When the electricity (and the lights) go out: transient changes in excitability

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Natural or artificially induced electrical activity changes can alter ion balance so as to briefly influence firing. An optogenetics study delineates one mechanism: Cl^- shifts causing seconds-long excitability changes after silencing.

Both native and exogenously imposed influences on neural activity, whether excitatory or inhibitory, can briefly alter the distribution of ions across neural membranes to produce similarly brief alterations in important parameters governing neuronal function such as resting potential and membrane excitability. These changes may persist for many seconds after cessation of activity[1] and have been observed under a range of experimental circumstances, including natural burst-firing as well as epileptic and migraine-epileptiform discharges and electrical stimulation[1,2]. Like electrical interventions, optogenetic interventions (using light-activated ion channels or pumps with genetically specified cell targeting) offer no exception to this rule, but rather provide an opportunity to causally test effects of modulating the distribution of individual ionic species with high temporal precision. Indeed, traditional methods (including electrical stimulation) for manipulating neural tissue have not permitted researchers to fully disentangle the causative role of individual ion species in regulating poststimulus excitability. In this issue of *Nature Neuroscience,* Raimondo *et al.*[3] carefully demonstrate one of the routes to ion-balance aftereffects, focusing on the role of Cl^- shifts in regulating postmodulation excitability.

This work provides meticulous quantitative assessment of a phenomenon initially predicted in 2007 (ref. 4), when it was cautioned with regard to the optogenetic halorhodopsin tool NpHR (which generates intracellularly directed Cl^- flux) that the resulting elevations in Cl^- can modulate the effects of endogenous GABAergic neurotransmission and that this factor must be considered in experimental design. Appropriate caution has indeed been exerted in the ensuing years, with investigators in general careful to ensure that experimental findings, behavioral exposures and electrophysiology that depend on inhibition of the target population are obtained or carried out during (and not immediately after) the optogenetic inhibition[5–9], with appropriately safe temporal windows. And, of course, similar considerations are indicated for most experimental interventions (whether pharmacological, electrical or optogenetic; for example, electrical stimuli and optogenetic channelrhodopsin tools will alter the distribution of Na^+, K^+, Ca^{2+} and H^+ ions, and optogenetic H^+ pumps produce outwardly directed H^+ flux). Here Raimondo *et al.*[3] focused specifically on the Cl^- aspect of this complex picture and quantitatively mapped the extent to which Cl^- transported by halorhodopsin indeed can cause the predicted shift in the $GABA_A$ receptor ($GABA_AR$) reversal potential, which in turn can affect excitability of expressing cells for several seconds after prolonged illumination.

Raimondo *et al.*[3] tested optogenetic silencers (eNpHR3.0 and Arch) expressed in organotypic hippocampal cultures or in acute hippocampal slices and began by quantifying changes in cell excitability following optogenetic silencing. Synaptically evoked spikes in opsin-expressing pyramidal neurons in the CA1 and CA3 regions were elicited by electrically stimulating the afferent Schaffer collaterals, before and after a 15-s epoch of opsin-activating illumination. Owing to accumulation of intracellular Cl^- influencing inhibitory synaptic transmission through Cl^--permeable $GABA_A$ receptors, the probability of the recorded neuron spiking in response to electrical stimulation could be increased for seconds after eNpHR3.0 (but not Arch)-mediated silencing. Indeed, by applying of puffs of GABA directly to the halorhodopsin-expressing somata, the authors confirmed that before laser activation GABA induced a small hyperpolarizing response, whereas immediately after an epoch of illumination the same GABA puff transiently evoked depolarization. The authors carefully quantified the change in $GABA_A$ reversal potential (E_{GABA_A}), observing ~8.8 mV of positive shift in E_{GABA_A} per 100 pA of eNpHR3.0 photocurrent. This photocurrent-induced modulation of E_{GABA_A} persisted for approximately 15 s after the laser was switched off, was highly correlated with photocurrent magnitude and increased with photocurrent duration. In contrast, for Arch-expressing cells (Arch does not directly move Cl^- ions), E_{GABA_A} after light persisted at approximately –70 mV, as expected.

