

# THE OTHER SIDE OF THE ENGRAM: EXPERIENCE-DRIVEN CHANGES IN NEURONAL INTRINSIC EXCITABILITY

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Modern theories of memory storage have largely focused on persistent, experience-dependent changes in synaptic function such as long-term potentiation and depression. But in addition to these synaptic changes, certain learning tasks produce enduring changes in the intrinsic excitability of neurons by changing the function of voltage-gated ion channels, a change that can produce broader, even neuron-wide changes in synaptic throughput. We will consider the evidence for persistent changes in intrinsic neuronal excitability — what we will call intrinsic plasticity — that is produced by training in behaving animals and by artificial patterns of activation in brain slices and neuronal cultures. These intrinsic changes might function as part of the engram itself, or as a related phenomenon such as a trigger for the consolidation or adaptive generalization of memories.

## SYNAPTIC STRENGTH

The amplitude of the postsynaptic potential that is evoked by a single shock to a population of axons.

## ASSOCIATIVITY

The property of long-term potentiation (LTP) whereby weak stimulation of a synaptic input, which will not elicit an increase in synaptic strength, can lead to the onset of LTP if strong stimulation is simultaneously applied to an independent input to the same postsynaptic cell.

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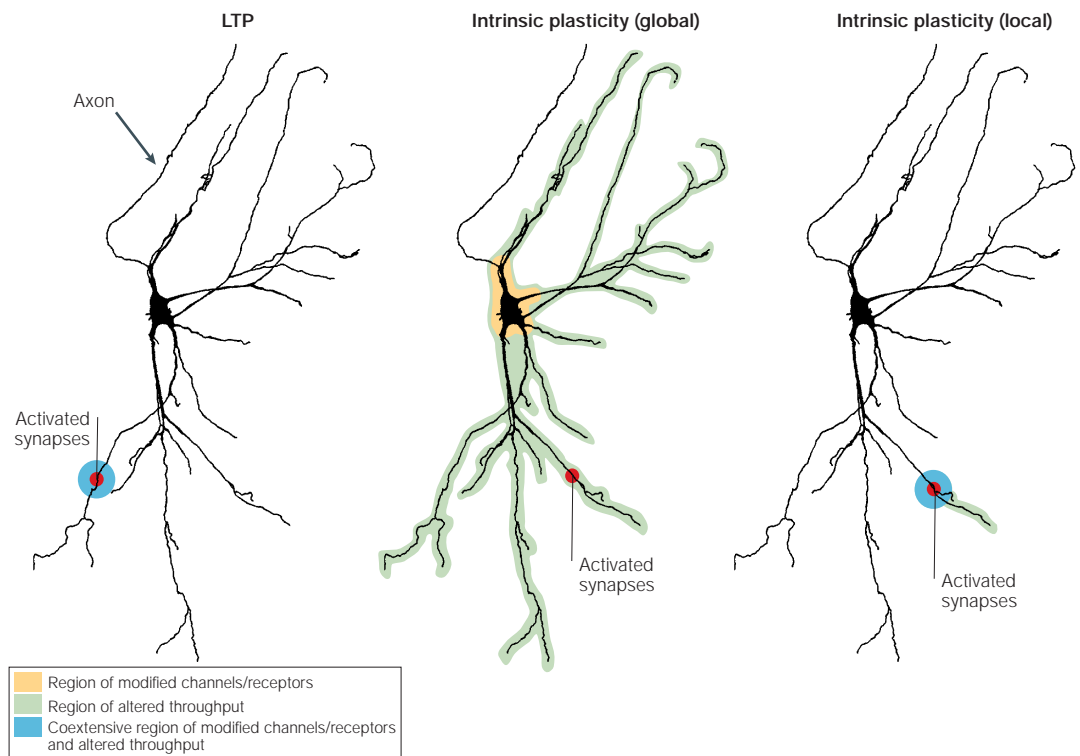
In recent years, several lines of evidence have supported the hypothesis that memory storage involves persistent, experience-dependent modulation of synaptic function. Synapses can be modified in many ways, but the function that has been investigated most assiduously has been SYNAPTIC STRENGTH. This alteration of synaptic strength typically takes the form of long-term potentiation or depression (LTP or LTD, respectively) of excitatory, glutamatergic synapses. Considerable interest in these phenomena has been generated by evidence that certain forms of behavioural training can produce LTP or LTD-like synaptic changes in defined brain regions, and that attempts to interfere with LTP/LTD have been found to block learning tasks<sup>1,2</sup>.

LTP and LTD have also been attractive model systems of memory storage because of their computational properties such as ASSOCIATIVITY and INPUT SPECIFICITY (FIG. 1). So, a memory storage system that uses input-specific changes in synaptic strength has a potentially enormous storage capacity, on the order of  $10^4$  synapses per neuron, multiplied by  $10^{11}$  neurons.

Whereas synapses mediate fast intercellular signalling in the nervous system, information is ultimately conveyed by action potentials, which are typically initiated

in the axon hillock and adjacent somatic membrane as the result of integrated synaptic activity in the dendrite and soma. So, an information-storage mechanism, in which a particular pattern of synaptic activity modulated voltage-gated ion channels in the axo-somatic compartment, would change the THROUGHPUT of all the synapses that impinge on the dendrites or soma of the postsynaptic neuron. As such, the storage capacity of this mechanism is considerably lower than synaptic alteration. Does this mean that information storage by intrinsic modulation is of such a low capacity as to be unimportant? We believe this is not the case.

First, it is important to remember that the real question is not whether we can build the storage capacity of the mammalian brain only with intrinsic plasticity, but what additional computations can be performed by adding intrinsic plasticity to the circuit? Second, intrinsic plasticity does not necessarily always involve axo-somatic ion channels. It has recently become clear that dendrites are also endowed with voltage-gated ion channels, and these channels influence both the conduction of synaptic signals to the axo-somatic region and the backpropagation of action potentials into the dendrite. If a particular pattern of synaptic



**Figure 1 | Input-specificity and candidate memory storage mechanisms.** Long-term potentiation (LTP) is typically evoked by brief, high-frequency stimulation of a small group of synapses, shown in red (left panel). This gives rise to persistent increases in the strength of these activated synapses and, perhaps, of some near neighbours on the postsynaptic cell, shown in blue. So, the probability of spike firing as a consequence of synaptic activation (the throughput function) is altered only for those synapses that express LTP. In the case of long-term changes in intrinsic excitability, there are two possible outcomes. The centre panel depicts global modulation in which activation of a delimited set of synapses (red) produces an increase in excitability by modulating voltage-gated ion channels in the axo-somatic membrane of the postsynaptic cell (yellow). Because these altered channels are interposed between all of the synapses that are received by the neuron and the axo-somatic spike initiation zone, this results in a global increase in synaptic throughput (green). The right panel shows an alternative form of intrinsic plasticity. In this case, activation of a small set of synapses (red) produces a delimited alteration of dendritic voltage-gated ion channels in the postsynaptic cell (blue). This could be driven by a local  $Ca^{2+}$  transient. This small region of altered voltage-gated channels could then alter synaptic throughput for a module consisting of adjacent synapses (blue) as well as those that are more distal on the same dendritic branch (green).

activation on a dendrite gave rise to a local change in voltage-gated channels (as has recently been suggested<sup>3</sup>), this could produce a change in throughput that is restricted to a certain dendritic module — bigger than a single synapse, but smaller than a whole neuron (FIG. 1). Therefore, the information-storage capacity of intrinsic plasticity might be larger than commonly assumed. Third, although it is easy to imagine that we need a ‘really big hard drive’ for memories stored over a lifetime of experience, this might not always be the case. It might be useful for some memories to be stored, in part, using mechanisms that are not synapse-specific. Whereas memories for facts and events (declarative memories) might need a high level of specificity, non-declarative memories might, in some cases, be rendered more useful by certain forms of generalization, as we discuss later.

Intrinsic plasticity produced by learning tasks  
**Invertebrate preparations.** Alkon and co-workers described one of the first examples of intrinsic plasticity elicited by behavioural training in the nudibranch mollusk *Hermisenda*. *Hermisenda*, like most

nudibranchs, shows positive phototaxis, a tendency to move towards light. However, when light and rotation are paired, the positive phototactic response is reduced or eliminated. This learning persists for weeks and is associative — it is not produced by light alone, rotation alone, or unpaired presentations of light and rotation<sup>4</sup>. In the *Hermisenda* eye, a photoreceptor (the type B cell) is excited by light and also by synaptic drive from STATOCYST cells that are activated by rotation. The type B cell sends inhibitory projections to type A cells, which excite identified interneurons. These interneurons in turn activate motor neurons to cause the foot-turning response that underlies positive phototaxis. When light and rotation are paired, the excitability of the type B photoreceptor is increased when challenged with either light or direct somatic current injection. This results in a greater number of action potentials evoked by either stimulus. When voltage-clamp recordings were made from type B cells, paired (but not unpaired) training resulted in a decrease of the transient  $K^+$  current  $I_A$ , and a  $Ca^{2+}$ -sensitive  $K^+$  current (but not a voltage-sensitive  $Na^+$  current)<sup>5</sup>. Like conditioning, this increase in excitability persists for weeks and is not produced by

**INPUT SPECIFICITY**

The property of long-term potentiation whereby strong synaptic stimulation only elicits an increase in synaptic strength at the activated pathway, leaving every other input unaffected.

**THROUGHPUT**

The probability of a single synapse evoking an action potential.

**STATOCYST**

Organ that mediates balance in many invertebrates. It consists of a fluid-filled sac that contains statoliths (minute calcareous particles) that stimulate sensory cells and help indicate position when the animal moves.

unpaired stimulation. The increase in excitability seems to be intrinsic to the type B photoreceptor (rather than a consequence of altered tonic synaptic drive or the release of a diffusible modulatory substance), as it persists even when this cell is surgically isolated from neighbouring cells. In individual animals, the amplitude of the increase in intrinsic excitability was positively correlated with the degree of phototactic suppression, pointing to a causal relationship. In the case of *Hermisenda*, the computational requirements of the type B photoreceptor cell are low: the conditioned stimulus — light — is a unitary input, so a global change in excitability is an appropriate memory mechanism.

