Variability, compensation and homeostasis in neuron and network function

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Abstract | Neurons in most animals live a very long time relative to the half-lives of all of the proteins that govern excitability and synaptic transmission. Consequently, homeostatic mechanisms are necessary to ensure stable neuronal and network function over an animal's lifetime. To understand how these homeostatic mechanisms might function, it is crucial to understand how tightly regulated synaptic and intrinsic properties must be for adequate network performance, and the extent to which compensatory mechanisms allow for multiple solutions to the production of similar behaviour. Here, we use examples from theoretical and experimental studies of invertebrates and vertebrates to explore several issues relevant to understanding the precision of tuning of synaptic and intrinsic currents for the operation of functional neuronal circuits.

Synaptic scaling
Process by which neurons regulate the strength of all of their synapses to help maintain a target activity level.

Conductance densities
The conductance density is conductance divided by surface area. Conductance for a given channel is calculated from the current and reversal potential, and the surface area is estimated from capacitance measurements.

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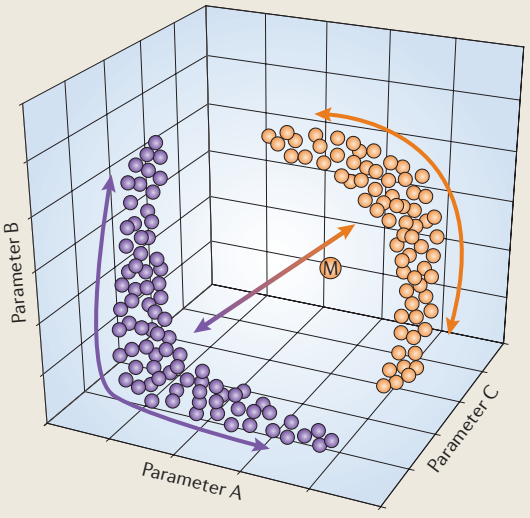
Humans, and other long-lived animals such as turtles and lobsters, have neurons that live and function well for decades. By contrast, ion channel proteins, synaptic receptors and the components of signal transduction pathways are constantly turning over in the membrane and being replaced, with half-lives of minutes, hours, days or weeks[1–5]. Therefore, each neuron is constantly rebuilding itself from its constituent proteins, using all of the molecular and biochemical machinery of the cell. This allows for plastic changes in development and learning, but also poses the problem of how stable neuronal function is maintained as individual neurons are continuously replacing the proteins that give them their characteristic electrophysiological signatures[6–10]. Turrigiano and Nelson[9] have elegantly reviewed a great deal of important recent work describing the homeostatic mechanisms by which Hebbian learning can, in principle, be appropriately balanced by stability mechanisms that allow neurons and synaptic connections to be maintained in operating ranges appropriate to neuronal function. In brief, this work argues that various mechanisms, including synaptic scaling[11,12] and changes in densities of individual ionic currents[13,14], slowly compensate for use-dependent changes in neuronal inputs such that neurons retain their target levels of excitability[15]. Even more recently, Davis[10] has provided an insightful discussion of what will be needed to understand the mechanisms that could give rise to the targets for homeostatic regulation[10].

Here, we focus on another set of issues that arise as one attempts to confront seriously the problems of homeostasis and network regulation: for neurons with many different types of voltage-dependent conductance, how tightly regulated do their conductance densities need to be to produce a characteristic electrophysiological activity pattern? In other words, how large are the multi-dimensional parameter spaces (BOX 1) that are consistent with a given activity pattern? How different are cells of the same type within the same animal, and across individual animals? How tightly regulated do the strength of synapses need to be to maintain proper network function? To what extent do specific combinations of membrane currents and synaptic conductances compensate for each other to maintain constant or appropriate network function? And how much drift in neuron and network parameters can occur before we see appreciable changes in behaviour over the lifetime of an animal?

Variability in channel densities

We now know that neurons express a large number of different classes of ion channel, each of which can be described in terms of its voltage and time-dependence of activation and inactivation[16]. Moreover, since the first descriptions of the transient outward current, I_A[17,18], and the Ca^{2+}-dependent K^+ current[19], it has been clear that many types of ion channel with different voltage dependencies and dynamics contribute to the specific firing pattern of a neuron. For example, it is well

Box 1 | **System properties that depend on a multidimensional parameter space**



In this schematic, three parameters, A, B and C, are plotted (for example, the conductances for three different ion channels). Individual neurons displaying one type of behaviour are shown in purple, and those with a different type of behaviour are shown in yellow. For a specific pattern of activity, one can find a region of parameter space within which modifications of parameters do not produce qualitative changes in behaviour (purple or yellow arrows). Leaving that region of parameter space is associated with qualitative alterations in behaviour. Both populations of neurons (purple and yellow) show individual neurons with widely different values of all three parameters. In a recent study of a large database of model neurons, virtually all neurons that were similarly classified were found to be connected in parameter space[40]. Therefore, homeostatic tuning rules that maintain a constant activity pattern could, in principle, operate to tune conductances so that an individual neuron remains within a given region of parameter space, although its values for one or more conductances may be substantially altered. Neuromodulators can move neurons from one behaviour to another, by bringing a cell from one parameter regime to another (purple/yellow gradient arrow)[41]. When the regions of parameter space are concave in structure, as is shown here, the means of the parameters may fail to capture the behaviour of the individuals in the mean (the mean of the yellow population is shown by the larger yellow circle marked M) (see also REF. 38).

Transient outward current
(I_A). This is caused by a voltage-gated K^+ channel that opens when the neuron is depolarized and then inactivates (closes) rapidly. To remove the inactivation, the neuron must be hyperpolarized. I_A often plays a part in determining the frequency of action potential firing.

Afterhyperpolarization
The membrane hyperpolarization that follows an action potential.

Window currents
A sustained current at a membrane potential that occurs if the voltage dependence of activation and inactivation overlap at that membrane potential.

K_{leak}
K^+ current active at hyperpolarized membrane potentials that contributes to the resting potential.

Hyperpolarization/cyclic nucleotide gated channels
(HCN channels). These are a family of mixed cation conductances that activate when the cell is hyperpolarized.

Inwardly rectifying potassium channels
(Kir2 channels). These are K^+ channels that pass inward current much better than outward current. These channels often play an important part in setting the resting potential by contributing an outward current when the neuron is close to its resting potential. However, when the neuron is depolarized, the outward current that develops is less than would be expected from the increase in driving force.

understood that I_A can influence a neuron's firing rate[20] and that Ca^{2+}-dependent K^+ currents can contribute to afterhyperpolarizations[21,22]. Nonetheless, although it is at some level obvious, it is less commonly appreciated that the influence of a given current on a neuron's activity is crucially dependent on all of the other channels present in the membrane[23]. A given process, such as spike repolarization, often involves the dynamics of a number of currents working together[24,25], rather than being primarily determined by a single current.