The authors took appropriate precautions to systematically repeat experiments in two preparations (organotypic cultures and acute hippocampal slices), with similar results. They also took care to preserve the intracellular ionic composition of cells by recording under gramicidin-perforated patch, loose-patch and cell-attached configurations. Although eNpHR3.0 and Arch were expressed under different promoters, photocurrents were similar in magnitude. The authors also included a single-compartment model of Cl^- homeostasis to explore a variety of theoretical cell and photocurrent parameters and verified that experimental findings matched those predicted. Finally, the authors demonstrated that $GABA_A$ receptor reversal potentials could be shifted by native GABA receptors as well as by opsin-induced currents: by incrementally depolarizing the cell while applying a puff of GABA, the authors were able to load Cl^- into the cell to variable extents. When membrane potential was returned to rest and GABA again focally applied, the polarity of the $GABA_A$ current was reversed, corresponding to the depolarizing shift in the $GABA_A$ reversal potential seen following eNpHR3.0-mediated silencing.

Raimondo *et al.*[3] focused on Cl^- ion balance, testing for changes in $GABA_A$-mediated chloride signaling and not for effects of altered intracellular or extracellular pH that are expected from prolonged H^+ currents. Especially in the setting of long, continuous light pulses, alterations in extracellular proton composition in principle[10,11] are likely to influence operation and excitability of adjacent nonexpressing cells (for example, through diverse and ubiquitous acid-sensitive cation channels). Moreover, previous studies *in vivo* and *in vitro* have tested for or observed changes in excitability of proton pump–expressing

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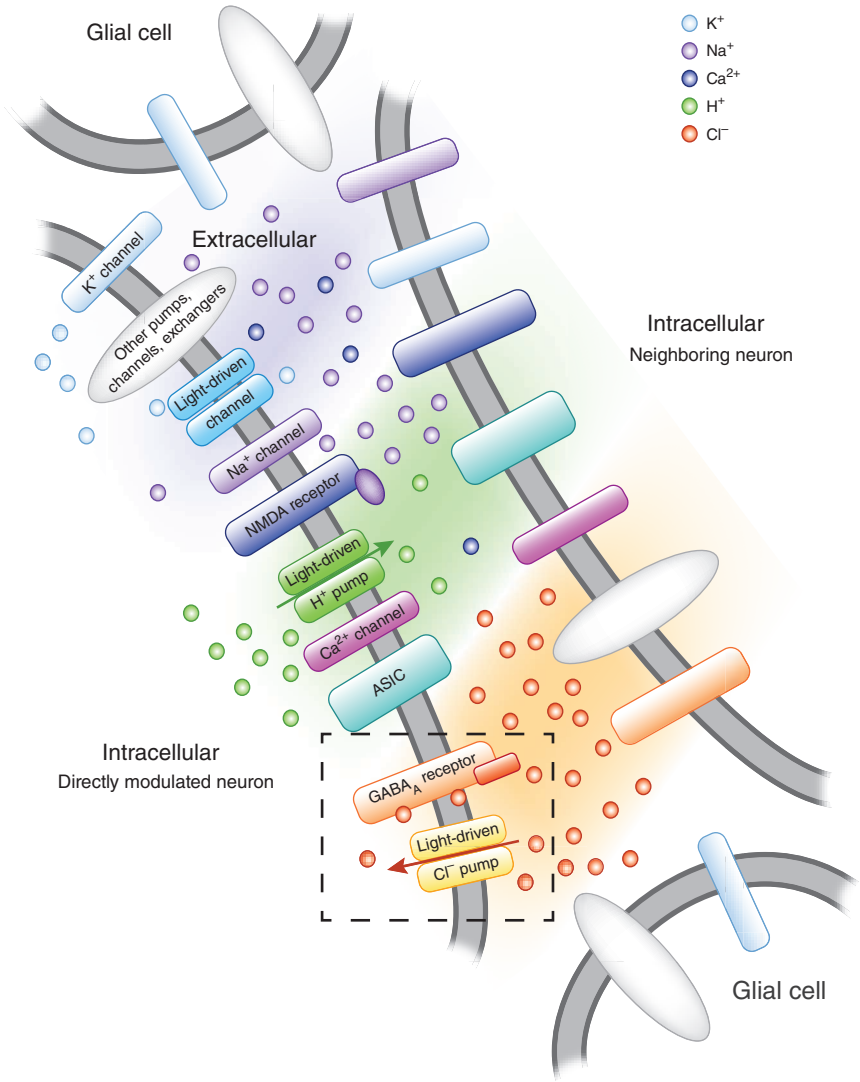