Another useful invertebrate preparation for studying associative learning is the terrestrial snail *Helix*. When an air puff is delivered to the PNEUMOSTOME as an unconditioned stimulus, its reflexive closure is elicited. A shell tap, which does not normally induce pneumostome closure, was used as a conditioned stimulus, and robust associative learning developed with 150 pairings of shell tap and air puff. When microelectrode recordings were made after training, the animals that received paired stimulation (but not controls) showed significant reductions in spike threshold and positive excursions in resting potential in four identified interneurons that drive pneumostome closure<sup>6,7</sup>.

The marine mollusk *Aplysia* has been useful for studying learning for many years. The gill/siphon withdrawal reflex in *Aplysia* undergoes non-associative learning (HABITUATION and SENSITIZATION) and associative conditioning. In the latter case, a brief siphon tap is used as the conditioned stimulus, a tail shock is used as the unconditioned stimulus, and siphon withdrawal is measured as the conditioned response. In a semi-intact preparation, it is possible to record from synaptically connected siphon sensory and motor neurons in the abdominal ganglion during training<sup>8</sup>. As learning develops, there is a commensurate increase in the strength of the sensory neuron–motor neuron synapse. This increase seems to reflect two different forms of synaptic potentiation: one triggered by activation of presynaptic cAMP-dependent protein kinase A (PKA), acting through presynaptic spike broadening, and second one triggered by postsynaptic NMDA (*N*-methyl-D-aspartate) receptors and a postsynaptic Ca<sup>2+</sup> transient<sup>8,9</sup>. However, in addition to these synaptic changes, the sensory neuron shows increases in intrinsic excitability, which develop in parallel with the conditioned response: the number of spikes that is evoked by direct current injection or a siphon tap is increased, as is the INPUT RESISTANCE. By contrast the intrinsic excitability of the motor neuron is not altered. Furthermore, ‘off-field’ sensory neurons that were not driven by the siphon tap did not show altered intrinsic excitability<sup>8</sup>. In this case, the increase in intrinsic excitability of the sensory neuron and the presynaptic component of the synaptic potentiation might be related, as an attenuation of axo-somatic K<sup>+</sup> channels of the sensory neuron can produce both effects.

Similar results have been reported in *Aplysia* sensory neurons from pleural–pedal ganglia after the acquisition of non-associative, tail-induced sensitization of the siphon withdrawal reflex<sup>10</sup>. In this case, depolarizing current injections into sensory neurons disclosed an increase in the number of evoked spikes and an increase in the afterdepolarization that followed an evoked single spike. Interestingly, changes in the intrinsic properties of motor neurons were also seen, with sensitized motor neurons having a more negative resting membrane potential and a reduction in spike threshold. Recordings from the identified interneuron LP117 showed no changes in intrinsic properties, ruling out a ganglion-wide alteration. As in the case of *Aplysia* associative conditioning, sensitization was also associated with a potentiation of the sensory–motor synapse, pointing to a common theme of combined intrinsic and synaptic plasticities in the engram for *Aplysia* reflexes.

Another semi-intact invertebrate preparation in which it has been possible to make intracellular recordings during the training process is in the medicinal leech *Hirudo*, which has a defensive withdrawal reflex that consists of whole-body shortening in response to light touch or mild shock. Repeated presentations of a mild shock result in habituation of this reflex — a suppression of the amplitude of whole-body shortening. If a strong conditioning shock is delivered to a different body segment than the test shock (in a manner that does not require pairing with the mild test shock), sensitization manifests as a persistent increase in the amplitude and duration of the shortening response. Sensitization seems to require a particular interneuron, the S-cell, and serotonergic drive to this interneuron (which is not serotonergic itself), as either lesion of this interneuron or depletion of serotonin blocks this phenomenon (see REF. 11 for review). Sensitization was accompanied by a gradual increase in S-cell intrinsic excitability that developed in parallel to the increase in the amplitude of the shortening reflex. It was measured as a decrease in spike threshold and an increase in the number of action potentials elicited by direct depolarizing current injection<sup>12</sup>. The effects of sensitization on both the shortening reflex and S-cell excitability could be mimicked by serotonin. Habituation of the shortening response developed in parallel with a decrease in S-cell intrinsic excitability, mimicked by a lower dose of serotonin. Although changes in S-cell excitability probably contribute to sensitization and habituation of the shortening reflex, it should be noted that shortening is not triggered by S-cell stimulation alone, leaving open the possibility of several plastic sites for these simple memories.

**Vertebrate preparations.** To our knowledge, the first report of intrinsic plasticity from behavioural training involved associative conditioning in cats<sup>13</sup>. In this study, an auditory click (conditioned stimulus) preceded a GLABELLA tap (unconditioned stimulus) and, after many pairings, a short-latency conditioned response, which consisted of a combined eyeblink and nose twitch,

#### PNEUMOSTOME

A small opening in the mantle of gastropods through which air passes.

#### HABITUATION

The cessation of a response upon repeated presentations of a stimulus.

#### SENSITIZATION

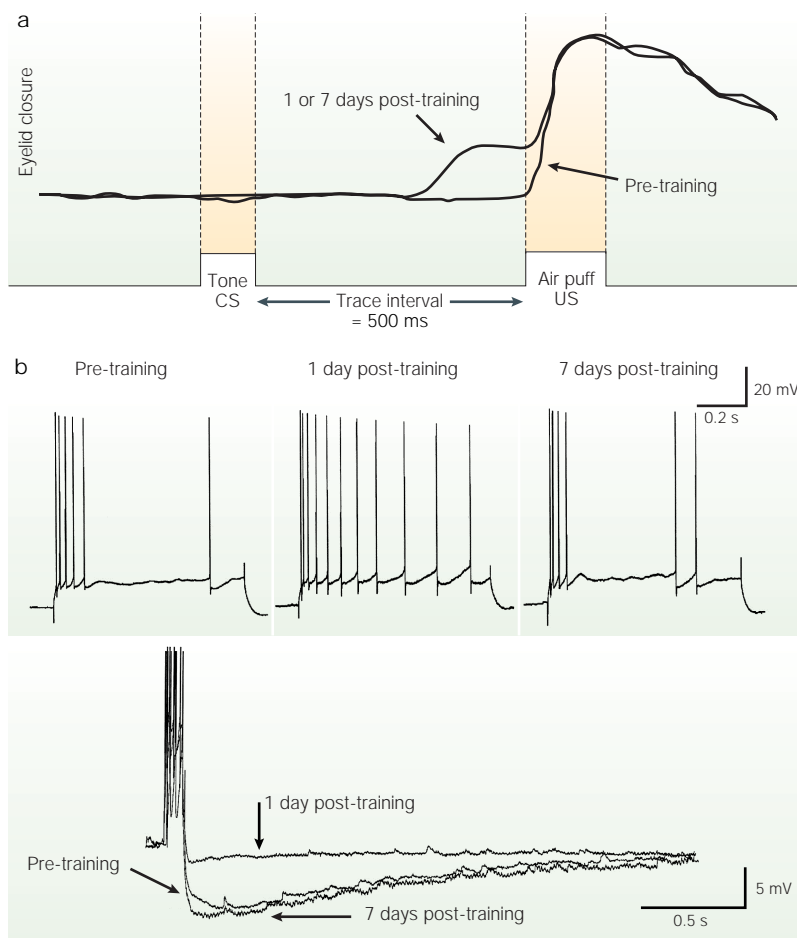
The unspecific augmentation of a behavioural response to a stimulus after the animal has been exposed to an injurious stimulus.

#### INPUT RESISTANCE

The voltage change that is elicited by the injection of current into a cell, divided by the amount of current injected.

#### GLABELLA

The smooth area between the eyebrows just above the nose.



**Figure 2 | Intrinsic plasticity evoked by associative eyelid conditioning.** **a** | Trace eyelid conditioning in rabbits is evoked by repeated pairings of a neutral tone (conditioned stimulus, CS) with an air puff (unconditioned stimulus, US), separated by 500 ms (trace interval). In an untrained animal, the only response is a reflexive blink in response to the air puff (the unconditioned response). In an animal that has received many pairings of these stimuli, a carefully timed eyeblink (the conditioned response) is evoked so that the eyelid is partially closed when the air puff arrives. Animals tested 7 days after training still retain a strong memory for this association. **b** | Hippocampal slices were prepared from rabbits either prior to training or 1 or 7 days post-training, and microelectrode recordings were made from CA1 or CA3 pyramidal neurons. One day after training, both CA3 and CA1 neurons showed increased intrinsic excitability as indexed by a reduction in spike accommodation during prolonged depolarizing current injection and a reduced post-burst afterhyperpolarization. Seven days after training, when memory for the association remains strong, intrinsic excitability has returned to baseline values. Modified, with permission, from REF. 20 © the Society for Neuroscience (1996).

was acquired. When intracellular recordings were made from the pericruciate sensorimotor cortex in awake cats, the amount of positive current that was required to evoke a spike was significantly lower in neurons from trained animals as compared with controls. This effect was confined to those cells that ultimately projected to the musculature involved in the conditioned response and persisted for at least 28 days (after which the study was terminated). When production of the conditioned response was extinguished by repeated presentation of the conditioned stimulus alone, the increase in excitability was not abolished. Retraining after extinction in this and other associative conditioning protocols results in a much faster rate of acquisition during retraining,

**AFTERHYPERPOLARIZATION**  
The membrane hyperpolarization that follows the occurrence of an action potential.

a phenomenon called 'savings'. It is tempting to speculate that the increase in excitability in these cortical neurons could have served as the engram for savings. It is worthwhile noting that, in intact tissue and in the absence of drugs, it is not possible to know whether this training-induced increase in excitability was truly intrinsic to the cortical neurons or was secondary to some other change, such as reduced tonic drive from inhibitory interneurons.

The rabbit has been a particularly useful preparation for studying associative conditioning of an eyelid-closure reflex. When a brief air puff (unconditioned stimulus) is delivered to the eye, a reflexive blink is elicited (the unconditioned response). When a neutral (conditioned) stimulus, such as a tone, is repeatedly paired with an air puff, the rabbit will learn to blink in a carefully timed manner, such that the eyelid is closed when the air puff arrives (the conditioned response). When a trace interval is imposed between the offset of the tone and the onset of the air puff, this task is then called 'trace eyelid conditioning' and becomes sensitive to hippocampal inactivation. The memory for trace eyeblink conditioning can last for weeks to months (FIG. 2a).