In particular, in many neurons the approach to threshold is influenced by multiple currents, because many voltage-dependent currents that are large when the neuron is either strongly depolarized or hyperpolarized can generate small currents close to the resting potential that can govern, in subtle but significant ways, the resting potential and response to small synaptic inputs. FIGURE 1 shows the voltage dependence of only a few of the currents that can contribute to the membrane potential in the range from –75 mV to –55 mV. Among others, these include the hyperpolarization-activated inward current (I_h)[26], the persistent Na^+ current (I_{NaP})[27], I_A[28] and the low-threshold Ca^{2+} current (I_{CaT})[29]. These currents are active in the –75 mV to –55 mV range, either as window currents or because they show little inactivation. FIGURE 1b shows data from several recent studies looking at the effect of pharmacological blockade of several of these currents on dendritic integration[30–34]. In all of these recordings from hippocampal CA1 neurons, the synaptic potentials were recorded at membrane potentials significantly below threshold, but their amplitudes were influenced by voltage-gated channels that contribute to the subthreshold conductance. Similarly, Day *et al.*[23] showed that dendritic excitability and response to excitatory inputs in mouse frontal cortex pyramidal neurons are also shaped by interactions among K_{leak}, hyperpolarization/cyclic nucleotide gated (HCN) and inwardly rectifying potassium (Kir2) channels.

A recent paper indicates that those wishing to eventually describe the intrinsic properties of neurons in terms of their underlying conductances should exercise caution[35]. Thalamocortical neurons have been characterized as having two highly different firing modes: a depolarized tonic mode and a hyperpolarized bursting mode. The switch between these two modes of firing was thought to occur predominantly as a consequence of changes in the neuromodulatory environment[36], which modifies I_h and the low-threshold Ca^{2+} current[37]. However, this bimodality is not present when these neurons are subjected to noisy synaptic stimulation[35]. Instead, they respond in a unimodal way that involves both single spike firing and bursting behaviour at all voltages. The results of this study demonstrate that measuring a neuron's intrinsic properties after isolating it from its synaptic inputs might not accurately predict the neuron's behaviour when it is embedded in an active network.

Intrinsic properties and channel densities. One of the consequences of the fact that neurons express many different ion channels is that similar intrinsic properties can arise from various combinations of conductance densities. This conclusion comes from both computational studies[38–40] and experimental work on a number of different preparations[25,41,42]. In a remarkable study, Swensen and Bean[43] voltage-clamped dissociated Purkinje neurons in culture to action potential wave-forms and measured the ionic currents during bursts (FIG. 2a,b).

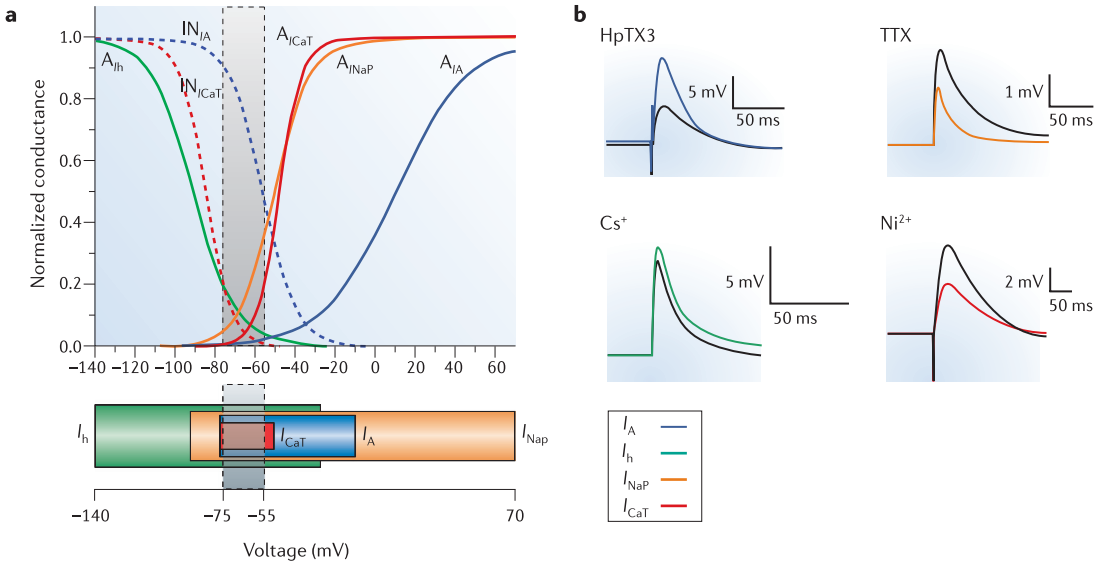


Figure 1 | **Conductances active below threshold can strongly influence neuronal activity and synaptic integration. a** | Top panel shows activation curves (A_{ICaT}, A_{Ih}, A_{IA}, A_{INaP}) and inactivation curves (IN_{ICaT}, IN_{IA}) for the low-threshold calcium current I_{CaT}, the transient potassium current I_A, the persistent sodium current I_{NaP} and the hyperpolarization-activated current I_h. The bottom panel shows a schematic of the voltage range in which these currents are persistently active. In both panels, the grey dashed box covers the resting membrane potential region (–75 to –55 mV). **b** | Effect of blocking I_A, I_h, I_{NaP} and I_{CaT} on the integration of dendritic excitatory postsynaptic potentials (EPSPs) in hippocampal CA1 pyramidal neurons. EPSPs were recorded close to resting membrane potential, between –70 and –58 mV depending on the cell. HpTX3, Cs^+, TTX or Ni^{2+} were used to block I_A, I_h, I_{NaP} and I_{CaT}, respectively. In the case of HpTX3, Cs^+ and Ni^{2+} applications, EPSPs were generated by extracellular stimulation of afferent fibres. In the case of TTX, EPSPs were stimulated by injection of current at distal sites in the apical dendrite (~250 µm). Black traces, control. Coloured traces, effects of pharmacological blockade. Panel **a**, data from REFS 26–29. Panel **b** modified, with permission, from REF. 31 © (2006) Elsevier Science; REF. 32 © (1998) Society for Neuroscience; REF. 33 © (2002) National Academy of Sciences; REF. 34 © (1997) American Physiological Society.

Different ratios of inward Na^+ and Ca^{2+} currents were found at the same membrane potential in different neurons with almost identical activity profiles[43].

A similar conclusion comes from recent studies on crab stomatogastric ganglion neurons[42] (FIG. 2c,d). Schulz *et al.*[42] first made voltage clamp measurements of several K^+ currents and then followed these with real-time quantitative PCR measurements for the K^+ channel mRNA from these same neurons. This allowed the correlation of the current densities with the levels of channel expression in the same neuron (FIG. 2c,d). Two of the K^+ currents showed strong correlations between mRNA copy number and current density in the same neuron, replicating at the single neuron level similar conclusions made from pooled data[44]. However, there were two- to fourfold variations in both of these measurements in the same types of neuron from different animals[42]. Interestingly, the two electrically coupled pyloric dilator neurons from the same animal showed highly correlated I_A and I_h values, whereas there remained considerable cross-animal variation in the mRNA expression for these currents in the pyloric dilator neurons. The two- to fourfold variation in conductance density measured from cell to cell has previously been reported[7,42,45], and even larger ranges of values have been seen for single-cell mRNA expression in vertebrate neurons of the same type[46].

