The new work discussed here [7,8] is a significant advance in the study of natural behaviour in a lab context. There are, however, still other important aspects of real-world behaviour that need to be brought into the lab. As discussed above, natural behaviours emerge from the complex interaction between brains, bodies and environments [6] and to capture the essential nature of complex real-world behaviours we have to complement free behaviour with natural reward schedules and ecologically relevant sensory statistics (Figure 1). Furthermore, real behaviours depend on multi-modal sensory information where the fine details of an animal's response to sensory information depends on species specific ecological tuning. Thus, we have to understand behaviour in the real world [17] before we can bring real-world behaviour into the lab.

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Neuronal Homeostasis: Voltage Brings It All Together

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Neurons generate consistent patterns of activity combining a large set of ionic currents and variable conductance levels. How they regulate this variability so that it does not run out of control seems to depend most prominently on neuronal activity itself.

Most neurons produce a specific and consistent pattern of activity, which they can maintain for extended periods of time. This activity is usually produced by the interplay of many ionic currents. The properties of these currents must be carefully balanced for the activity to remain within functional boundaries, and this needs to be accomplished homeostatically as conditions, inputs and perturbations change over time. Furthermore, neurons in a population of a given cell type often generate their characteristic (often nearly identical) patterns of activity even though they express widely different ionic conductance levels [1,2]. In order to be able to do this, ionic current subsets in a cell appear to be co-regulated so that their relative amplitudes are maintained, which generates correlated levels of conductance in populations of identical neurons [3,4]. How do cells coordinate the expression of the multitude of ionic currents on their membrane needed to produce their cell type-specific pattern of activity? At least two broad mechanisms have been proposed: activity feedback and neuromodulatory input, with evidence supporting each [5,6]. Activity

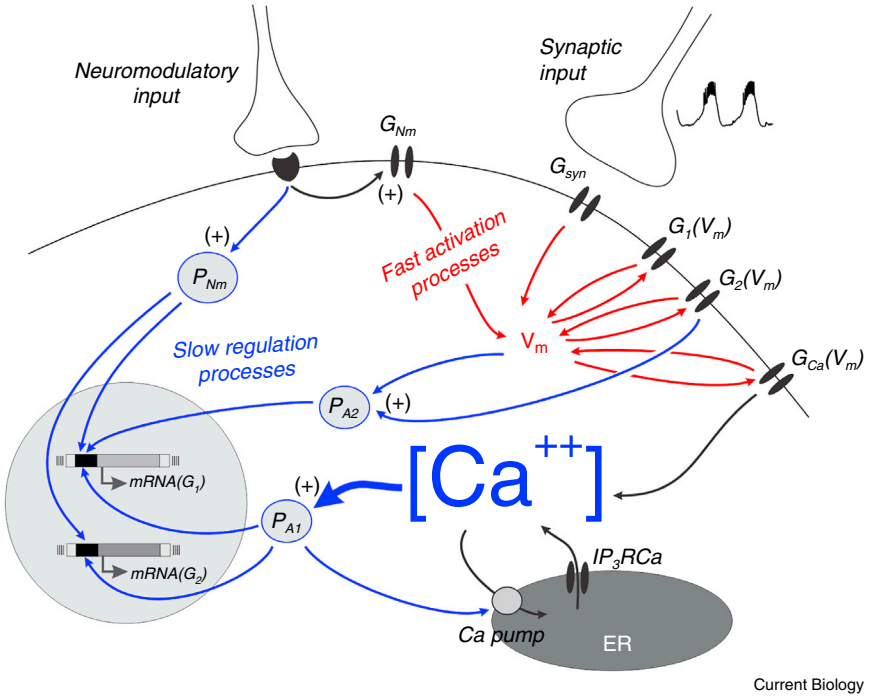

Figure 1. Homeostatic ion channel regulation pathways.
Represented is a neuron that receives synaptic and neuromodulatory input and regulates the expression of multiple ion channels to control their relative levels under the influence of activity. G_1 and G_2 are generic voltage-gated ion channel populations whose mRNA levels are regulated via transcription regulatory pathways in the nucleus (light grey circle). G_{Ca} are Ca^{++} channels, which could also be regulated (not indicated), but whose main role here is to be one of the two sources of intracellular Ca^{++}. The second is the endoplasmic reticulum (ER; dark gray oval) via IP_3-dependent Ca^{++} channels, for example. Ca^{++} is assumed to be the main transducer of neuronal activity levels and is shown occupying a prominent place. Ca^{++} regulates an activity-dependent signaling pathway (P_{A1}), but different pathways regulated by either Ca^{++} or other factors (P_{A2}) can exist. P_{A2} is shown as being regulated by channel protein presence on the membrane as suggest by Mee *et al.* [18], and by voltage, but these could be separate paths too. Voltage (V_m) is directly affected by ion channel activity (red arrows) and affects voltage-gated channels directly at very fast time scales (red). Neuromodulatory input can activate specific ion channels (G_{Nm}) as well as regulate transcription. Ca^{++} and neuromodulator-dependent regulation of transcription is a slow process (blue), and pathways activated by each may interact at multiple levels (here only shown at the transcription regulation level).