Disterhoft and co-workers trained rabbits in trace eyelid conditioning, and prepared hippocampal slices for intracellular recording 24 hours later. They found that pyramidal neurons in areas CA1 and CA3, but not granule cells of the dentate gyrus, show an increase in the number of spikes evoked by depolarizing current injection and a reduction in the amplitude of the AFTERHYPERPOLARIZATION evoked by a spike burst<sup>14-16</sup> (FIG. 2b,c). Voltage-clamp analysis of CA1 cells from conditioned animals<sup>17</sup> showed a decrease in the  $Ca^{2+}$ -sensitive  $K^+$  current  $I_{AHP}$  (perhaps in  $sI_{AHP}$  as well), but not in the  $K^+$  currents  $I_C$  or  $I_{M1}$ , or the hyperpolarization-activated cation current  $I_h$ . This increase in intrinsic excitability was not found for animals that received unpaired tone and air puff stimulation, nor was it found in a subpopulation of animals that were unable to learn the task<sup>18</sup>. The observation that these changes persist in hippocampal slices makes it unlikely that they result from tonic activity in an extrahippocampal structure. Furthermore, conditioning-specific decreases in  $I_{AHP}$  could be recorded from voltage-clamped CA1 pyramidal neurons bathed in the  $Na^+$ -channel blocker tetrodotoxin (TTX) to suppress action potentials. This result indicates that the alteration is truly intrinsic to the recorded cell and not the result of tonic synaptic drive from other neurons in the slice<sup>17,19</sup>.

Does the training-induced increase in intrinsic excitability recorded in CA1 and CA3 pyramidal neurons represent a part of the engram for trace eyelid conditioning? One observation that bears on this question is that >50% of all the cells sampled in these regions of the dorsal hippocampus showed intrinsic changes. In our view, this reduces the probability that these intrinsic alterations constitute a portion of the memory trace *per se*, and makes it more likely that they have some permissive function in memory. Consistent with this view is the finding that 7 days after training, when the memory for trace eyelid conditioning is still strong,

the increases in intrinsic excitability of CA1 and CA3 pyramidal neurons are no longer present<sup>20,21</sup>. One possibility is that transient changes in intrinsic excitability render the hippocampal network more excitable, thereby increasing the probability that LTP and LTD occur in the hippocampus proper or in sites that receive hippocampal projections, and that these synaptic alterations ultimately constitute the long-term engram.

Associative eyelid conditioning that is performed using a tone (conditioned stimulus) and a co-terminating air puff or weak periorbital shock (unconditioned stimulus) is called 'delay eyelid conditioning'. This task is not sensitive to hippocampal lesions, but is quite sensitive to inactivation of both the cerebellar cortex and cerebellar deep nuclei (see REF. 22 for review). When cerebellar slices were cut from rabbits that had acquired delay eyelid conditioning, and microelectrode recordings were made from Purkinje cells, a set of familiar alterations occurred: a reduced spike threshold and an attenuated afterhyperpolarization when compared with controls<sup>23,24</sup>. The degree of reduction of the dendritic spike threshold was positively correlated with the performance in the delay eyelid task in animals that received paired training. Unlike the alterations found after trace eyelid conditioning in hippocampal pyramidal neurons<sup>20,21</sup>, the intrinsic plasticity in Purkinje cells was still present 30 days after training. Also, unlike the hippocampal case where intrinsic alterations were widespread, Purkinje cells with altered excitability were only found in a defined microzone of the cerebellar lobule HVI.

A similar set of findings has emerged from studies by Barkai and co-workers on an OPERANT CONDITIONING task. In this protocol, thirsty rats are trained to discriminate odour pairs for a water reward. After training, recordings were made from layer 2 pyramidal cells of the piriform cortex. When rats were trained to discriminate one pair of odours, a reduction in both post-burst afterhyperpolarization and spike ACCOMMODATION during a prolonged depolarization was seen when compared with pyramidal cells from naive or control rats<sup>25–27</sup>. This intrinsic change was evident 1–3 days after training, but had decayed to baseline values by 5–7 days after training. Interestingly, rats take a long time to learn discrimination of the first odour pair, but the subsequent pairs are acquired about fivefold more quickly if they are presented soon after the first pair (see REF. 28 for review). This has been called 'rule learning' and is a form of non-declarative memory that is independent of the memories of any particular odours. Interestingly, if we train a rat to discriminate one odour pair and then interpose a 5–7-day delay before training with the second odour pair, rule learning decays and the rat will behave as if it were untrained. However, a 1–2-day delay does not produce this effect. As this is roughly the same time course as the decay of intrinsic excitability in layer 2 pyramidal cells, it is tempting to speculate that the increase in intrinsic excitability underlies rule learning by rendering the circuit in piriform cortex hyperexcitable.

**Some general conclusions.** When considering this literature several themes emerge. First, across a large number of organisms and learning tasks, training produces increases in excitability that are manifest as a reduction in spike threshold, spike accommodation and amplitude of burst-evoked afterhyperpolarization, all of which point to modulation of K<sup>+</sup> channels as one potential mechanism. Second, in at least some cases we can be confident that these changes are intrinsic to the recorded neurons rather than resulting from alterations in tonic synaptic drive. Third, in at least some cases training-induced intrinsic plasticity is accompanied by persistent changes in synaptic strength in the same neurons or in related portions of a local circuit. Fourth, in many cases the degree of intrinsic plasticity is positively correlated with some measure of learning, pointing to the possibility of a causal relationship between intrinsic plasticity and behaviour.

The exact nature of this relationship is an area where some speculation is warranted. It is likely that in some situations training-induced intrinsic plasticity constitutes the engram for that training or for a part of it. This might be appropriate in cases such as the *Hermisenda* type B photoreceptor, for which the computational requirements are low. However, there are also cases in which there are dissociations between intrinsic plasticity and memory. These include hippocampal intrinsic plasticity after trace eyelid conditioning and intrinsic plasticity of the piriform cortex after odour-discrimination learning, in which memory for the training persists longer than the intrinsic plasticity. Also, intrinsic plasticity of the pericruciate cortex persists even after conditioned responses have undergone extinction. In cases in which intrinsic plasticity decays within a few days, it is tempting to speculate that it might be involved in creating a hyperexcitable state to promote consolidation of memory by other means, such as LTP and LTD.

In all of these cases, intrinsic plasticity might form the neural substrate for some form of adaptive generalization of memory. For various forms of associative conditioning this might involve the phenomenon of savings, in which the rate of acquisition on retraining is higher than the naive state. A related phenomenon called 'conditioned-stimulus generalization' might also result from intrinsic plasticity. In this situation, associative conditioning to one conditioned stimulus (such as a tone) together with an unconditioned stimulus (such as an air puff) is followed by extinction of learning after repetitive presentation of the tone alone. Following this, pairing of a new conditioned stimulus (such as a light) with an air puff will result in a rapid rate of acquisition that exceeds the naive state. A similar phenomenon is rule learning for odour discrimination; training to discriminate one odour pair results in an enhancement of the learning rate for subsequent pairs, if they follow within the same time period in which intrinsic excitability persists. So, the more global nature of intrinsic plasticity at a neuronal level (its ability to affect throughput from a larger number of synapses than LTP and LTD) might be useful for the adaptive generalization of non-declarative memory.

**OPERANT CONDITIONING**  
Form of conditioning in which the subject learns from the consequences of its actions, thereby modifying its behaviour.

**ACCOMMODATION**  
The cessation of spike firing despite constant depolarization above firing threshold.

Intrinsic plasticity and other forms of experience Neural plasticity is a broad term that includes learning, but it also includes other phenomena such as the adaptation of neural circuits to altered forms of sensory input or even trauma. In the free-swimming *Xenopus* tadpole, a 4-h exposure to an enhanced visual motion stimulus (a flashing bank of light-emitting diodes) gives rise to a set of changes in the optic tectum. These changes include increased dendritic growth rate<sup>29</sup> and a new form of depression of retinotectal synapses that involves increased synthesis of intracellular polyamines, which then block Ca<sup>2+</sup>-permeable AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors<sup>30</sup>. In addition to these morphological and synaptic changes, the intrinsic excitability of tectal neurons was also increased; there was a reduction in spike threshold and spike accommodation<sup>31</sup>. In voltage-clamp recordings, tectal neurons from visually stimulated tadpoles had significantly larger voltage-gated Na<sup>+</sup> currents, but no change in transient and sustained K<sup>+</sup> currents or an INWARD PLATEAU CURRENT. The intrinsic plasticity seemed to be a consequence of polyamine-mediated synaptic depression, as inhibition of ornithine decarboxylase, the limiting step in polyamine synthesis, blocked the increase in intrinsic excitability that was produced by visual stimulation. It is to be expected that the consequences of synaptic depression and the increases in intrinsic excitability would offset. Interestingly, this was not the case: four hours of enhanced visual stimulation resulted in a decreased rate of background firing in tectal cells and in an increased number of spikes evoked by a brief whole-field visual stimulus, resulting in a markedly improved signal-to-noise ratio.

Another model in which the brain adapts to altered sensory input is vestibular compensation after damage to one vestibular labyrinth or vestibular nerve (see REF. 32 for review). Immediately after unilateral vestibular trauma, a large asymmetry is present in the basal firing rate of neurons in the vestibular nucleus, resulting in inappropriate activation of vestibulo-ocular and vestibulo-spinal reflexes. However, these symptoms are reduced over time, and this is correlated with an increase in the basal firing rate of neurons in the vestibular nucleus on the lesioned side. In the guinea pig and rat, this seems to result, in part, from an increase in the intrinsic excitability of a subpopulation of neurons in the medial vestibular nucleus called type B cells<sup>33–36</sup>. This increase in excitability is manifest as an increase in the basal firing rate, a positive excursion in the resting potential and an increase in the amplitude of a fast after-hyperpolarization. Interestingly, when the ipsilateral cerebellar flocculus (the source of extrinsic inhibition to the relevant medial vestibular nucleus neurons) is lesioned together with the labyrinth in rat, vestibular compensation (as measured behaviourally) and compensatory increases in excitability (as measured in medial vestibular nucleus brain slices) are blocked<sup>37</sup>. The mechanism by which the flocculus drives compensatory increases in excitability in the medial vestibular nucleus seems to involve a stress response that is mediated by glucocorticoids. If rats are kept under stable anaesthesia

for 4–6 hours after labyrinthectomy to reduce acute stress, or if they are treated with a glucocorticoid receptor antagonist either systemically<sup>38</sup> or with injection into the ipsilateral flocculus<sup>37</sup>, then compensatory increases in excitability in the medial vestibular nuclei are blocked. Similar to many cases that we described earlier, intrinsic plasticity is also accompanied by synaptic changes. In vestibular compensation, there is a decrease in the efficacy of GABA ( $\gamma$ -aminobutyric acid) synapses in the medial vestibular nucleus<sup>39,40</sup>.