The range of conductances seen in neurons with similar activity patterns flies in the face of the intuitions that we have collectively developed from years of experience with neuromodulators and pharmacological agents. It is not uncommon to apply a neuromodulator or a drug and see a dramatic effect on neuronal activity when a given current is blocked or enhanced by 20–50%[47,48] (FIG. 3a). Similarly, in a model, modification of only one conductance can also produce a significant change in behaviour[49,50]. How can we reconcile the apparent sensitivity of many neurons to rapid pharmacological treatments with new data indicating that individual neurons within a class can differ by as much as two- to fourfold in the densities of many of their currents? The answer can be seen in computational models that show that a number of different compensating combinations of conductances can result in similar activity patterns[38,51]. In contrast to pharmacological manipulations, which manifest on a timescale too rapid for compensation to occur, slow mechanisms that function during development and over days and weeks can result in a set of compensating conductances that give rise to a target activity pattern.

Several studies have now directly compared the effects of short-term pharmacological blockade of a current with long-term deletions of the same current[43,52] (FIG. 3). FIGURE 3a,b compares the effects of acute tetrodotoxin (TTX) application with a comparison of recordings from Purkinje neurons from wild-type animals and those from a Na^+-channel knockout, and shows that although low concentrations of TTX produced the

Pyloric dilator neurons
There are two electrically coupled pyloric dilator neurons in each stomatogastric ganglion. These neurons are also electrically coupled to the anterior burster neuron, and the anterior burster and pyloric dilator neurons together form the pacemaker kernel for the pyloric rhythm. The pyloric dilator neurons are also motor neurons that innervate muscles that dilate the pyloric region of the stomach.

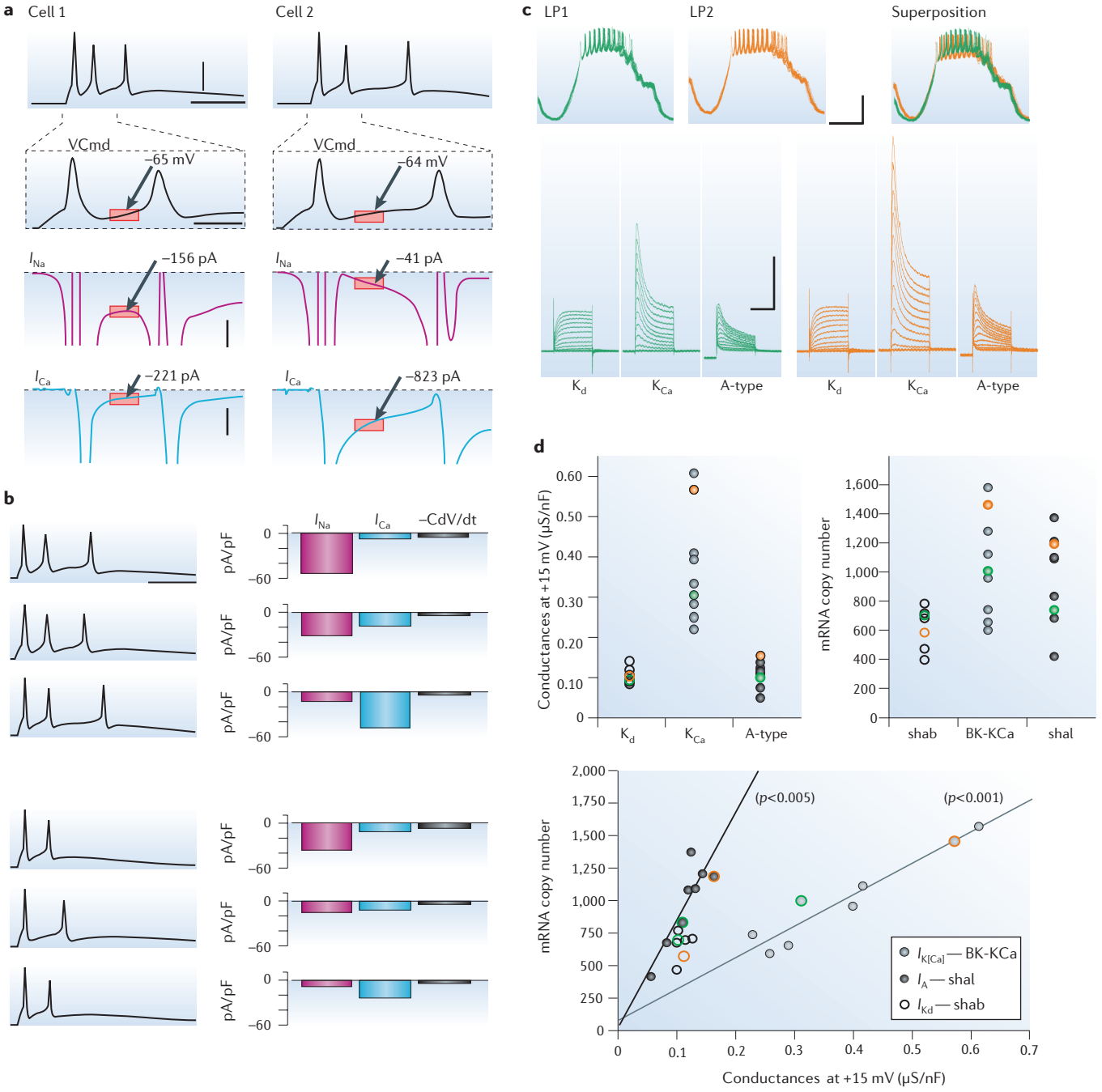


Figure 2 | **Neurons with similar intrinsic properties have different ratios of conductances. a** | Bursts of action potentials evoked in two Purkinje cells in response to a brief depolarizing pulse of current. Part of the recording including the first two spikes (voltage command (VCmd), expanded traces) was used to voltage-clamp these neurons and record the sodium and calcium currents (I_{Na} and I_{Ca}) evoked during the burst of action potentials (lower traces). The amplitude of these currents was measured in the interval between the first two spikes (orange box). The Na^+ current was larger in Cell 1. The Ca^{2+} current was larger in Cell 2. Vertical scale bars, 50 mV for voltage traces, 100 pA and 600 pA for Na^+ and Ca^{2+} currents, respectively; horizontal scale bars, 10 ms for top trace, 2 ms for expanded trace, I_{Na} and I_{Ca} recordings. **b** | Left panels show voltage recordings from three different three-spike bursters and three different two-spike bursters. Right panel shows bar plots of the amplitude of the I_{Na} and I_{Ca} currents, and the net ionic current (–CdV/dt) corresponding to the traces on the left. Horizontal scale bar, 10 ms. **c** | Top panel shows voltage recordings from two lateral pyloric (LP) cells during spontaneous activity of the pyloric network. Top right panel shows superposition of the traces shown on the left. Bottom panels show recordings of the delayed rectifier (K_d), the calcium-activated potassium (K_{Ca}) and the transient potassium (A-type) currents in LP 1 (left) and LP 2 (right). Vertical scale bars, –60 to –50 mV for top traces, 50 nA for lower traces; horizontal scale bars, 200 ms for top traces, 100 ms for lower traces. **d** | Maximal conductance values for K_d, K_{Ca} and the A-type current in 9 different LP cells (top left). Number of copies of the mRNAs corresponding to these three currents (shab, BK-KCa and shal, respectively) in the same nine LP cells presented in the previous plot (top right). Lower panel shows the correlation between maximal conductance and mRNA copy numbers for the three channels. Coloured points correspond to cells presented in **c**. Panels **a** and **b** modified, with permission, from REF. 43 © (2005) Society for Neuroscience. Panels **c** and **d** modified, with permission, from REF. 42 © (2006) Macmillan Publishers Ltd.

