2+} oscillation model). Whether or not such a mechanism could explain the much longer periodicities seen in GnRH neurons remains to be seen. Since each of the bursts last for 4–5 min, it is possible that the endoplasmic reticulum (ER) may become severely depleted and this could desensitize the release channels. It may take a long time to re-load the stores sufficiently to resensitize the channels to enable them to trigger the next burst.

2. The burst of Ca^{2+} may then activate a non-selective cation channel to provide the pacemaker depolarization necessary to trigger the action potentials.
3. The action potentials that occur during each burst have a frequency that ranges from about 0.1 to 0.7 Hz. Each action potential depends upon a combination of channels contributing both inward and outward currents. There is an inward Na^+ current (I_{Na}) carried by tetrodotoxin-sensitive voltage-dependent Na^+ channels and an inward Ca^{2+} current carried by voltage-operated L-type Ca^{2+} channels. The entry of external Ca^{2+} will help to load up the store and could thus contribute to the release of internal Ca^{2+} by the InsP_3 Rs. The repolarization phase depends on inactivation of these inward currents and the activation of

outward currents that include one of the inward rectifier K^+ (K_{ir}) channels and the small-conductance (SK) Ca^{2+} -activated K^+ channels.

4. The SK channels seem to be particularly important in determining the pattern of action potentials within each burst. They appear to be activated both by the global elevation of Ca^{2+} resulting from the internal release of Ca^{2+} and perhaps by the local Ca^{2+} microdomains produced by the L-type Ca^{2+} channels.
5. The burst of action potentials travel down the axon to trigger the release of GnRH from the terminals located in the median eminence (Module 10: Figure hypothalamic pituitary regulation).
6. The activity of these GnRH neurons is exquisitely sensitive to the kisspeptin released from the kisspeptin neurons (Module 10: Figure kisspeptin neuronal circuit). Kisspeptin acts on the GPR54 receptors that are coupled through $\text{G}_{\text{q/11}}$ and phospholipase C (PLC) to generate the second messengers inositol 1,4,5-trisphosphate (InsP_3) and diacylglycerol (DAG).
7. The InsP_3 then acts on the InsP_3 receptor (InsP_3R) to release Ca^{2+} from the endoplasmic reticulum (ER). What is not clear at present is just how this kisspeptin regulatory network is integrated into the oscillatory system responsible for producing the periodic bursts of Ca^{2+} and neural activity. As mentioned earlier, the pacemaker mechanism that controls the onset of each burst may be located in the kisspeptin neuron.

Module 10: | Figure GnRH neuron



GnRH neuronal activation hypothesis.

The proposed mechanism for gonadotropin-releasing hormone (GnRH) activation. GnRH is released during bursts of neuronal activity that occur with periodicities of 30–60 min. Each burst seems to depend on the spontaneous release of Ca^{2+} from the intracellular stores. This pulse of Ca^{2+} causes the pacemaker depolarization that initiates each burst of action potentials. See text for further details.

Alternatively, the kisspeptin neuron may activate the GnRH neuronal oscillatory mechanism by sensitizing the InsP_3Rs such that they can initiate the spontaneous Ca^{2+} release process described above (see point 1).

Gonadotropin-releasing hormone (GnRH)

Gonadotropin-releasing hormone (GnRH) is synthesized and released by the GnRH neurons (Module 10: Figure hypothalamic pituitary regulation). The GnRH comes in two forms: GnRH-I, which is released from the hypothalamic GnRH neurons and GnRH-III which is found in neurons in the midbrain where it controls sexual behaviour. The release of GnRH from the hypothalamic neurons is pulsatile (Module 10: Figure GnRH neuron) and the frequency of release determines how it functions on the gonadotrophs. The normal frequency of 1 burst/1 h induces the release of LH whereas a slower frequency (1 burst/3–4 h) releases FSH. GnRH-I acts on the gonadotrophs to set up Ca^{2+} oscillations that stimulate hormone release (Module 10: Figure gonadotroph regulation).

Posterior pituitary

The posterior pituitary is the site where the hormones oxytocin and vasopressin are released to the blood from the nerve endings of the magnocellular neurons (MNCs) (Module 10: Figure hypothalamic pituitary regulation). The axons of the oxytocin neurons and the vasopressin neurons travel down to the posterior pituitary where they split up to form the axonal swellings and terminals that release the large-dense-core vesicles (LDCVs). The released

hormone is then carried away by the web of blood vessels formed from a branch of the hypophyseal artery (Module 10: Figure magnocellular neurons). Each neuron has approximately 2000 of these swellings and terminals. When the action potential invades these swellings and terminals, activation of voltage-operated channels (VOCs) allow Ca^{2+} to enter to trigger exocytosis and hormone is released. The probability of release from these swellings and terminals is very low (0.0025), which means that each neuron releases about five vesicles for every spike.

Once hormones have been released from the swellings and terminals, they have to gain access to the blood by diffusing into the perivascular space and then into the fenestrated capillaries. This transfer into the blood is regulated by the pituicytes, which can undergo considerable morphological changes dependent on the degree of stimulation.

Pituicytes

Pituicytes are glial-like cells that surround the nerve swellings and terminals of the magnocellular neurons (MCNs) in the posterior pituitary (Module 10: Figure magnocellular neurons). They regulate the way hormones gain access to the blood vessels after they have been released from the nerve terminals. At low rates of hormone release, pituicyte extensions wrap around the nerve endings to reduce their access to the perivascular space (neuron 2 in Module 10: Figure magnocellular neurons). By contrast, when secretion is increased during periods of dehydration (for the vasopressin neurons) or lactation and parturition (for the

oxytocin neurons), these long extensions are retracted thus increasing the number of contacts between the nerve endings and the basement membrane facing the blood vessels (see neurons 1, A and B).

The way in which the arms of the pituicytes extend during low rates of hormone secretion and retract when hormone secretion is high seems to depend on actin remodelling. The Rho signalling pathway is particularly important in regulating the formation and depolymerization of the actin filaments responsible for extension and retraction respectively. The products released by the nerve endings seem to play a role in regulating these morphological changes. For example, the ATP that is packaged together with vasopressin or oxytocin seems to provide one of the stimuli for retraction. Ecto-ATPases convert ATP into adenosine, which then acts through A1 receptors to inhibit the activity of Rho leading to actin depolymerization. Paradoxically, the vasopressin that is released together with ATP has the opposite effect. It is not clear how the opposite effects of these two stimuli function to co-ordinate the opposing effects of retraction and extension.

Growth hormone-releasing hormone (GHRH)

Growth hormone-releasing hormone (GHRH) is synthesized by hypothalamic neurons and is released into the hypophyseal artery and travels down to the anterior pituitary where it acts on the somatotrophs to control the release of growth hormone (GH) (Module 10: Figure hypothalamic pituitary regulation). GHRH acts on the growth hormone-releasing hormone receptor (GHRH-R), which is a G protein-coupled receptor (GPCR) that activates the cyclic AMP signalling pathway (Module 10: Figure somatotroph regulation). GHRH may also play a role as an endogenous sleep-regulatory molecule.

GHRH is metabolized by dipeptidyl peptidase type 4 (DPP4) and by trypsin-like endopeptidases.

Somatostatin (Sst)

Somatostatin (Sst) is a peptide hormone that functions to regulate a number of cellular processes. There are six somatostatin receptors (sst_1 , sst_{2A} and sst_{2B} , sst_3 , sst_4 , sst_5 , sst_6), which are typical G protein-coupled receptors (GPCRs) (Module 1: Table G protein-coupled receptors). Cortistatin (CST) resembles Sst in structure and can act through the sst receptors.

Many of the actions of Sst are based on its ability to inhibit a variety of cellular processes:

- It can inhibit hormone secretion by various anterior pituitary cells such as prolactin (PRL) secretion by the lactotrophs (Module 10: Figure lactotroph regulation) and growth hormone secretion by the somatotrophs (Module 10: Figure somatotroph regulation).
- Various cells in the gastrointestinal tract release Sst to control intestinal function. For example, D cells in the antral region of the stomach secrete Sst, which inhibits the activity of both the G cells (Module 7: Figure stomach structure) and also the acid-secreting parietal cells (Module 7: Figure HCl secretion).

- Sst may act to modulate neurotransmission. For example, hippocampal interneurons project to the outer molecular layer of the dentate gyrus, which is part of the hippocampus (Module 10: Figure hippocampus), where they innervate dendrites of the granular cells near the input of the perforant pathway. They release Sst that acts to reduce cognition by inhibiting hippocampal long-term potentiation (LTP).

Acromegaly and certain endocrine tumours can be treated by analogues of Sst.

Anterior pituitary

The anterior pituitary is an endocrine gland responsible for secreting a wide range of hormones. These different hormones are synthesized and released by separate groups of cells that are lined up on an anastomizing web of blood vessels derived from the hypophyseal artery (Module 10: Figure hypothalamic pituitary regulation). The endothelial cells that line the capillaries have typical fenestrae that facilitate the exchange of molecules between the endocrine cells and the blood (Module 10: Figure anterior pituitary structure). The blood vessels not only bring in the neurohormones that control the activity of the pituitary cells, but they also carry the released hormones away into the general circulation. The anterior pituitary also contains an extensive web of interconnected folliculostellate (FS) cells.

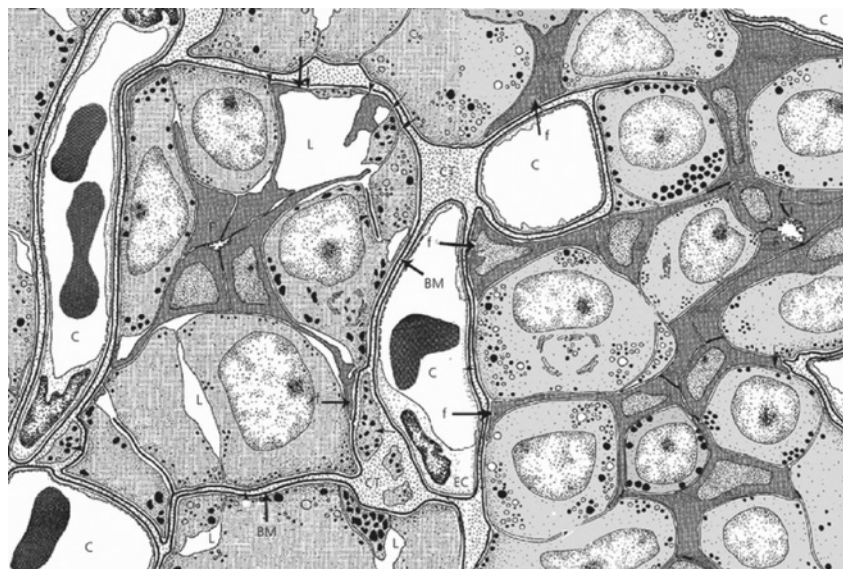
The anterior pituitary contains five endocrine cells that are present in different proportions:

- Corticotrophs (10%) that release adrenocorticotrophic hormone (ACTH)
- Gonadotrophs (10%) that release follicle-stimulating hormone (FSH) and luteinizing hormone (LH)
- Lactotrophs (25%) that release prolactin (PRL)
- Somatotrophs (50%) that release growth hormone (GH)
- Thyrotrophs (5%) that release thyrotropin (TSH)

These proportions are not fixed but can vary depending on many factors such as developmental age and oestrus cycle.

Corticotrophs

The corticotrophs, which represent about 15% of the endocrine cells in the anterior pituitary, are responsible for releasing adrenocorticotrophic hormone (ACTH) (Module 10: Figure corticotroph regulation). There is a circadian rhythmicity of ACTH release with a peak in the early morning followed by a gradual decline during the day to reach its lowest level in the middle of the night. This rhythmicity is controlled directly by clock neurons in the suprachiasmatic nucleus (Module 6: Figure circadian clock location), which send neural afferents to the hypothalamic neurons that synthesize and release corticotropin-releasing factor (CRF) (Module 10: Figure hypothalamic pituitary regulation). CRF functions to control both the transcription of pro-opiomelanocortin (POMC) and the release of pre-formed ACTH through the following sequence of events (Module 10: Figure corticotroph regulation):

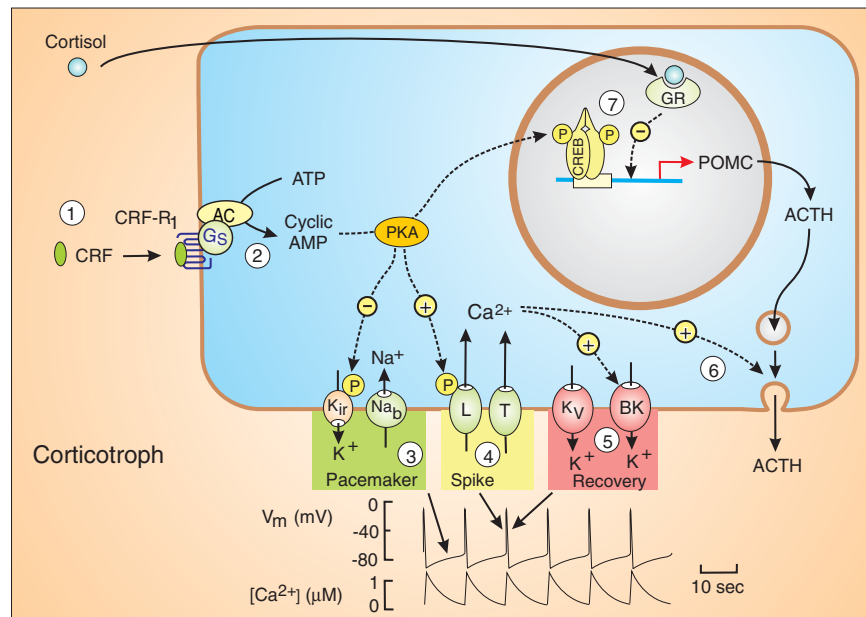
Module 10: | Figure anterior pituitary structure**Endocrine and folliculostellate cells in the anterior pituitary.**

The anterior pituitary has three main cell types: the chords of endocrine cells containing numerous dense vesicles, fenestrated endothelial cells (EC) that line the capillaries (C) and the darker folliculostellate (FS) cells (f) which surround the endocrine cells. Reproduced from Deneff, C. (2008) Paracrinicity: the story of 30 years of cellular pituitary crosstalk. *J. Neuroendocrinol.* 20:1–70. Copyright (2008), with permission from John Wiley and Sons, Inc; see Deneff 2008.

1. The main regulator of ACTH release is corticotropin-releasing factor (CRF), which acts on the corticotropin-releasing factor (CRF) receptor 1 (CRF-R₁), which is a typical G protein-coupled receptor (GPCR) (Module 1: Table G protein-coupled receptors).
2. The CRF-R₁ stimulates adenylyl cyclase (AC) to initiate the cyclic AMP signalling pathway (Module 2: Figure cyclic AMP signalling). One of the main functions of cyclic AMP is to control a membrane oscillator in the plasma membrane that consists of at least five channels that interact with each other to generate oscillations in the level of Ca²⁺ that are triggered by periodic action potentials (see the traces at the bottom of Module 10: Figure corticotroph regulation).
3. A key element of such membrane oscillators is the pacemaker depolarization responsible for the slow membrane depolarization that activates the Ca²⁺ channels, which introduce Ca²⁺ into the cell. Inhibition of an inwardly rectifying K⁺ channel (K_{ir}) seems to be particularly important for initiating the pacemaker depolarization. The latter may be facilitated by a background Na⁺ conductance (Na_b).
4. Once the slow pacemaker depolarization reaches the threshold for the two voltage-operated-channels (VOCs), these open during the upstroke of the spike to introduce the Ca²⁺ that then triggers exocytosis. The main Ca²⁺ entry channel belongs to the Ca_v1 family of L-type channels. The corticotrophs also have one of the T-type channels, but the function of these is unclear. It is possible that they may contribute to the pacemaker depolarization as they do in many other membrane oscillators. Cyclic AMP may also act to enhance the activity of the L-type channels as it does for the modulation of the Ca_v 1.2 L-type channel in the heart (Module 3: Figure Ca_v1.2 L-type channel). This modulation depends on the phosphorylation of the L-type channel by protein kinase A (PKA).
5. The recovery of the spike depends on the opening of two K⁺ channels, a voltage-dependent K⁺ (K_v) channel and the large-conductance (BK) channels. The latter may be particularly important during the recovery process because they will respond to the build up of Ca²⁺ by initiating the recovery process. As the Ca²⁺ concentration returns back towards the resting level, the hyperpolarizing effect of these BK channels will decline and this will contribute to the pacemaker depolarization that develops to trigger the next spike.
6. The elevation of Ca²⁺ during each spike triggers Ca²⁺-dependent exocytosis to release ACTH into the circulation.
7. The transcription of the pro-opiomelanocortin (POMC) precursor of ACTH is also under hormonal regulation. The CRF that activates secretion uses the same second messenger cyclic AMP to activate transcription of POMC. Phosphorylation of the transcription factor CREB by protein kinase A (PKA) seems to be the most likely mode of action. Cortisol acting through the glucocorticoid receptor (GR), which is a typical nuclear receptor, acts to inhibit expression of the POMC gene.

Adrenocorticotrophic hormone (ACTH)

Adrenocorticotrophic hormone (ACTH), also known as corticotropin, is synthesized and released by

Module 10: | Figure corticotroph regulation**Regulation of ACTH secretion by corticotrophs.**

Release of adrenocorticotrophic hormone (ACTH) is regulated by corticotropin-releasing factor (CRF) that acts through the cyclic AMP signalling pathway to induce the firing of regular action potentials (APs). The latter are responsible for opening L-Type (L) voltage-operated Ca^{2+} channels that generate the Ca^{2+} signals responsible for triggering the release of ACTH. Cyclic AMP acting through protein kinase A (PKA) also activates the gene for pro-opiomelanocortin (POMC), which is the precursor of ACTH. Cortisol acts through the glucocorticoid receptor (GR) to inhibit POMC transcription.

corticotrophs (Module 10: Figure corticotroph regulation). The ACTH precursor pro-opiomelanocortin (POMC) is processed into ACTH and β -lipocortin by prohormone convertase 1. The primary action of ACTH is to stimulate the synthesis and release of cortisol from the zona fasciculata cells and aldosterone from the zona glomerulosa cells (Module 7: Figure glomerulosa cell signalling).

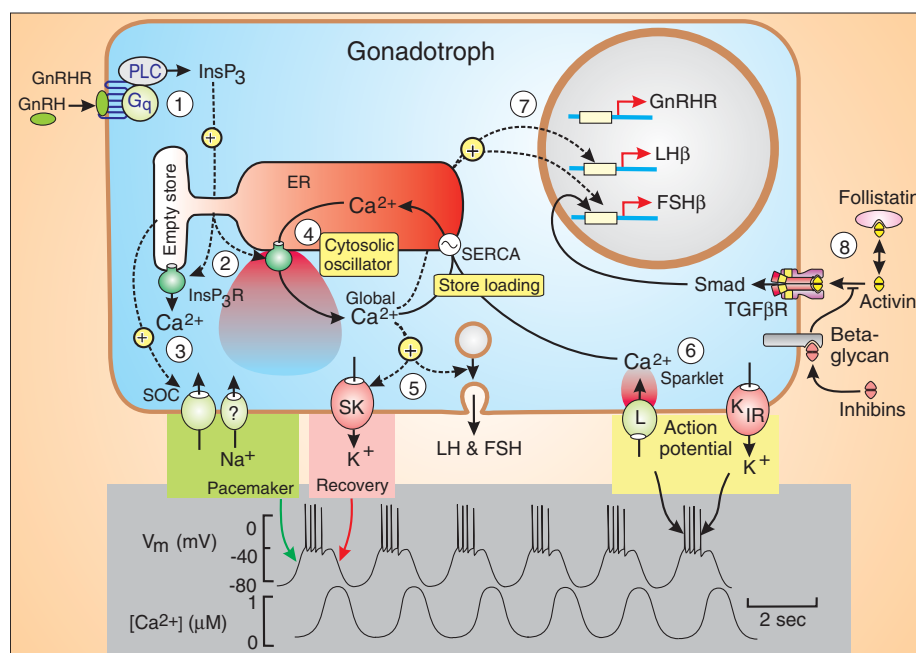
Gonadotrophs

The gonadotrophs, which make up approximately 10% of the anterior pituitary, regulate gonadal function in both sexes by releasing both luteinising hormone (LH) and follicle-stimulating hormone (FSH) (Module 10: Figure gonadotroph regulation). The levels of LH and FSH vary during the menstrual cycle with peak levels occurring at ovulation. The primary regulator of gonadotrophs is gonadotropin-releasing hormone (GnRH), which is released from the GnRH neurons at a frequency ranging from 30 to 60 min depending on species (Module 10: Figure GnRH neuron). The release of LH and FSH from the gonadotrophs occurs in phase with pulses of GnRH. Each pulse of GnRH acts on the gonadotrophs to induce the rapid fluctuations in both membrane potential and intracellular Ca^{2+} that controls the release of LH and FSH through the following sequence of events (Module 10: Figure gonadotroph regulation):

1. GnRH acts on the GnRH receptor (GnRHR), which is coupled through the G protein $G_{q/11}$ to phospholipase C resulting in the formation of InsP_3 .

2. InsP_3 then acts on InsP_3 receptors (InsP_3Rs) to release Ca^{2+} from the endoplasmic reticulum (ER). This release seems to have two consequences in that it can promote both an increase in Ca^{2+} entry and the induction of a cytosolic oscillator that provides the periodic Ca^{2+} pulses that drive exocytosis.
3. Release of Ca^{2+} from ER regions near the membrane, where the InsP_3 levels are highest, will empty the store thus providing a signal for the activation of store-operated Ca^{2+} channels (SOCs) (Module 3: Figure capacitative Ca^{2+} entry). This influx of Ca^{2+} may help to depolarize the membrane during the pacemaker phase (see section 5 below) and it will also provide a supply of Ca^{2+} to load up the internal store during the interval between each phase of release.
4. The lower levels of InsP_3 deeper in the cell will sensitize the InsP_3Rs to activate a cytosolic oscillator that sets up very regular fluctuations in intracellular Ca^{2+} (Module 6: Figure gonadotroph Ca^{2+} oscillations). The mechanism of Ca^{2+} oscillations in gonadotrophs remains to be established. One possibility is that these oscillations are driven by the consequence of periodically loading and unloading the store of Ca^{2+} within the ER. When the intracellular store of Ca^{2+} was measured during the course of an oscillations, the luminal level of Ca^{2+} fell during the upstroke of the Ca^{2+} spike and then gradually recovered during the interval leading up to the next spike (Module 10: Figure gonadotroph ER Ca^{2+} oscillations) It is this increase in the luminal level of Ca^{2+} that may function to sensitize the InsP_3Rs to initiate the release of Ca^{2+} (Module 6: Figure Ca^{2+} oscillation model).

Module 10: | Figure gonadotroph regulation



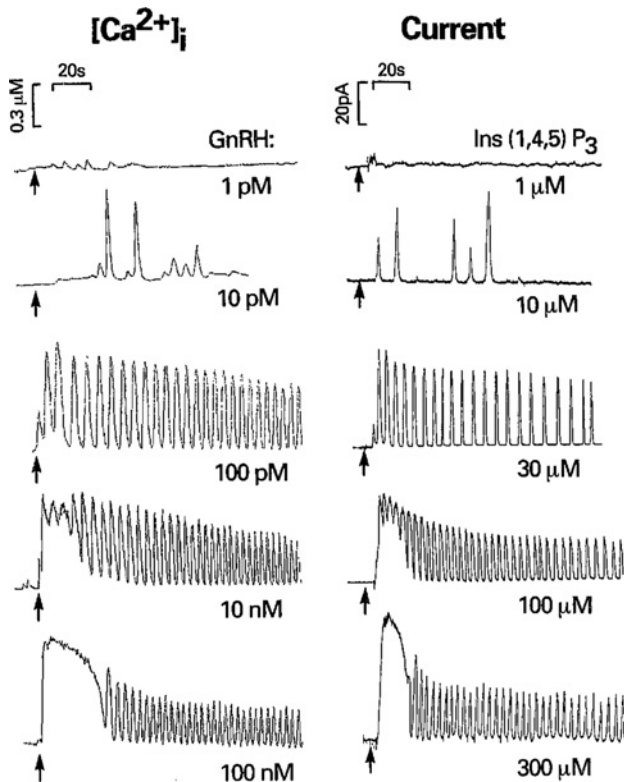
Regulation of LH and FSH secretion by gonadotrophs.

Release of luteinising hormone (LH) and follicle-stimulating hormone (FSH) is regulated by gonadotropin-releasing hormone (GnRH), which acts through the GnRH receptor (GnRHR) to stimulate the formation of inositol 1,4,5-trisphosphate (InsP₃). The latter then induces a cytosolic oscillator that releases a periodic burst of Ca²⁺ from the endoplasmic reticulum (ER). See text for further details. Some of the information used to construct this figure was taken from Vergara et al 1995.