Finally, let us consider some examples in which experience-dependent changes in intrinsic plasticity are clearly maladaptive. Febrile seizures, the most common form of developmental seizure, have been studied in a model system in which rats were briefly heated with warm air to produce hyperthermia for 30 minutes. This resulted in a complex seizure that last for ~20 minutes, and involved the hippocampus and amygdala<sup>41</sup>. Hippocampal slices were then prepared 1–9 weeks later, and recordings were made from CA1 pyramidal neurons<sup>42</sup>. This revealed a negative shift in the voltage dependence of activation for the hyperpolarization-activated cation conductance  $I_h$ . As these recordings were made in the presence of glutamate- and GABA-mediated transmission blockers and in the presence of TTX, they probably reflect an intrinsic alteration of the CA1 pyramidal cell.

When *in situ* hybridization was performed on hippocampal slices from rats with febrile seizure, an alteration in the pattern of  $I_h$  channels was seen<sup>43</sup>: a reduction in HCN1 (hyperpolarization-activated and cyclic nucleotide-gated 1), and an increase in HCN2. This change in expression was not seen in rats in which seizures were not generated. It will be interesting to determine in a heterologous expression system whether this change in the HCN1/HCN2 ratio can mimic the negative shift in the voltage-dependence of  $I_h$  produced by febrile seizures.

A functional consequence of this alteration of  $I_h$  is that hyperpolarizing stimuli, such as those that arise from a burst of GABA-mediated inhibitory postsynaptic potentials (IPSPs), are much more likely to evoke spiking as the hyperpolarization terminates (a phenomenon called rebound excitation). In the case of febrile seizures, this is particularly interesting as they also result in a potentiation of GABA-dependent drive to CA1 pyramidal cells<sup>41</sup>, which by itself would be expected to be antiepileptogenic, but becomes epileptogenic when coupled with enhancement of  $I_h$ .

Intrinsic changes have also been observed in another model of epilepsy in which chronic temporal lobe seizures are evoked in adult rats by a single dose of the muscarinic agonist pilocarpine. Recordings were made from hippocampal CA1 pyramidal neurons<sup>44</sup> and subicular neurons<sup>45</sup> 2–6 weeks after pilocarpine treatment. In both locations, neurons from rats with chronic seizures showed a conversion in firing mode from tonic firing to bursting, which was accompanied by development of a spike afterdepolarization. Pharmacological experiments showed that expression of bursting and the enhanced afterdepolarization could be

INWARD PLATEAU CURRENT  
A current that inactivates slowly, resulting in a sustained depolarization.

attenuated by blockers of T-type  $\text{Ca}^{2+}$  channels. Consistent with this finding, patch-clamp recordings disclosed a selective increase of T-type  $\text{Ca}^{2+}$  current in these bursting cells<sup>46</sup>.

These examples show that experience can produce a wide range of persistent changes in intrinsic neuronal excitability, involving diverse effectors such as voltage-gated  $\text{K}^+$ ,  $\text{Na}^+$  and  $\text{Ca}^{2+}$  channels, as well as  $I_h$ .

Slow changes in intrinsic excitability in culture  
So far, the forms of training and environmental alteration that produce changes in intrinsic excitability are characterized by the fact that the behavioural effects accrue slowly, over hours to days — for example, asymptotic acquisition of an associative eyelid response typically requires hundreds of trials. Can similar slowly developing changes in intrinsic excitability be observed *in vitro*? Most work on this front has been done in neuronal cultures, in which chronic treatments, recording and imaging are technically straightforward.

In the spiny lobster *Panulirus* and the crab *Cancer*, stomatogastric ganglion (STG) neurons function in a well-defined circuit to generate gastric mill rhythms (see REF. 47, for review). *In vivo*, where they are exposed to synaptic and neuromodulatory drive, these neurons fire in bursts. When deprived of this input by pharmacological blockade *in vivo* or immediately after being placed in culture, these neurons are mostly silent and respond to depolarizing current or to the offset of hyperpolarizing current with tonic firing. However, after 2–4 days in culture, still isolated from synaptic input, these neurons changed their activity from tonic to burst firing, thereby re-acquiring aspects of their *in vivo* firing pattern<sup>48</sup>. In lobster STGs, this involved upregulation of several inward currents, including voltage-sensitive  $\text{Ca}^{2+}$  currents, a rapidly inactivating voltage-sensitive  $\text{Na}^+$  current, a slowly-inactivating  $\text{Na}^+$  plateau current, and downregulation of the transient  $\text{K}^+$  current  $I_A$  and of a DELAYED OUTWARD-RECTIFIER  $\text{K}^+$  CURRENT<sup>49</sup>. This modulation was bi-directional, as application of repetitive hyperpolarizing current pulses for one hour changed the firing pattern of bursting cultured neurons back to tonic firing. This firing-mode transition was dependent on the frequency of applied hyperpolarizing pulses and the occurrence of rebound bursts evoked by those pulses, and could be blocked by intracellular application of a fast  $\text{Ca}^{2+}$  chelator, indicating a dependence on  $\text{Ca}^{2+}$  influx<sup>48</sup>.

Persistent changes in intrinsic excitability can also be evoked in cultured vertebrate neurons by activity deprivation. Pyramidal neurons of the rat visual cortex that were cultured in the presence of TTX for two days showed an increased firing frequency and a lowered spike threshold<sup>50</sup>. This increase in intrinsic excitability was mediated by upregulation of a  $\text{Na}^+$  current and downregulation of tetraethylammonium ion-sensitive  $\text{K}^+$  currents that include the delayed outward-rectifier  $\text{K}^+$  current and the  $\text{Ca}^{2+}$ -sensitive  $\text{K}^+$  current), whereas  $I_A$  and tetraethylammonium ion-insensitive  $\text{K}^+$  currents were unchanged. Chronic TTX treatment also caused an increase in the intrinsic excitability of inhibitory interneurons.

Activity deprivation produced by synaptic blockade might also lead to intrinsic plasticity in presynaptic neurons. In a *Xenopus* motoneuron–myocyte culture system, chronic blockade of cholinergic transmission caused a broadening of action potentials and a decrease in repetitive firing in the presynaptic motoneuron<sup>51</sup>. The action potential broadening was due to an increase in the duration of its falling phase, and the decrease in repetitive firing resulted from an increase in the REFRACTORY PERIOD. Whole-cell patch recordings and recordings of outside-out MACROPATCHES that were pulled from the cell soma showed that synaptic blockade induced a global decrease in a delayed outward-rectifier  $\text{K}^+$  conductance by shifting the CURRENT-VOLTAGE RELATION to more depolarized potentials. This modulation probably underlies both the effects on spike broadening and refractory period.

Several studies have tried to chronically upregulate activity in neuronal cultures. In cultured crab STG neurons, several hours of low frequency stimulation (1 Hz) with depolarizing current pulses decreased the magnitudes of the delayed outward-rectifier current  $I_{\text{kd}}$  and the  $\text{Ca}^{2+}$ -sensitive  $\text{K}^+$  current  $I_{\text{K(Ca)}}$ , but increased the magnitude of  $I_A$ . This change was observed in the inferior cardiac neuron, but not in the lateral pyloric neuron (another type of STG neuron), indicating a cell-type-specific modulation of excitability. Spontaneous reversion of the currents was seen after the stimulation was stopped, and the effect was abolished when  $\text{Cd}^{2+}$  blocked  $\text{Ca}^{2+}$  influx<sup>52</sup>.

Another study using cultured rat myenteric neurons found that persistent depolarization of neurons (produced by elevated extracellular  $\text{K}^+$  concentration) caused a long-term reduction in the macroscopic voltage-sensitive  $\text{Ca}^{2+}$  current without changing the voltage-dependence of activation or inactivation<sup>53</sup>. However, the opposite effect was seen when cultured rat hippocampal neurons were repetitively stimulated by exposure to glutamate or elevated extracellular  $\text{K}^+$  for 1 h daily. This treatment led to a specific enhancement of the high- (but not the low-) voltage-activated  $\text{Ca}^{2+}$  current without changes in voltage dependence or kinetics. This effect was blocked by the translation inhibitor cycloheximide, indicating a requirement for new protein synthesis<sup>54</sup>.

Last, a study using cultured mouse dorsal root ganglion neurons found that both the high- and low-voltage-activated  $\text{Ca}^{2+}$  currents could be persistently down-regulated by chronic (40–70 h) electrical stimulation in a manner that is dependent on the frequency and pattern of stimulation<sup>55</sup>. So, both chronic enhancement and chronic blockade of activity in neuronal cultures can give rise to alterations in intrinsic excitability, and these alterations are diverse, including functional changes in postsynaptic  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Na}^+$  channels, and in the cultured *Xenopus* neuromuscular junction, in presynaptic  $\text{K}^+$  channels.

Rapid changes in intrinsic excitability  
Recently, several studies have indicated that persistent changes in intrinsic excitability can be rapidly induced (see also BOX 1). Our group has used intracellular

#### DELAYED OUTWARD-RECTIFIER $\text{K}^+$ CURRENT

A slowly activating and very slowly inactivating voltage-gated  $\text{K}^+$  conductance that preferentially passes  $\text{K}^+$  out of the cell.

#### REFRACTORY PERIOD

The period after a spike during which a neuron cannot fire a new action potential.

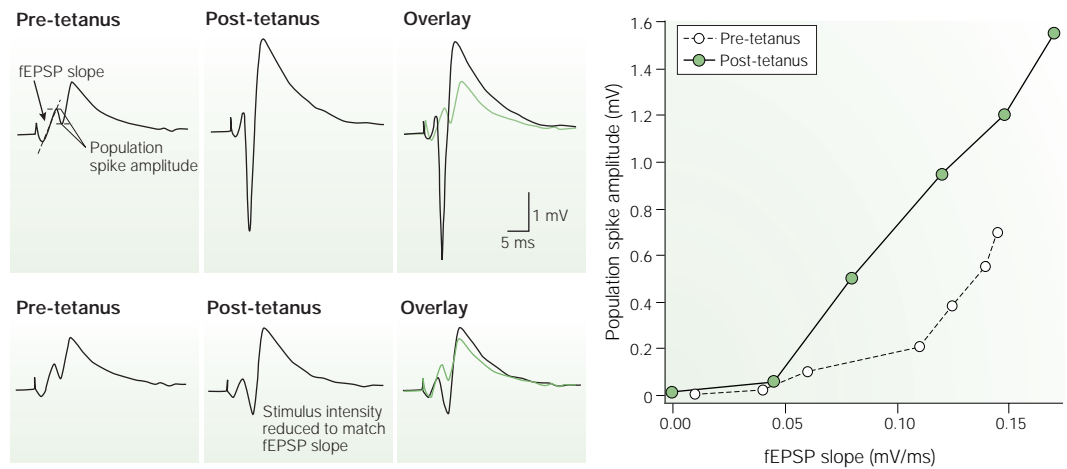
#### MACROPATCHES

Giant membrane patches that are commonly obtained to study membrane currents of cells that are too large to record with conventional patch-clamp methods.