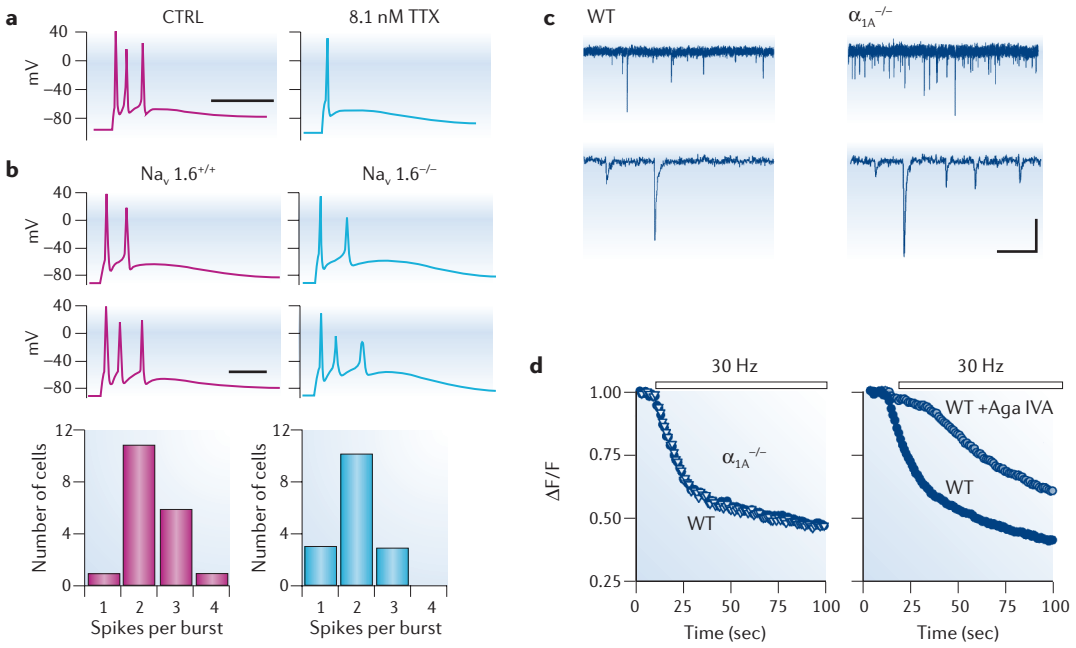


Figure 3 | **Comparison of short-term pharmacological manipulations and long-term genetic deletions. a** | Bursts of action potentials recorded from a Purkinje cell in control condition (CTRL, left trace) and in the presence of a blocker of sodium current (8.1 nM TTX, right trace). Scale bar, 20 ms. **b** | Top four traces show bursts in wild-type animals (NaV1.6$^{+/+}$, left) and in animals in which the sodium channel NaV1.6 subunit has been knocked out (NaV1.6$^{-/-}$, right). Horizontal scale bar, 10 ms. Lower panel, histogram showing the number of cells as a function of the number of spikes per burst in NaV1.6$^{+/+}$ and NaV1.6$^{-/-}$ animals (left and right, respectively). **c** | Spontaneous synaptic activity is increased in P/Q Ca^{2+} channel subunit α1A knock-out (α1A$^{-/-}$) (right) compared with wild-type (WT) animals (left). Vertical scale bar, 10 pA; horizontal scale bar, 1 s and 100 ms for top and bottom traces, respectively. **d** | Synaptic release from hippocampal neurons in culture as measured by a change in the FMI-43 fluorescence (ΔF/F). Left panel shows the evoked synaptic release following a 30 Hz stimulation of the neuron in wild-type animals and P/Q Ca^{2+} channel subunit α1A knock-out (α1A$^{-/-}$). Right panel shows that pharmacological blockade (using Aga IVA) of the P/Q Ca^{2+} channels in wild-type animals reduces the synaptic release evoked by a 30 Hz stimulation of the neuron. These two examples show the discrepancy between the effect of pharmacological blockade and genetic knockout of an ion channel. Although pharmacological blockade of the channel has a dramatic effect on the intrinsic or synaptic activity of the cell, compensatory mechanisms operate when the gene is knocked out so that this deletion has a surprisingly mild effect on the activity of the cell. It is plausible that such efficient compensation mechanisms might underlie the negative phenotype of some knockout animals. Panels **a** and **b** modified, with permission, from REF. 43 © (2005) Society for Neuroscience. Panels **c** and **d** modified, with permission, from REF. 52 © (2004) The National Academy of Sciences.

expected effects, the knockout and wild-type neurons showed very similar behaviour[43]. FIGURE 3d compares the effects of pharmacological blockade of a Ca^{2+} channel on evoked synaptic release with data from a knockout of the channel. Again, the acute pharmacological treatment was effective, whereas the knockout showed no physiological phenotype[53]. These and other experiments show significantly less effect on activity patterns of long-term deletion of a current than would be expected from short-term pharmacological blockade[43,52,54].

The involvement of slow compensatory mechanisms in the regulation of neuronal excitability has also been demonstrated by recent overexpression studies in which *shal* mRNA (encoding I_A) was injected into pyloric dilator neurons from the lobster stomatogastric ganglion[55,56]. This resulted in several-fold increases in I_A but no change in activity because the overexpression of I_A was accompanied by a compensating increase in expression of I_h. This demonstrates that slow developmental and homeostatic mechanisms can 'find' multiple solutions of correlated and compensating values of membrane conductances consistent with a given activity pattern, even while rapid pharmacological treatments that vary the value of one current at a time result in altered activity[57]. Nonetheless, it is important that not all manipulations can give rise to compensations. When mRNA for I_h was injected into the pyloric dilator neurons[58] it gave rise to a fourfold increase in I_h, which did alter the neurons' behaviour, showing that the regulatory relationship between I_A and I_h expression is not reciprocal. Interestingly, in a modelling study, deletions of I_h were also not completely compensated for by homeostatic regulation[7]. This illustrates that the challenge for the future is to discover, for each neuronal type, what its compensatory mechanisms are, and how these might differ from one cell type to another.

The paradox posed by neuromodulation. The astute reader will already have recognized that there is an essential conundrum associated with the idea that neurons with similar activity patterns under 'control conditions'

can show variable sets of underlying conductances. If a neuromodulator were to modify one or more conductances, then would the effects of the neuromodulator be expected to vary considerably depending on the starting values of the conductances? Simply stated, it might be imagined that a neuromodulator that altered one of the Ca^{2+} channels of a neuron might produce large or small effects depending on the conductance density of that channel. Is it possible to obtain consistent actions of neuromodulators even when the initial conductance densities are different? This is one of the most important questions for the future, but even today there are some interesting observations relevant to this issue. First, the effects of a given neuromodulator on cells of the same type may often show considerable cell-to-cell or preparation-to-preparation variation[59]. Second, many neuromodulators act simultaneously on more than one voltage-dependent current in a target neuron[60,61], or at several targets within a network[61–63]. By acting simultaneously on multiple targets, it is possible that consistency of neuromodulatory action is maintained as the various modulatory actions may be cooperative or synergistic. Third, some neuromodulators might move neurons 'tangentially' in parameter space (BOX 1), ensuring that they will have relatively constant actions despite differences in the neurons' starting sets of conductances[41]. Moreover, it is likely that the subset of possible solutions to generating similar behaviour is restricted by the constraints of maintaining neuromodulator action.