- The large global elevation in Ca²⁺ that occurs during each spike has a number of consequences. It triggers bursts of LH and FSH release that occur in phase with each pulse of Ca²⁺. This is an example of exocytosis triggered by Ca²⁺ release from internal stores (Module 4: Figure Ca²⁺-dependent exocytosis). The microdomain of Ca²⁺ that forms near the mouth of the InsP₃ receptor is high enough to trigger exocytosis. These pulses of Ca²⁺ are also responsible for driving the oscillation in membrane potential (V_m) characterized by regular fluctuations of depolarization and hyperpolarization (see trace in Module 10: Figure gonadotroph regulation). Each increase in Ca²⁺ activates the small-conductance (SK) Ca²⁺-activated K⁺ channels causing the membrane to hyperpolarize during the recovery phase, but as the Ca²⁺ is removed from the cytoplasm, the SK channels close and the membrane begins to depolarize. This pacemaker depolarization will be assisted by inward currents flowing through various channels such as the SOC channels described earlier (section 3 above).
- The plateau phase of each burst contains a number of action potentials that result from the opening of one of the Ca_v1 family of L-type channels, whereas the recovery phase depends on the opening of an inwardly rectifying K⁺ channel (K_{ir}). These action potentials are very brief and give rise to small localized Ca²⁺ sparklets that are not able to trigger exocytosis. However, this entry of Ca²⁺ may help to activate the InsP₃Rs and may also provide a source of Ca²⁺ to load up the internal store for each global pulse of Ca²⁺.
- The genes for LH, FSH and components of the signalling system such as the GnRHR are also regulated by GnRH. Just how transcription of these genes is regulated is still being determined, but there are indications that the global Ca²⁺ pulses may act to stimulate the transcription of the FSHβ and LHβ genes.
- The transcription of FSH is also regulated by activin operating through a Smad signalling pathway (Module 2: Figure Smad signalling). The activin stimulates a transforming growth factor-β (TGF-β) receptor that activates the Smads that carry information into the nucleus to induce the transcription of FSH. Follistatin that is synthesized by the gonadotrophs operates in an autocrine manner by binding to activin to inhibit its activity. The inhibins that are synthesized and released by the gonads (Module 10: Figure kisspeptin neuronal circuit) function through a negative-feedback loop to inhibit the secretion of FSH. The inhibins bind to the accessory receptor betaglycan (Module 2: Table Smad signalling toolkit) that interferes with the ability of activin to stimulate the TGF-β receptor.

Lactotrophs

The lactotrophs, which make up approximately 25% of the anterior pituitary, release the hormone prolactin (PRL). The lactotrophs are spontaneously active and control is exercised primarily by tonic inhibition by various hormones such as dopamine, somatostatin (Sst) and endothelin-1 (ET-1) (Module 10: Figure lactotroph regulation). This spontaneous activity is characterized by regular

Module 10: | Figure gonadotroph Ca^{2+} oscillations**Gonadotroph Ca^{2+} oscillations.**

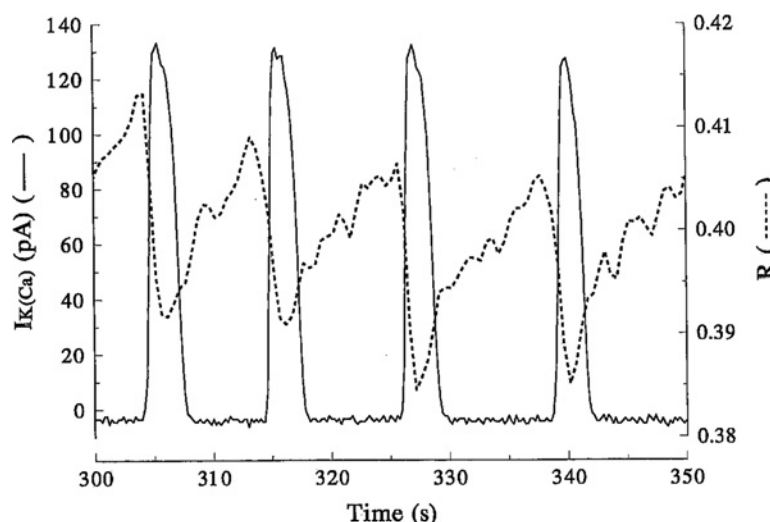
Oscillations in intracellular $[\text{Ca}^{2+}]_i$ and membrane current in gonadotrophs stimulated with different concentration of gonadotropin-releasing hormone (GnRH) (left-hand panels) or perfused with different concentrations of InsP_3 . Reproduced from Stojilković, S., Kukuljan, M., Tomić, M., Rojas, E. and Catt, K.J. (1993) Mechanisms of agonist-induced $[\text{Ca}^{2+}]_i$ oscillations in pituitary gonadotrophs. *J. Biol. Chem.* 268:7713–7720. Copyright (1993), with permission from the American Society for Biochemistry and Molecular Biology; see Stojilković et al 1993.

oscillations in the intracellular level of Ca^{2+} , which are induced by regular fluctuations in membrane potential (V_m) (see traces in Module 10: Figure lactotroph regulation). With regard to how prolactin (PRL) release is controlled, therefore, there are two questions to consider: what is the nature of the spontaneous mechanism that generates the Ca^{2+} signal that triggers exocytosis and how is this spontaneous activity regulated? The following description of how lactotroph secretion is controlled will begin with the mechanism of spontaneous release:

1. The spontaneous electrical activity depends on the cyclic AMP signalling pathway, which seems to be maintained through an autocrine mechanism driven by the release of vasoactive intestinal peptide (VIP) that then acts on the VPAC_1 receptors (Module 1: Table G protein-coupled receptors) to increase the formation of cyclic AMP.
2. Cyclic AMP seems to be responsible for switching on a membrane oscillator by activating a background Na^+ conductance (Na_b), which initiates the pacemaker depolarization.
3. Depolarization of the membrane activates one of the Ca_v1 family of L-type channels that open repetitively

during the plateau phase of the membrane depolarization. Unlike the large action potentials that occur during the plateau phase in gonadotrophs (Module 10: Figure gonadotroph regulation), these lactotroph APs are much smaller and thus do not reach the level of depolarization that will activate the voltage-dependent K^+ channels necessary to fully hyperpolarize the membrane (Module 10: Figure lactotroph regulation). A close apposition between these L-type Ca^{2+} channels and the large-conductance (BK) channels is responsible for truncating this hyperpolarizing response. The local domain of Ca^{2+} near the mouth of the L-type channel activates the nearby BK channels causing a small inward current that allows a partial hyperpolarization such that the membrane remains sufficiently depolarized to allow the L-type channels to re-open. A rapid succession of such brief APs riding on the crest of the depolarizing wave is responsible for the gradual increase in the intracellular level of Ca^{2+} necessary to induce exocytosis.

4. Termination of each burst depends upon this global elevation of Ca^{2+} that then activates a sufficient number of the peripheral BK channels that leads to the recovery phase. As the Ca^{2+} concentration declines, these BK channels will switch off and this will facilitate the depolarizing effect of the Na_b channels to bring about the pacemaker that initiates the next cycle.
5. The global elevation of Ca^{2+} that occurs during each burst is then responsible for activating the release of prolactin (PRL).
6. The spontaneous membrane events responsible for triggering exocytosis (steps 1–5) can be inhibited by various hormones such as dopamine, somatostatin (Sst) and endothelin-1 (ET-1) through a number of mechanisms. Dopamine and somatostatin, acting through D_2 and sstr receptors respectively, result in the dissociation of G_i into $\text{G}_{i\alpha}$ and $\text{G}_{i\beta\gamma}$, which then act on the channels responsible for the pacemaker depolarization. The $\text{G}_{i\alpha}$ acts to inhibit adenylyl cyclase thus reducing the level of the cyclic AMP that drives the membrane oscillator. The $\text{G}_{i\beta\gamma}$ also has an inhibitory effect because it stimulates an inward rectifying K^+ channel (K_{ir}) and the resulting hyperpolarization reduces the pacemaker depolarization that is necessary to drive the oscillator.
7. Endothelin-1 (ET-1), which is another potent inhibitor of prolactin (PRL) release, does not effect the generation of the Ca^{2+} signal but it inhibits the process of exocytosis. ET-1 stimulates the ET-A receptor to activate the G protein G_Z to release $\text{G}_Z\beta\gamma$ that then inhibits release by interacting with the exocytotic machinery responsible for exocytosis.
8. Lactotrophs are also sensitive to various hormones [galanin, endothelin-1, thyrotropin releasing hormone (TRH)], which act through the phosphoinositide signalling pathway. The ability of oestradiol to stimulate lactotroph proliferation and prolactin (PRL) gene expression depends on its ability to increase the synthesis and release of galanin, which then acts in an autocrine manner to stimulate the GalR2 receptor that acts to increase the formation of InsP_3 and the release of internal Ca^{2+} . Oestradiol also stimulates the release $\text{TGF-}\beta 3$

Module 10: | Figure gonadotroph ER Ca^{2+} oscillations**Oscillations of gonadotroph ER Ca^{2+} concentrations.**

Oscillations of Ca^{2+} within the lumen of the ER (dashed line) recorded simultaneously with the outward current caused by the opening of the Ca^{2+} -activated K^{+} channels (continuous line), which is a direct measure of the intracellular level of Ca^{2+} . Reproduced from Tse, F.W., Tse, A. and Hille, B. (1994) Cyclic Ca^{2+} changes in intracellular stores of gonadotropes during gonadotrophin-releasing hormone-stimulated Ca^{2+} oscillations. *Proc. Natl. Acad. Sci. U.S.A.* 91:9750–9754; Copyright (1994), with permission from the National Academy of Sciences; see Tse et al 1994.

that then acts on the folliculostellate (FS) cells to release basic fibroblast factor (bFGF) that feeds back to induce lactotroph proliferation.

9. The signalling mechanisms that activate release are also responsible for stimulating longer-term responses such as an increase in the expression of prolactin (PRL) and also the proliferation of lactotrophs. Just how these responses are controlled is still being worked out but there are indications that there may be a role for the periodic increases in Ca^{2+} that are generated by the membrane oscillator.

Galanin

Galanin is a peptide that functions as an autocrine regulator of prolactin (PRL) gene expression and may also control lactotroph proliferation (Module 10: Figure lactotroph regulation). Oestradiol controls the production and release of galanin that thus fluctuates during the oestrous cycle.

Galanin released together with GABA from the ventrolateral preoptic (VLPO) neurons is one of the main sleep-inducing systems (Module 10: Figure sleep/wake cycle regulation).

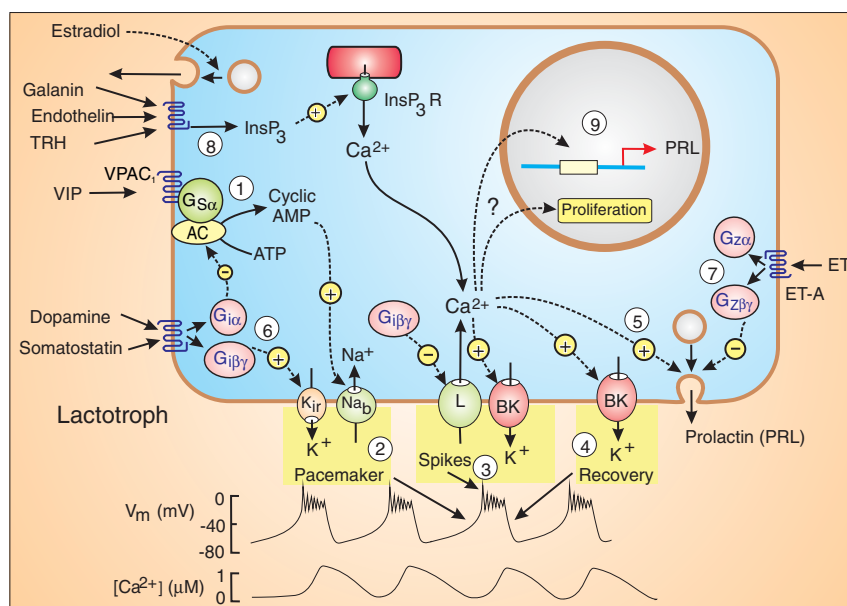
Somatotrophs

The somatotrophs, which make up approximately 50% of the anterior pituitary, are the major endocrine cell type in the anterior pituitary. They respond to growth hormone-releasing hormone (GHRH) by releasing growth hormone (GH) (Module 10: Figure hypothalamic pituitary regulation). GH is secreted from the somatotrophs at a frequency of about 1–2 h. In the case of males, these pulses are much larger early at night especially during the phase of slow wave sleep, whereas in females they tend to be

more continuous throughout the day. This pattern of GH release appears to be driven by the rate at which GHRH is released from the hypothalamic neurons (Module 10: Figure hypothalamic pituitary regulation). A number of signalling systems operate to control GH release by the somatotrophs as described in the following section (Module 10: Figure somatotroph regulation):

1. The growth hormone-releasing hormone (GHRH) regulates somatotroph activity either by initiating or by increasing the frequency of a membrane oscillator responsible for the regular bursts in membrane potential (V_m). This oscillator, which is very similar to that found in the lactotrophs, depends on the GHRH-H acting on the GHRH receptor (GHRH-R) to induce the cyclic AMP signalling pathway.
2. Cyclic AMP switches on a membrane oscillator by activating a background Na^{+} conductance (Na_b) that initiates the pacemaker depolarization.
3. The pacemaker depolarization depends on a balance between the activity of outward currents such as those produced by an inward rectifying K^{+} (K_{ir}) channel and the inward depolarizing current conducted by Na_b . Enhancing the latter by cyclic AMP will switch the balance towards membrane depolarization giving rise to the pacemaker, which is an essential feature of the membrane oscillator.
4. This pacemaker depolarization activates one of the Ca_v1 family of L-type channels that open repetitively during the plateau phase of the membrane depolarization to give rise to a series of action potentials (APs). These somatotroph APs resemble those seen in lactotrophs (Module 10: Figure lactotroph regulation) in that they are rather small and do not reach the level of depolarization that will activate the voltage-dependent

Module 10: | Figure lactotroph regulation



Regulation of prolactin (PRL) secretion by lactotrophs.

Lactotrophs have a membrane oscillator that spontaneously generates repetitive slow waves of membrane depolarization (see V_m trace) that is responsible for the periodic bursts of Ca^{2+} that trigger the release of prolactin (PRL). See text for further details.

K^+ channels necessary to fully hyperpolarize the membrane. A close apposition between these L-type Ca^{2+} channels and the large-conductance (BK) channels is responsible for truncating this hyperpolarizing response. The local domain of Ca^{2+} near the mouth of the L-type channel activates the nearby BK channels causing a small hyperpolarization such that the membrane remains sufficiently depolarized to allow the L-type channels to re-open quickly. A rapid succession of such brief APs riding on the crest of the depolarizing wave is responsible for the gradual build up of the intracellular level of Ca^{2+} necessary to induce exocytosis (Module 10: Figure somatotroph regulation).

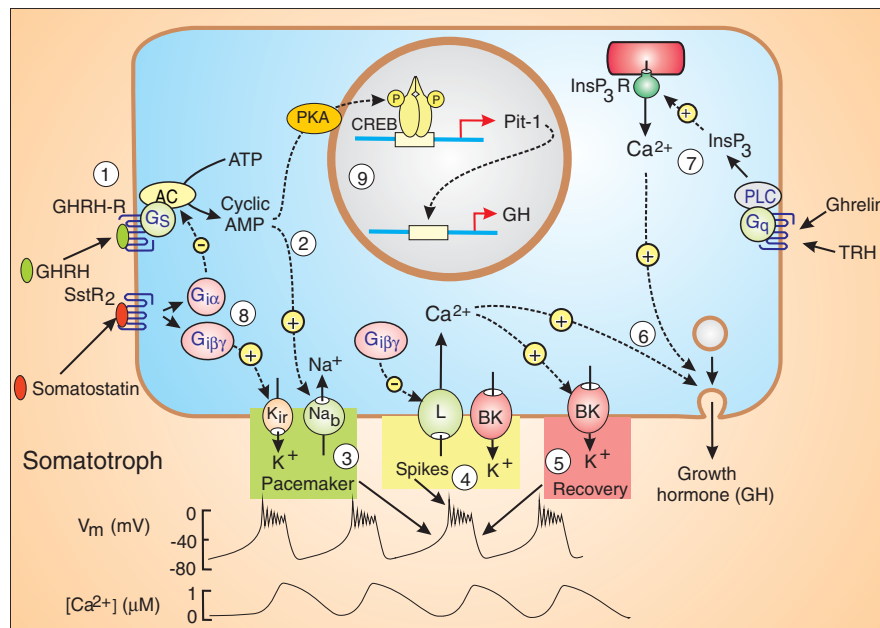
- Termination of each burst depends upon the global elevation of Ca^{2+} reaching a level to activate a sufficient number of the peripheral BK channels to provide the larger hyperpolarization responsible for the recovery phase. As the Ca^{2+} concentration declines, these BK channels will switch off and this will facilitate the depolarizing effect of the Na_b channels to bring about the next pacemaker phase.
- The global elevation of Ca^{2+} that occurs during each burst is then responsible for activating the release of growth hormone (GH). A remarkable feature of this Ca^{2+} signal is that it appears to be coordinated throughout the population of somatotrophs. Despite the fact that these somatotrophs are distributed throughout the anterior pituitary, they are connected to each other through adherens junctions to create a large-scale network. The physical connectivity seems to ensure that large groups of somatotrophs synchronize their Ca^{2+} signals to deliver a co-ordinated pulse of GH.

- Various hormones, such as ghrelin and thyroid stimulating hormone (TSH) can also stimulate secretion. They also act by increasing the intracellular level of Ca^{2+} , but in this case they act through the inositol 1,4,5-trisphosphate ($InsP_3$)/ Ca^{2+} signalling cassette.
- The membrane oscillator is strongly inhibited by somatostatin (Sst) acting through a number of mechanisms. Somatostatin binds to sstR2, which is a G protein-coupled receptor (GPCR), that is coupled to the G protein G_i . The G_i dissociates into the $G_{i\alpha}$ and $G_{i\beta\gamma}$ subunits, both of which act to inhibit the membrane oscillator using different mechanisms. The $G_{i\alpha}$ acts to inhibit adenylyl cyclase thus reducing the level of the cyclic AMP that drives the membrane oscillator. The $G_{i\beta\gamma}$ also has an inhibitory effect because it stimulates K_{ir} and the resulting hyperpolarization reduces the pacemaker depolarization that is necessary to drive the oscillator. In addition, the $G_{i\beta\gamma}$ inhibits the activity of the L-type channel thus reducing the entry of the external Ca^{2+} that drives secretion.
- Transcription of the growth hormone (GH) gene is regulated by the pituitary-specific transcription factor (Pit-1), which controls the development and differentiation of somatotrophs. The transcription factor CREB may increase the expression of Pit-1 that then goes on to regulate the expression of the GH gene.

Thyrotrophs

The thyrotrophs, which make up approximately 5% of the anterior pituitary, release thyroid-stimulating hormone (TSH), which is also known as thyrotropin. (Module 10: Figure thyrotroph regulation). TSH is secreted in pulses

Module 10: | Figure somatotroph regulation



Regulation of growth hormone (GH) secretion by somatotrophs.

Somatotrophs have a membrane oscillator that spontaneously generates repetitive slow waves of membrane depolarization (see V_m trace) that are responsible for the periodic bursts of Ca^{2+} that triggers the release of growth hormone (GH). Growth hormone-releasing hormone (GHRH) acts through the cyclic AMP signalling pathway to control the background Na^+ (Na_b) channel responsible for the pacemaker depolarization. See text for further details.

lasting for 2–3 h with a nocturnal surge before sleep. Release is curtailed when sleep begins. This release of TSH by the thyrotrophs is induced by thyrotropin-releasing hormone (TRH) which is synthesized and released by hypothalamic neurons (Module 10: Figure hypothalamic pituitary regulation). The control of TSH synthesis and secretion is illustrated in Module 10: Figure thyrotroph regulation:

1. Thyrotropin-releasing hormone (TRH) acts through a G protein-coupled receptor (GPCR), which is coupled through the G protein $G_{q/11}$ to phospholipase C (PLC) resulting in the formation of $InsP_3$.
2. $InsP_3$ then acts on $InsP_3$ receptors ($InsP_3Rs$) to release Ca^{2+} from the endoplasmic reticulum (ER).
3. Ca^{2+} released from the endoplasmic reticulum (ER) stimulates the release of thyroid-stimulating hormone (TSH). This is an example of exocytosis triggered by Ca^{2+} release from internal stores (Module 4: Figure Ca^{2+} -dependent exocytosis).
4. The increase in Ca^{2+} also functions to increase the expression of TSH particularly by increasing the transcription of the β -subunit.
5. The secretion of TSH is inhibited by a negative-feedback loop operated by the thyroid hormone triiodothyronine (T_3), which is produced locally through the action of type II deiodinase (Dio2) that converts thyroxine (T_4) into the active 3,5,3'-tri-iodothyronine (T_3). This T_4 into T_3 conversion seems to take place in the folliculostellate (FS) cells. The T_3 enters the thyrotrophs to activate the thyroid hormone receptor (TR). The T_3 -TR complex binds to the T_3 response elements

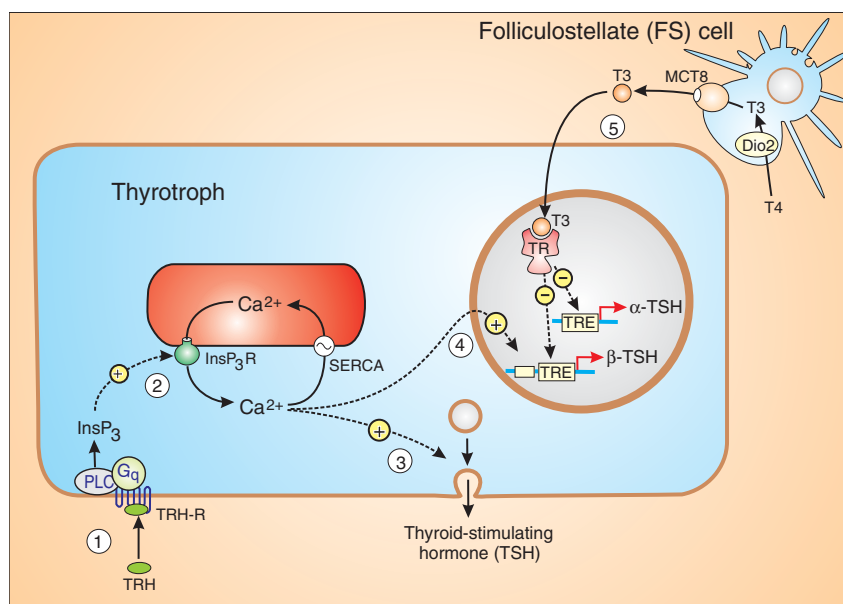
(TRE) on the promoter region of the two TSH genes with the main effect being on the expression of the β -TSH subunit. The monocarboxylate transporter 8 (MCT8) is a specific T_3 transporter that enables T_3 to cross membranes. There are indications that MCT8 may function to enhance the efflux of T_3 after its formation from T_4 in the FS cells. Severe psychomotor retardation has been linked to mutations in the MCT8 gene.

Thyroid-stimulating hormone (TSH)

Thyroid-stimulating hormone (TSH), which is also known as thyrotropin, is released from thyrotrophs (Module 10: Figure thyrotroph regulation). TSH consists of two subunits: an α -subunit (92 amino acids) that is common to some of the other pituitary hormones such as follicle-stimulating hormone (FSH), luteinising hormone (LH) and chorionic gonadotropin (CG) and a specific β -subunit (118 amino acids). Glycosylation of arginine residues on the two substrates is essential for the folding and heterodimerization of the two subunits.

Thyrotropin-releasing hormone (TRH)

Thyrotropin-releasing hormone (TRH), which is also known as thyroid-releasing hormone, is released from hypothalamic neurons and functions to control the release of thyroid-stimulating hormone (TSH) from thyrotrophs (Module 10: Figure thyrotroph regulation). TRH is a tripeptide (Glu-His-Pro) that acts on the TRH receptor (TRH-R) (Module 1: Table G protein-coupled receptors).

Module 10: | Figure thyrotroph regulation**Regulation of TSH secretion by thyrotrophs.**

Release of thyroid-stimulating hormone (TSH) by thyrotropin-releasing hormone (TRH) depends on the activation of phospholipase C (PLC) to produce inositol 1,4,5-trisphosphate (InsP₃). The latter then releases Ca²⁺ from the endoplasmic reticulum to induce the exocytosis of the vesicles containing TSH. Ca²⁺ may also act to switch on the expression of the β-TSH gene. The latter is also under negative control by T₃, which is produced locally from T₄ by an enzyme located on the folliculostellate (FS) cells.

Folliculostellate (FS) cells

Folliculostellate (FS) cells form a three-dimensional interconnected network that surrounds the hormone-secreting endocrine cells of the anterior pituitary (Module 10: Figure hypothalamic pituitary regulation). Some of the FS cells come together to form a 'hub' with a central lumen, hence the 'folliculo-' part of their name. The stellate part of their name is derived from the fact that they have long thin processes that ramify between the endocrine cells and they also make contacts with the basement membrane surrounding the capillaries (Module 10: Figure anterior pituitary structure). The stellate projections of neighbouring FS cells are connected to each other through gap junctions thus forming an interconnected network of cells that extends throughout the anterior pituitary. The precise function of the FS cells is still not clear but they have been implicated in a number of functions related to the support and co-ordination of the hormone-secreting endocrine cells:

- They may initiate a local inflammatory response in that they show many properties of immune cells such as monocytes and dendritic cells. Like these immune cells, the FS respond to lipopolysaccharide (LPS) by releasing pro-inflammatory cytokines such as tumour necrosis factor α (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6).
- The FS cells express receptors for a large number of stimuli such as acetylcholine, vasoactive intestinal peptide (VIP), pituitary adenylyl cyclase-activating peptide (PCAP), angiotensin II, norepinephrine, adenosine and thyroid stimulating hormone (TSH). Some of these are

released from the endocrine cells suggesting that the two cell types are in close communication and this might account for the intercellular Ca²⁺ waves that have been described in the FS cells (see below).