#### CURRENT-VOLTAGE RELATION

A plot of the changes in ionic current as a function of membrane voltage.

Box 1 | The difficult case of E-S potentiation



In the original description of long-term potentiation (LTP), it was noted that the increase in the POPULATION SPIKE amplitude was greater than could be accounted for simply by the LTP-evoked increase in the population excitatory postsynaptic potential (EPSP)<sup>86</sup>. Furthermore, in some cases tetanic stimulation resulted in increases in the population spike even when the population EPSP was unchanged. This phenomenon was subsequently termed the non-synaptic component of LTP<sup>86–92</sup>, and later came to be known as EPSP–spike or E–S potentiation<sup>93,94</sup>. In field recordings, E–S potentiation is generally defined as a leftward shift in the curve that relates the amplitude of the population spike to the slope of the population EPSP at various stimulus intensities (see figure), indicating that a given field EPSP slope produces a larger population spike<sup>93,94</sup>. The intracellular correlate of E–S potentiation is an increased probability of firing for given EPSP amplitude<sup>93–95</sup>. More recently, it has also been shown that induction of long-term depression (LTD) in the hippocampus can be accompanied by E–S depression<sup>83</sup>.

The mechanisms underlying E–S potentiation are still controversial. Two main hypotheses have been put forward. The first states that it is owing to a decrease in the ratio of inhibitory to excitatory drive. This could either take the form of an absolute decrease in inhibitory drive or an increase in inhibitory drive that was less than that for excitatory drive<sup>88,95,96–98</sup>. The second is that E–S potentiation results from an increase in the intrinsic excitability of the postsynaptic neuron through modulation of postsynaptic voltage-gated conductances<sup>92,99–107</sup>. A straightforward prediction of the first hypothesis is that blockade of inhibitory synaptic transmission should occlude E–S potentiation. In SCHAFER COLLATERAL synapses in hippocampal slices, application of the GABA<sub>A</sub> (γ-aminobutyric acid subtype A) antagonist picrotoxin was found to cause E–S potentiation. Furthermore, picrotoxin-induced and tetanus-induced E–S potentiation occluded each other. In some cases, this occlusion/blockade was complete, arguing for a single mechanism that is mediated by inhibitory drive<sup>97</sup>, whereas in other cases, varying degrees of partial blockade have been seen<sup>83,98,108,109</sup>. Most recently, a 60% blockade of E–S potentiation by a GABA<sub>A</sub> receptor antagonist has been confirmed in CA1 pyramidal neurons using whole-cell recording<sup>110</sup>. Another approach to this question used a partially lesioned hippocampal slice preparation in which E–S potentiation was absent in reinnervated circuitry where feedforward inhibition was impaired<sup>88,96</sup>. Further evidence for the role of GABA-mediated drive includes observations that changes in inhibitory synaptic transmission occurred concomitantly with E–S potentiation. In the CA1 region of guinea pig hippocampal slices, the ratio of intracellular inhibitory postsynaptic potential (IPSP) peak to field EPSP slope was decreased following induction of E–S potentiation, indicating a relative decrease in inhibitory drive<sup>97</sup>. It was also observed that excitatory synapses on neighbouring interneurons undergo LTD after stimulation that causes LTP at synapses onto projection neurons<sup>111</sup>. More recently, it was found that E–S potentiation in CA1 pyramidal neurons is accompanied by a calcineurin-dependent LTD of GABA<sub>A</sub>-mediated IPSPs, and that blockade of calcineurin both pharmacologically and in mutant mice blocks both LTD of IPSPs and E–S potentiation<sup>112</sup>.

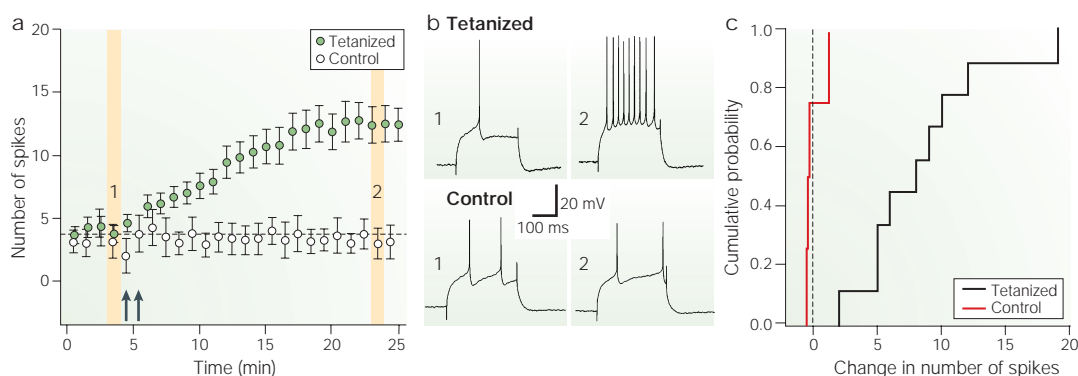
However, several lines of evidence indicate that increases in intrinsic excitability might also underlie E–S potentiation. Most directly, E–S potentiation can be observed in the presence of GABA<sub>A</sub> antagonists in many preparations<sup>83,98,101,103,104,107–109</sup>. In addition, field potential recordings in the CA1 region of the kainic acid-lesioned rat hippocampus — a preparation designed to eliminate inhibitory interneurons — showed that E–S potentiation remained intact<sup>92,106</sup>. Studies using intracellular recording in the CA1 region of rat hippocampal slice found that E–S potentiation was accompanied by a decrease in the spike threshold as evoked by direct depolarizing current, a decrease in spike frequency accommodation and a decrease in spike latency<sup>95,113</sup>. The reduction in firing threshold significantly correlated with the change in E–S potentiation, indicative of a possible causal relationship<sup>110</sup>. Another line of evidence comes from reports that have found little or no decrease in inhibitory drive on postsynaptic neurons after tetanic stimulation. Several have even found increased inhibitory drive<sup>99,109,114,115</sup>. In summary, it is probable that reduction in the ratio of inhibitory to excitatory drive cannot fully account for E–S potentiation at many synapses, and the balance is mediated by intrinsic plasticity.

POPULATION SPIKE

The summated action potential of the postsynaptic neurons that respond to a given stimulus as recorded with an extracellular electrode.

SCHAFER COLLATERALS

Axons of the CA3 pyramidal cells of the hippocampus that form synapses with the apical dendrites of CA1 neurons.



**Figure 3 | Intrinsic plasticity evoked by brief synaptic stimulation in the deep cerebellar nuclei.** **a** | High-frequency stimulation of excitatory synapses produced a sustained increase in intrinsic excitability of deep cerebellar nucleus neurons in a rat brain slice preparation. This figure shows the averaged time course of the number of spikes evoked during 200-ms-long depolarizing current pulses. Control experiments (open circles) show that a stable baseline can be recorded for at least 25 minutes. Application of a synaptic conditioning tetanus (at arrowheads, filled circles) resulted in a slowly developing increase in the number of spikes evoked by the test stimulus. Error bars = standard error of the mean. **b** | Representative traces from tetanized and control experiments at the time points indicated by numbered yellow bars in part **a**. **c** | A cumulative-probability distribution shows the resulting increase in the number of spikes measured at 20–25 minutes for the tetanized and control groups that are shown in **(a)**. Modified, with permission, from *Nature Neuroscience* REF. 56 © Macmillan Magazines Ltd (2000).

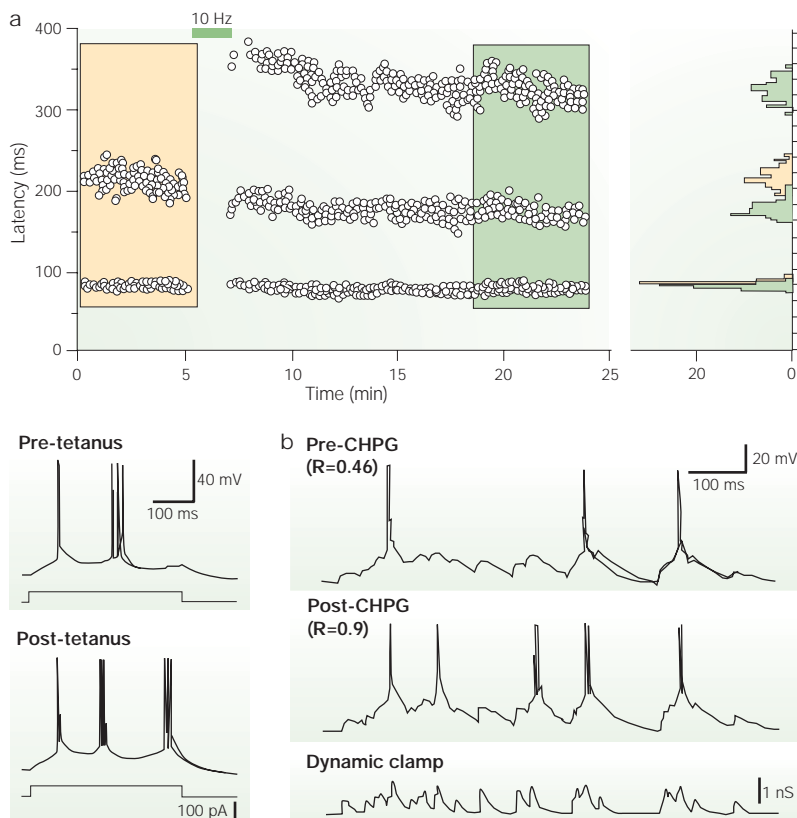
recording in rat cerebellar slices to show that persistent changes in intrinsic excitability can occur within minutes of stimulation in the cerebellar deep nuclei<sup>56</sup>, a structure that has been implicated in delay eyelid conditioning. Brief high-frequency stimulation delivered to glutamatergic mossy fibre inputs of deep cerebellar nucleus neurons caused a rapid and persistent increase in intrinsic excitability, as shown by an increase in the number of spikes evoked by a depolarizing test pulse and a decrease in the first-spike threshold (FIG. 3). Repeated application of strong intracellular depolarizing pulses can also induce an increase in intrinsic excitability with a similar time course (but smaller amplitude). The synaptically induced increase in intrinsic excitability was blocked by an NMDA receptor antagonist, and the high-frequency-induced increase was abolished by a blocker of voltage-sensitive  $\text{Ca}^{2+}$  channels, indicating that a  $\text{Ca}^{2+}$  transient might be required, but that this can arise from either mode of influx. Interestingly, this increase in intrinsic excitability had a secondary effect on the kinetics of the excitatory postsynaptic potentials (EPSPs) — their broadening being attributed to an increase in the duration of the falling phase.