Target activity levels. What types of mechanism could result in individual neurons that can regulate the balance of their conductances to maintain a given activity pattern over the neuron's lifetime? One possibility is that each neuron could have a target activity pattern[6,7,10], determined as part of its identity, in much the same way that its neurotransmitter phenotype, anatomical form and synaptic partners are determined early in development. This type of mechanism would require some way of encoding a target activity rate, mechanisms to sense the activity rate, and mechanisms to modify channel density and distribution as a function of sensor values. Alternatively, instead of a target activity pattern, each type of neuron could have a genetically determined set of ion channels, which would need to be carefully regulated, in either a single or a coordinated manner, to ensure that appropriate activity resulted. Davis[10] provides a discussion of recent work in *Drosophila* and other systems relevant to the biological mechanisms that could be responsible for setting targets for homeostasis. At present, there are data consistent with both views, and it is likely that neuronal activity is controlled by a mixture of activity-dependent[13] and activity-independent mechanisms[55].

The evidence indicating that neurons have a target activity level is largely circumstantial and derives from experiments in which modifications of a neuron's intrinsic properties result from activity perturbations[13,43,64] in such a way as to restore the neuron's activity to its starting condition. Although theoretical studies can never be thought of as evidence, models in which activity is the controlled variable and conductance density is determined by negative feedback regulation using sensors of neuronal activity easily compensate for various perturbations and recover a given target activity level[6–8,65] or the ability to encode information[66]. In the theoretical studies, concentrations of intracellular Ca^{2+} are used as a monitor of a neuron's activity, and local and global fluctuations in Ca^{2+} and other signal transduction molecules are thought to couple to a series of processes that control channel synthesis and/or degradation. This is consistent with a large body of experimental evidence that demonstrates that Ca^{2+}, cyclic AMP, and other signal transduction pathways are involved in a large number of activity-dependent processes, including the regulation of channel and receptor synthesis and degradation[52,67–72].

Nonetheless, it is more difficult to understand how a 'target activity level' can be encoded. It can be thought to emerge from the equilibrium resulting from the combination of Ca^{2+} and other signal transduction mechanisms that control excitability. This would demand that different target activity levels would have either different sets of proteins that respond to signal transduction molecules, or some other mechanism to set differential target levels of activity. This means that in complex vertebrate brains the issue of cell identification might require knowledge of the expression of a specific set of genes involved in determining the neuron's activity profile[73–75], to eventually include not only the ion channels themselves, but all of the molecules that help a neuron sense its own activity, as well as all of the molecules that are involved in transcriptional regulation, insertion and degradation of ion channels. The vagueness of this model illustrates how important it will be in the future to obtain detailed information about the correlated levels of all of the transcription factors that regulate channel and receptor expression and function.

It is now becoming clear that the regulation of some channels is inextricably linked[2,55,56,76]. Therefore, to understand how neuronal excitability is regulated, it will be necessary to determine which sets of channels are coordinately regulated, either by direct interaction at the gene expression level, or by some protein level mechanism. That said, the variation of conductance densities seen in models and biological cells with similar activity is consistent with the idea that individual neurons can 'wander around in solution space' to find one of many possible solutions that gives rise to a characteristic target activity level[8]. It is important to reiterate here that the variations in conductance density that can be found in model neurons with similar behaviour might be much larger than those found in a population of biological neurons. The two- to fourfold ranges of conductance densities we have previously discussed are larger than most of us would have expected, but they also represent a smaller region of parameter space than can be found in neuronal models with similar activity[40,77,78].

Variability in synaptic strength

There is now a considerable body of literature dealing with homeostatic mechanisms that influence the long-term regulation of synaptic strength in both invertebrate and vertebrate systems[9,11,12,52,53,79–82], and this has been the

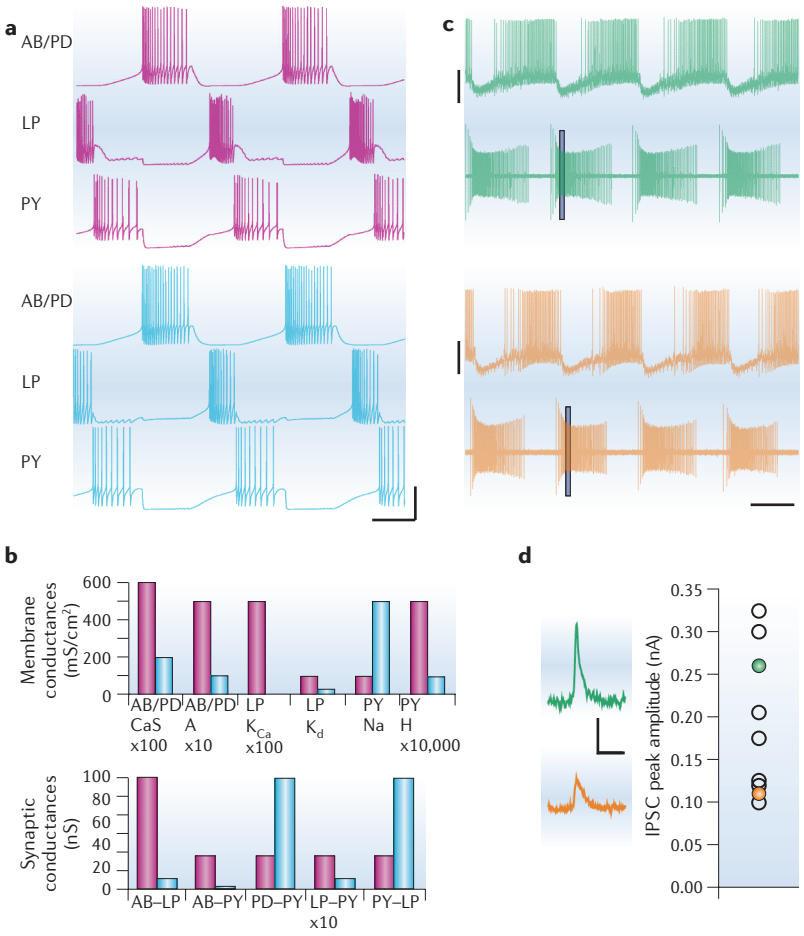


Figure 4 | **Similar network behaviour with different underlying conductances.** **a** | Triphasic pyloric rhythms generated by two different model networks. The activity of electrically coupled anterior burster (AB) and pyloric dilator (PD) neurons, as well as lateral pyloric (LP) and pyloric (PY) neurons are shown. Vertical scale bar, 50 mV; horizontal scale bar, 0.5 s. **b** | Maximal conductances for 6 different intrinsic conductances and 5 different synaptic conductances for the model networks presented in **a**. Intrinsic conductance values for the slow calcium current (CaS) and the A-type current in AB/PD, calcium-activated current (K_{Ca}) and the delayed-rectifier current (K_d) in LP, the sodium current (Na) and the hyperpolarization-activated current (H) in PY. Synaptic conductances are shown for the AB to LP, AB to PY, PD to PY, LP to PY and PY to LP synapses. **c** | Intracellular (top trace) and extracellular (lower trace) recordings showing the activity of two reciprocally inhibitory leech heart interneurons. Two different networks are shown in two different colours. Vertical scale bar, –60 to –40 mV; horizontal scale bar, 5 s. **d** | Left panel shows inhibitory postsynaptic currents (IPSCs) in the intracellularly recorded interneurons presented in panel **c**. IPSCs were recorded intracellularly during ongoing activity of the extracellularly recorded interneuron in the early phase of the bursts (grey boxes in panel **c**). Vertical scale bar, 100 pA; horizontal scale bar, 20 ms. Right, variability of the IPSC amplitude measured in 9 different preparations. Coloured points correspond to cells presented in **c** and **d** (left). Panels **a** and **b** modified, with permission, from REF. 78 © (2004) Macmillan Publishers Ltd. Panels **c** and **d**, data from REF. 92.