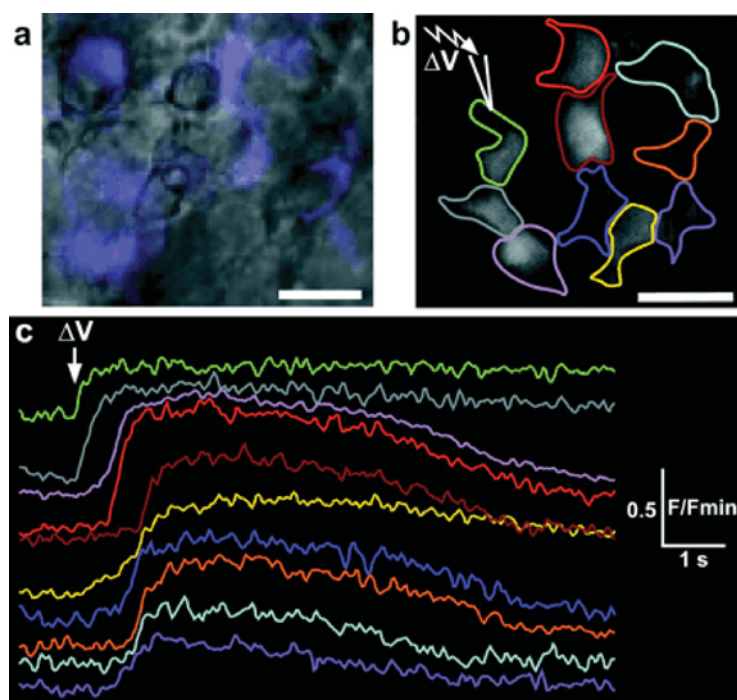
- The FS cells are excitable in that they display rapid membrane depolarizations resembling action potentials that result in brief pulses of Ca²⁺. Such depolarizations can spawn an intercellular Ca²⁺ wave that spread throughout the cellular network travelling at a speed of 120 μm/s (Module 10: Figure FS Ca²⁺ wave). These waves may thus provide a long-range communication system to co-ordinate the activity of the endocrine cells.
- The type II deiodinase (Dio2) that converts T₄ into T₃ is located on the surface of FS cells. This role in providing T₃ is particularly important for the control of thyrotrophs (see step 5 in Module 10: Figure thyrotroph regulation).
- The FS cells may play a role in lactotroph proliferation. When lactotrophs are stimulated with oestradiol, they release TGF-β₃ that then acts on the FS cells to release basic fibroblast factor (bFGF) that feeds back on to the lactotrophs to induce proliferation.

Pineal gland

The pineal gland contains pinealocytes that synthesize and secrete the hormone melatonin, which has an important role in promoting sleep.

Melatonin

Melatonin (*N*-acetyl-5-methoxytryptamine), which is the hormone that it released from the pineal gland, has a number of functions. It can promote sleep by acting on the

Module 10: | Figure FS Ca^{2+} wave**Folliculostellate (FS) intercellular Ca^{2+} wave.**

The top two panels show the location of the FS cells. The coloured outlines (b) indicate the FS cells (shown in panel a) that have been labelled with a fluorescent Ca^{2+} indicator used to measure changes in intracellular Ca^{2+} . The bottom panel (c) illustrates how a depolarizing pulse (ΔV) delivered to the green cell initiates an immediate increase in the level of Ca^{2+} that then spread progressively as a wave to the other cells. Reproduced from Fauquier, T., Guérineau, N.C., McKinney, R.A., Bauer, K. and Mollard, P. (2001) Folliculostellate cell network: a route for long distance communication in the anterior pituitary. *Proc. Natl. Acad. Sci. U.S.A.* 98:8891–8896. Copyright (2001), with permission from the National Academy of Sciences; Fauquier et al. (2001).

suprachiasmatic nucleus (SCN) to reduce the firing rate of the SCN neurons.

Melatonin can also protect the mitochondria against lipid peroxidation particularly the lipid cardiolipin CL, which provides a scaffolding role to support the many membrane components that participate in oxidative phosphorylation (Module 5: Figure cardiolipin). Melatonin is small enough to insinuate itself into the lipid bilayer where it can inactivate oxidants such as the hydroxyl radical ($\text{OH}\cdot$) and peroxynitrite (ONOO^-).

The levels of melatonin in the plasma decline with age and this could provide a link between mitochondrial dysfunction and ageing.

Sensory systems

Signalling mechanisms play a central role in the operation of sensory systems, where they function in the process of signal transduction and adaptation. The following examples illustrate different ways in which cell signalling pathways are employed in sensory perception:

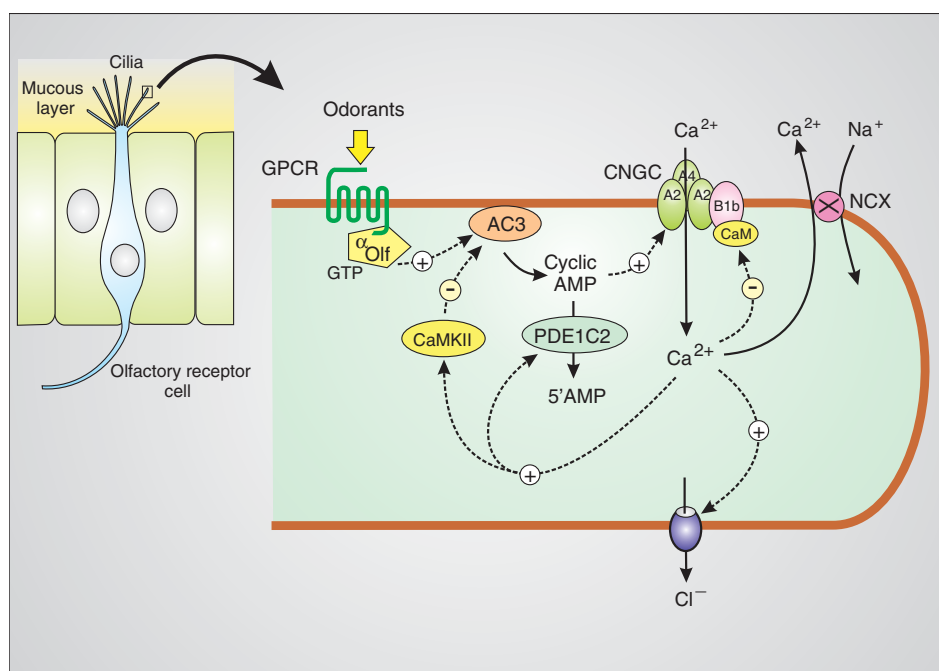
- Hearing
- Hypoxia-sensing mechanisms
- Itch
- Olfaction
- Osmoreception
- Photoreception

- Nociception
- Temperature sensing
- Taste
- Touch

Olfaction

The olfactory receptor cell is responsible for detecting odorants in the air passing over the olfactory epithelium in the nasal cavity. These odorants bind to receptors located on the fine cilia that extend out from the apical surface into the mucous layer (Module 10: Figure olfaction). The process of sensory transduction depends upon the cyclic AMP signalling pathway. The process begins by binding to one of the large population of odorant G protein-coupled receptors (GPCRs) to the heteromeric G protein G_{olf} , which is specific for these olfactory receptor cells. The α_{olf} subunit then activates the type 3 adenylyl cyclase (AC3) to generate cyclic AMP. The latter then acts on the cyclic nucleotide-gated channel (CNGC), which is a tetramer composed of channel subunits (CGNA2 and CGNA4) together with a single modulatory CNGB1b subunit (Module 3: Figure cyclic nucleotide-gated channels). The channel subunits have a cyclic nucleotide-binding domain (CNBD) that detects the increase in cyclic AMP, resulting in the opening of the channel. The channels are particularly permeable to Ca^{2+} , resulting in a rapid increase in the concentration of Ca^{2+} .

Module 10: | Figure olfaction



Signal transduction mechanism of olfactory neurons responding to odorants.

The olfactory receptor cell is embedded in the olfactory epithelium, with its long thin apical cilia extending out into the mucous layer. Olfactory transduction occurs within these cilia, as outlined in the text.

within the narrow confines of the cilia. This increase in Ca^{2+} within the cilia has been observed in Ca^{2+} imaging experiments (Module 10: Figure Ca^{2+} signals in olfactory cilia). There were also increases in Ca^{2+} in other parts of the olfactory cells, but these responses always followed the early rise in ciliary Ca^{2+} .

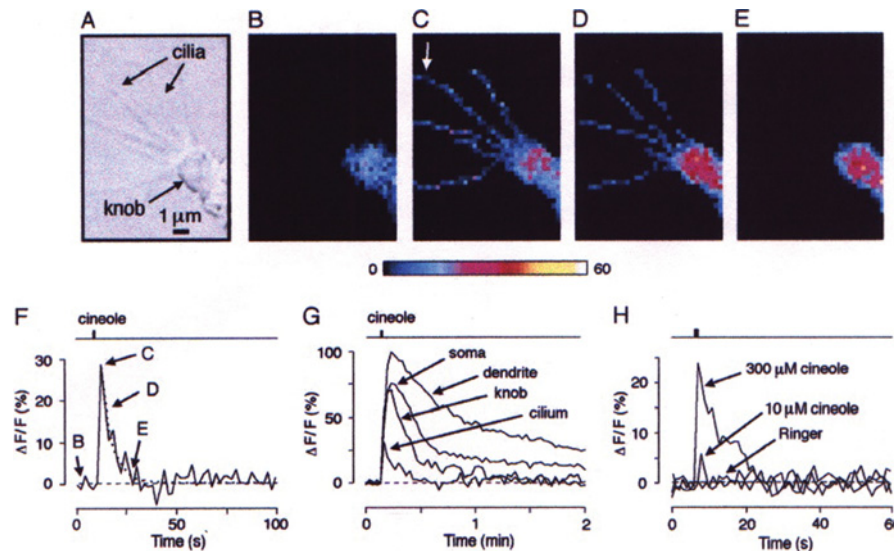
The increase in Ca^{2+} within the cilia has two important functions (Module 10: Figure olfaction). Firstly, the influx of Ca^{2+} is a key component of the transduction process because the inward Ca^{2+} current depolarizes the membrane and thus converts the odorant stimulus into the electrical signal that is sent off to the brain. The membrane depolarization provided by the CNGC is amplified by the Ca^{2+} -sensitive Cl^- channels (CLCAs). The olfactory receptor cell is unusual in having a high concentration of Cl^- ions, which means that their Cl^- equilibrium potential is more positive than the resting membrane potential. The increase in the level of Ca^{2+} opens these Cl^- channels to depolarize the membrane further, thus amplifying the depolarization initiated by the cyclic AMP-dependent opening of the CNGC.

The second important function of the Ca^{2+} that enters the cilia is to activate a process of adaptation. Olfactory receptor cells adapt quickly to a maintained level of an odorant. The increase in Ca^{2+} switches off the transduction processes at two levels. Firstly, there is a negative feedback of Ca^{2+} on the CNGC. The modulatory CNGB1b subunit has calmodulin (CaM) bound to the N-terminal region (Module 3: Figure cyclic nucleotide-gated channels). When Ca^{2+} binds to this resident CaM, the channel is rapidly inactivated. Secondly, the increase

in Ca^{2+} also feeds back to switch off the generation of cyclic AMP by inhibiting its formation and by accelerating its degradation (Module 10: Figure olfaction). The type 3 adenylyl cyclase (AC3) (Module 2: Table adenylyl cyclases) is inactivated following its phosphorylation by the Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII). In addition, Ca^{2+} acts through CaM to activate the phosphodiesterase PDE1C2, which is a splice variant expressed in these olfactory receptor cells. The combined action of Ca^{2+} to inhibit the AC3 while promoting the activity of PDE1C2 severely curtails the cyclic AMP signalling pathway to switch off the transduction process. Once transduction ceases, the Ca^{2+} signal is removed through the operation of a $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX).

Osmoreception

The osmotic pressure of the blood is kept constant at about $280 \text{ mosmol} \cdot \text{kg}^{-1}$ by balancing water intake and excretion. These two processes are regulated by the CNS control of thirst and the release of vasopressin to control water excretion. These different control mechanisms depend on osmosensory neurons capable of responding to osmotic stimuli. Most of these osmosensitive neurons, which respond to small changes in fluid osmolarity, are located in three main regions: the organum vasculosum of the lamina terminalis (OVLT), the subfornical organ (SFO) and the median preoptic nucleus (MnOP). The cell bodies of the OVLT and SFO neurons lie outside the blood-brain barrier and are thus ideally suited to monitor the osmotic pressure of the blood. The neurons send both glutamatergic and GABAergic projections to the

Module 10: | Figure Ca^{2+} signals in olfactory cilia

Olfactory cilia respond to odorants by generating rapid Ca^{2+} transients.

Olfactory receptor cells have fine cilia that extend out from the knob (A). Under resting conditions, there is no detectable signal in these fine cilia (B), but 2 s after adding the odorant cineole, there was a rapid increase within the cilia (C) that was still present 4 s later (D), but which had declined back to the resting level by 16 s (E). The time course for this response, which was taken from the cilium marked by a white arrow in C, is shown in F. G shows that the odorant also caused increases in Ca^{2+} in other cellular compartments. H shows that the amplitude of the Ca^{2+} transient was related to the odorant concentration. Reproduced, with permission, from Leinders-Zufall, T., Greer, C.A., Shepherd, G.M. and Zufall, F. (1998) Imaging odor-induced calcium transients in single olfactory cilia: specificity of activation and role in transduction. *J. Neurosci.* 18:5630–5639. Copyright (1998) by the Society for Neuroscience; see Leinders-Zufall et al. 1998.

supraoptic nuclei (SON) and paraventricular nuclei (PVN) in the hypothalamus where they innervate the vasopressin neurons (Module 10: Figure vasopressin neuron). There are indications that members of the vanilloid transient receptor potential (TRPV) family such as TRPV4 may function in osmosensing by providing the changes in membrane potential that alters the firing rates of the excitatory and inhibitory osmosensitive neurons.

Photoreception

The eye contains two types of photoreceptors, the rods and the cones, which are specialized to detect light. The rod and cone structure reveals an enormous amplification of the membranes containing the proteins responsible for light detection and phototransduction. There is a central role for cyclic GMP in phototransduction, during which light triggers a signalling cascade that uses the second messenger cyclic GMP to regulate the opening of cyclic nucleotide-gated channels (CNGCs) responsible for the change in electrical activity that sends a message to the brain.

Rod and cone structure

Rods and cones are ciliary photoreceptors. Their overall structure is fairly similar. They have an outer segment, which is part of a modified cilium where the outer body is full of stacked membranes containing the light detectors (rhodopsin) and components of the phototransduction system (Module 10: Figure rod and cone structure). In the case of rods, this amplification is achieved by having internal discs, which are stacked on top of each other. On

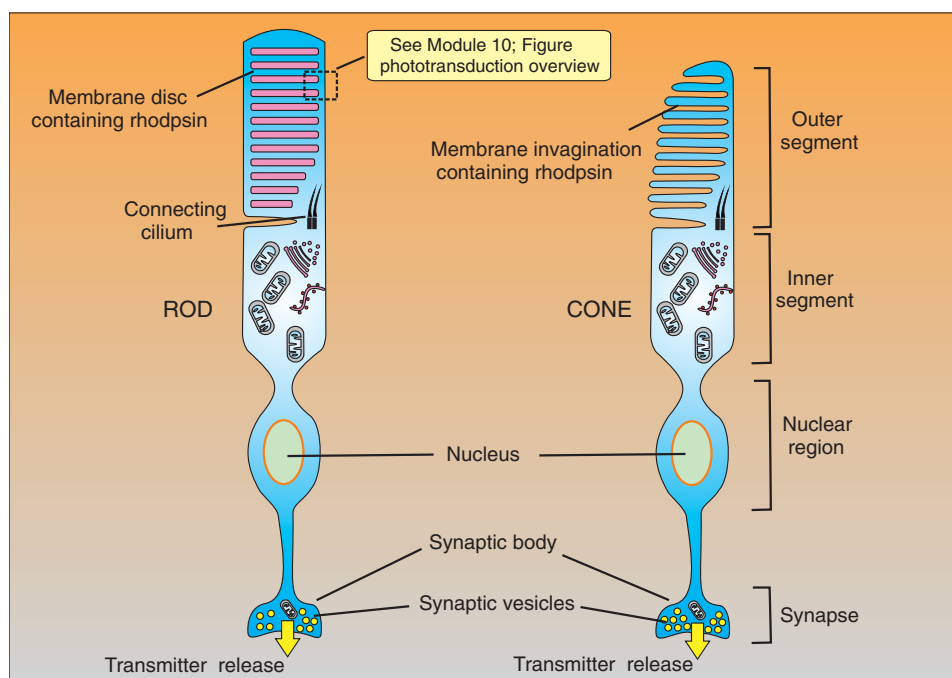
the other hand, the cones multiply up the amount of membrane by having many invaginations (Module 10: Figure ultrastructure of rods and cones).

Phototransduction

There are subtle differences in the way rods and cones detect light. Cones are adapted to function during daylight conditions (photopic vision) in that they are less sensitive to light and can thus operate over a wide range of light intensities. On the other hand, the rods operate at much lower light levels (scotopic vision) and are sufficiently sensitive to detect single photons.

The term phototransduction, which is similar in both rods and cones, refers to the process whereby the absorption of photons is converted into an electrical signal. In the case of photoreceptors, the electrical signal is membrane hyperpolarization, which shuts off the tonic release of transmitter that occurs continuously in the dark. The first point to consider is therefore the nature of the ionic events responsible for the dark current that depolarizes the membrane in the absence of light (Module 10: Figure photoreceptor ionic currents). It is the influx of Na^+ and Ca^{2+} into the outer segment that is responsible for the membrane depolarization that drives transmitter release in the dark. There is a unique role for cyclic GMP in visual transduction, because light reduces this dark current by shutting off the cyclic nucleotide-gated channels (CNGCs) by inducing a rapid hydrolysis of cyclic GMP (Module 10: Figure phototransduction overview).

One of the remarkable features of phototransduction is its enormous dynamic range. Single photons can be

Module 10: | Figure rod and cone structure**The structural organization of rods and cones.**

Rods and cones are specialized neuronal cells designed to detect light and to relay information to the nervous system by releasing transmitters from the synaptic region. The photon-detection region is localized in the outer segment, where there is a large increase in the membranes containing components of visual transduction. In the case of rods, there are large numbers of membrane discs that are stacked on top of each other. Membrane amplification in the cones is achieved by invaginations of the plasma membrane. Cellular organelles such as the mitochondria, endoplasmic reticulum (ER) and Golgi are packed into the inner segment, and this may ensure that they do not interfere with the optics of the outer segment. Rhodopsin located on the membranes in the outer segment absorbs light, which triggers a phototransduction cascade that decreases the amount of transmitter being released from the synapse at the opposite end of the cell. The box indicates a region of the outer segment of the rod that is magnified in Module 10: Figure phototransduction overview to illustrate the process of visual transduction.

detected in the dark-adapted eye when the photoreceptors are at their most sensitive. However, the photoreceptors can continue to respond to light levels that are 5 logarithmic units higher. To achieve this dynamic range, photoreceptors must balance an enormous capacity to amplify low-amplitude light signals while retaining an ability to detect light signals against a continuous background illumination. To achieve the latter, there is a light-adaptation mechanism that enables them to adjust the sensitivity of the transduction process to variable light intensities.

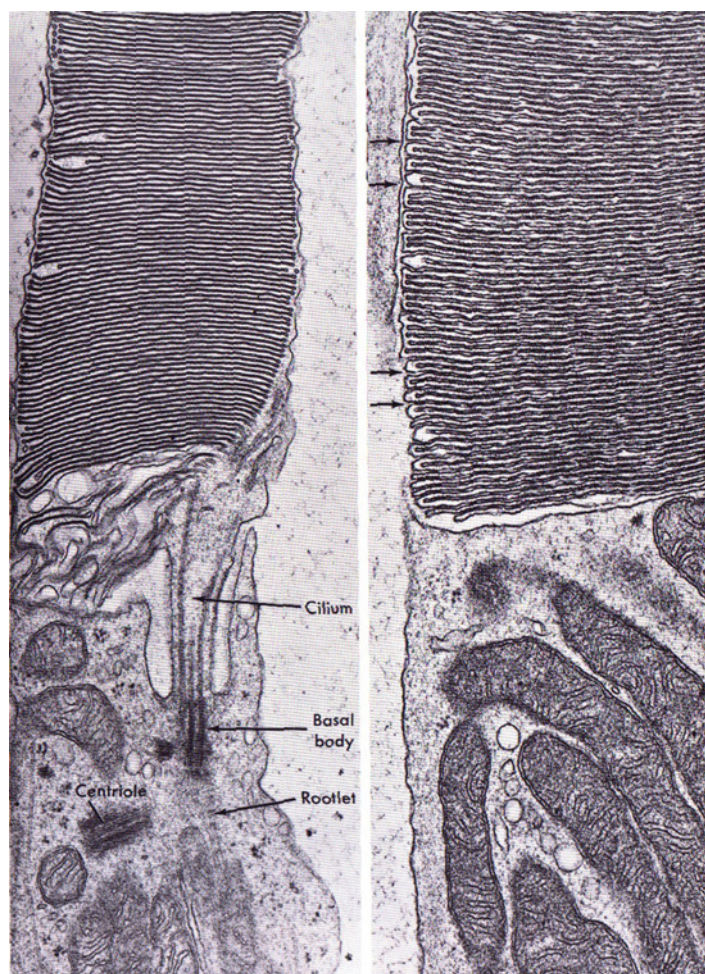
Cyclic GMP in phototransduction

The cyclic GMP signalling pathway is the light-sensitive signalling system that is used to transduce a light signal into a change in membrane potential. The major components of the phototransduction cascade are rhodopsin (Rh), transducin (G_t), phosphodiesterase 6 (PDE6), guanylyl cyclase (GC), cyclic nucleotide-gated channels (CNGCs) and the $\text{Na}^+/\text{Ca}^{2+}\text{-K}^+$ exchanger (NCKX). These components interact with each other during the ON reaction, when the capture of a photon by rhodopsin initiates a sequence of events that ends with the closing of the CNGCs (Module 10: Figure phototransduction overview):

1. The phototransduction process begins when a photon is captured by rhodopsin (Rh), which undergoes conver-

sion into an activated form, Rh^* . The very high density of rhodopsin molecules (3 mM, which translates into 3×10^9 molecules/rod) greatly increases the efficiency of photon capture. Since Rh is highly mobile within the two-dimensional plane of the disc membrane, each Rh^* can make multiple contacts with transducin (G_t).

2. The activated Rh^* interacts with the heterotrimeric protein transducin to induce an exchange reaction, whereby the GDP bound to the $G_t\alpha$ subunit is exchanged for GTP to liberate the active $G_t\alpha/\text{GTP}$ complex that dissociates from the membrane. This is the first step in the amplification process, because each Rh^* activates approximately 150 transducin molecules. Transducin is present in the membrane at a concentration of 0.3 mM, which is about one-tenth that of Rh, and this enables each Rh^* to interact with a large number of transducin molecules during its brief lifespan.
3. The activated $G_t\alpha/\text{GTP}$ complexes then stimulate PDE6, which rapidly hydrolyses cyclic GMP to GMP.
4. Cyclic GMP functions to activate the cyclic nucleotide-gated channel (CNGC), which is responsible for the influx of Na^+ and Ca^{2+} that maintains the dark current. The light-induced reduction in cyclic GMP closes the channel, and this results in the membrane hyperpolarization that inhibits transmitter release. Na^+ carries approximately 85% of the current, while Ca^{2+} carries the remaining 15%.

Module 10: | Figure ultrastructure of rods and cones

Ultrastructural organization of inner segment membrane systems in rods (left) and cones (right).

Membrane amplification in the rods is achieved by stacking flattened discs on top of each other. In the cones, membrane elaboration is achieved by invaginations of the plasma membrane (arrows point to the openings of these invaginations). Note that the outer segment is devoid of organelles that are packed into the lower inner segment, where the mitochondria are particularly prevalent. Reproduced from Bloom and Fawcett 1968; the original figure was supplied by T. Kuwabara.

5. The Ca^{2+} that enters through the CNGC is extruded from the cell by a $\text{Na}^+/\text{Ca}^{2+}\text{-K}^+$ exchanger (NCKX) to set up a Ca^{2+} cycle across the plasma membrane, which plays an important role in the recovery process and may also function in adaptation.
6. One of the processes of visual adaptation depends upon the ability of Ca^{2+} , acting through the guanylyl cyclase-activating protein (GCAP), to regulate the activity of particulate guanylyl cyclase (pGC). When the CNGC is open, the Ca^{2+} concentration is high, and this inhibits pGC. During a light stimulus, the CNGC closes and in the absence of entry, the exchanger is able to reduce the Ca^{2+} concentration, and this stimulates the pGC to increase the concentration of cyclic GMP.