In a study on the rat cerebellar slices, THETA-BURST stimulation (TBS) to the mossy fibres potentiated the intrinsic excitability of granule cells, which was expressed as an increase in the input resistance and a decrease in the spike threshold for spikes evoked by either direct current injection or EPSPs<sup>57</sup>. This long-lasting increase in intrinsic excitability was often evoked together with LTP of the mossy fibre–granule cell synapse. The induction of both phenomena depended on membrane depolarization during TBS and activation of NMDA receptors. However, a weak TBS could induce intrinsic plasticity but not LTP, whereas a stronger stimulus induced both. So, the increase in intrinsic excitability is distinct from LTP even though they can often co-occur.

Rapid intrinsic excitability changes are not restricted to targets of the cerebellar mossy fibres. A study of layer V neurons in the rat entorhinal cortex found that, in the presence of  $\text{GABA}_A$ , glutamate receptor antagonists and a muscarinic receptor agonist, a 5-s long depolarizing pulse can induce a persistent state of steady firing in neurons that were previously silent<sup>58</sup>. Remarkably, repeated depolarizing pulses led to accumulating, graded increases in basal firing frequency that were persistent and stable, and repetitive application of hyperpolarizing current pulses led to graded decreases in firing frequency. Persistent graded increases in firing frequency could also be induced by repetitive activation of excitatory synapses (with blockade of inhibitory transmission) and, conversely, persistent graded decreases in firing frequency could be induced by repetitive activation of inhibitory synapses (in the presence of glutamate receptor antagonists). These persistent changes in basal firing frequency were dependent on the muscarinic agonist carbachol and were abolished by application of atropine, a muscarinic antagonist. Other manipulations that could block the increase in basal firing included removal of external  $\text{Ca}^{2+}$ , intracellular application of a  $\text{Ca}^{2+}$  chelator, application of L-type  $\text{Ca}^{2+}$  channel blockers and application of a blocker of  $\text{Ca}^{2+}$ -activated non-specific cation current. These results indicate that the graded changes in firing frequency might depend on  $\text{Ca}^{2+}$  influx and activation of a  $\text{Ca}^{2+}$ -sensitive cationic current. This phenomenon might be used as a substrate for ‘working memory’, an idea that is consistent with the role of the entorhinal cortex and of muscarinic drive in the temporal lobe memory system.

A study of intrinsic plasticity in layer V pyramidal neurons in slices of rat cortex revealed a persistent increase in intrinsic excitability after 10 Hz burst stimulation of layer II/III glutamatergic inputs for 3–4 minutes<sup>59</sup>. The increase in excitability was manifest as an increase in the number of spikes evoked by direct depolarizing

THETA BURSTS  
Rhythmic neural activity with a frequency of 4–8 Hz.



**Figure 4 | Intrinsic plasticity in neocortical pyramidal cells produces an improvement in the temporal precision and reliability of throughput.** **a** | Tetanic stimulation of glutamatergic inputs in the presence of GABA<sub>A</sub> (γ-aminobutyric acid subtype A) and glutamate receptor antagonists increased the number of spikes evoked by a depolarizing current pulse and induced a long-lasting reduction of the temporal jitter of the spike discharge, as shown by the three superimposed traces before and after synaptic tetanus. The graph on the left plots the latency of each spike against time, and the histogram on the right is a comparison of the distribution of the spike latency before (yellow) and after (green) the tetanus in a time window defined by the rectangles. **b** | Representative traces showing action potentials evoked by a barrage of excitatory postsynaptic potentials simulated with the dynamic clamp technique. Three successive trials before and 40 minutes after application of the mGluR5 agonist CHPG (2-chloro-5-hydroxyphenylglycine) are shown. CHPG is used because it produces an effect that mimics and occludes that of synaptic stimulation. The reliability (R), determined by the number of action potentials in each peak of activity, was increased after CHPG application. Modified, with permission, from REF. 59 © Society for Neuroscience (in the press).

current injection, and a reduction in the post-burst afterhyperpolarization, the first spike threshold and the first interspike interval (FIG. 4a). This phenomenon was blocked by application of MPEP (2-methyl-6-(phenylethynyl)-pyridine), a specific antagonist of the metabotropic glutamate receptor mGluR5, and mimicked by mGluR5 agonists such as ACPD (1-amino-1,3-cyclopentanedicarboxylic acid) and CHPG (2-chloro-5-hydroxyphenylglycine). Voltage-clamp recordings showed a reduction in the amplitude of the pharmacologically isolated  $I_{AHP}$ , which is mediated by small conductance Ca<sup>2+</sup>-dependent K<sup>+</sup> (SK) channels. Application of apamin, an SK channel blocker, mimicked and partially occluded the excitability increase, indicating that both synaptically and CHPG-induced increases in intrinsic excitability might be mediated by down-regulation of SK channels. Furthermore, application of EBIO (1-ethyl-2-benzimidazolinone), a drug that up-regulates SK channel function, had the opposite effect. Using DYNAMIC CLAMPING

**DYNAMIC CLAMPING**  
Recording configuration in which the current that is injected into the cell mimics a specific pattern of synaptic activation.

to present recurring patterns of simulated EPSPs to neurons, it was determined that the CHPG-induced increase in intrinsic excitability functioned to increase the probability that a given EPSP would fire a spike, thereby increasing the reliability of synaptic throughput (FIG. 4b). These results indicate that changes in intrinsic excitability might be expressed as changes in the reliability and timing of spike trains.

Persistent changes in intrinsic excitability can occur not only after increasing neuronal activity, but also after decreasing it. In a recent study in mouse brainstem slices, a 5-minute periodic stimulation of GABA synapses impinging on medial vestibular nucleus neurons was found to cause long-term increases in the spontaneous firing rate and in the gain of the firing response. This was expressed as an increase in the slope of the relationship between input current and the number of spikes evoked by depolarizing current<sup>60</sup>. A reduction in the afterhyperpolarization and an increase in the input resistance accompanied this effect, but the action potential threshold was unchanged. Direct periodic hyperpolarization of the neuron with blockade of both excitatory and inhibitory synaptic drive also induced this increase in intrinsic excitability, indicating that its expression is intrinsic to the recorded vestibular nucleus neuron and not dependent on network properties. Ca<sup>2+</sup> imaging showed that silencing the neuron caused a reduction in intracellular Ca<sup>2+</sup>, indicating that decreases in Ca<sup>2+</sup> concentration might have caused the increase in intrinsic excitability. Furthermore, intracellular application of the Ca<sup>2+</sup> chelator BAPTA, a brief reduction in extracellular Ca<sup>2+</sup> concentration, or a brief application of a non-specific Ca<sup>2+</sup> channel blocker (CdCl<sub>2</sub>) all induced this effect, indicating that transient decreases in intracellular Ca<sup>2+</sup> concentration can persistently increase neuronal excitability. Iberiotoxin, a specific blocker of large conductance Ca<sup>2+</sup>-dependent K<sup>+</sup> (BK) channels, also increased spontaneous firing rate and gain, and occluded subsequent increases in intrinsic excitability, pointing to the involvement of a reduction in BK currents.

It is also possible to observe rapidly-induced intrinsic plasticity in cultured neurons. When dual recordings were made from two connected pyramidal neurons in a hippocampal culture, repetitive correlated spiking resulted in both LTP (as previously shown), and in a rapid and persistent increase in intrinsic excitability of the presynaptic neuron<sup>61</sup>. This was seen as an increase in the number of spikes evoked by current pulses in the presynaptic neuron, a decrease in the first spike threshold, a decrease in the interval between the first and second evoked spike, and a decrease in the variance of this measure. This effect required activation of NMDA receptors and subsequent postsynaptic Ca<sup>2+</sup> influx. Presynaptic application of protein kinase C (PKC) blockers (which did not affect LTP) abolished the increase in presynaptic excitability. The increase in excitability seems to be intrinsic to the presynaptic neuron, as it can be recorded during blockade of both glutamate- and GABA-mediated transmission. In addition, recordings from the presynaptic neuron

Table 1 | Potential molecular signals and substrates for neuronal intrinsic plasticity

Receptors/ion channels	Effectors	Enzymes	Final functional targets
Voltage-sensitive Ca <sup>2+</sup> channels	Ca <sup>2+</sup>	PKC	K <sup>+</sup> channels: $I_{A^+}$ , $I_{K^+}$ (delayed outward rectifier), $I_{AHP^+}$ , $sI_{AHP}$ and $I_{K(Ca)}$ BK type
NMDARs	G-proteins	CaMKII	Na <sup>+</sup> channels: fast inactivating and plateau
mGluRs		Adenylyl cyclase	Ca <sup>2+</sup> channels: R-type and T-type
mAChRs		Guanylyl cyclase	Others: $I_h$ and $I_{CAN}$
5HTRs		nNOS	
		PKA	
		PKG	
		MAPK	
		Protein phosphatases	

5HTR, 5-hydroxytryptamine (serotonin) receptor; BK, large conductance Ca<sup>2+</sup>-dependent K<sup>+</sup> channel; CaMKII, Ca<sup>2+</sup>/calmodulin-dependent protein kinase II; mAChR, acetylcholine receptors (muscarinic subtype); MAPK, mitogen-activated protein kinase; mGluR, metabotropic glutamate receptor; NMDAR, *N*-methyl-D-aspartate receptor; nNOS, neuronal nitric oxide synthase; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G.

after pairing stimulation showed a negative shift in the voltage-dependence of Na<sup>+</sup> channel activation and a positive shift in inactivation, which probably underlie the observed decrease in spike threshold. So, intrinsic plasticity of presynaptic neurons can be evoked slowly by chronic receptor blockade in cultured *Xenopus* neuromuscular junctions<sup>51</sup> and rapidly by a spike-timing dependent mechanism in hippocampal cultures<sup>61</sup>.