Long-term potentiation (LTP). A long-lasting increase in the amplitude of synaptic potentials as a result of specific patterns of presynaptic stimulation. LTP is often thought to be a cellular correlate of changes in networks underlying learning.

subject of numerous recent reviews[9,12,80,83]. The focus of much of this work is to understand how slow, baseline regulation of synaptic strength can rebalance the effects of processes such as long-term potentiation (LTP) and long-term depression (LTD), building on a long intellectual tradition that assumes that stable changes in network performance, such as those in learning, depend on the encoding of learned information in synaptic weights.

So far, most of what we know about homeostatic mechanisms for regulating synaptic strength comes from work with excitatory synapses, although activity-dependent homeostatic and/or long-term regulation of inhibition is now beginning to get the attention it deserves[83–87]. The results of these studies show that, like excitatory synapses, which can be bidirectionally scaled by activity manipulations, inhibitory synaptic events can also be bidirectionally scaled by alterations in activity[83,85]. Although synaptic scaling is often associated with changes in the number of postsynaptic receptors[9,10,85], recent studies suggest that changes in the expression of the vesicular transporters for glutamate and GABA (γ-aminobutyric acid)[86,88] might also be involved in homeostatic regulation of synaptic strength.

Networks, synaptic and intrinsic properties. Ultimately, what matters for an animal is not the strength at any given synapse or the intrinsic properties of any single neuron, but how an animal's networks produce behaviour. How, then, does network performance depend on the properties of the synaptic connections and intrinsic properties of the neurons in the network? Recent theoretical work argues that similar network function can arise from widely disparate underlying mechanisms, in much the same way that similar patterns of activity at the single neuron level can arise from different combinations of membrane currents.

Prinz *et al.*[78] recently constructed more than 20 million models of the network that generates the lobster pyloric rhythm. They first searched for those models that matched the behaviour of the biological network[89] by a variety of measures. FIGURE 4a shows the output of two of the model networks, along with the values of a number of their underlying parameters (FIG. 4b). Note that although these two networks are producing very similar triphasic motor patterns, many of the synaptic strengths and voltage-dependent conductances that give rise to these patterns are quite different. This demonstrates that, in principle, there can be various combinations of compensating synaptic and intrinsic properties that, together, can produce similar network performance.

To what extent are the intuitions gained from the work of Prinz *et al.*[78] likely to hold for biological networks? We have already discussed data that deal with the variability in intrinsic properties in the same type of neuron in different animals, but what is known about the variance in synaptic strength in small networks of identified neurons in which it is possible to identify the same physiological synapse from animal to animal? In many studies of neuromodulation or dynamics of synaptic strength, the raw data were normalized to remove the across-animal variance[90] in the control population. In other studies the actual values are presented well enough to see the apparent scatter, or it is possible to recover and plot the raw data itself[91,92]. FIGURE 4c,d shows data from a recent study on the half-centre oscillator formed by reciprocal inhibition between the pair of HNs (heart interneurons) in the leech[92]. These two preparations had similar network periods (time between onset of a burst and the succeeding burst), whereas the strengths of the inhibitory

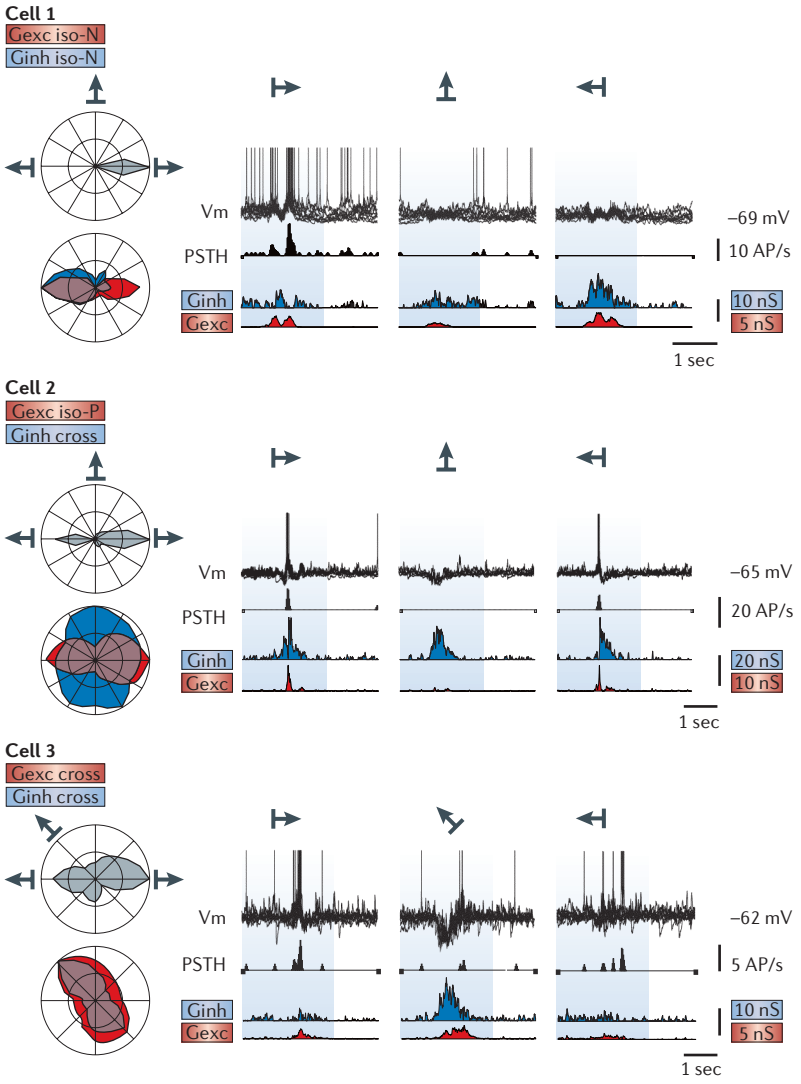


Figure 5 | **Variability of tuning of inhibitory and excitatory synaptic inputs in neurons in the cat primary visual cortex.** Three different cells are presented. Left panel, from top to bottom, shows orientation maps for the spiking response (grey, top polar map), the excitatory and the inhibitory synaptic responses (red and blue, respectively; lower polar map). The preferred direction for the spiking response is always at 0 degrees on the polar map. Right panel shows intracellular recordings (Vm) of the cell for three orientations of the stimulus (black arrows above traces); PSTH (peristimulus time histogram) in number of action potentials per second (AP/s); value of the synaptic inhibitory conductance (Ginh, blue) and excitatory conductance (Gexc, red) during the stimulus presentation. For cell 1, the preferred direction of the maximal inhibitory and excitatory conductances are opposite to the preferred direction of the spiking response (Gexc and Ginh iso-N for iso-oriented in non-preferred direction). For cell 2, Gexc is iso-oriented in the preferred direction (iso-P), and the maximal inhibitory synaptic response is cross-oriented with respect to the spiking response (Ginh cross). For cell 3, both the excitatory and inhibitory synaptic responses are cross-oriented. These results imply that the preferred orientation of the output of the neuron cannot be determined by simply looking at the preferred orientation of the excitatory or inhibitory synaptic inputs. Modified, with permission, from REF. 105 © (2003) Elsevier Science.