A more complete description of phototransduction has to include the OFF reactions that reverse the transduction process during the recovery of a light response. The ON and OFF reactions of the different signalling components form a series of interacting cycles, as illustrated in Module 10: Figure phototransduction:

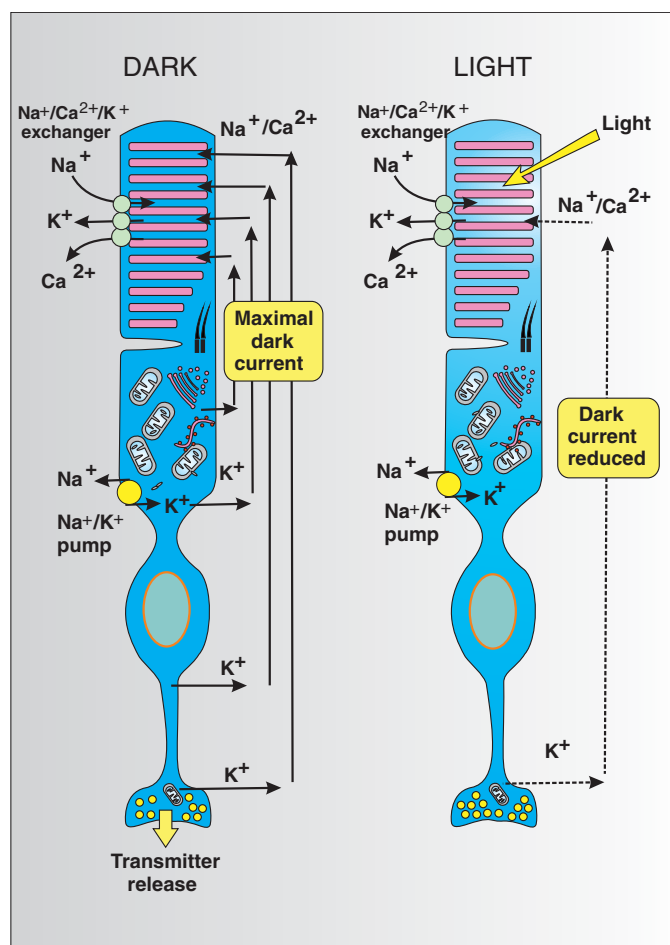
Rhodopsin cycle

1. The chromophore 11-*cis*-retinal, which is covalently attached to rhodopsin (Rh), responds to light by undergoing an isomerization to the all-*trans* form. The latter then functions as an agonist to convert rhodopsin into its active form (Rh^*), which is responsible for triggering the transducin cycle (see below).
2. The inactivation of activated Rh (Rh^*), which is the OFF reaction of the rhodopsin cycle, begins with the phosphorylation of Rh^* by rhodopsin kinase, which is also known as GRK1 and is one of the G protein receptor kinases (GRKs). The neuronal Ca^{2+} sensor protein recoverin is thought to act by inhibiting this kinase.
3. Removal of the phosphate returns Rh to its sensitive state.

Transducin cycle

The transducin cycle represents the first step in the amplification process, since each activated rhodopsin (Rh^*) is

Module 10: | Figure photoreceptor ionic currents



The ionic mechanisms responsible for the dark current in rods.

In the dark, a maximal dark current circulates through the cell. It is driven by the entry of Na^+ and Ca^{2+} through cyclic nucleotide-gated channels (CNGCs) in the outer segment, while charge compensation is achieved by K^+ leaving from the lower segments. The resulting membrane depolarization is responsible for the tonic release of transmitter in the dark. The passive fluxes of Na^+ and K^+ that are mainly responsible for the dark current are reversed by the sodium pump, whereas the $\text{Na}^+/\text{Ca}^{2+}-\text{K}^+$ exchanger removes Ca^{2+} from the cell. Light rapidly reduces this dark current by hydrolysing the cyclic GMP that keeps the CNGCs open in the dark. The reduction in current results in membrane hyperpolarization and a reduction in the release of transmitter.

capable of activating approximately 150 transducin (G_t) molecules.

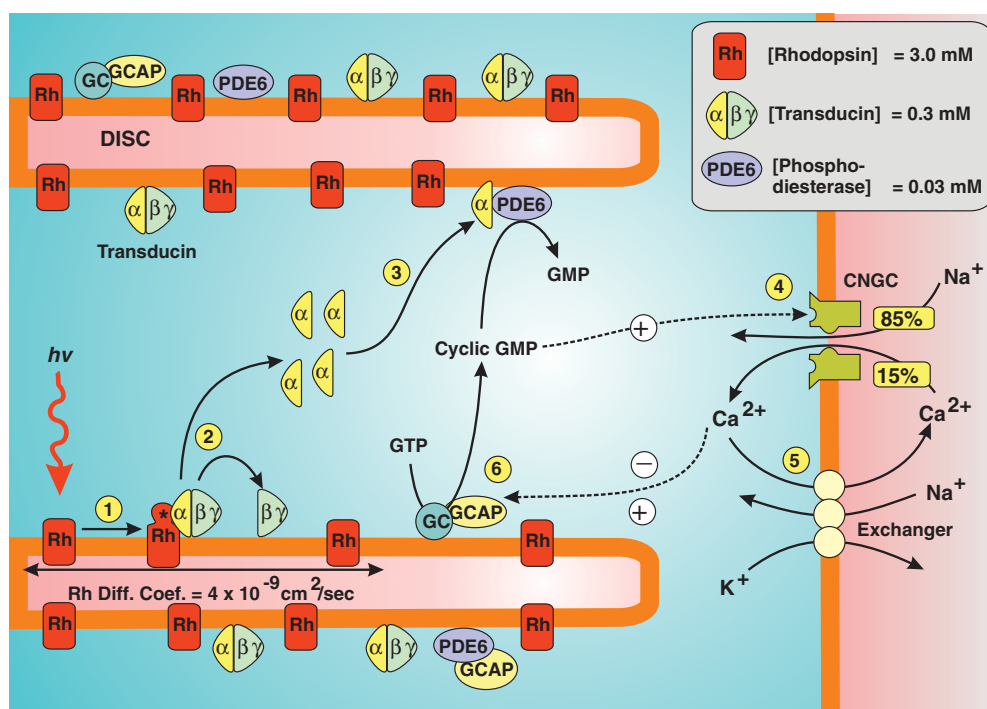
- When G_t/GDP binds to Rh^* , it undergoes a GDP-for-GTP exchange reaction that results in dissociation of the heterotrimeric complex into $G\beta\gamma$ and $G\alpha_t/\text{GTP}$.
- The activated $G\alpha_t/\text{GTP}$ then binds to the γ -subunit of the type 6 phosphodiesterase (PDE6), resulting in a dramatic increase in its enzymatic activity (see below).
- Inactivation of the light response is largely dependent upon the speed with which the activated $G\alpha_t/\text{GTP}/\text{PDE6}$ complex is switched off, and this depends on hydrolysis of GTP to GDP by the GTPase activity of $G\alpha_t$. The intrinsic GTPase activity of $G\alpha_t$ is low, but this activity is greatly enhanced by a regulator of G protein signalling 9 (RGS9), which is a GTPase-activating protein (GAP), acting together with the long splice variant type 5G protein β subunit ($G\beta 5L$). As soon as the GTP is hydrolysed to GDP, the inactive $G\alpha_t/\text{GDP}$ complex dissociates from PDE6.

- The inactive $G\alpha_t/\text{GDP}$ recombines with $G\beta\gamma$ to reconstitute the G_t/GDP heterotrimeric complex that is then able to re-enter the transducin cycle.

PDE6 cycle

- The phosphodiesterase 6 (PDE6) that hydrolyses cyclic GMP is kept inactive by two γ subunits that bind tightly to the α and β catalytic subunits. The latter contain allosteric cyclic GMP-binding GAF domains that bind cyclic GMP and help to keep the enzyme inactive by facilitating the binding of the γ subunits. In order to ensure an appropriate signal-to-noise ratio, the PDE6 is kept as inactive as possible to avoid spontaneous activity through the co-ordinated action of the γ subunits' binding of cyclic GMP.
- The activated $G\alpha_t/\text{GTP}$ overcomes this inhibition by pulling away the inhibitory γ subunits, and the activated phosphodiesterase 6 (PDE6) can then hydrolyse cyclic GMP (Step 5).

Module 10: | Figure phototransduction overview



Organization of the major components of phototransduction.

An important aspect of phototransduction is the way the components of the signalling cascade are organized within the confines of the highly membranous outer segment (see boxed region in Module 10: Figure rod and cone structure). This figure describes the organization found in rods, where much of the transduction takes place on the discs. The organization in the cones is much the same, except that transduction occurs on the invaginations of the plasma membrane. The phototransduction cascade proceeds through a series of discrete steps, as described in the text.

 Ca^{2+} cycles

Ca^{2+} cycles are operating continuously in both the light and the dark. The cycles depend upon Ca^{2+} coming in through the CNGC and being extruded by the $Na^{+}/Ca^{2+}-K^{+}$ exchanger (NCKX). In the dark, the high level of cyclic GMP activates the cyclic nucleotide-gated channel (CNGC), which gates both Na^{+} (85%) and Ca^{2+} (15%). The Ca^{2+} that enters is extruded to set up a Ca^{2+} cycle, which maintains an elevated dark-adapted level of Ca^{2+} .

10. During the light response, when the cyclic GMP falls and the channel closes, there is no Ca^{2+} influx and the continued operation of the exchanger results in a fall in the intracellular level of Ca^{2+} .
11. The reduction in the level of Ca^{2+} that occurs in the light performs an important adaptive function, because it results in the activation of particulate guanylyl cyclases (pGCs) such as pGC-E and pGC-F to restore the cyclic GMP back to the level that will reopen the CNGCs to depolarize the membrane again. This adaptive response resulting from the fall in Ca^{2+} is carried out by the Ca^{2+} -sensitive guanylyl cyclase-activating protein (GCAP).

Hearing

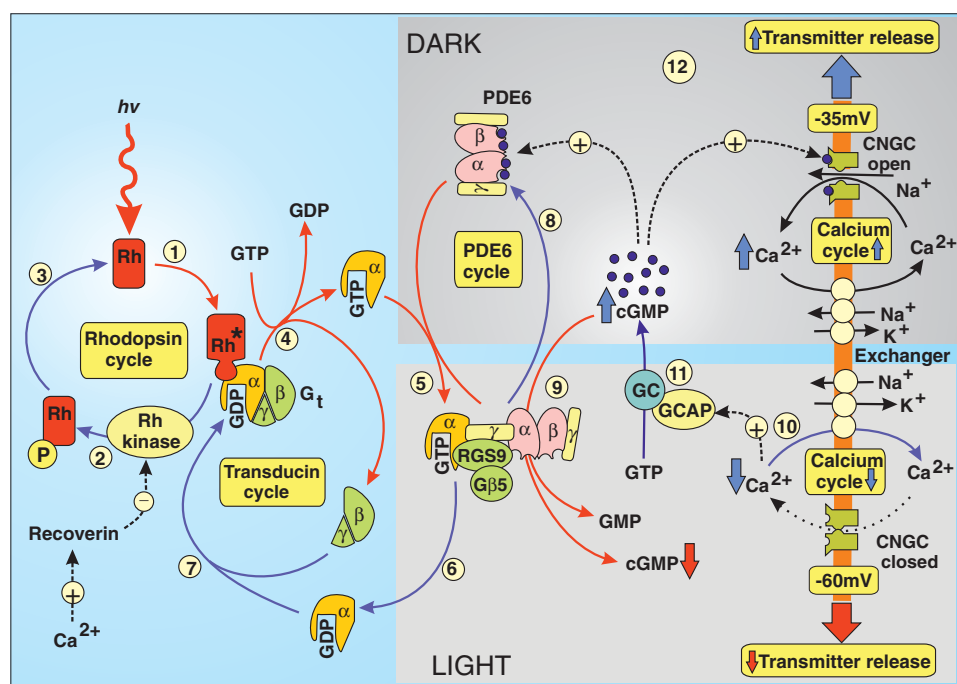
The process of hearing is located in the inner ear, where sound-induced vibrations are converted into electrical signals by hair cells. They possess an apical hair bundle of stereocilia

responsible for hair cell mechanoelectrical transduction, whereby small changes in movement are transduced into the electrical signals responsible for hair cell transmitter release that excites the afferent nerve fibres to transfer acoustic information to the brain. There are different populations of hair cells, which are specialized to carry out separate functions. Inner hair cells are specialized for acoustic detection, whereas the outer hair cells function in hair cell amplification through a process of electromotility. As for many other sensory systems, there is a process of hair cell adaptation that enables the transduction system to operate over a wide range of sound intensities. In addition to detecting sound, the inner ear is also adapted to detect motion by specialized hair cells, such as the vestibular hair cells, located in the semicircular canals.

Inner ear

The inner ear is specialized to detect motion in its fluid-filled channels (Module 10: Figure inner ear). The vestibular and saccular hair cells detect motion of the head, which sets up a flow of fluid in the semicircular canals. Detection of sound occurs in the snail-shaped cochlea, which is about 35 mm long in humans. Airborne sounds set up a vibratory motion in the eardrum, which is then transmitted by the ossicles to the oval window of the fluid-filled cochlea. These vibrations of the oval window then set up a wave that travels down the cochlea and begins to flex the basilar membrane, which causes a shift in the position of

Module 10: | Figure phototransduction



Summary of the ON and OFF reactions of the phototransduction cascade in rods and cones.

Rods and cones have a similar transduction process, which begins with the arrival of a photon (top left) and culminates in membrane hyperpolarization and a decrease in transmitter release following closure of the cyclic nucleotide-gated channel (CNGC) (bottom right). In the dark, there is a high level of cyclic GMP (cyclic GMP), which keeps the CNGC open, thereby depolarizing the membrane to maintain a tonic release of transmitter. The signalling events that occur during a light response can be divided into a series of interacting cycles that contain both ON reactions (red arrows) and OFF reactions (blue arrows), as described in the text.

the tectorial membrane and imparts a shearing force to the stereocilia of the hair cells. The point along the length of the basilar membrane where the amplitude of the wave is maximal is a function of the frequency of the tone (Module 10: Figure cochlear amplifier). High sound frequencies produce a maximum displacement at the base, whereas the lower sound frequencies are recorded at the apical end of the cochlea. The basilar membrane thus functions as a frequency sorter so that each frequency will set up a maximal vibration at a particular point down the length of the cochlea. The vibrations set up by different frequencies then activate the hair cells responsible for reporting that particular sound frequency to the brain.

Hair cells

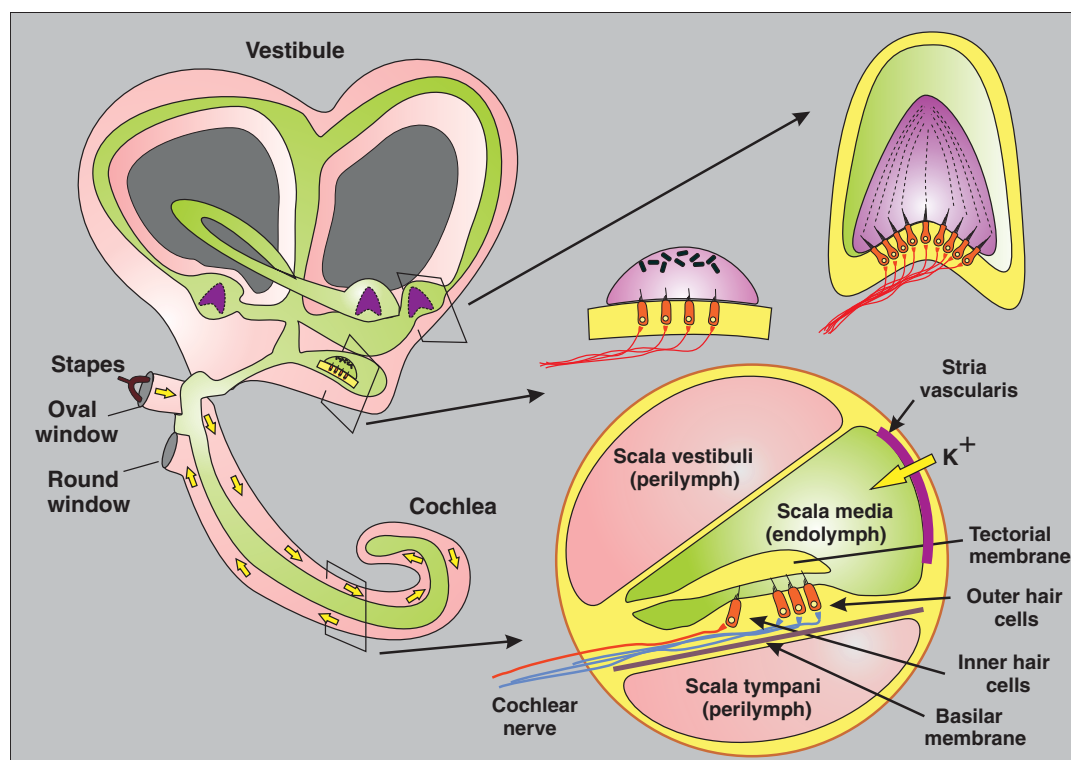
The cochlea contains approximately 16000 hair cells. These are arranged in four uniform rows that run down the length of the cochlea (Module 10: Figure organ of Corti). Hair cells are specialized mechanotransducers capable of transforming minute deformations of their stereocilia into an electrical signal. The two major hair cell types in the ear are the inner and outer hair cells. The hair cells located in the organ of Corti are responsible for hearing. Hair cells are typical epithelial cells with tight junctions in the apical region separating the endolymph (green), which is rich in K^+ , from the perilymph (red) bathing the basal and lateral membranes (Module 10: Figure hair cell). The apical region contains a number of stereocilia that project into the endolymph. The precise V-shaped orientation of hair

cell stereociliary bundles (see panel g in Module 8: Figure PCP in insects and vertebrates) depends on the developmental process of planar cell polarity (PCP). The tips of the tallest stereocilia are embedded in the tectorial membrane. These stereocilia have a large number of actin filaments that extend from their tips and are embedded in the cuticular plate. They also have a number of rigid lateral links and ankle links that tie them all together and ensure that they all move together when responding to a mechanical deformation. The critical components with regard to hair cell mechanoelectrical transduction are the tip links, which are thin fibres that connect the tips of each stereocilium to the lateral side of its tallest partner.

The molecular components of the tip link complex are still being characterized. Some of the components belong to the superfamily of cadherins (Module 6: Figure cadherin superfamily). For example, there are indications that cadherin-23 may be a component of the helical filament that connects the two stereocilia (Module 10: Figure tip link). Isoforms of protocadherin-15 may be responsible both for attaching the main filament to the ends of the two stereocilia and may be part of the lateral links. Mutations in cadherin-23 and protocadherin-15 cause the deaf/blindness Usher syndrome.

The inner hair cells have an extensive afferent connection (red) with a presynaptic active zone on the hair cell that contains the neurotransmitter vesicles that are released during sensory transduction. Hair cells also have an efferent innervation (blue), which is particularly evident in the

Module 10: | Figure inner ear

**Structural organization of the inner ear.**

The vestibule has the semicircular canals that function in movement detection. Flow of fluid in the canals excites vestibular and saccular hair cells. Detection of airborne sounds is carried out by the cochlea, which has three fluid-filled compartments: the scala vestibuli, scala media and scala tympani. The stapes, which is one of the small bony ossicles, vibrates the oval window to set up a wave that travels down the length of the scala vestibuli (yellow arrows) and back through the scala tympani, where pressure is adjusted by vibration of the round window. This travelling wave sets up a vibration in the basilar membrane. The latter supports the organ of Corti, which contains the inner and outer hair cells. The stereocilia that project from the apical surface of the hair cells interact with the tectorial membrane, which is made up of a collagenous gel. The endolymph is rich in K^+ , which is secreted into the scala media by the stria vascularis.

outer hair cells, where neural stimuli activate a cochlea amplification system. The postsynaptic region of this efferent input contains an extensive endoplasmic reticulum that contains the type 1 ryanodine receptor (RYR1). The basolateral membranes of the outer hair cells have a high density of prestin molecules that function as a motor protein to change the shape of these cells during cochlear amplification.

The process of mechanotransduction is localized in the stereocilia that project from the surface of the hair cells (Module 10: Figure hair cell). While both the inner and outer hair cells share a similar hair cell mechanoelectrical transduction mechanism, they differ in how they use the resulting membrane depolarization. In the case of the inner hair cells, which are primarily responsible for hearing, the membrane depolarization results in hair cell transmitter release from presynaptic active zones on the basolateral surface that then activates the afferent fibres of the sensory neurons. By contrast, the outer hair cells use the depolarization to activate a process of electromotility, which is responsible for hair cell amplification.

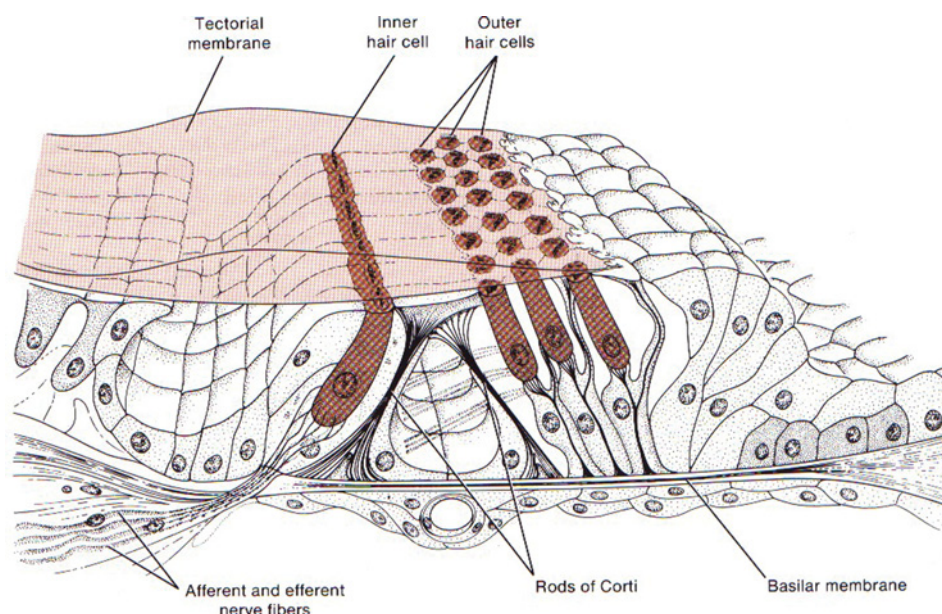
Many of the hair cell functions are carried out by Ca^{2+} signals restricted to localized regions of the cell (marked in yellow in Module 10: Figure hair cell):

1. Mechano-electrical transduction results in pulses of Ca^{2+} that are localized to the tips of the stereocilia (Module 10: Figure stereocilia Ca^{2+} signals).
2. ATP acting through P2Y receptors stimulates the production of inositol 1,4,5-trisphosphate ($InsP_3$) that releases Ca^{2+} from Hensen's body in the apical region of hair cells.
3. The depolarization produced during mechano-electrical transduction acts through L-type channels to produce the localized pulse of Ca^{2+} to trigger the release of transmitter at the presynaptic active zones (Module 10: Figure hair cell presynaptic Ca^{2+} signals).
4. Efferent stimulation releases acetylcholine that acts through nicotinic acetylcholine receptors (nAChRs) to introduce Ca^{2+} that creates a local domain of Ca^{2+} , which may be augmented by a process of Ca^{2+} -induced Ca^{2+} release from the endoplasmic reticulum (ER) lying immediately below the membrane.

Hair cell mechano-electrical transduction

The remarkable aspect of the hair cell mechanotransduction system is its sensitivity. For example, it can respond to vibrations as small as 0.3 nm, which is the diameter of an atom. In effect, the sensory system is operating at the level of thermal noise when it is attempting to detect

Module 10: | Figure organ of Corti



The structural organization of the organ of Corti.

Four orderly rows of hair cells, located in the organ of Corti, run the whole length of the cochlea. There is a single row of inner hair cells and three rows of outer hair cells. When viewed from the surface, the stereocilia of the outer hair cells are arranged in a V-shape, with the tallest stereocilia at the apex. The tectorial membrane that lies over the surface of the hair cells makes contact with the stereocilia. These hair cells are connected to both afferent and efferent nerve fibres. Reproduced from Eckert, R. and Randall, D. (1983) *Animal Physiology: Mechanisms and Adaptation*, with permission from W.H. Freeman and Company; see Eckert and Randall 1983.

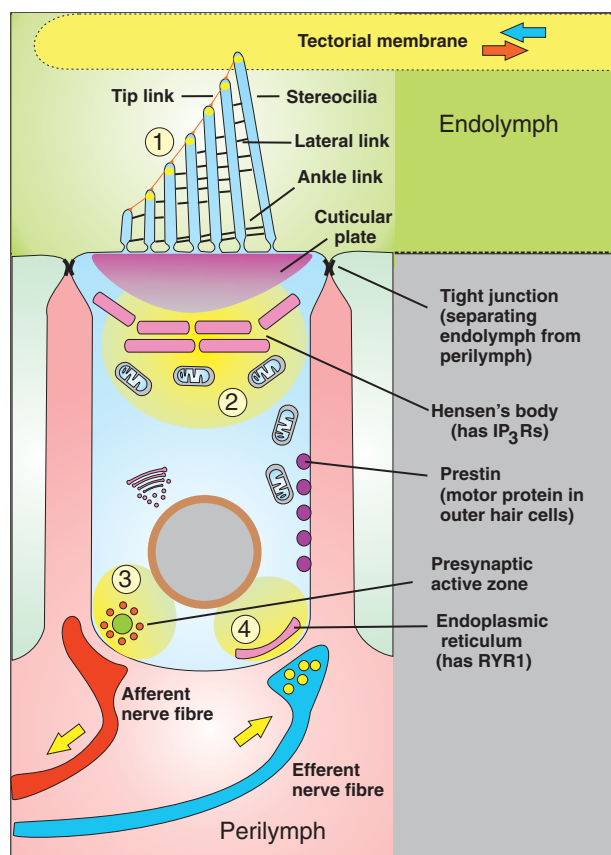
such low-intensity sounds. The operation of the hair cell amplification systems helps to detect sounds at these very low intensities. Another unusual property of the hair cells is their ability to detect sounds at very high frequencies (up to 20 kHz in humans). The transduction system must thus be able to detect vibrations, which are in the atomic range and can occur at very high frequencies. Unlike the other sense organs that employ diffusible second messengers, which are time-consuming, the hair cell employs a multicomponent molecular system that directly converts the energy of a mechanical stimulus into channel opening.