Molecular substrates for intrinsic plasticity  
Investigations into intrinsic plasticity are still in a largely descriptive phase, reminiscent of our understanding of synaptic plasticity 20 years ago. However, there is some information on the molecular underpinnings of intrinsic plasticity in certain situations. Initially, let us consider rapid changes in intrinsic excitability evoked by brief electrical stimulation and the general signalling cascades that might underlie them. In many cases, the patterns of synaptic activation or direct depolarization that are used to evoke intrinsic plasticity are the same as those used to induce LTP and LTD. Indeed, LTP and LTD are often co-expressed with intrinsic plasticity (BOX 1). Therefore, it is not surprising that, like LTP and LTD, Ca<sup>2+</sup>-dependent signalling is a prime candidate for the induction mechanism of intrinsic plasticity (TABLE 1). Intracellular Ca<sup>2+</sup> levels have long been regarded as a biochemical correlate of electrical activity. Ca<sup>2+</sup> influx into neurons can be caused at glutamatergic synapses by activation of Ca<sup>2+</sup>-permeable NMDA receptors. Voltage-sensitive Ca<sup>2+</sup> channels can also cause Ca<sup>2+</sup> influx and are most commonly activated by depolarization after AMPA or NMDA receptor activation or spiking activity. In some neurons with high densities of  $I_h$  and low-threshold Ca<sup>2+</sup> channels, a train of inhibitory postsynaptic potentials (IPSPs) can produce a hyperpolarization that, when finished, evokes 'rebound spiking' and consequent Ca<sup>2+</sup> influx<sup>62,63</sup>. So, Ca<sup>2+</sup> influx from several routes can trigger intrinsic plasticity. An intriguing exception to this model is the observation that brief GABA<sub>A</sub> receptor-mediated hyperpolarization can produce a reduction in free internal Ca<sup>2+</sup>, which triggers an increase in intrinsic excitability in vestibular nucleus neurons<sup>60</sup>.

Ca<sup>2+</sup> release from intracellular stores can also be caused by activation of the appropriate second messengers in response to various neurotransmitters. Of particular relevance are the group I mGluRs (mGluR1 and mGluR5) and the muscarinic receptors M1, M3 and M5, all of which are coupled through G-proteins to the activation of phospholipase C and consequent production of 1,2-diaclyglycerol and inositol-1,4,5-trisphosphate (InsP3), which mobilizes Ca<sup>2+</sup> from internal stores. The Ca<sup>2+</sup> signals that are provided by both influx and mobilization from internal stores are converted into physiological responses through several Ca<sup>2+</sup>-sensing molecules. Ca<sup>2+</sup> that is bound with calmodulin can directly activate a number of kinases (and potentially phosphatases), most notably PKC and Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII). Ca<sup>2+</sup> can also activate other kinases indirectly through cascades that involve adenylyl cyclase/cAMP/PKA or neuronal nitric oxide synthase/guanylyl cyclase/cGMP/cGMP-dependent protein kinase. These signals might alter intrinsic excitability by changing the function of voltage-sensitive ion channels through direct phosphorylation or through the phosphorylation of associated proteins. Owing to their rapid onset, phosphorylation/dephosphorylation are suited to subserve rapid bidirectional modification of intrinsic excitability. However, it is unclear whether there is an upper limit on the duration of plasticity that is mediated by phosphorylation changes (see REF. 64 for a discussion of this issue in relation to LTP and LTD).

It should be noted that there are many variations on this general scheme. For example, both Ca<sup>2+</sup>-sensitive K<sup>+</sup> channels and some voltage-sensitive Ca<sup>2+</sup> channels are modulated directly by Ca<sup>2+</sup>/calmodulin (although these effects are probably brief). Likewise, some of the products of Ca<sup>2+</sup>-activated cascades can exert their action directly on ion channels in a phosphorylation-independent manner. For example, cAMP modulates  $I_h$  directly, and nitric oxide can nitrosylate and alter the function of Ca<sup>2+</sup>-sensitive K<sup>+</sup> channels. Ca<sup>2+</sup>-independent signalling might also participate in intrinsic excitability. Group II and III mGluRs can modulate voltage-gated Ca<sup>2+</sup> and K<sup>+</sup> channels in a Ca<sup>2+</sup>-independent fashion through

G-protein  $\beta\gamma$ -subunits, and classical modulatory neurotransmitters such as serotonin and noradrenaline can also alter intrinsic excitability through  $\text{Ca}^{2+}$ -independent means. Neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3) can be released in an activity-dependent manner and can also persistently alter intrinsic excitability through  $\text{Ca}^{2+}$ -independent signalling. So far, however, this has only been observed for slowly developing changes (hours to days) that require protein synthesis.

Rapid changes in intrinsic excitability induced by synaptic stimulation have been blocked by NMDA receptor antagonists in many cases<sup>56,57,61</sup>. However, there are other situations in which activation of mGluR5 (REF. 59) or muscarinic receptors<sup>58</sup> seems to be the initial trigger. Indeed, in neurons of the cerebellar deep nuclei, activation of NMDA receptors by synaptic stimulation or voltage-sensitive  $\text{Ca}^{2+}$  channels by repeated direct depolarization suffice to elicit intrinsic plasticity<sup>56</sup>. It might be that, in many cases, any stimulus that produces a sufficient  $\text{Ca}^{2+}$  transient in the appropriate subcellular location would induce intrinsic plasticity.

Little is known about the second messengers that couple the initial events driven by neural activity to the expression of intrinsic plasticity. In hippocampal cultures, presynaptic application of a PKC inhibitor blocked the presynaptic expression of intrinsic plasticity. Because the induction of this plasticity required activation of postsynaptic NMDA receptors, a requirement for an unidentified retrograde messenger has been suggested<sup>61</sup>. Interestingly, PKC has been implicated in the intrinsic excitability that is produced by several forms of behavioural training. PKC-activating drugs (such as phorbol esters and synthetic diacylglycerols) can produce changes in intrinsic conductances, which mimic and occlude those produced in rabbit hippocampal CA1 neurons by trace eyelid conditioning, odour discrimination learning<sup>27</sup> and light-rotation conditioning<sup>65</sup>.

Regardless of the mechanism of induction, intrinsic plasticity must ultimately result from changes in the properties, number or distribution of ion channels on the neuronal surface. So far, the studies that have tried to identify the ion channels that are modulated during changes in neuronal excitability have used two strategies. The first is to mimic and thereby occlude intrinsic plasticity by using a particular channel blocker. For example, apamin, a blocker of the SK-type  $\text{Ca}^{2+}$ -sensitive  $\text{K}^+$  channels mimicked and occluded the subsequent effect of synaptic stimulation on intrinsic excitability of neocortical pyramidal cells<sup>59</sup>. Similarly, iberiotoxin (but not apamin) mimicked and occluded intrinsic plasticity evoked by brief hyperpolarization of neurons in the medial vestibular nucleus<sup>60</sup>. This approach depends on having a highly specific toxin for the channel of interest and is limited to those forms of intrinsic plasticity that are mediated by channel attenuation. Furthermore, it cannot distinguish between different forms of channel modulation (such as a decrease in unitary conductance, a change in voltage-dependence of activation or a reduction in channel number). The second approach consists of voltage-clamp recordings using a step and

drug protocol that is appropriate for isolating individual conductances with drugs or electrophysiological protocols. In this way, it has been possible to identify a number of specific conductance changes after training or stimulation. Examples include a negative shift in the voltage-dependence of  $I_h$  activation after febrile seizures in rat CA1 pyramidal cells<sup>42</sup> and a negative shift in the voltage-dependence of fast-inactivating  $\text{Na}^+$  channel activation after spike-timing-dependent intrinsic plasticity in hippocampal cultures<sup>61</sup>. In most cases, these effects on pharmacologically isolated membrane currents do not allow for a unique molecular identification of the channel involved. This would require coupling our present forms of analysis with strategies to interfere with specific channel proteins, as has recently been attempted using mutant mice<sup>66-68</sup>.

Analysis of slow changes in intrinsic excitability induced by chronic treatments is a difficult task. Chronic application of receptor antagonists, ion-channel blockers, enzyme inhibitors or protein synthesis inhibitors tend to produce large confounding baseline effects. One approach to get around these problems has been to investigate the potential role of slowly acting signals such as trophic factors. Chronic treatment of cultured cortical neurons with TTX produced an increase in intrinsic excitability that was blocked by BDNF and mimicked by a TrkB (tyrosine receptor kinase B) fusion protein (which functions as a BDNF antagonist), indicating a possible role for BDNF withdrawal in this process<sup>69</sup>. Similarly, chronic treatment of *Xenopus* neuromuscular junction cultures with a nicotinic cholinergic antagonist resulted in presynaptic action potential broadening, which could be blocked by co-application of NT3 (REF. 51). Analysis of the membrane currents altered by chronic treatments has been more productive as voltage-clamp recording from cultured neurons is straightforward.

#### Conclusion

**Some computational issues.** Beyond simple considerations of throughput, how might the induction of intrinsic plasticity affect the subsequent function of circuits and synapses? In most of the chronic experiments that we have considered, intrinsic plasticity seems to act to maintain homeostasis. Manipulations that reduce network activation (such as the TTX blockade of action potentials or the use of antagonists of excitatory neurotransmitters) tend to produce changes that increase the probability and duration of spike firing (such as attenuation of voltage-sensitive  $\text{K}^+$  channels and enhancement of voltage-sensitive  $\text{Na}^+$  or  $\text{Ca}^{2+}$  channels). Conversely, treatments that enhance network activation (such as raising external  $\text{K}^+$  concentrations or the use of repetitive stimulation with field electrodes) mostly decrease the probability and duration of spike firing. It should be noted that intrinsic plasticity is only one of several properties that are regulated to maintain network homeostasis, such as synaptic structure, number and strength (see REFS 70,71 for review). It has been suggested that homeostatic processes function to maximize information storage by keeping the dynamic range of signalling within useful limits<sup>72,73</sup>. Many questions

remain about slow, homeostatic processes. Do they always require *de novo* protein synthesis or can they be mediated by post-translational changes? What is their duration? Can homeostatic processes act locally (at an individual synapse, for example) or must they always be neuron-wide? Does training, which often takes many trials, produce intrinsic plasticity that is reminiscent of slow homeostatic change or the rapid changes that are evoked by brief electrical stimulation?