Long-term depression (LTD). A long-lasting decrease in synaptic strength that is induced by specific patterns of presynaptic activation.

postsynaptic currents (IPSCs) in the two preparations varied about 2.5-fold, and the strengths of the IPSCs in the entire population studied varied more than threefold (FIG. 4c,d). In the stomatogastric ganglion of *Panulirus interruptus* synaptic transmission between both the lateral pyloric and pyloric dilator neurons and between pyloric dilator and lateral pyloric neurons measured in different animals varied from about three- to fourfold[90,93]. Similarly, synaptic transmission between lateral pyloric and pyloric dilator neurons in *Homarus americanus* varied over a two- to threefold range[91].

There are two possible interpretations of these several-fold variations in synaptic strength in these small networks that are governed by inhibition. Follower neurons fire on rebound from inhibition, and their recovery from inhibition is crucially dependent on the strength of the inhibition[94], and on their intrinsic currents[48,93,95–97]. Because of this, it is possible that in different animals different values of synaptic strength are balanced appropriately by different sets of intrinsic currents — so that the recovery from inhibition remains constant across animals — or that the strength of the synapse is not very important, once it is large enough[96], as is the case with inputs to some oscillators[51,91]. Again, it is important to reiterate that although the ranges of parameters seen in the biological measurements are much larger than many would have predicted, they are smaller than those observed in less constrained modelling studies[78].

Diversity and variability in large circuits

One of the main impediments to understanding how circuits in the vertebrate nervous system work is that it is extremely difficult to identify many of the component neurons. For example, understanding the central pattern generating circuits in the spinal cord has lagged behind understanding those in smaller invertebrate ganglia because identification of the spinal interneurons has been difficult. Recent studies investigating the expression of transcription factor networks in the spinal cord hold promise for eventual cell identification[98]. Similarly, major molecular attempts to determine how to identify classes of interneurons in the vertebrate brain are now underway[73]. Because similar activity profiles can arise from different underlying mechanisms (as detailed above), and because the same neuron can have different activity states depending on its prior history of activity[99], electrical activity *per se* is an inadequate criterion for cell identification.

There are interesting issues relevant to the roles of neuronal diversity in signal propagation and stabilization in large vertebrate networks[100]. Here, we use the term 'diversity' to describe the heterogeneity of a given network in terms of the number of different neuronal types, whereas 'variability' is used to indicate the variance in parameters within a given neuronal type. In a series of papers, Soltesz and colleagues have studied the impact of both diversity and variability of interneurons on network dynamics[100–104]. Computational modelling and dynamic clamp studies showed that increasing the variability in parameters (cellular or synaptic) in a given population of interneurons or the diversity of subpopulations of interneurons can both strongly decrease the level of synchrony in the network. Therefore, the diversity of interneuronal species in a given network might be a crucial factor regulating the excitability of the network, and preventing disorders such as epilepsy[100], which has been related to increased synchrony in neuronal networks. This suggests that other neurological disorders could

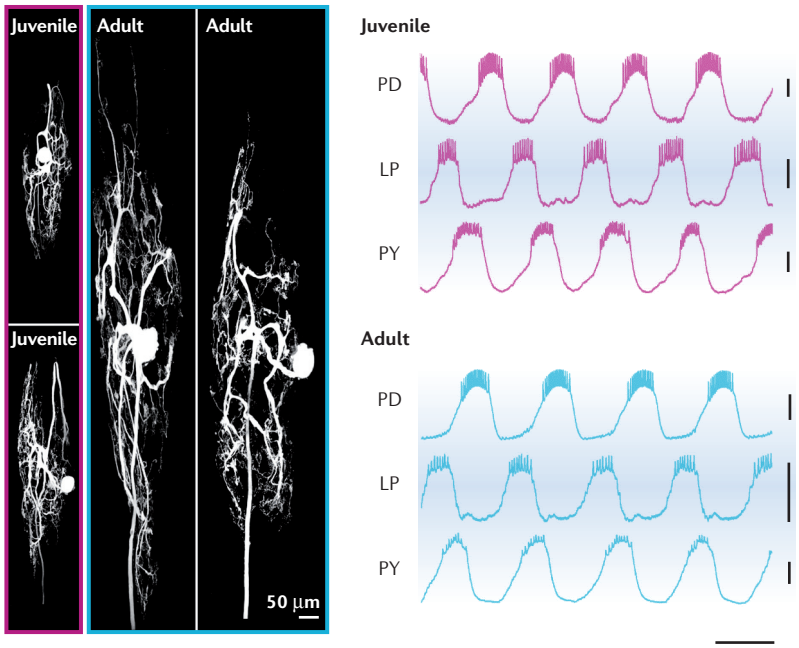


Figure 6 | **Constancy of network performance despite major size changes during growth.** Left panel shows dye fills of identified pyloric dilator (PD) neurons from the lobster, *Homarus americanus*. Right panel shows simultaneous intracellular recordings from the PD, lateral pyloric (LP) and pyloric (PY) neurons in a juvenile and adult preparation, showing similar waveforms and motor patterns. Vertical scale bars, 10 mV; horizontal scale bars, 1 s. Modified, with permission, from REF. 89 © (2005) Society for Neuroscience.

result from alterations in the extent to which individual neurons in a population maintain a significant variance in their underlying properties. This is particularly intriguing because it argues that the variance in conductances seen at the level of single neurons might not be a result of homeostatic regulation alone, but might also have a major positive physiological function.

Sensory responses and network structures. Trying to understand how network dynamics depend on the underlying synaptic and intrinsic properties is relatively easy in well-defined small networks, but becomes much less well-defined when large vertebrate networks are considered. Therefore, it is more difficult to experimentally address in larger cortical networks some of the questions that are easy to pose in small networks. However, there are indications that widely different underlying structures might also produce similar network performance in large vertebrate circuits.