This assembly of molecules appears to act as a mechanical electrical converter capable of transforming small displacements of the stereocilia into an electrical signal. As shown in A in Module 10: Figure tip link, this molecular array begins with anchor filaments in the tip of one stereocilium, and then proceeds through the tip links (cadherin 23), the mechanosensitive channel (possibly TRPA1) with its ankyrin repeats (the gating spring) and a rafting protein (green) that holds together 50–100 myosin 1a molecules, which are attached to actin. The tip links are composed of multiple subunits of cadherin 23 organized into a right-handed helix with a period of 60 nm. The tip link is 10 nm in diameter and approximately 150 nm long. One end of the tip link is attached to anchoring filaments in the tips of the stereocilia, whereas the other end is attached to the channel located in the side of a neighbouring stereocilium. Following mechanical displacement (B), there is an increase in the tension in the rigid tip link, which then pulls on the channel causing it to open to allow K^+ and Ca^{2+} to enter the stereocilium to create the membrane

depolarization responsible for releasing transmitter from the basolateral presynaptic zone. Following closure of the channel, the microdomain of Ca^{2+} that forms in the tip of the stereocilium dissipates rapidly by diffusion and extrusion by the type 2 isoform of the plasma membrane Ca^{2+} ATPase (PMCA) Ca^{2+} pumps that have a density of 2000 PMCA2 molecules/ μm^2 .

A key component of the linear molecular assembly that carries out mechanoelectrical transduction in hair cells is the channel, which opens in response to a mechanical displacement of the stereocilia. The Ca^{2+} that flows in through the channels creates a microdomain localized at the tip of the stereocilium (Region 1 in Module 10: Figure hair cell). These are typical elementary events that can be visualized in line scans of single stereocilia (Module 10: Figure stereocilia Ca^{2+} signals). This microdomain seems to be responsible for closing the channel, which, like many other Ca^{2+} channels, is inactivated by Ca^{2+} . Once the channel closes, the microdomain rapidly dissipates by diffusion down the length of the stereocilium, where numerous PMCA2 pumps return Ca^{2+} to the surrounding endolymph.

The nature of the mechanosensitive channel is still uncertain. A likely possibility is the TRPA1 channel, which has all the properties necessary to carry out this role. Like some of the other transient receptor potential (TRP) channels (Module 3: Figure TRP channel family), these TRPA1 channels have ankyrin repeats in their N-terminal regions, which function as a gating spring. This molecular spring has an important function in maintaining a specific tension on the TRPA1 channel such that its open probability (P_o)

Module 10: | Figure hair cell**Structure of a hair cell.**

This is a generic structure incorporating features of both the inner and outer hair cells. The yellow regions marked 1–4 represent microdomains of Ca^{2+} that carry out different functions during hair cell mechanoelectrical transduction. See the text for details.

is maintained at 0.1 when the channel is at rest. This resting tension on the channel is of particular significance with regard to hair cell adaptation. As the channel opens, K^+ and Ca^{2+} flow into the stereocilia to create the membrane depolarization that triggers hair cell transmitter release from the presynaptic active zone (see Region 3 in Module 10: Figure hair cell).

Mutation of the PMCA2 isoform that is responsible for extruding Ca^{2+} from the hair cells results in hearing loss.

Hair cell transmitter release

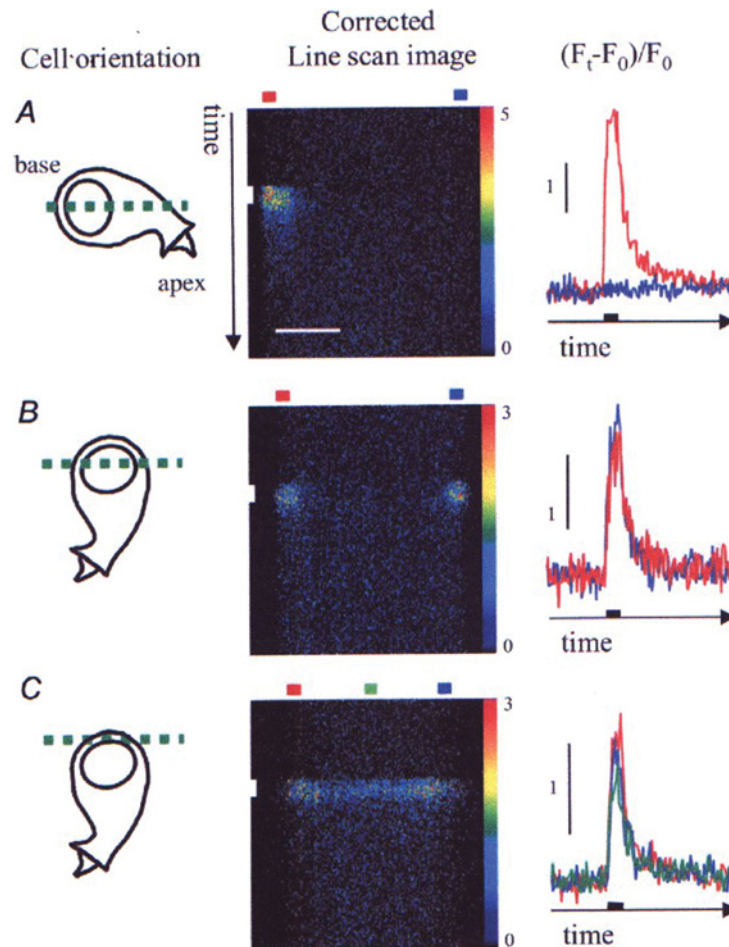
The inner hair cell that is primarily responsible for acoustic detection has a rich afferent innervation. The membrane depolarization that occurs during mechanoelectrical transduction depolarizes the hair cell to trigger transmitter release from the presynaptic active zones (see Region 3 in Module 10: Figure hair cell). There is a tonic release of transmitter from these active zones that can be adjusted either up or down, depending on the direction of movement of the hair cell bundle. Exocytosis is triggered by L-type channels that create a local domain of Ca^{2+} that is restricted to the basal region of the cell, where the afferent dendrites are located (Module 10: Figure hair cell presynaptic Ca^{2+} signals). The Ca^{2+} sensor

responsible for triggering this release appear to be otoferin, which is embedded in the membrane and has three cytoplasmic C2 domains that are responsible for responding to the local elevation of Ca^{2+} to trigger transmitter release.

Hair cell adaptation

Like many other sensory cells, the hair cell displays a process of adaptation, which is still somewhat of a mystery. Adaptation enables the transduction system to operate over a range of sound intensities. The question is therefore how the system avoids being saturated at a particular sound intensity so that it can respond to additional inputs. The gating spring hypothesis suggests a mechanism whereby the sensitivity of the transducing mechanism can be adjusted over a range of intensities, as illustrated in Module 10: Figure hair cell adaptation:

- In the absence of any mechanical displacement, a small tension is applied to the transducing system by the myosin 1a motors that attempt to climb up the actin filament (direction of the yellow arrow). These motors pull on the ankyrin spring that then applies a weak force to the TRPA1 channels such that their open probability (P_o) is held at a value of 0.1, which is the most sensitive point because it lies close to the steep part of the displacement–response relationship (i.e. at point A on the blue curve in the top graph).
- In response to mechanical displacement of the cadherin 23 tip link, force is applied to the channel, P_o increases and the channel opens to allow K^+ and Ca^{2+} to enter as part of the transduction step. The system moves to position B on the activation curve. The microdomain of increased Ca^{2+} that develops around the mouth of the TRPA1 channel is then responsible for inducing two possible types of adaptation. One form of adaptation, which can take place very quickly, depends upon a rapid Ca^{2+} -dependent inactivation of the channels. The other form of adaptation is slower, and depends upon the transducing system resetting itself to a new equilibrium position (i.e. position C on the orange curve).
- In order to adjust to a new equilibrium position, the myosin motor must detach from the actin through a process that may depend upon the increase in Ca^{2+} (as shown in B). Once it is detached, the ankyrin spring pulls the motor back down the actin to a new position, where it once again can attach to actin when the Ca^{2+} signal declines (one of the actin monomers is shown in white to show this downward displacement of myosin). When the Ca^{2+} microdomain dissipates, the myosin motor can reattach and can begin to move upwards once again, extending the ankyrin spring to restore the tension such that the same P_o value of 0.1 is re-established on the displacement–response curve (position C on the orange curve), even though this is now displaced to the right. Through this adaptation process, the transducing system can maintain its sensitivity over a very wide range of signal intensities.

Module 10: | Figure hair cell presynaptic Ca^{2+} signals

Depolarization-induced Ca^{2+} transient localized to the basal region of an inner hair cell.

The panels on the left illustrate the location of the line scans (dashed green lines) that are shown in the middle panel. The white bar on the line scan represents the time when the cell was depolarized. The coloured bars at the top represent the bands that were used to construct the time course (red and blue traces on the right) of the Ca^{2+} signals in the different cellular locations. Reproduced from Kennedy, H.J. and Meech, R.W. (2002) Fast Ca^{2+} signals at mouse inner hair cell synapse: a role for Ca^{2+} -induced Ca^{2+} release. *J. Physiol.* 539:15–23, with permission from Blackwell Publishing; see Kennedy and Meech 2002.

Hair cell amplification

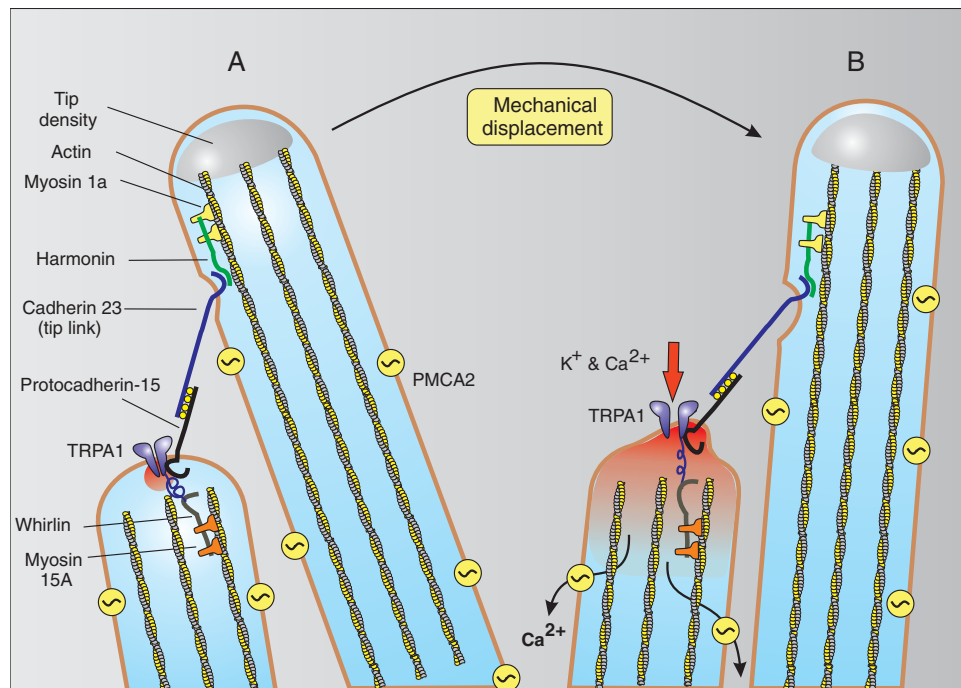
Hair cells in the cochlea are noted for their extreme sensitivity: they are able to detect very small vibrations equivalent to the diameter of an atom that are very close to those induced by thermal noise. In order to detect such low-intensity signals, the outer hair cells have developed a unique cochlea amplification system. There is thus a division of labour in that the outer hair cells amplify the acoustic signal for the inner hair cells to detect. The active amplification system is frequency-specific, which means that each region of the cochlea has hair cells that participate in both the amplification and the detection of specific frequencies (Module 10: Figure cochlear amplifier).

The outer hair cells use a fast positive-feedback process based on electromotility to enhance the incoming stimulus. The nature of this amplification system is still a matter of debate. Two motile mechanisms have been detected, somatic electromotility and hair bundle motility.

Somatic electromotility

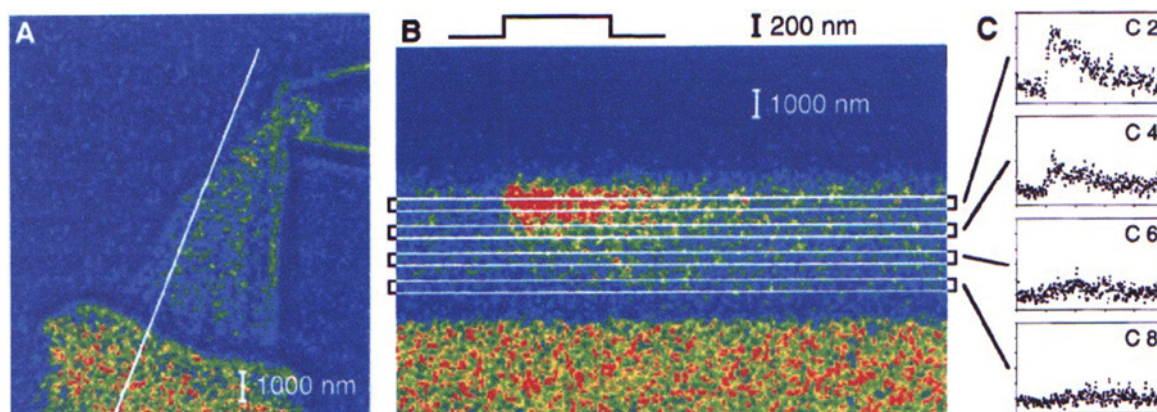
Somatic electromotility is driven by changes in membrane potential that arise from two mechanisms. Firstly, the outer hair cells, just like the inner hair cells, use their stereocilia to detect movement of the tectorial membrane to open the TRPA1 channels to produce the membrane depolarization that drives contraction of these cells. As its name implies, somatic motility involves a large-scale movement of the outer cell body, which is activated by changes in membrane potential: depolarization causes the cell to shorten, whereas hyperpolarization causes it to elongate. The motile mechanism is somewhat unusual in that it operates at very high frequencies (20 kHz), and it is based on the motor protein prestin located on the lateral plasma membrane in outer hair cells (Module 10: Figure hair cell). Prestin molecules are 10 nm in diameter, and are closely packed on the membrane at a density in excess of 4000/ μm^2 . These molecules contract and expand

Module 10: | Figure tip link



Hair cell tip links and mechanoelectrical transduction.

A gating spring hypothesis proposes that the minute displacements of the stereocilia induced by sound are converted into an electrical signal by a linear array of molecules that act to open the TRPA1 channels. The resting condition is shown in A, where the ankyrin spring maintains a small tension on the channel to allow some Ca^{2+} to enter to create a small microdomain of Ca^{2+} (shown in red). During deformation, the lower stereocilium uses the tip link to pull the channel open to create a large inward current carried by K^+ and Ca^{2+} . The latter forms a much larger microdomain that can be visualized in line scan recordings (Module 10: Figure stereocilia Ca^{2+} signals).

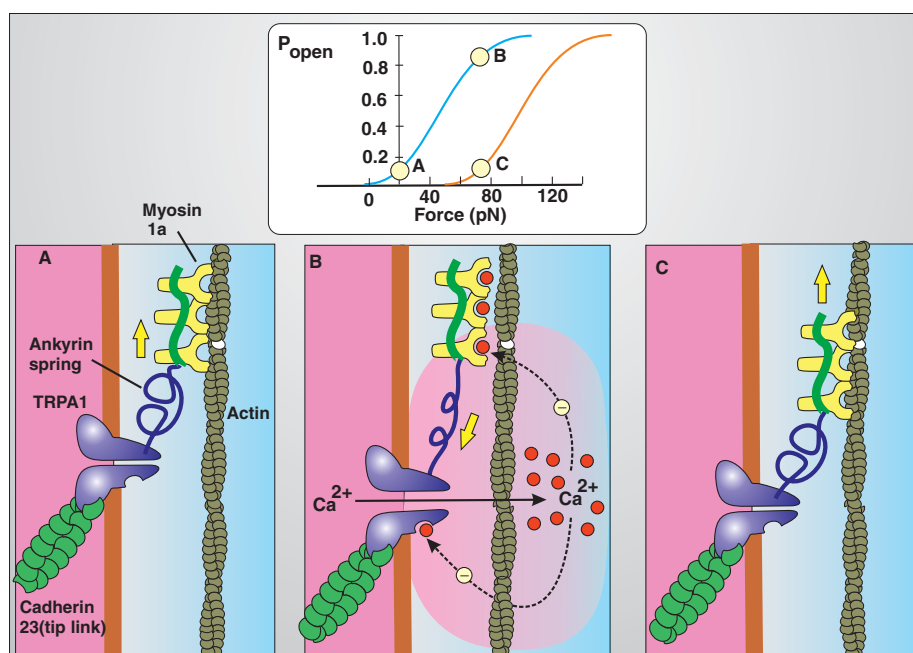
Module 10: | Figure stereocilia Ca^{2+} signalsVisualization of the highly localized Ca^{2+} transient in a single stereocilium following mechanical deformation.

The diagonal white line in A marks the region that was scanned to create the line-scan image shown in B that ran for 500 ms (i.e. along the abscissa). Note the rapid increase in Ca^{2+} that began as soon as the tip was displaced by 200 nm for a period of 100 ms (time register at the top). The panels in C illustrate the time course of Ca^{2+} transients recorded at different points along the length of the stereocilium. It is clear that the microdomain was localized to the tip of the stereocilium (panels C2 and C4), and declined rapidly down the length of the stereocilium. Reproduced, with permission, from Lumpkin, E.A. and Hudspeth, A.J. (1998) Regulation of free Ca^{2+} concentration in hair-cell stereocilia. *J. Neurosci.* 18:6300–6318. Copyright (1998) by the Society for Neuroscience; see Lumpkin and Hudspeth 1998.

in response to changes in membrane potential, and, when acting collectively, they can alter the length of the cell very rapidly. This motility is then imparted to the basilar membrane to amplify the small vibrations set up by the incoming sounds.

The second way in which the membrane potential in outer hair cells can be modified is through the efferent innervation. The main efferent input to the cochlea terminates on the outer hair cells, where it releases acetylcholine that then acts through

Module 10: | Figure hair cell adaptation



A hair cell adaptation hypothesis based on the mechanical properties of the transducing mechanism.

The three panels at the bottom depict how the transducing system might adjust during adaptation, as described in the text.

nicotinic acetylcholine receptors (nAChRs). Hair cells have the heteromeric $\alpha 9\alpha 10$ form of the nAChR, which gates Ca^{2+} that then activates the small-conductance (SK) channels to produce a rapid membrane hyperpolarization. In addition, there is a slower response that appears to depend upon the release of Ca^{2+} from the internal store located in the endoplasmic reticulum (ER) that lies immediately below the synapse (Module 10: Figure hair cell postsynaptic structure). This ER has type 1 ryanodine receptors (RYR1s), which are thought to amplify the Ca^{2+} signal coming in from the nAChRs through a process of Ca^{2+} -induced Ca^{2+} release (CICR), to create a microdomain of Ca^{2+} in the postsynaptic region of the hair cell (region 4 in Module 10: Figure hair cell).

This efferent signalling system appears to control the frequency selectivity and sensitivity of sound perception. It may act to reduce the operation of the feedback force and thus the gain of the cochlear amplifier. In this way, it may protect the ear against overstimulation.

Hair bundle motility

The other motile mechanism that seems to operate in cochlear amplification is driven by hair bundle motility, and is closely integrated with the mechanisms of hair cell adaptation. There appear to be two mechanisms. One is a very fast mechanism that can operate in microseconds that derives its energy from the Ca^{2+} -dependent closure of the transducing channel. This mechanism is fast enough to account for the very high frequencies necessary for amplification. The other is a slower mechanism that may depend upon the operation of the myosin motor, and may play a role in adjusting the position of the hair bundle so as to maximize the function of the fast amplifier.

Nociception

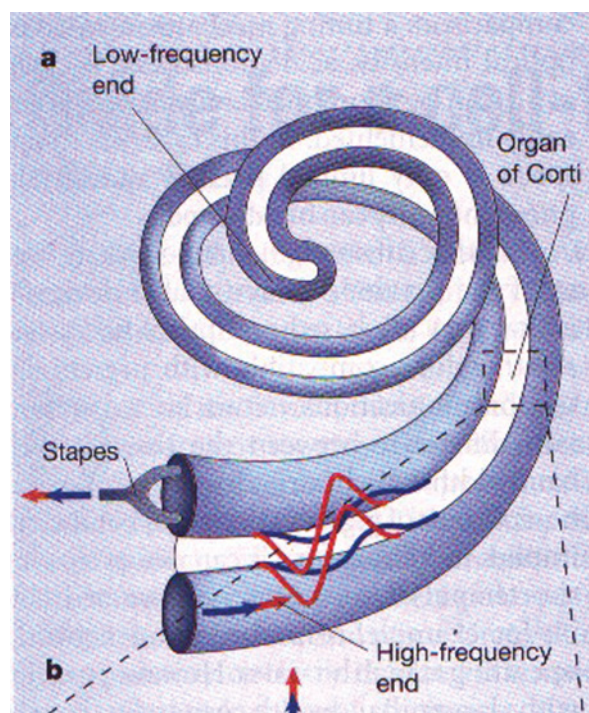
The process of nociception concerns the way noxious stimuli, such as thermal (intense heat or cold), mechanical or chemical stimuli are detected by specialized sensory neurons. During a process of pain perception, noxious stimuli are detected by the peripheral endings of these neurons that then send information to the brain, where it is registered as the unpleasant sensation of pain. This acute pain is of physiological significance in that it warns us of tissue damage. On the other hand, chronic pain appears not to serve any defensive function and has a number of pathological manifestations such as hyperalgesia, allodynia and neuropathic pain.

Sensory neurons

The peripheral nervous system contains different sensory neurons. Some of these have their cell bodies in the dorsal root ganglion (DRG), and send out their sensory endings to the skin. Other sensory neurons located in the DRG and in the visceral ganglia, such as the nodose, jugular and petrosal ganglia, receive information from the vasculature, heart, lungs, kidney and gut. There are two types of sensory neuron, the A-type fibres and the C-type fibres. A-type fibres come in three types:

- $\text{A}\alpha$ and $\text{A}\beta$ fibres are large-diameter fast-conducting myelinated neurons that function in proprioception.
- $\text{A}\delta$ fibres have less myelination and function in nociception to detect thermal, mechanical and chemical stimuli.

C-type fibres are slow-conducting unmyelinated neurons that function in nociception by detecting thermal, mechanical and chemical stimuli.

Module 10: | Figure cochlear amplifier**Operation of the cochlear amplifier.**

Sound-induced vibration of the stapes sets up vibrations that occur in different regions of the organ of Corti, depending on their frequency. High frequencies are detected at the base, whereas lower frequencies occur at the apex. The blue lines represent the oscillations that occur in response to a soft sound in the absence of amplification, whereas the red lines illustrate how this oscillation is increased and sharpened by the cochlear amplifier. Reproduced by permission from Macmillan Publishers Ltd: *Nature*, Kros, C. (2005) *Aid for hair force*. 433:810–811. Copyright (2005); <http://www.nature.com>; see Kros 2005.

It is the A δ and C-type fibres that are the main nociceptors (specialized sensory neurons) that are responsible for pain reception.

Pain perception

The perception of pain depends on the Nav1.2 channel that has two main functions: the primary detection of painful stimuli at the nerve endings of the sensory neurons, which is followed by the neural processing of this information in the brain. Here we consider the signalling mechanisms that are responsible for the initial process of detecting and transducing painful stimuli into the inward currents that depolarize and excite the nerve endings of sensory neurons.

The A δ and C fibres of the nociceptors have nerve endings that respond to a range of modalities, such as thermal (intense heat or cold), chemical (irritants) or mechanical (intense pressure). We know most about the mechanism of nociception that occurs in the skin, especially when there has been tissue damage and inflammation (Module 10: Figure inflammatory soup). A remarkable feature of many of these nociceptors is their ability to detect more than one modality. This is achieved by having channels such as vanilloid transient receptor potential 1 (TRPV1) that can detect different modalities, such as temperature and chem-

icals (pH and capsaicin). Such polymodal reception is also achieved when neurons express channels that are capable of responding to a number of different modalities.