has been known for some time to homeostatically regulate ionic conductance levels [7,8], but confounding factors (modulatory and synaptic input) have sometimes obscured the specific role of activity. New work by Santin and Schulz [9] reported in a recent issue of *Current Biology* has addressed this issue head on by using the voltage clamp technique in dynamic mode to impose back on neurons their own prerecorded activity after all modulatory and synaptic input, as well as their own spontaneous activity has been completely removed, thus fully separating activity from these factors.

To do this, Santin and Schulz evaluated the effect of activity on the expression of pair-wise correlations of ion channel mRNA levels in identified crab stomatogastric ganglion pyloric dilator (PD) neurons. First, they recorded the spontaneous activity of these cells, then blocked activity, and synaptic inputs and neuromodulator release onto them (using tetrodotoxin, TTX), completely isolating them from these influences. They determined mRNA counts of 13 ion channel genes and evaluated their correlated levels under three different conditions: control (no TTX), TTX-isolated, and in isolated neurons plus their own activity imposed back with voltage clamp. The mRNA counts of all genes in the neurons from these three sets were plotted against each other and pairwise correlations between them were determined. From the 78 possible unique correlated pairs, 33 were found to be significantly correlated in control conditions, most of which were lost when activity was eliminated for 8 hours. Most (21) correlations were restored when their activity (and only activity) was played back onto these cells. These results almost certainly eliminate any factors other than activity as possibly regulating the balance of ion channel mRNA levels in the restored pairs. However, new questions arise, some mentioned by the authors: is voltage the most important factor in regulating ionic channel correlations in different cell types (one third of them don't appear to be regulated by activity)? Is activity a more important factor in cells with regular activity patterns, such as the PD neurons in this study, than in neurons with more irregular patterns? What is the role of neuromodulatory input? Why do we not see negative correlations in these data? What is the role of these correlations in generating activity? And, of course, what are the cellular mechanisms that translate activity into ionic channel co-regulation?

The data of Santin and Schulz allow us to valuate some theoretical predictions made before [10,11]. O'Leary and collaborators have proposed an elegant activity-dependent mechanism that generates linear correlations among currents that homeostatically adjust activity to within close boundaries of a given pattern [11]. Hudson and Prinz [10] predicted that the majority of correlations should involve Ca^{++} currents, and both studies predicted that up to 30% negative correlations should be observed. Activity as a key factor and the existence of positive, linear, activity-dependent correlations in PD neurons are clearly confirmed. However, most correlations do not involve Ca^{++} currents, and no negative correlations were observed. Not all positive correlations can achieve homeostasis of activity. Think for example of two similar K^+ currents (e.g., Shal and BKCa, or Shal and Shaker). While homeostasis can be achieved by negative correlations of same sign currents (e.g., [12]), that is not what we see here: half of the correlated pairs are between currents of the same sign. So, what might these correlations be controlling? In neurons with simpler physiology, such as neurons that only spike tonically [13], neurons that generate stereotypical action potentials

[14] or neurons that are specifically tuned to respond to a narrow stimulus frequency [15], a very specific feature of activity needs to be regulated. For that, a pair of conductances might suffice [13–15]. But for neurons with more complex activity patterns, such as the bursting of PD neurons, there are many features of activity that may need to be regulated (slow wave shape, amplitude, and frequency, action potential frequency, etc.). One subset of correlated conductances may specifically control one activity feature but also encroach into others. Is there a hierarchy of activity features that must be obeyed when it comes to adjusting the co-expression of conductance subsets? This was the best explanation of what was observed by Zhao *et al.* in this same cell type [16]. If so, can multiple subsets of conductances separately regulate corresponding features of activity? Perhaps part of the answer lies in the different correlation relationships (slopes) for each channel pair. It is important also to consider that mRNA correlation levels are not the same as correlations of ionic currents (or conductances), which are ultimately what determines neuronal activity. Many steps separate the transcript product (mRNA) from the protein product (functional channels), including translation regulation, post-translational modifications and membrane expression. In fact, when conductance levels are measured in the same neurons in which mRNA levels are measured, the variance of the correlations is much higher, and the slopes can be substantially different [17]. This suggests that what we observe at the transcript level does not necessarily neatly translate to the expression of activity. And yet, activity feedback determines these correlations at the transcript level. Thus, the functional significance of the correlations observed as well as the mechanisms that control them remain a wide open field of inquiry.