In contrast to chronic treatments, which alter activity levels, brief electrical stimulation is more likely to produce changes in intrinsic excitability that are not homeostatic. For example, strong electrical stimulation of cerebellar mossy fibre–granule cell synapses produces both LTP and a persistent reduction in spike threshold<sup>37</sup>. These potentiating effects can be mutually reinforcing. As we have discussed, persistent changes in axosomatic voltage-sensitive channels have the capacity to alter the throughput of all the synapses on a neuron, and alterations of dendritic voltage-sensitive channels have the capacity to alter throughput that is restricted to a particular dendritic domain (FIG. 1). In addition to changing throughput, intrinsic plasticity can also alter METAPLASTICITY. This is largely due to the role of intrinsic conductances in the initiation of axo-somatic spikes and their subsequent backpropagation into dendrites. Whereas postsynaptic spiking is not an absolute requirement for the induction of LTP and LTD, many forms of LTP and LTD can be readily induced by pairing backpropagating spikes with synaptic activation in the dendrites at various intervals (see REFS 74,75 for review). So, intrinsic plasticity, which serves to alter the threshold for axosomatic spikes generation, or the pattern or duration of firing, can change how these spikes contribute to the induction of LTP and LTD.

Perhaps even more interesting is the intrinsic plasticity that might modulate dendritic voltage-sensitive channels. Various drugs can attenuate dendritic voltage-gated K<sup>+</sup> channels, such as  $sI_{AHP}$ , either directly<sup>76</sup> or indirectly through group I mGluRs<sup>77–79</sup>. These treatments can cause weak high-frequency stimulation, which fails to induce LTP in control conditions, to succeed in inducing it<sup>76–79</sup>. Drugs that facilitate another dendritic K<sup>+</sup> channel,  $I_{A}$ , can have the opposite effect, blocking LTP induction from a suprathreshold high-frequency train<sup>80</sup>. Furthermore, in a recent experiment, dendritic recordings were made on pharmacologically isolated CA1 pyramidal neuron in mouse hippocampal slices, and repetitive axonal stimulation was used to evoke trains of backpropagating action potentials in the middle apical dendrite<sup>81</sup>. In agreement with previous studies, the later action potentials in the train were reduced in amplitude when dendritic recordings were taken. However, after injection of a series of brief depolarizing currents to the dendrites, all spikes in the train were shown to have the same amplitude. Lowering the concentration of Ca<sup>2+</sup> in the extracellular medium, the intracellular application of a Ca<sup>2+</sup> chelator or the use of a CaMKII inhibitor blocked this effect, indicating the possible involvement of the Ca<sup>2+</sup>/CaMKII pathway. In this experiment, it is unclear if the dendritic depolarization produced a change in intrinsic excitability that spread throughout the dendrite

or was confined to a local domain. The latter possibility has been suggested in a recent study that used synaptic activation instead of direct dendritic current injection. High-frequency stimulation delivered to a set of distal synapses on the apical dendrite of a hippocampal CA1 cell produced a persistent local increase in the Ca<sup>2+</sup> transient evoked by a brief train of somatic spikes<sup>3</sup>.

In a separate study, a train of backpropagating action potentials produced a persistent reduction in the Ca<sup>2+</sup> transients that were evoked by a single backpropagating action potential<sup>82</sup>. Interestingly, this phenomenon, which resulted in approximately a 40% decrease in the amplitude of the Ca<sup>2+</sup> transient, was induced in a spine-specific, all-or-none fashion. Pharmacological experiments showed it to result from attenuation of R-type voltage-sensitive Ca<sup>2+</sup> channels. Once induced, this Ca<sup>2+</sup>-transient attenuation reduced the probability and amplitude of subsequent LTP induction by an EPSP-spike timing protocol. In this way, a local change in dendritic voltage-sensitive ion channels can give rise not only to a local domain of altered throughput (FIG. 1), but also to a local domain of altered metaplasticity.

To this point, we have mostly considered a single dimension of intrinsic plasticity — excitability. Treatments that reduced threshold, accommodation or the post-burst afterhyperpolarization were considered to increase excitability. However, many lines of evidence have led to the conclusion that it is not just the rate of spiking (as captured in the term excitability) that encodes salient information in networks of neurons, but also spike timing, the particular pattern in which spikes are evoked. In this context, we should note that both chronic manipulations, such as placing lobster STG neurons in culture<sup>48</sup>, and brief activation, such as high-frequency stimulation of cerebellar mossy fibre-deep nuclear neurons synapses (W.Z. and D.J.L., unpublished observations) can give rise to a change in the pattern of spiking. In the former case, STG neurons slowly convert their firing pattern from tonic to burst firing. In the latter, deep nuclear neurons that burst in response to depolarization are modulated to fire in a more tonic fashion. So, intrinsic plasticity can contribute to shaping both a rate code and a timing code for neural information.

**Future directions.** These are early days in the study of neuronal intrinsic plasticity. At the time of writing this review, a PubMed search on synaptic plasticity yielded >6,000 references, whereas most of the work on intrinsic plasticity is cited herein (115 references). One observation that has emerged from the study of synaptic plasticity is that, even if one only considers LTP and LTD in the mammalian brain, there are several molecular mechanisms that are operative for each phenomenon. In many cases, multiple forms of LTP or LTD co-exist at the same synapse. Although still in its infancy as a field, intrinsic plasticity is likely to be even more diverse (TABLE 1). It might be that most voltage-sensitive conductances expressed in neurons are subject to persistent use-dependent modulation.

#### METAPLASTICITY

Term that has been coined to refer to the higher-order plasticity of synaptic plasticity. In other words, how synaptic activity or other stimuli modify the properties of synaptic plasticity itself.

To examine how this field might develop, we will use the model system that is studied in our laboratory as a typical example. At present, we know that brief burst-stimulation applied to glutamatergic mossy fibre synapses gives rise to a persistent increase in the intrinsic excitability of deep cerebellar nuclear neurons (FIG. 3). This manifests as a reduction in spike threshold, an increase in the number of spikes evoked by depolarizing current injection and, in some cases, an alteration in the pattern of spiking (REF. 56, and W.Z. and D.J.L., unpublished observations). This set of changes is similar to that seen at many other cell types, including the cerebellar granule cell<sup>57</sup> and the neocortical pyramidal cell<sup>59</sup> (FIG. 4). It might be triggered by NMDA-receptor activation or by voltage-sensitive Ca<sup>2+</sup> channels activated by repeated direct depolarizing current injection or rebound depolarization after trains of IPSPs. At present, this is our understanding. There is much more that we would like to know.

First, some parametric considerations. What is the duration of intrinsic plasticity in the deep cerebellar nuclei (this is central to formulating hypotheses about the potential role of this phenomenon in cerebellar motor learning)? This is a real technical challenge, as measurement of intrinsic excitability often requires intracellular recording techniques that cannot be maintained longer than 1–2 h. Can it be actively reversed by some other pattern of activity? This is a general problem that will come up in all of the model systems discussed here. At present, there are only a handful of examples of use-dependent bidirectional changes in intrinsic excitability<sup>12,58,83</sup>. Is this form of intrinsic excitability always expressed as a change in axo-somatic ion channels, which produce neuron-wide changes in throughput, or can it sometimes be expressed at local dendritic sites<sup>3,82</sup>, which could alter throughput in a discrete dendritic module (FIG. 1)? This will require dendritic recordings or optical imaging to be resolved. And perhaps most importantly, can these changes be produced by patterns of activity that can be recorded *in vivo* during the acquisition phase of cerebellar motor learning tasks?

Next to be considered is the identification of the initial and intermediate signals. In the case of the deep cerebellar nuclei, although we know that activation of voltage-gated Ca<sup>2+</sup> channels or NMDA receptors is sufficient, it is still possible that other receptors such as group I mGluRs, or receptors for serotonin or noradrenaline are involved. This question, together with the role of second messengers, will be best addressed through a pharmacological screen, perhaps supplemented with the use of mutant mice lacking various receptors or enzymes.

A central issue is the identification of the conductances that underlie this effect. In other cases, occlusion with a specific channel blocker has been informative, and this can be a good initial strategy. Ultimately, however, voltage-clamp recordings of pharmacologically isolated candidate currents will be required for their detailed characterization (for example, to distinguish an effect on voltage-dependence of activation from a change in unitary conductance,). These might pose as considerable technical challenges if the altered conductances are located in fine dendrites or axons.

We also need to understand the processes by which second messengers give rise to a persistent alteration of voltage-gated conductances. In some cases, this might be straightforward and involve direct phosphorylation of an ion channel, whereas in others, a complex cascade of events involving protein–protein interactions and trafficking might be involved. When a substrate for phosphorylation seems to participate, a useful strategy has been to attempt to rescue the phenomenon in cells from a null mutant mouse by transiently expressing the missing protein that harbours point mutations at candidate phosphorylation sites. This strategy has recently been employed to identify the crucial phosphorylation events in the induction LTD<sup>84</sup> and LTP<sup>85</sup> at the cerebellar parallel fibre–Purkinje cell synapse.

Finally, it is important to envision the experiments that are required to test the role of intrinsic plasticity in a behavioural setting. Let us imagine (with no experimental basis yet) that intrinsic plasticity in the cerebellar deep nuclei involves a cascade in which a local Ca<sup>2+</sup> transient stimulates CaMKII to phosphorylate the SK1 subunit of Ca<sup>2+</sup>-sensitive K<sup>+</sup> channels at an identified serine. The cerebellar deep nuclei are vital for certain forms of motor learning such as delay eyelid conditioning. An initial experiment to test the hypothesis that intrinsic plasticity in the deep nuclei is required for delay eyelid conditioning might involve injecting a CaMKII inhibitor or a SK channel blocker into the deep nuclei during the acquisition phase of this task. Although a negative result in this experiment would be informative, an impairment in acquisition of delay eyeblink conditioning could be just as easily attributed to non-specific effects such as the increased basal firing rate under apamin, or the blockade of CaMKII-mediated phosphorylation events unrelated to the SK conductance. Ultimately, a much more stringent test of this hypothesis would involve an inducible conversion of the crucial substrate residue in the cells of interest. In the long term, this is one way to come full circle to a molecular understanding of memory.

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