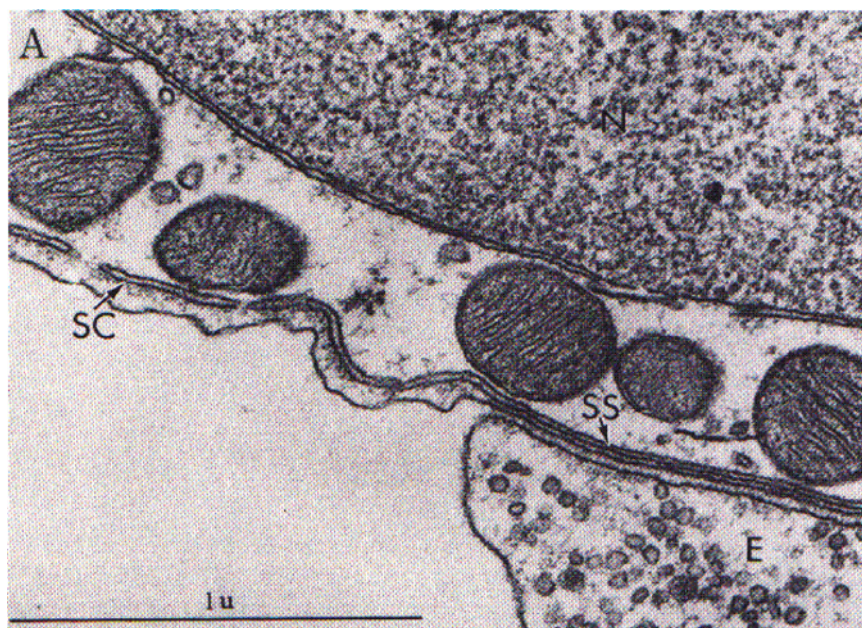
The large numbers of painful stimuli present in the 'inflammatory soup' surrounding a wound are detected by sensory ion channels located in the nerve endings of the sensory neurons. Members of the transient receptor potential (TRP) ion channel family, such as TRPV1 and melastatin-related TRP 8 (TRPM8), are particularly important. Noxious cold is detected by TRPM8 channels, whereas noxious heat is detected by TRPV1 channels (Module 3: Figure temperature sensing). The TRPV1 channels are also sensitive to an increase in H⁺ released from damaged cells. The P2X3 receptors detect ATP released from damaged cells and from the endothelium. In addition, it has been proposed that keratinocytes may also release ATP in response to noxious heat acting on TRPV4 channels. The inward currents flowing through the P2X3 and TRP ion channels produce generator potentials, which are amplified by the opening of a sensory neuron-specific Na⁺ channel (Nav1.7) coded for by the SCN9A gene that triggers the sensory neuronal action potentials that convey information to the neurons in the spinal cord that then relay information to the brain.

An important aspect of nociception is hyperalgesia, which is an example of chronic pain caused by an increased pain sensation that occurs in response to many of the stimuli that are released at inflammatory sites. These hyperalgesic stimuli [bradykinin, 5-hydroxytryptamine (5-HT), histamine, nerve growth factor (NGF) and chemokines] modulate the sensitivity of the pain receptors.

Some of the endings of sensory neurons have typical exocytotic sites that release agents such as substance P and calcitonin gene-related peptide (CGRP) that can enhance the sense of pain. Substance P induces mast cell degranulation to release histamine and 5-HT, whereas CGRP causes vasodilation of blood vessels. The latter also release bradykinin and 5-HT. Sympathetic fibres contribute to the inflammatory soup by releasing noradrenaline (norepinephrine) and ATP. Fibroblasts release nerve growth factor (NGF) and prostaglandin E₂ (PGE₂). The ATP-sensitive P2X3 receptors are also important receptors responding to the extracellular ATP that is released into sites of inflammation. The way in which some of these modulators adjust the sensitivity of the pain receptors is shown in Module 10: Figure nociception.

Pain transmission

Once the sensory nerve terminals perceive painful stimuli, the sensory nerve terminal action potential is transmitted to the nerve cord where it activates the neurons that carry information to the brain. The synapses in the spinal cord responsible for this pain transmission pathway are under control by a number of signalling systems. One of these depends on a complex series of neuronal-microglia interactions (Module 7: Figure neuronal chemokine function). The endocannabinoid signalling pathway may also play a role. The membrane depolarization associated with the firing of the relay neuron will increase the level of Ca²⁺ and this will stimulate the formation of anandamide

Module 10: | Figure hair cell postsynaptic structure

Organization of the endoplasmic reticulum in the postsynaptic region of the efferent fibres (E) in outer hair cells.

The endoplasmic reticulum (ER) is organized into flattened subsynaptic cisternae (SS) that lie immediately below the nerve ending (E). The SS extend out to subsurface cisternae (SC) away from the synaptic region. Reproduced from *Int. Rev. Cytol.*, Vol. 42, Kimura, R.S., The ultrastructure of the organ of Corti, pp. 173–222. Copyright (1975), with permission from Elsevier; see Kimura 1975.

(Module 1: Figure anandamide). The anandamide then acts through the endocannabinoid retrograde signalling mechanism (Module 10: Figure endocannabinoid retrograde signalling) to activate the CB1 receptors that inhibit transmitter release (Module 7: Figure neuronal chemokine function) and thus play a role in analgesia.

Chronic pain

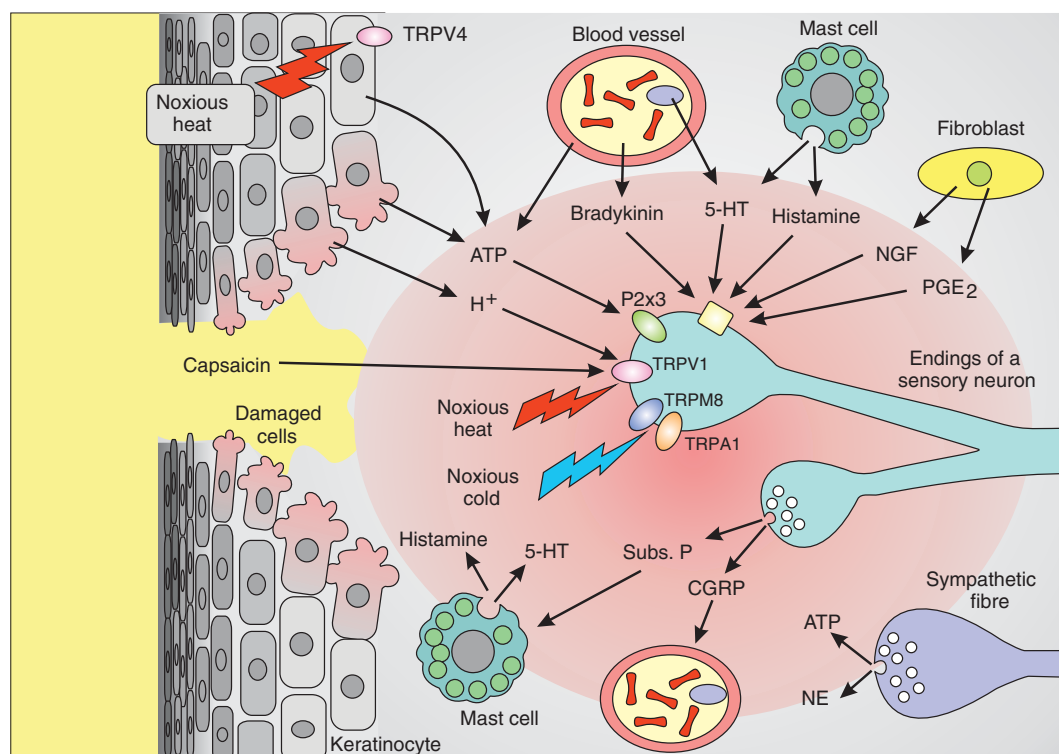
Chronic pain is a pathological form of pain that is intermittent or constant and is divorced from the normal role of pain to prevent injury. Such pain seems to arise through phenotypic modifications that enhance the sensitivity of the sensory neurons and/or the relay neurons that normally send information about painful stimuli via the spinal cord to the pain centres in the brain. In some way, the activation threshold of the neurons within this pain pathway is lowered sufficiently for them to become constitutively active in the absence of any overt painful stimuli. In some cases, such chronic pain is associated with severe inflammatory conditions such as atherosclerosis, arthritis and chronic inflammatory bowel disease. In other cases, such pain develops through nerve injury caused by diabetes or post-herpetic neuralgia. Chronic pain associated with these different pathologies can have different manifestations such as familial episodic pain syndrome, hyperalgesia, allodynia and neuropathic pain.

Hyperalgesia

The increase in pain sensation that characterizes hyperalgesia results from an increase in the sensitivity of the ion

channels responsible for the membrane depolarization of the nociceptor nerve endings (Module 10: Figure nociception). An example of such hyperalgesia is the burning sensation that one experiences when taking a hot shower when suffering from sunburn. The inflammation caused by the sunburn sensitizes the vanilloid transient receptor potential 1 (TRPV1) channels so that their temperature threshold is reduced so that a normal warm sensation begins to register as a painful stimulus. The TRPV1 channels are modulated by adjusting the level of PtdIns4,5P₂ that normally acts to inhibit this channel (Module 2: Figure PtdIns4,5P₂ regulation of TRP channels). Hydrolysis of this lipid by phospholipase Cγ (PLCγ), which is activated by nerve growth factor (NGF) receptors or by PLCβ activated by bradykinin receptors, sensitizes the TRPV1 receptors. In addition, the diacylglycerol (DAG) resulting from the hydrolysis of PtdIns4,5P₂ can activate protein kinase C (PKC) to phosphorylate the P2X receptors to enhance their sensitivity (Module 3: Figure P2X receptor structure). The generator potentials, resulting from the inward flow of current, initiates the action potentials in the sensory neurons. These generator potentials are amplified by the opening of a sensory neuron-specific tetrodotoxin-insensitive Na⁺ channel (Nav1.7) coded for by the *SCN9A* gene that triggers the sensory neuronal action potentials that convey information to the brain. The sensitivity of the Nav1.7 channel is modulated by the cyclic AMP signalling pathway that is either activated by prostaglandin E₂ (PGE₂) or inhibited by opiates.

Mutations of the *SCN9A* gene that codes for the Nav1.7 channel results in either erythromalgia, which is a dramatic

Module 10: | Figure inflammatory soup**Signalling mechanisms operating in the skin to induce pain stimuli in endings of the sensory neurons.**

The endings of sensory neurons have a dual function in nociception in that they not only have receptors to detect noxious stimuli, but they can also release agents such as substance P and calcitonin gene-related peptide (CGRP) that contribute to the sensation of pain. The detection of a range of noxious stimuli (thermal, mechanical or chemical) is carried out by a variety of ion channel receptors (TRPA1, TRPV1, TRPM8 and P2X3) that respond to a great number of painful stimuli present in the local environment that has been referred to as an 'inflammatory soup' (central red region).

increase in pain sensation, or in a congenital insensitivity to pain.

Allodynia

Allodynia is a form of chronic pain that develops as a result of injury or disease and is characterized by a heightened sensitivity to normal innocuous stimuli. An example is touch allodynia that can be caused simply by the touch of clothing. Such forms of allodynia probably arise through sensitization of the sensory neurons through mechanisms similar to those described for hyperalgesia.

Neuropathic pain

Neuropathic pain is caused by aberrant processing of somatosensory information in either the peripheral or central nervous system. There appears to be an up-regulation of the pain processing pathways brought on by nerve injury or by neural degeneration that accompanies diseases such as diabetes or post-herpetic neuralgia. In contrast with hyperalgesia and allodynia, where the aberrant sensitization mechanisms seem to occur in the nerve endings of the sensory neurons, the processes responsible for neuropathic pain seem to be located further downstream in the peripheral and central nervous system. This sensitization may develop through neural-microglia interactions carried out

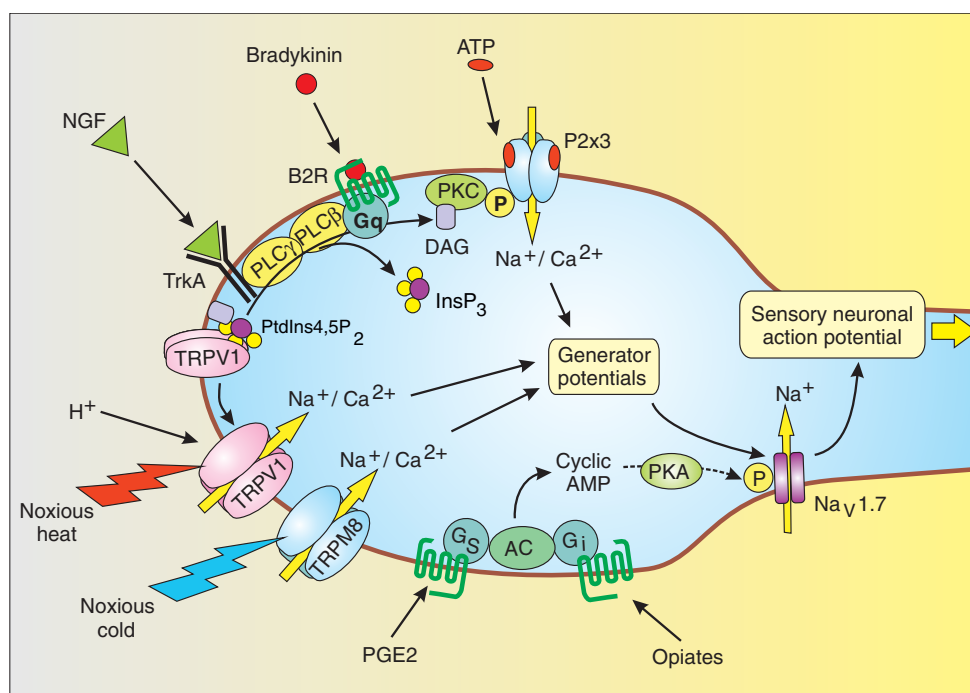
by a chemokine signalling network (Module 7: neuronal chemokine function).

A decrease in the expression of the K_{ir}4.1 channel in the satellite glial cells may contribute to the onset of neuropathic pain by decreasing the spatial buffering of K⁺.

Touch

Some of the sensory neurons that innervate the skin (Module 7: Figure skin) function as mechanosensitive neurons to sense touch. These touch-sensitive neurons have a variety of peripheral terminals that end in either the dermis surrounding the hair cells or in the epidermis where they lie in between the keratinocytes. Some of the thickly myelinated A β fibres are associated with Merkel cells (Module 10: Figure Merkel cell), which have been implicated in touch sensing. Just how touch is detected by these sensory neurons is largely unknown. There are suggestions that the mechanotransduction mechanism may depend on mechanosensitive channels that produce the generator depolarization that triggers action potentials in the sensory neurons. There is some doubt as to the exact location of these mechanosensitive channels. They may be located either in the nerve terminals that end in the skin or they might occur in specialized cells, such as the Merkel

Module 10: | Figure nociception

**Transduction and modulation of pain stimuli.**

The nerve endings of sensory neurons have a number of ion channels that respond to a variety of pain stimuli. Noxious cold and heat are detected by transient receptor potential channels TRPM8 and TRPV1 respectively. The TRPV1 channels are also sensitive to the H^+ released at sites of tissue injury during inflammatory responses (Module 10: Figure inflammatory soup). ATP acts on P2X3 channels. The sensitivity of these different ion channels can be modulated by a variety of signalling mechanisms, as described in the text.

cells or the keratinocytes that sense mechanical deformation and then transmit information to the sensory neurons.

The mechanosensitive channels may also play a role in the process of Itch, which is triggered by a wide range of factors that are released in the vicinity of the dendritic terminals of the sensory neurons that ramify throughout the skin.

Itch

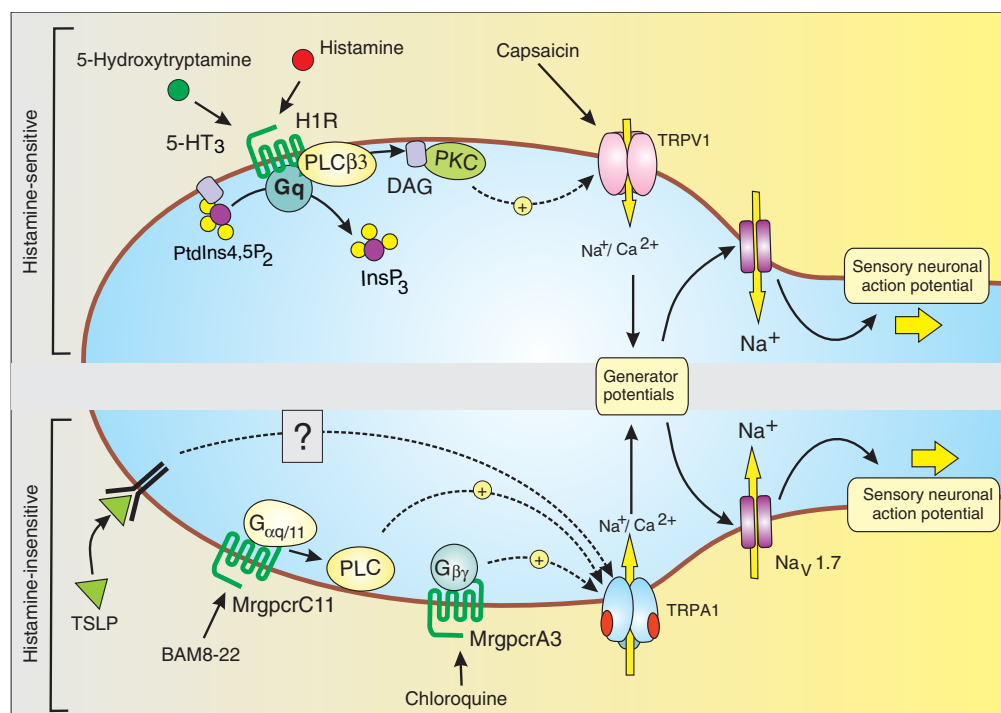
Itch, which is also known as pruritus, is a skin sensation that triggers a desire to scratch. There are different forms of Itch: pruriceptive, neuropathic, neurogenic and psychogenic. Pruriceptive itch is induced by a wide range of pruritic factors that appear in the dermis through a wide range of processes such as toxins from insect bites or through a variety of inflammatory disorders. With regard to the latter, the inflammatory disorder atopic dermatitis (AD) is a chronic itch syndrome. Another chronic itch condition is neuropathic itch that is caused by nerve injury such as that induced by herpes viruses such as varicella zoster. As its name implies, neurogenic itch does not arise at the sensory nerve endings but originates within the CNS and is often associated with renal disease and kidney failure. The causes of psychogenic itch are complex and are often associated with various mental disorders.

Most attention here will be focused on the sensory transduction mechanisms located in the dendrites of the sensory

neurons that innervate the skin (Module 7: Figure skin). Of these skin sensory neurons, it is the slow-conducting unmyelinated C-type fibres that are responsible for detecting the many and varied chemical (pruritic) stimuli that activate the Itch sensation. Many of these pruritic stimuli are also present in the inflammatory soup that surrounds a wound or at sites of inflammation and are responsible for pain perception, but many of these also act as pruritic stimuli to function in Itch (Module 10: Figure inflammatory soup). There appears to be a number of neuronal subsets that are defined by their sensitivity to pruritic stimuli. These subsets are activated by low concentrations of these stimuli but at high concentrations additional nociceptive receptors are activated to produce pain that effectively occludes the sensation of Itch. The close relationship between Itch and pain is reflected by the fact that they act through very similar signal transduction pathways.

The different subsets of sensory neurons responsible for the Itch sensation fall into two main groups: the histamine-sensitive Itch neurons and the histamine-insensitive Itch neurons. The sensory circuitry located in the dendritic ending of the neurons have the following components: the receptors that detect the pruritic stimuli, transducing mechanism that act to open the ion channels (TRPV1 and TRPA1) that create the generator potential, which is the depolarization necessary to activate the sodium channels to initiate an action potential that

Module 10: | Figure Itch signal transduction mechanisms



Itch transducing mechanisms.

The Itch circuitry seems to depend on two main pathways; the histamine-sensitive and the histamine-insensitive pathways. Pruritic stimuli such as histamine and 5-hydroxytryptamine act on receptors that stimulate phospholipase C3β (PLC3β) to open the TRPV1 channels. The histamine-insensitive pathway responds to other stimuli such as chloroquine, bovine adrenal medulla 8-22 peptide (BAM8-22) or thymic stromal lymphopoietin (TSLP) that act through different mechanisms to stimulate the opening of TRPA1 channels. The inward currents that flow through the TRPV1 and TRPA1 channels induce the generator potential that triggers the action potential that transfer information to the brain.

conveys information to the brain (Module 10: Figure Itch signal transduction mechanism).

Histamine-sensitive Itch neurons

As their name implies, this subset of Itch-sensitive sensory neurons respond to the histamine that is released from Mast cells in the skin (Module 11: Figure mast cell signalling). In addition to histamine, mast cells release a very large number of other components some of which can also activate Itch such as 5-hydroxytryptamine (5-HT) acting through the 5-hydroxytryptamine type 3 (5-HT₃). Histamine induces Itch by acting on the H1R, which is coupled through G_q to activate phospholipase C3β (PLC3β) (Module 10: Figure Itch signal transduction mechanism). Just how the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PtdIns4,5P₂) results in the opening of the TRPV1 channel is somewhat uncertain. One possible mechanism may depend on the mechanism of PtdIns4,5P₂ regulation of ion channels and exchangers (Module 2: Figure PtdIns4,5P₂ regulation of TRP channels). PtdIns4,5P₂ normally acts to inhibit this channel but upon its hydrolysis by PLC3β the channel opens. In addition, the diacylglycerol (DAG) resulting from the hydrolysis of PtdIns4,5P₂ can activate protein kinase C (PKC) to phosphorylate and activate TRPV1 (Module 10: Figure Itch signal transduction mechanism). The depolarizing current resulting from the inward flow of Na⁺ and Ca²⁺ induces the generator potential

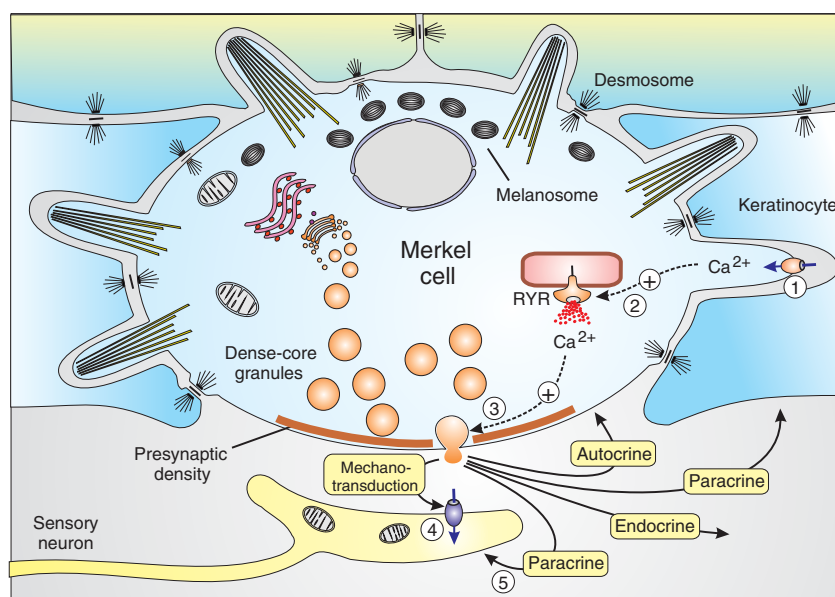
that initiates the action potentials in the sensory neurons. These generator potentials are amplified by the opening of a sensory neuron-specific tetrodotoxin-insensitive Na⁺ channel (Na_v1.7) coded for by the *SCN9A* gene that triggers the sensory neuronal action potentials that convey information to the brain.

Histamine-insensitive Itch neurons

The subset of histamine-insensitive Itch neurons respond to a number of different pruritics such as chloroquine (CQ) and bovine adrenal medulla 8-22 peptide (BAM8-22, which is a proteolytically cleaved product of proenkephalin A). These neurons, which are also sensitive to components that are generated during allergic inflammation, may also be the target of thymic stromal lymphopoietin (TSLP), which is a cytokine that has been implicated in Atopic dermatitis (AD).

A characteristic feature of these neurons is that they express members of the Mas-related G protein-coupled receptor (Mrgpr) family, which belongs to the very large group of G protein-coupled receptors (GPCRs) (Module 1: Table G protein-coupled receptors). Some of these Mrgprs have been linked to specific pruritic stimuli. For example, the antimalarial drug chloroquine (CQ), which induces an acute itching sensation, acts through the MrgprA3 receptor to activate TRPA1 through a mechanism that seems to depend on G_{βγ}, but does not require PLC activation. On the other hand, the MrgprC11 receptor, which

Module 10: | Figure Merkel cell



Structure and proposed functions of Merkel cells

Merkel cells have all the structural hallmarks of a secretory cell in that they contain dense-core granules. The release of these granules has been implicated in a number of functions such as mechanotransduction (sense of touch) or as part of a hormonal signalling system operating through autocrine, endocrine or paracrine mechanisms.

responds to the endogenous pruritic stimulus BAM8-22, activates TRPA1 through a mechanism based on $G_{q/11}$ and phospholipase C (PLC) (Module 10: Figure Itch signal transduction mechanism).

Merkel cells

Ovoid Merkel cells (10–15 μm) are located in the basal epidermal layer of the skin where they make close contact with the keratinocytes (Module 10: Figure Merkel cell). The surface facing the keratinocytes has numerous microvilli (approximately 2.5 μm in length) that interdigitate with the keratinocytes. These microvilli contain intermediate filaments made up of cytokeratin. The cytokeratin 20 (CK20) is a marker for Merkel cells. The distribution of Merkel cells varies between anatomical sites, but the highest densities are found in hairy and glabrous skin.