Studies such as those of Santin and Schulz beg the question of what cellular mechanisms sense voltage and how they regulate subsets of currents to produce the correlation relationships observed (Figure 1). Since the first studies suggesting that activity regulates ion channel expression, intracellular Ca^{++} level changes have been assumed to be the universal activity sensor as many intracellular signaling pathways are Ca-dependent [5]. Recent studies have revealed several highly specific mechanisms that homeostatically regulate ion channel expression, which may or may not involve Ca^{++}. For example, Mee *et al.* showed activity-dependent regulation of the *para*-encoded Na^{+} channel by a path requiring the translation repressor Pumilio [18]. This regulation in turn requires cofactors Nanos and Brain Tumor (Brat) in some cell types, and only Nanos in others, to form a repressor complex that results in the translational regulation of yet another mRNA (*hunchback*) and ultimately *para*. Pumilio furthermore also regulates *Shal*, which codes for a transient K^{+} current similar to that observed in PD neurons [19]. What the link is between changes in activity and activation of Pumilio is not known (perhaps Ca^{++} level changes). In a different study, Kulik *et al.* have characterized two separate and independent pathways that homeostatically regulate action potential firing after perturbation of the *Shal*-encoded K^{+} channel [13]. One pathway requires the Krüppel transcription factor and involves the enhancement of several other K^{+} channels (*slo, Shab*, and *Shaker*), while the other involves a Krüppel-independent enhancement of the delayed rectifier K^{+} channel. Interestingly, the Krüppel-independent pathway is activated by the expression of a non-conducting Shal channel, while the Krüppel-dependent pathway is activated by the removal of the Shal channel from the membrane [13]. Thus, it appears that these cells can detect both the change in current flow through the membrane as a separate signal from the absence of a specific channel protein in the membrane. Does this suggest that each channel protein regulates its own replacement pathway? Does this happen via effects on activity, or is there a direct link from channel protein to transcription regulatory molecules? The complexity and exquisite level of control involved is quite remarkable, and one can well imagine that the disturbance of some of these pathways may be involved in some neuropathologies.

These different pathways show high degrees of specificity and it is unclear how activity alone can select pathways with such a degree of precision (but see [20]). Santin and Schulz's work, however, suggests that many channel pairs are regulated simultaneously, which may hint at a lack of specificity in this system [9]. However, this brings us back to what exactly is being regulated among the many features of activity expressed by neurons whose activity patterns are as complex as those of PD neurons. Additionally, current correlation in PD neurons has also been shown to be dependent on neuromodulatory input [6], which may in part explain why some mRNA pairs in the Santin and Schulz study do not recover their correlation by restoring activity. What cellular mechanism might be involved in that form of co-regulation of ion channels remains to be determined. It is also intriguing to consider whether interactions between activity- and neuromodulator-dependent signaling pathways exist, which would add another large degree of complexity to the question of how neurons balance their channel populations to maintain functional activity patterns.

There is much work that needs to be done to unravel the questions addressed here, and possibly many others. Work on invertebrate neurons should continue to lead the way given the high level of tractability of these systems, including the ability to precisely identify cell types, to address these questions. However, work on mammalian and other vertebrate species is quickly advancing and should reveal interesting alternative mechanisms.

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Neurobiology: REM-Sleep-Promoting 'Goldilocks' Neurons

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REM sleep is a paradoxical state accompanied by suspended thermoregulation that is preferentially expressed under optimal ambient temperatures. Komagata and colleagues now demonstrate that activity in hypothalamic melanin concentrating hormone neurons is essential for the temperature-dependent modulation of REM sleep.

We spend one third of our lives disconnected from the environment, in a state that prevents us from working, feeding, parenting or defending ourselves — i.e. asleep. We generally think of sleep as one homogenous state of inactivity; however, if you could watch yourself sleeping, you would notice periodic changes in behavior as you cycle through the two major sleep states found in mammals and birds: non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. During NREM sleep, breathing is slow, and your limbs and eyes are peacefully at rest. However, as you transition from NREM to REM sleep, breathing becomes fast and irregular, your limbs twitch, and your eyes dart around rapidly. Interestingly, these behavioral changes are associated with dramatic changes in brain activity, muscle tone, and thermoregulation [1]. During NREM sleep, neurons in the neocortex pulse on and off, resulting in large electroencephalogram (EEG) slow waves that wash across the neocortex [2] like the wave performed by fans in a sports stadium. By contrast, during REM sleep most of the brain becomes continuously active, like fans standing in excitement, resulting in EEG activity remarkably similar to wakefulness. The awake-like brain activity occurring during sleep led to this state also being called paradoxical sleep. Strangely, despite the presence of eye and limb twitching, skeletal muscle tone is absent, rendering our body virtually paralyzed. And, if that were not bizarre enough, even though mammals and birds are endotherms with fine-tuned mechanisms for regulating body temperature, including the capacity to shiver when too cold or pant when too hot in NREM sleep, they suspend thermoregulation during REM sleep [3–5]