The orientation and/or directional selectivity of a neuron in the primary visual cortex is defined by the selectivity of its excitatory and inhibitory synaptic inputs. The results of recent work indicate that different combinations of orientation selectivity of the excitatory and inhibitory synaptic inputs can lead to the same orientation selectivity for the spiking response of the neuron[105]. By using whole-cell patch clamp *in vivo* to record from cells in the primary visual cortex of the cat, Monier *et al.*[105] were able to separate the inhibitory and excitatory components of the synaptic input received by a cortical neuron during visual stimulation. Using this analysis, they have shown that excitatory and inhibitory inputs can have the same or different orientation selectivity and that the neuron's selectivity can be oriented differently from those of its synaptic inputs (FIG. 5). The authors of this and other studies have proposed that this diversity of combinations arises from the location of the neuron in the orientation maps present at the level of a cortical column[106,107]. These data show that in the neocortex there are diverse structural solutions for producing a given sensory response, and the choices among these different solutions are constrained by the global anatomical structure of the cortical network.

Network performance during growth. Direct evidence that multiple solutions must exist to produce similar network performance also comes from studies of growing animals. Many species, including fish, lobsters and turtles, grow considerably after achieving their mature body plan. In at least some cases, as the animals grow, their behaviour remains essentially constant[89,108], although the nervous system and muscles that underlie that behaviour are expanding greatly in size[89,108–110]. For example, the response properties of cricket interneurons remain quite constant over the animal's maturation despite the fact that additional sensory neurons are added to the synaptic input to these neurons[108]. The dye-filled neurons and recordings shown in FIG. 6 come from juvenile and adult lobsters, and demonstrate that despite the remarkable change in size, the resulting motor patterns are almost indistinguishable[89]. This suggests that as each neuron grows, all of the network's parameters must be 'retuned' so that the new membrane capacitance is properly balanced with the membrane resistances, distances to synaptic contacts, channel and receptor densities[110–112] and so on, to maintain appropriate network performance.

Target network performance. An animal's success in its environment does not depend on the strength of a single synapse or the number of channels in a given neuron. Instead, an animal's ability to feed, walk, fight and breathe depends on the overall performance of the networks that allow the animal to behave. Therefore, it is possible that the target for homeostatic regulation might not be the behaviour of individual neurons alone[9], but also network performance. How might this be achieved? In some cases it is possible that cell-autonomous or local tuning rules might be sufficient to produce adequate network performance[9]. For example, if the stomatogastric ganglion is deprived of its neuromodulatory input it first loses its ongoing patterns of rhythmic activity, then, subsequently (over days) its rhythmic activity returns[113–118]. This recovery is associated with increases in excitability in individual network neurons, as channel expression is altered. In this case, it could be sufficient for each neuron to modify the densities of its own ion channels and receptors using a cell-autonomous target for network recovery to occur. However, in other cases, adequate network performance might require an as yet unidentified global sensor of network performance to provide error signals for tuning of the network.

Synaptic weights
The strengths of synaptic potentials are often called synaptic weights. This term is commonly used in computational and network modelling studies.

Pyloric rhythm
One of the motor patterns produced by the crustacean stomatogastric ganglion. The pyloric rhythm is an example of a central pattern generator, and consists of an oscillatory motor discharge with a frequency of ~1 Hz. It is one of the best understood small circuits.

Half-centre oscillator
An oscillatory circuit produced by reciprocal inhibition. Half-centre oscillators are thought to be important components of many central pattern-generating circuits.

Lateral pyloric neuron
Each stomatogastric ganglion has a single lateral pyloric neuron, which fires in alternation with the pyloric dilator neurons in the pyloric rhythm. The lateral pyloric neuron provides the only feedback from the pyloric circuit to the pacemaker neurons, and is also a motor neuron that innervates the constrictor muscles of the stomach.

Central pattern generator
A neural circuit that produces rhythmic motor patterns without requiring timed sensory input.

Many preparations show spontaneous network activity early in development[119–126], often considerably before the behaviours subserved by these networks are needed. For example, rhythmic movements are produced by embryos of both vertebrates and invertebrates. One possibility is that spontaneous network activity provides tuning signals necessary for the correct development of adult networks. In an interesting new study, researchers blocked spontaneous activity in the chick embryo for 2 days and found compensatory changes in overall network activity and in both GABAergic and glutamatergic synaptic events[127]. Here again, the question is whether there is some sensor of overall network activity, or whether changes in network performance are an emergent property that results from the implementation of local synaptic tuning rules[127].

Conclusions

Variability and compensation are not specific networks of neurons[128,129]. Such properties have also been described for biochemical and genetic regulation networks[10,130–133]. These networks, like those described above, are able to adapt to modifications of their components to maintain the same output, even if one of their components is deleted. This has sometimes been attributed to redundancy, but is much more likely to be a consequence of the potential for compensation among components with different properties[128]. In fact, unlike in engineering, in which redundant systems often consist of two or three copies of identical devices, such as electrical generators or navigation systems, in biological systems there are probably no truly redundant processes, but system robustness and flexibility are achieved simultaneously through overlapping functions and compensation. This gives the organism more resistance to mutations, and possibly more evolutionary potential[133], as organisms can move towards beneficial solutions in altered environments.

For years, the limitations of their experimental tools have so concerned most reductionist biologists that they have assumed that much variation in measurements from cell to cell, day to day, and animal to animal are an outcome of measurement error, although, of course, we know that individuals differ in almost any property we can describe. Improvements in computational power and imaging methods[134,135] now offer new ways to look at the complex correlations that will allow us to understand the mechanisms by which compensations in complex networks occur. This will give us new understanding of how networks can be both robust and flexible at the same time.

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Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
Marder's laboratory: http://www.bio.brandeis.edu/faculty01/marder.html
Access to this links box is available online.

BIOGRAPHIES

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Jean-Marc Goaillard is a postdoctoral fellow who joined the Marder laboratory at Brandeis University, Massachusetts, USA, in 2002. Goaillard received his Ph.D. in 2002 from the Université Pierre et Marie Curie, Paris, France. For his thesis work, he studied the regulation of intrinsic properties by neuromodulators in vertebrate cardiac muscle and neurons using simultaneous cyclic AMP imaging and electrophysiological recordings. Goaillard is interested in homeostasis and neuromodulation, and his long-term goal is to study these problems in epilepsy.

ON-LINE SUMMARY

• Whereas neurons may live for scores of years, ion channels and receptors turnover in the membrane in minutes, hours, days or weeks. This means that neurons are constantly rebuilding themselves and neuronal circuits are in a constant state of molecular flux.
• Homeostatic mechanisms that help to regulate intrinsic excitability and synaptic strength are needed to stabilize circuit performance.
• Computational models have demonstrated that similar activity patterns can be produced by different underlying mechanisms.
• Experimental work indicates that the densities of ion channels can vary by as much as two- to fourfold across neurons of the same type in different animals, and that mRNA expression in the same neuron type can also vary in about the same range.
• Intuitions about channel function that are developed on the basis of rapid pharmacological manipulations may fail to predict the results of long-term genetic manipulations of the same channel because of slow, compensatory mechanisms.
• Much future work is needed to define the combinations of parameters that can give rise to a desired pattern of activity in neurons and networks, to discover the molecular mechanisms that regulate target activity levels, and to uncover the mechanisms by which compensatory regulation of channel expression occurs.

ToC blurb (max. 40 words)

Many neuronal and network behaviours are surprisingly stable in the face of ongoing fluctuations in channels and receptors. The authors discuss issues relevant to the homeostatic regulation of synaptic and intrinsic currents necessary for stable neuronal and network activity.

Further information

Marder's laboratory: http://www.bio.brandeis.edu/faculty01/marder.html