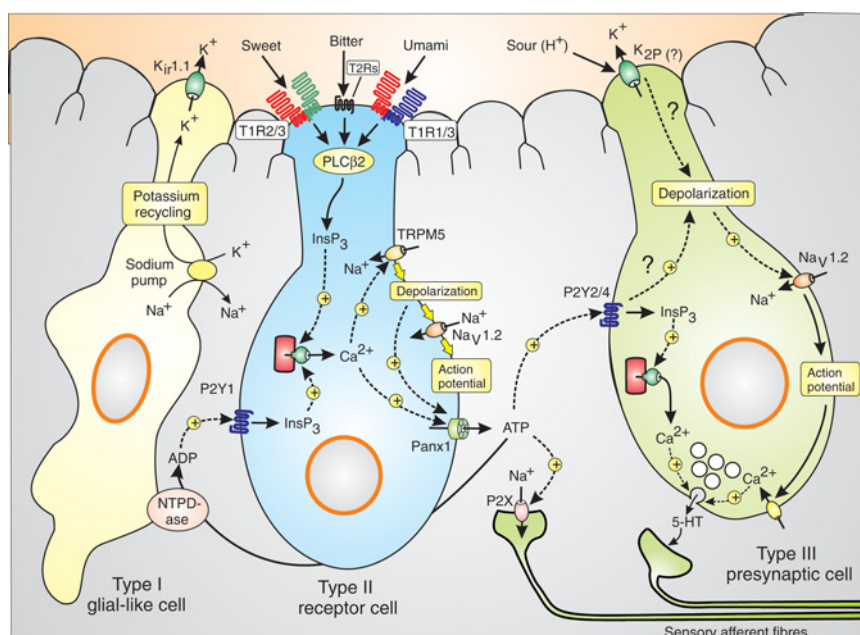
Merkel cells have a large number of chromogranin-containing dense-core granules that are congregated at the basal surface where the membrane has densities typical of those found at neuronal presynaptic areas. These granules contain a variety of agents such as calcitonin gene-related protein (CGRP), vasoactive intestinal peptide (VIP), somatostatin (SST) and 5-hydroxytryptamine (5-HT).

Many Merkel cells, but not all, are innervated by the $A\beta$ -afferent terminals of sensory neurons. These sensory neurons form a large neuronal terminal that covers most of the exposed surface of the Merkel cell. They are particularly evident in 'touch domes' where congregations of approximately 50 Merkel cells are innervated by a few sensory neurons. It is this structural organization that has led to the suggestion that Merkel cells may play a role in mechanotransduction.

There is still some uncertainty concerning the function of Merkel cells. Their structure clearly indicates that they are secretory cells capable of releasing a range of bioactive agents, but what do these do? At present there are two main hypotheses, which are not mutually exclusive. First, they may function as an endocrine cell that releases agents that either feedback to regulate their own activity (autocrine) or diffuse away to regulate other cell types either in the local community (paracrine) or further afield (endocrine). Such a function may well apply to those Merkel cells that are not innervated. Secondly, they may function as mechanotransducers as part of the sense of touch. Such a possibility is certainly in line with the fact that they are innervated by the $A\beta$ -afferent terminals of sensory neurons that are known to play a role in the sense of touch. How might this mechanotransduction system operate? The following hypothesis suggests a possible mechanism as outlined in steps 1–5 in Module 10: Figure Merkel cell:

1. The microvilli with their central filaments that project into the keratinocytes are ideally positioned to sense deformations in the skin. This deformation may act to open mechanosensitive channels to allow ions such as Na^+ or Ca^{2+} to enter the cytoplasm. Ca^{2+} is an interesting possibility because it may either act to trigger exocytosis or it may act indirectly to trigger further release.
2. There is some evidence to indicate that the Merkel cells display the process of Ca^{2+} -induced Ca^{2+} release (CICR), which will function to amplify any Ca^{2+} signals entering from the outside.

Module 10: | Figure taste receptor cells



Taste receptors cells.

Within each cup-shaped taste bud there are three main taste receptor types. Type I glial-like cells appear to act by supporting the activity of the other two cell types. Type II receptor cells respond to sweet, bitter or umami stimuli by recruiting the InsP₃/Ca²⁺ signalling pathway to release the transmitter ATP that has a number of actions. Type III presynaptic cells respond to ATP by inducing the action potentials responsible for releasing the transmitter 5-HT that acts to stimulate the sensory afferent fibres. This figure is based on information contained in Figure 3 of Chaudhari and Roper (2010). I would like to thank Nirupa Chaudhari and Stephen Roper for all their help in designing this figure.

3. The increase in Ca²⁺ then triggers the release of the dense-core granules.
4. A transmitter such as glutamate released from the Merkel cell activates cation channels in the nerve terminal of the sensory neuron to provide the depolarization to trigger an action potential to convey information to the brain.
5. An alternative explanation is that the channels in the sensory terminal are the mechanotransducers and the agents released by the Merkel cell act in a paracrine manner to modulate the activity of the nerve terminal.

Taste

Taste receptor cells, which are specialized neuroepithelial cells, are located within the cup-shaped taste buds that are found on the tongue. These receptor cells form an epithelial layer that is connected by tight junctions thus separating the small apical surface at the taste pore from the basolateral membrane that faces the interstitial space (Module 10: Figure taste receptors). The sense of taste functions to discriminate between nutritionally relevant and potentially toxic compounds in our food. In addition to the primary taste modalities (sweet, umami, sour, salty and bitter), taste receptors can also detect other properties such as fatty and metallic substances. Within each taste bud, there are approximately one hundred taste receptor cells of which there are three main types that interact with

each other to set up a sophisticated sensory system for detecting an extraordinary variety of tastes:

- Type I glial-like cells
- Type II receptor cells
- Type III presynaptic cells

Type I glial-like cells

The type I glial-like cells are the main cell type in the taste bud even though they do not play a direct role in taste reception, but they do have an important supporting role acting like glial cells. They have large cytoplasmic lamellae that wrap around the other two cell types and one of their primary functions is to restrict the spread of the transmitters that are released during taste reception. For example, the Type I cell expresses the ecto-nucleoside triphosphate diphosphohydrolases (NTPDase) on their surface and this nucleotidase hydrolyses the ATP released from the Type II cells (Module 10: Figure taste receptor cells). The type I cells may thus isolate the type II cells enabling them to transduce taste stimuli independently from their neighbours in much the same way that glial cells insulate neurons.

Another proposed function of the type I cells is to regulate the level of K⁺ within the restricted interstitial space within the taste bud. During intense activity when the type III presynaptic cells are firing action potentials, an accumulation of extracellular K⁺ would reduce the excitability

of the type II and type III cells. The type I cells may drain this excess K^+ away through a K^+ recycling pathway that depends on uptake by a sodium pump operating in tandem with $K_{ir}1.1$ channels on the apical membrane that allows K^+ to leak out into the pore of the taste bud.

While most evidence favours a glial-like supporting role, the type I cells may have a function in detecting salt, but the mechanism remains to be determined.

Type II receptor cells

The type II receptor cells play a major role in taste reception in that they detect sweet, bitter and umami stimuli (Module 10: Figure taste receptor cells). It is the apical membrane that has the receptors for sensing these different taste modalities, whereas the basolateral membrane is responsible for releasing the neurotransmitter ATP that excites the nerve terminals of the primary sensory afferent fibres that transmit information back to the brain. The taste receptors that detect these stimulants belong to the large family of G protein-coupled receptors (GPCRs) (Module 1: Table G protein-coupled receptors). Umami compounds are detected by the T1R1–T1R3 heterodimer, whereas sweet compounds activate T1R2–T1R3 heterodimers. These T1R1, T1R2 and T1R3 receptors, which form heterodimers have enormously extended N-termini. Bitter compounds are sensed by the large family of T2Rs, which are coded for by approximately 30 genes that do not form dimers and also lack the extended N-termini. Each bitter-responsive cell expresses approximately eight different T2Rs, and this heterogeneity greatly enhances the ability to discriminate between different bitter compounds. Each type II receptor cell has a specific set of these taste receptors enabling them to respond to the different taste modalities.

The sensory transduction pathway activated within each specific taste receptor cell is very similar in that it recruits the inositol 1,4,5-trisphosphate ($InsP_3$)/ Ca^{2+} signalling cassette (Module 10: Figure taste receptor cells). The activated receptors are coupled through α -gustducin ($G\alpha_{gust}$) (Module 2: Table heterotrimeric G proteins), which is a taste-selective G protein that acts to stimulate phospholipase C β 2 (PLC β 2) to generate $InsP_3$. The latter then acts on $InsP_3$ receptors on the endoplasmic reticulum (ER) to release Ca^{2+} into the cytoplasm. The increase in cytoplasmic Ca^{2+} activates two transduction processes. First, Ca^{2+} activates hemichannels, probably formed from pannexin 1 (Pannx1) (See Module 3: Figure hemichannels and gap junctions), to induce release of the transmitter ATP that then has both paracrine and autocrine actions. Secondly, Ca^{2+} activates TRPM5 channels to depolarize the membrane, which also contributes to the activation of the Pannx1 channel. These type II taste receptors also express Nav1.2 channels and these will also respond to depolarization by initiating action potentials.

The paracrine actions depend on ATP activating P2Y2 or P2Y4 receptors on the type III presynaptic cell that then relays information to the afferent sensory fibres. In addition, the ATP can also act through P2X receptors to excite sensory afferent terminals directly (Module 10: Figure taste receptor cells). The autocrine action of ATP de-

pends on its hydrolysis to ADP, which is carried out by the NTPDase located on the surface of the type I glial-like cells. The ADP then acts on the P2Y1 receptors on the type II receptor cells to generate $InsP_3$ that feeds into the transduction processes that generate Ca^{2+} .

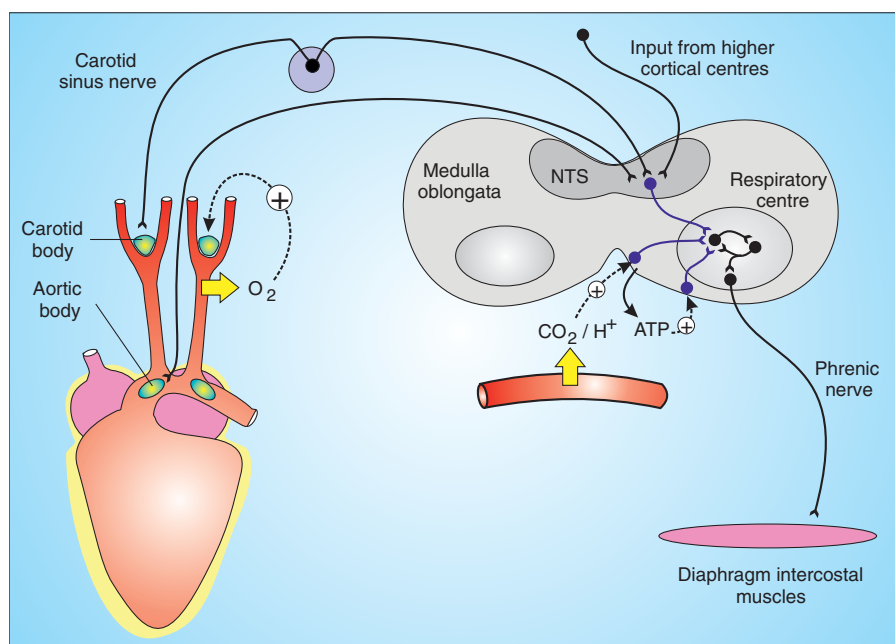
Type III presynaptic cells

The type III presynaptic cells appear to function in taste transduction by relaying information to the sensory afferent nerve fibres (Module 10: Figure taste receptor cells). They express many of the proteins normally associated with synapses. For example, they synthesize and package 5-hydroxytryptamine (5-HT) into synaptic vesicles that form synaptic-like junctions with the postsynaptic endings of the sensory fibres. These 5-HT-containing synaptic vesicles may be released through two mechanisms. The presynaptic cells are excitable in that they can generate action potentials based on the opening of Nav1.2 channels to provide the depolarization to activate the voltage-operated Ca^{2+} channels responsible for releasing 5-HT. What is not clear is just what induces the depolarization necessary to trigger these action potentials. One possibility is that it may be induced in response to sour stimuli (Module 10: Figure taste receptor cells). The apical membrane may have K^+ channels capable of responding to the H^+ in sour stimuli. Likely candidates are certain members of the family of two-pore domain K^+ (K_2P) channels that are sensitive to acid such as the TASK-1, TALK-1 and TWIK-1 channels. These channels close in response to an increase in acidity and this may provide the depolarization necessary to trigger action potentials.

These type III presynaptic receptor cells trigger action potentials in response to the sweet, bitter and umami compounds that activate the type II receptor cells and it seems that this information is relayed through the release of ATP that then activates P2Y2/4 receptors on the presynaptic cells. Just how these P2Y receptors act to trigger the action potentials has not been established. Another possibility is that these P2Y2/4 receptors produce $InsP_3$ that releases Ca^{2+} that could activate 5-HT secretion independently of action potentials through the mechanism of exocytosis triggered by Ca^{2+} release from internal stores (Module 10: Figure taste receptor cells).

Hypoxia-sensing mechanisms

There are a number of mechanisms responsible for ensuring that cells receive a constant supply of oxygen. The normal oxygen tension (pO_2) of the blood is approximately 100 mmHg. There are sensory systems that detect the onset of hypoxia, which is characterized by a decrease in pO_2 to levels below 80 mmHg. In addition, there are sensory systems for detecting the associated increase in CO_2/H^+ levels (hypercapnia). Respiration is controlled by an integration of sensory information coming in from the peripheral and central chemoreceptors (Module 10: Figure breathing control). O_2 -sensing systems are mainly located in peripheral chemoreceptors such as those found within the carotid body, whereas CO_2/H^+ -sensing systems are found in the central chemoreceptors located within the medullary respiratory centre. These gas-sensing

Module 10: | Figure breathing control**Location of the gas sensing systems that control breathing.**

The peripheral chemoreceptors located in the carotid and aortic bodies respond to small decreases in the level of O_2 by exciting sensory neurons that relay information to the respiratory centre in the medulla oblongata via the nucleus tractus solitarius (NTS). The respiratory centre contains the central chemoreceptors that are particularly sensitive to increases in either CO_2/H^+ or ATP levels, which act to accelerate the neuronal respiratory circuits that controls breathing. This respiratory centre communicates with the diaphragm and intercostal muscles via the phrenic nerve. The structure and function of the carotid body is illustrated in Module 10: Figure carotid body chemoreception, whereas the respiratory centre in the pre-Bötzinger region is illustrated in Module 10: Figure respiratory pacemaker circuit.

mechanisms serve to optimize gas exchange across the lungs by inducing rapid changes in both breathing and blood pressure in response to hypoxia. The peripheral chemoreceptors that respond to the decrease in oxygen are specialized sensory cells that feed information into the central chemoreceptors located in the medullary respiratory network that controls breathing.

When hypoxia is more severe (i.e. when pO_2 falls below 40 mmHg), there is a more general cellular response that develops over a much longer time scale of minutes to hours. This more general adaptive response depends upon the activation of gene transcription as the cell attempts to overcome the lack of oxygen. One of the prominent genes activated by this lack of oxygen is the hypoxia-inducible factor (HIF) (Module 4: Figure HIF activation), which plays a particularly important role in activating the process of angiogenesis).

 O_2 sensing

A small decrease in pO_2 is sensed by peripheral chemoreceptors, which are closely associated with the major blood vessels leaving the heart (Module 10: Figure breathing control). They are thus ideally suited to monitor the gaseous content of the blood in transit to the rest of the body.

Peripheral chemoreceptors

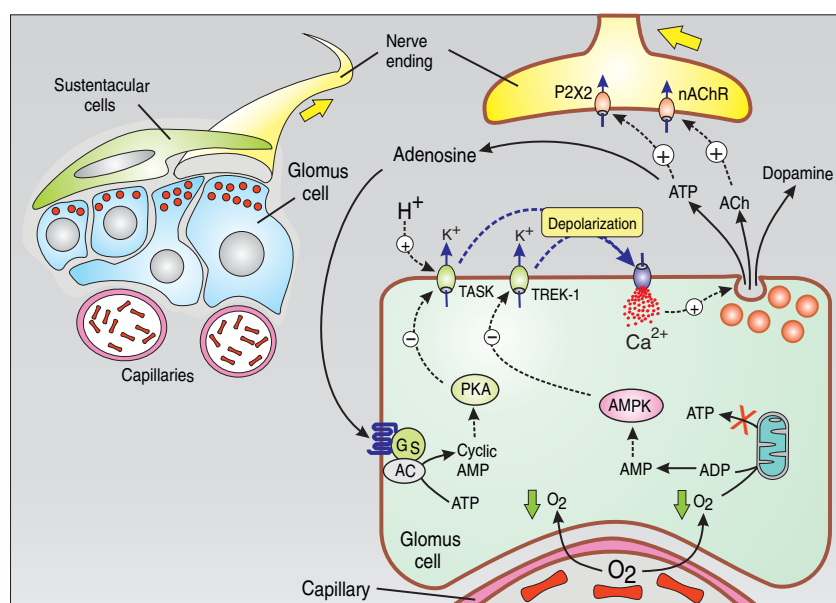
These arterial chemoreceptors, which are particularly sensitive to pO_2 , are found in the carotid and aortic bodies. They play a critical role in detecting the early onset of

hypoxia by activating the sensory nerves that carry information into the central respiratory centres that bring about the rapid increases in ventilation and arterial pressure (Module 10: Figure breathing control). Most attention has focused on the carotid body.

Carotid body

The two carotid bodies are located in the bifurcation of the common carotid arteries (Module 10: Figure breathing control). Within the carotid body there are clusters of glomus cells surrounded by a dense network of capillaries (Module 10: Figure carotid body chemoreception). These glomus cells are O_2 -sensitive neurosecretory cells that are adapted to sense both hypoxia and metabolic acidosis that develops when O_2 levels are low. They have a large population of secretory granules in the outer surface layers facing the sites where there is close apposition to the afferent nerve terminals. This 'synaptic' region between the glomus cells and the sensory nerve endings is often ensheathed by extension of the sustentacular cell, which appears to function like a glial cell.

There is still some debate as to the exact mechanism used by glomus cells to sense a decline in O_2 . Possible O_2 -sensing candidates include various haem-containing proteins such as mitochondrial cytochrome *c*, nitric oxide synthase (NOS), haem oxygenase 1 and 2 (HO-1 and -2) and NADPH oxidases. Another candidate for detecting the decline in O_2 is the mitochondrion that forms ATP. In hypoxic conditions, the accumulation of AMP

Module 10: | Figure carotid body chemoreception**Structure and function of the O₂-sensing carotid body.**

The panel on the left illustrates the functional organization of the four major components of the carotid body. O₂ levels within the capillaries are sensed by the glomus cells that release a range of transmitters to excite the sensory nerve endings that then relay action potentials to the brain (yellow arrow). The signalling mechanisms that respond to metabolic acidosis (an increase in H⁺) or hypoxia (an increase in AMP) act to inhibit various K⁺ channels (see the text for further details). The location of the carotid body is shown in Module 10: Figure breathing control.

may activate the AMP signalling pathway. The activated AMP-activated protein kinase (AMPK) may then phosphorylate the TREK-1 K⁺ channels leading to closure and membrane depolarization.

With regard to detecting metabolic acidosis, much attention has focused on the TASK subfamily of the two-pore domain K⁺ (K_{2p}) channel family (Module 3: Table two-pore domain K⁺ channels), which close when *p*O₂ declines. The TASK-1 and TASK-3 channels have an external proton sensor that detects an increase in external acidity. Closure of K⁺ channels is critical for sensory transduction because it provides the membrane depolarization that opens Ca²⁺ channels to trigger exocytosis of the transmitters that excite the nerve endings.

Whatever the link between a decrease in O₂ and the closure of K⁺ channels turns out to be, there is a general consensus that the resulting depolarization triggers the exocytosis of stored vesicles containing a range of transmitters such as ATP, acetylcholine (ACh), dopamine, 5-hydroxytryptamine (5-HT) and substance P. These transmitters then excite the afferent nerve endings to send information back to the brain (yellow arrows in Figure 10 carotid body chemoreception). There appears to be a central role for ATP acting on ionotropic P2X receptors, such as P2X2, and ACh acting on nicotinic acetylcholine receptors (nAChRs). The action of ATP is complicated by the fact that it is converted into adenosine that can feed back to modulate the glomus cells. The adenosine binds to A_{2A} receptors that act through the cyclic AMP signalling pathway to phosphorylate and inactivate the TASK K⁺

channels. Some of the other transmitters, such as dopamine, may play a more regulatory role by adjusting the sensitivity of the nerve endings.

The afferent nerve endings within the carotid body transmit information to the respiratory centre in the CNS that controls respiration (Module 10: Figure breathing control).

CO₂/H⁺ sensing

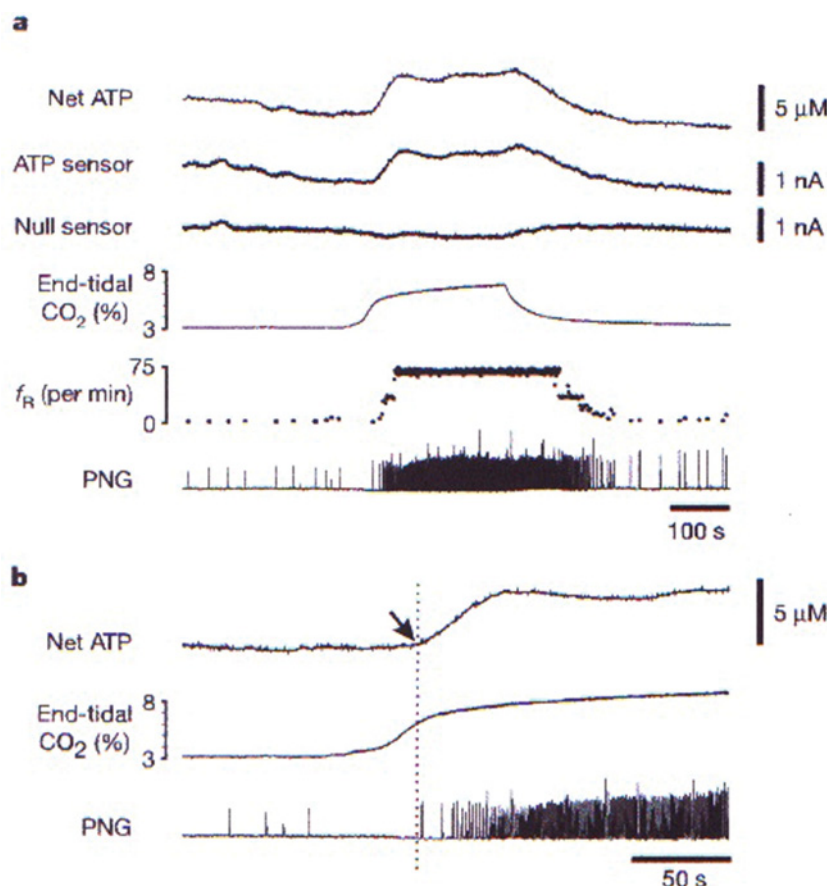
In addition to the peripheral chemoreceptors that are responsible for O₂ sensing, there are central chemoreceptors that can detect the corresponding increase in the levels of CO₂/H⁺. This response to hypercapnia plays an important role in accelerating the medullary respiratory centre. If the level of inspired CO₂ is increased, there is an immediate increase in phrenic nerve activity (Module 10: Figure hypercapnia accelerates respiration). The next question to consider is how the central chemoreceptors function to sense the increase in CO₂/H⁺.

Central chemoreceptors

Central chemoreceptors are distributed throughout the brainstem. However, the main location seems to be concentrated in the ventral region of the medulla oblongata such as the retrotrapezoid nucleus (RTN) where they are a component of the respiratory centre (Module 10: Figure ventral respiratory column).

Respiratory centre

The medullary respiratory centre consists of neuronal networks that function as a central pattern generator

Module 10: | Figure hypercapnia accelerates respiration**CO₂ accelerates the breathing rhythm.**

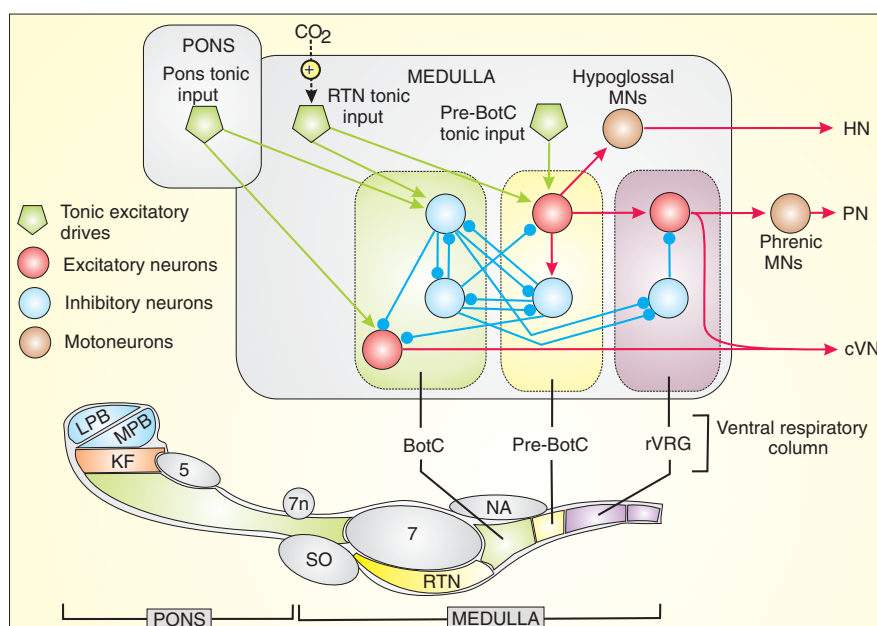
a. An increase in the level of inspired CO₂ (end tidal CO₂) results in the acceleration of phrenic nerve activity (PNG). In addition, there is an increase in the concentration of ATP on the ventral surface of the medulla close to where the medullary respiratory centre is located (Module 10: Figure breathing control). b. An enlarged region to show the relationship between the onset of the increase in the concentration of ATP on the surface (arrow) and the delayed onset in nerve activity. Reproduced by permission from Macmillan Publishers Ltd: Gourine, A.V., Llaudet, E., Dale, N. and Spyer, K.M. (2005) ATP is a mediator of chemosensory transduction in the central nervous system. *Nature* 436:108–111. Copyright (2005); <http://www.nature.com>; see Gourine et al. 2005.

(CPG) that produces the motor signals that are conveyed down the hypoglossal, phrenic and vagal nerves to control the pattern of breathing (Module 10: Figure breathing control). These neuronal networks are distributed along the ventral respiratory column (VRC) located within the medulla (Module 10: Figure ventral respiratory column). The VRC has three main regions: the Bötzinger complex (BötC), the pre-Bötzinger complex (pre-BötC) and the rostral ventral respiratory group (rVRG). The pre-BötC generates the inspiratory rhythm, whereas BötC drives expiration. There is still much to learn about the location and function of the different rhythm generators, which are coupled together in an integrated network to control the normal respiratory rhythm. It is also important for these breathing patterns to be co-ordinated with the other motor activities responsible for swallowing, phonation and gasping. The model shown at the top of Module 10: Figure ventral respiratory column illustrates the complex arrangement of excitatory and inhibitory neurons responsible for generating the various patterns of output signals that leave via the different motor neurons. In addition, the oscillatory circuits located within the BötC and pre-BötC are con-

trolled by tonic excitatory drives emanating from other neuronal groups including the pons, the retrotrapezoid nucleus (RTN) and from the pre-BötC regions. The RTN functions in chemoreception in that it responds to changes in metabolic state, which is usually signalled through an elevation of CO₂.

The pre-BötC, which is responsible for driving inspiration, has the 'kernel' excitatory circuit of glutamatergic neurons that has a central role as the main 'pacemaker' mechanism capable of interacting with the other circuit components. The organization of the pre-BötC respiratory pacemaker circuit is shown in Module 10: Figure pre-BötC respiratory circuit. The pacemaker pre-BötC neurons (blue and green cells), which are located bilaterally in the pre-BötC region (shown in pink), have an axon that branches soon after leaving the soma. The shorter branch has local collaterals that innervate the pre-motoneurons (preMNs), whereas the longer branch crosses the mid-line to innervate pre-BötC neurons on the opposite side. The prominent dendritic trees tend to face the ventral medullary surface. The preMNs (red cells), which are innervated by the pre-BötC neurons, have axons that

Module 10: | Figure ventral respiratory column



Brain stem respiratory network.

The figure at the bottom illustrates the different regions of the brainstem. Breathing patterns are generated by the ventral respiratory column that consists of the Bötzinger complex (BotC), the pre-Bötzinger complex (Pre-BötC) and the rostral ventral respiratory group (rVRG). Many tonic input 'drives' from the pons, glutamatergic neurons in the RTN and raphe serotonergic (5-HT) neurons that also contain substance P (SP), all of which are proposed to modulate the activity of this core circuitry. The figure at the top represents a model of the brain stem respiratory network showing how interactions between excitatory and inhibitory neurons generates the patterns of electrical activity conveyed down the motor neurons that leave via the hypoglossal nerve (HN), phrenic nerve (PN) or the central vagus nerve (cVN). The inhibitory neurons in the BotC and pre-BötC form a mutually interconnected ring-like inhibitory local circuit that is critical for generating and co-ordinating inspiratory/expiratory activity. The figure and model were adapted from Smith et al (2007).

project to the XII motor nucleus where they innervate the XII motoneurons. The latter then send their axons out through the XII nerve. Other axons project to the rVRG neurons that in turn make excitatory connections with the phrenic motoneurons and other inspiratory spinal motoneurons.

The respiratory mechanisms that control breathing pose two main cell signalling questions. First, what is the nature of the respiratory pacemaker mechanism that generates the breathing rhythms? Secondly, how is the modulation of the respiratory pacemaker mechanism carried out? Such modulation is essential to optimize gas exchange across the lungs to ensure that the O_2 tension of the blood remains constant in the face of changing demands.

Respiratory pacemaker mechanism

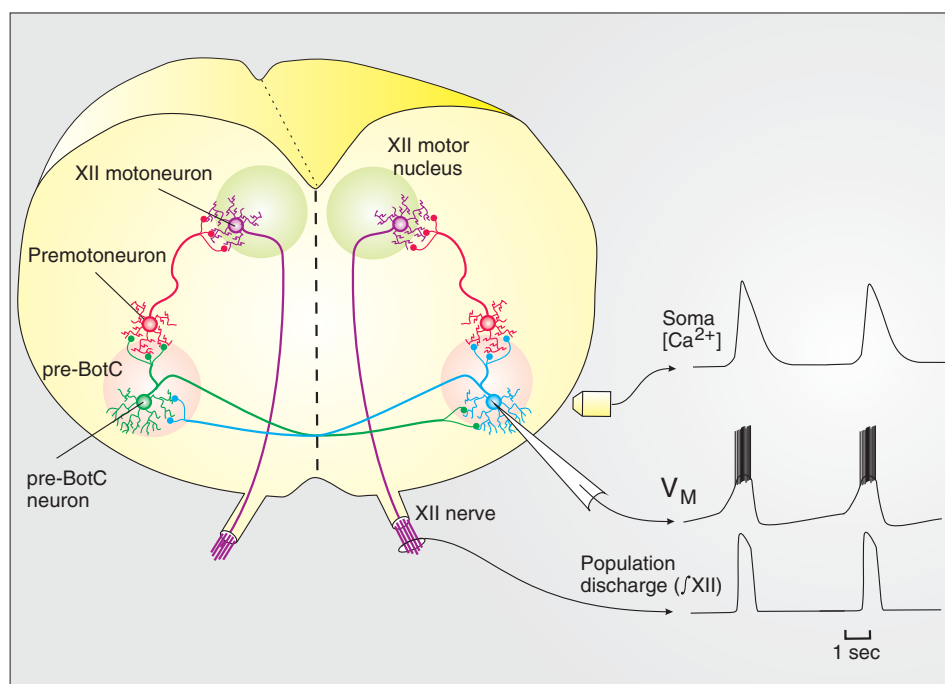
There is considerable debate about whether the respiratory rhythm is generated by a network oscillator or through an endogenous neuronal pacemaker mechanism. Most current evidence favours an endogenous excitatory network pacemaker mechanism, which has been referred to as the 'group pacemaker hypothesis', located in the pre-BötC neurons (Module 10: Figure pre-BötC respiratory circuit) that is controlled by tonic drives and phasic synaptic inhibition forming a pacemaker network system (Module 10: Figure ventral respiratory column).

The pacemaker mechanisms have been analysed by isolating the pre-BötC and the local circuit innervating hy-

poglossal motoneurons in living slice preparations from neonatal rodents. Recent evidence from studies in these and other reduced preparations suggest that groups of neurons might form modules consisting of interconnected clusters of neurons that make up a pacemaker circuit (Module 10: Figure respiratory pacemaker circuit). These pacemaker modules are connected with other modules to generate the pacemaker rhythm that drives inspiration. As indicated above, these pre-BötC pacemaker systems are modulated by inputs from other circuit components (e.g. inhibitory circuits and tonic drives) (Module 10: Figure ventral respiratory column).

The basis of the 'group pacemaker hypothesis' is that all the neurons within the circuit have an inherent rhythmicity such that when one neuron becomes active it rapidly recruits its neighbours through a synaptic mechanism to produce a synchronous output signal. The activity of individual neurons within the pre-BötC pacemaker circuit has been monitored by measuring both the electrophysiological properties and the changes in intracellular Ca^{2+} in both the dendrites and soma (Module 10: Figure respiratory pacemaker circuit). The membrane potential (V_M) displays typical bursting behaviour (Module 10: Figure respiratory pacemaker neurons). There is a characteristic pacemaker depolarization that precedes the firing of action potentials. If synaptic transmission is blocked by applying a cocktail of transmitter receptor blockers, some of the neurons fall silent whereas others, like those shown in

Module 10: | Figure pre-BötC respiratory circuit



Respiratory circuit from the pre-Bötzinger region of the brain stem.

Pre-Bötzinger (pre-BötC) neurons (green and blue) generate the respiratory rhythm that is passed via a local circuit to the premotoneurons and XII motoneurons (purple) that leave the brainstem through the XII nerve. The concentration of Ca^{2+} recorded from the pre-BötC begins to increase before the onset of the pacemaker depolarization that precedes the onset of the burst of action potential. The bottom trace recorded the population discharge from the XII nerve. The way in which the pre-BötC neurons are connected together to form the respiratory pacemaker circuits is shown in Module 10: Figure respiratory pacemaker circuit. This figure was drawn from information published in Koizumi and Smith (2008) and Koizumi et al. (2008).

Module 10: Figure respiratory pacemaker neurons, continue to burst.

The pacemaker depolarization that leads up to each burst often has two distinct phases (Module 10: Figure respiratory pacemaker circuit). Immediately after a burst, the membrane repolarizes and then begins to depolarize slowly (slow ΔV) and then there is a sudden increase in the rate of depolarization (fast ΔV) to give the inspiratory drive potential that triggers the next burst of action potentials. Onset of the fast ΔV coincides with the early small increase in the concentration of the Ca^{2+} within the dendrites and is followed by a much larger Ca^{2+} transient in the soma that is a consequence of the Ca^{2+} that enters during the burst of action potentials (Module 10: Figure respiratory pacemaker circuit). This burst occurs in phase with the electrical activity of the inspiratory motoneurons (e.g. recorded as the population discharge in the XII nerve), which is the output signal from the pre-BötC pacemaker oscillator.

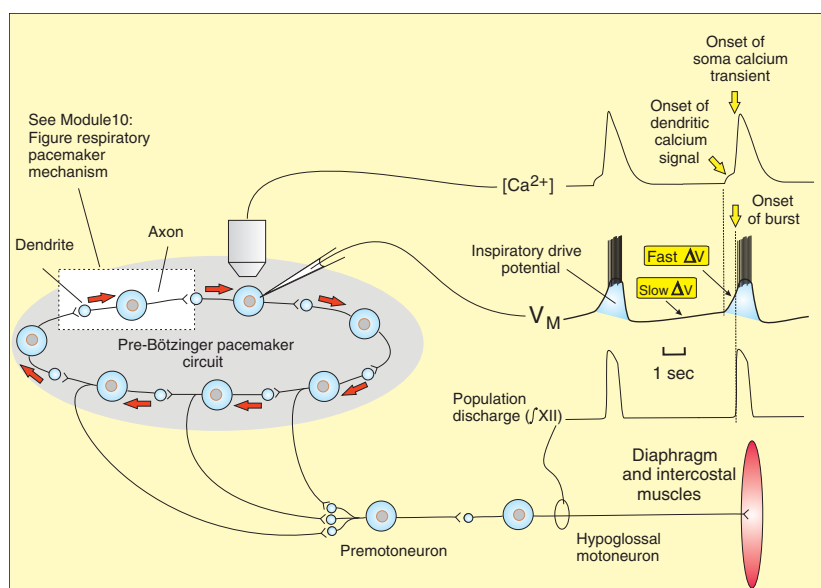
A characteristic of many pacemaker mechanisms is the presence of a persistence inward current that provides the pacemaker depolarization. In the case of the respiratory pacemaker responsible for the 'inspiratory drive potential' that triggers the regular bursts of activity, the pacemaker depolarization seems to have two components: an initial slow depolarization (slow ΔV) followed by a sudden increase in the rate of depolarization (fast ΔV) preceding the onset of the burst (Module 10: Figure respiratory pacer-

maker circuit). These slow and fast phases of the pacemaker depolarization seem to depend on two separate mechanisms.

There are two inward currents that could contribute to the slow ΔV : a persistent Na^+ current (I_{NaP}) and a Ca^{2+} current (I_{Ca}). These currents may function in the two types of pacemaker neurons that have been located in the pre-BötC respiratory centre. One type uses I_{NaP} and is insensitive to Cd^{2+} (see the left hand traces in Module 10: Figure respiratory pacemaker neurons). The other type appears to use I_{Ca} to provide the depolarizing drive and is thus very sensitive to Cd^{2+} , which blocks voltage-activated Ca^{2+} channels and completely inhibits pacemaker activity (see right-hand traces). These different pacemaker neurons contribute to the group pacemaker mechanism that generates the sharp population discharge (Module 10: Figure respiratory pacemaker circuit).

The fast ΔV that precedes the onset of the burst seems to reflect the mechanism that synchronizes the group of pacemaker neurons. In order to generate such a sharp motor output, all the neurons participating in the oscillatory mechanism must burst in synchrony with each other. Such synchronization within the respiratory network depends on the neurons being connected to each other through their excitatory glutamatergic synapses. These synapses are unusual in that they have a strong positive-feedback component that operates periodically to ensure that all the participating neurons fire together to produce a

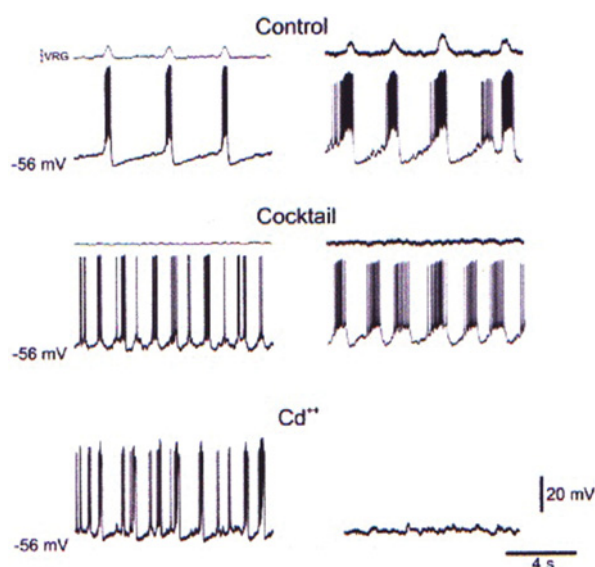
Module 10: | Figure respiratory pacemaker circuit



Pre-Bötzinger pacemaker circuit.

Imaging studies indicate that the pre-Bötzinger region consists of a collection of small circuits containing relatively few neurons. These interconnected neurons pass information to the premotoneurons responsible for activating the hypoglossal nerve (XII) that controls the diaphragm and intercostal muscles. Imaging studies indicate that Ca^{2+} increases in two steps: an initial small increase in the dendrites, associated with the rapid depolarization (fast ΔV), followed by a larger transient in the soma associated with the burst of action potentials. The blue shading on the membrane voltage (V_M) trace represents the inspirational drive potential during which the membrane is depolarized sufficiently to induce the action potentials responsible for the XII respiratory motor output. The pacemaker mechanism operating within each neuron is described in Module 10: Figure respiratory pacemaker mechanism. This figure was constructed based on information contained in Ramirez et al. 2004, Feldman and Del Negro 2006, Hartelt et al. 2008 and Mironov 2008.

Module 10: | Figure respiratory pacemaker neurons



Recordings of pacemaker neurons from the medullary respiratory centre.

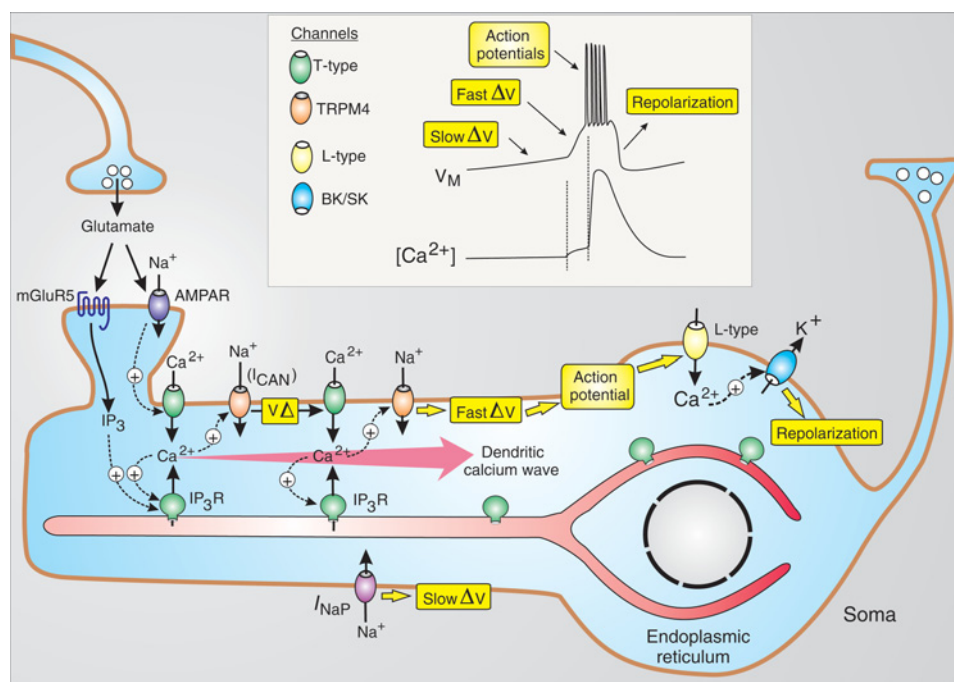
Pacemaker neurons fall into two classes, the Cd^{2+} -insensitive neurons (left) and the Cd^{2+} -sensitive neurons (right). The bursting behaviour persists after adding a cocktail of transmitter inhibitors, which prevent the neurons from interacting with each other. Note the pacemaker depolarization that precedes each burst of activity. Reprinted from Peña, F., Parkis, M.A., Tryba, A.K. and Ramirez, J.-M. (2004) Differential contribution of pacemaker properties to the generation of respiratory rhythms during normoxia and hypoxia. Neuron 43: 105–117, Copyright (2004), with permission from Elsevier; see Peña et al. 2004.

synchronous and robust output signal. The positive-feedback mechanisms that operate during a brief period of time (i.e. during the fast ΔV Module 10: Figure respiratory pacemaker circuit) enable pacemaker neurons to rapidly communicate with each other as a group oscillator.

The feed forward mechanism triggered by synaptic signalling seems to depend on the rapid activation of a Ca^{2+} -activated non-specific cation current (I_{CAN}), that depends on the melastatin-related transient receptor potential 4 (TRPM4) and 5 (TRPM5) channels. This activation of (I_{CAN}), which generates the fast ΔV that synchronizes the bursts of all the neurons within the pacemaker circuit (Module 10: Figure respiratory pacemaker circuit), coincides with the onset of a dendritic Ca^{2+} signal that is triggered by glutamate acting through two parallel pathways (Module 10: Figure respiratory pacemaker mechanism). In one pathway, glutamate acts on AMPARs to trigger a small depolarization that then triggers a voltage-operated Ca^{2+} channel (VOC), most likely to be the Ca_v3 family of T-type channels, to create the Ca^{2+} signal that activates I_{CAN} . The other mechanism depends on glutamate acting on mGluR5 to form $InsP_3$ that then acts on $InsP_3$ Rs to release Ca^{2+} that can also activate I_{CAN} . Having two mechanisms for initiating the pacemaker activity provides a fail-safe mechanism to ensure initiation of the fast phase of membrane depolarization (fast ΔV).

The initial depolarization occurring within the synaptic region appears to be amplified by using the same

Module 10: | Figure respiratory pacemaker mechanism



Pacemaker mechanisms of pre-Bötzinger complex pacemaker neurons.

The 'group pacemaker hypothesis' is based on the individual pre-Bötzinger complex neurons interacting with each other through a glutamatergic mechanism operating through both AMPARs and metabotropic mGluR5 receptors to ensure that they are coupled to their neighbours so that the circuit can generate a unified output signal. Pacemaker activity also depends on positive-feedback interactions through a number of channels as described in the text. This Figure was constructed based on information contained in Peña et al., 2004, Ramirez et al., 2004, Feldman and Del Negro, 2006, Pace et al., 2007 and Del Negro et al., 2011.

channels as described above. The depolarization activates Ca^{2+} channels located further down the dendrite and the resulting entry of Ca^{2+} can then trigger local InsP_3Rs to release Ca^{2+} to activate local TRPM4 channels and so on (Module 10: Figure respiratory pacemaker mechanism). The positive-feedback mechanism driven by the electrotonic depolarization results in a fast dendritic Ca^{2+} wave that travels at approximately $780 \mu\text{m/s}$. The fast ΔV resulting from these positive-feedback interactions produces the 'inspiratory drive potential' responsible for triggering the bursts of action potentials that occur almost synchronously in all the neurons within the pacemaker circuit.

The next question to consider is what terminates the burst and what controls the frequency of the oscillatory cycle. Each burst of action potentials is terminated by a rapid membrane repolarization. This hyperpolarization may depend on inactivation of the inward channels, such as NaP and TRPM4, and the activation of Ca^{2+} -dependent K^+ channels such as the large-conductance (BK) channels and the small-conductance (SK) channels (Module 10: Figure respiratory pacemaker mechanism). As Ca^{2+} is returned to the ER through the SERCA pumps or removed from the cell by the PMCA pumps, the intracellular level will decline and the Ca^{2+} -dependent K^+ channels will switch off and this will enhance the ability of the slow inward currents to depolarize the membrane to set the stage for the next burst. Inspiratory burst termination may

also depend on the synaptic inhibition from BötC circuit components responsible for the inspiratory/expiratory phase switch.

Adjusting the sensitivity of InsP_3Rs , which may contribute to the generation of the dendritic Ca^{2+} wave, may provide a mechanism to modulate the pacemaker rhythm. Reduction in the activity of these release channels can occur either through Ca^{2+} -dependent desensitization or through a reduction in the level of Ca^{2+} within the lumen of the ER. It is known that the level of Ca^{2+} within the ER lumen is particularly important in inositol 1,4,5-trisphosphate receptor (InsP_3R) modulation (Module 3: Figure InsP_3R regulation). Luminal Ca^{2+} levels change sensitivity and this can determine whether or not they can participate in amplifying the dendritic Ca^{2+} wave and thus the fast ΔV (Module 10: Figure respiratory pacemaker mechanism). Immediately after a burst, the luminal level of Ca^{2+} will be low reducing the likelihood of triggering this positive-feedback process. Indeed, the depletion of Ca^{2+} will be most severe near the synapse close to the mGluR5 receptors where the level of InsP_3 will be highest. Such an inactivation mechanism, based on store depletion, would help to explain why the neurons within the circuit are weakly coupled during most of the interburst interval. During this interburst interval, the internal store will fill up and the InsP_3Rs will re-sensitize such that the neurons within the circuit can participate in driving another burst of activity. Re-charging the internal store could thus

Module 10: | Figure SP and respiration

Substance P (SP) accelerates neurons of the respiratory centre.

The drawing on the left illustrates the location of the respiratory centre in the neonatal mouse medulla where recordings were made of the population activity (VRG) and the membrane potential (V_m) from a whole cell patched-clamped neuron. Reproduced from *J. Neurosci.*, Vol. 24, Peña, F. and Ramirez, J.-M. (2004) Substance P-mediated modulation of pacemaker properties in the mammalian respiratory network, pp. 7549–7556. Copyright (2004); with permission from the Society for Neuroscience; see Peña and Ramirez, 2004.

play a role in setting oscillator frequency and could thus contribute to the modulation of the respiratory pacemaker mechanism.

Modulation of the respiratory pacemaker mechanism

An important feature of the respiratory pacemaker mechanism is its flexibility in that its output can be altered to respond to changing demands on the respiratory system. There are two main regulatory inputs (Module 10: Figure breathing control). Firstly, there are chemosensitive neurons located in the retrotrapezoid nucleus (RTN) region on the surface of the medulla that respond to an increase in the level of CO_2/H^+ by an increase in their firing rate (Module 10: Figure ventral respiratory column). These neurons then interact with the pre-BötC neurons responsible for generating the respiratory rhythms. Just how CO_2/H^+ are sensed remains to be determined. For example, it is not clear whether there are separate sensing mechanisms for CO_2 and H^+ or whether they act in combination. Whatever the mechanism turns out to be, it is evident that it ultimately functions to reduce the activity of K^+ channels to create the membrane depolarization necessary to increase neuronal firing rates to increase the frequency of the rhythm generator. One of the consequences of an increase in CO_2/H^+ is a local release of ATP, which precedes the increase in firing rate (see lower traces in Module 10: Figure hypercapnia accelerates respiration). This ATP then acts through P2X2 receptors to depolarize the dendrites of neurons that act to accelerate the rhythm of the pacemaker circuit. The second major regulatory input into the respiratory centre comes from the neuronal input originating from the peripheral chemoreceptors and from the higher pontine and cortical circuits, the latter of which are involved in volitional control of breathing (Module 10: Figure breathing control).

These various regulatory inputs release transmitters such as substance P (SP), acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) from the raphe serotonergic neurons, and substance P (SP) (e.g. from the 5-HT neurons that co-localize SP), that function to regulate the respiratory rhythm. For example, when SP is applied to the respiratory centre, there is an acceleration of the firing rate of the respiratory network (Module 10: Figure SP and respiration).

The SP, which acts through the tachykinin receptor neurokinin 1 (NK1), appears to accelerate oscillatory activity by enhancing the persistent I_{NaP} current that contributes to pacemaker activity. 5-HT acting through 5-HT_{2A} receptors controls the activity of the Cd^{2+} -insensitive neurons that may use I_{NaP} . Both the NK1 and 5-HT_{2A} receptors are coupled to $\text{G}_{q/11}$ that acts to stimulate phospholipase C β to generate both InsP_3 and diacylglycerol (Module 2: Figure InsP_3 and DAG formation). These transmitters may also function to accelerate the respiratory rhythm by raising the resting level of InsP_3 , which would increase the sensitivity of the InsP_3 receptors that seem to participate in generating the dendritic Ca^{2+} wave (Module 10: Figure respiratory pacemaker mechanism).

It is important to understand these regulatory mechanisms because defects in their function can lead to respiratory distress including sudden infant death syndrome (SIDS) and Rett syndrome.

References

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