Figure 1 Interactions among unmodulated and directly modulated elements of neuronal circuitry. A directly modulated neuron is shown at left and an unmodulated neighboring neuron at right, along with two glial cells. The directly modulated cell may be stimulated synaptically, electrically (for example, through a distant axonal projection) or optogenetically (through its expressed light-driven channel, proton pump or chloride pump). Blue shading denotes a domain wherein efflux of K^+ ions from the directly modulated cell (arising from electrically driven or light-driven action potential bursts) may depolarize neighboring cells. Green shading denotes a zone wherein pH changes from electrical activity, light-driven channel activity or proton pump activity may modulate pH-sensitive channels (such as neurotransmitter receptors, voltage-gated channels and ASIC-type (acid-sensing ion channel) cation conductances) on the same or neighboring cells. Orange shading marks a domain wherein Cl^- shifts driven by native $GABA_A$ receptors or light-driven Cl^- pumps may modulate Cl^- driving forces affecting operation of Cl^--conducting channels, pumps or exchangers. Dashed box indicates the domain studied by Raimondo *et al.*[3]. Although interactions such as these come into play during natural neural activity as well as during electrical or optical interventions, many other potential interactions exist among native or introduced ion conductance regulators, as well as between directly modulated and nearby circuit elements.

tissues that outlive the light stimulus in a way that cannot be intrinsic to pump currents (based on known photocurrent kinetics) but instead are consistent with changes in ion balance and are similar in magnitude across classes of silencers[11–13]. Stimulation- or inhibition-mediated ion fluxes should be viewed in the context not only of the targeted cells but also in the context of neighboring cells and the many downstream ion fluxes generated by native ion channels that could be affected by stimulus-mediated currents in active neural tissue (**Fig. 1**).

In 2009, Tønnesen *et al.*[14] used NpHR to interrupt epileptiform activity in hippocampal slices and explicitly tested for changes in reversal potential of inhibitory currents in the presence of NpHR photocurrents; they observed no depolarizing effects or other evidence of NpHR-mediated increases in excitability. Although a lower-expressing version of NpHR was used by Tønnesen *et al.*[14], they observed potent interruption of strong seizure-like activity, thereby indicating that a substantial window for efficacy without aftereffect exists for NpHR-related tools, for which photocurrents can be experimentally tuned by altering expression and/or illumination level depending on the needs of the preparation. Indeed, several published *in vivo* experiments using high and effective levels of NpHR activation have identified little or no post-light changes in excitability[5–9], perhaps in part owing to differences between the *in vitro* physiology of Raimondo *et al.*[3] and the *in vivo* physiology of other studies involving temperature and ionic clearance mechanisms. Cell type differences may also be relevant; indeed, in 2007 Zhang *et al.*[15] found that muscle cells were more susceptible to prolonged NpHR-induced aftereffects than neurons and hypothesized that this difference might reflect tissue differences in Cl^- homeostatic capacity. Other differences could also stem from variation in experimental design, such as the use of GABA puffs after the illumination by Raimondo *et al.*[3], which would recruit both dendritic and perisomatic $GABA_A$ receptors, versus electrically induced inhibitory postsynaptic currents during the illumination by Tønnesen *et al.*[14].

The study by Raimondo *et al.*[3] represents a very important step in our understanding of the interaction between neurons and the ionic environment. Moreover, even though postillumination excitability changes (when present) last only for seconds, this study underscores the point that optogenetic interventions, like electrical and natural bursts of activity, can influence the cellular environment in ways that should be taken into account when designing experiments, and particular caution should be exerted when interpreting the postintervention epoch. Finally, this study sparks important consideration of the dynamic interplay among circuit elements contributing to the complex tapestry of ion movement and cellular interactions crucial for brain circuit structure and function.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details are available at http://www.nature.com/doifinder/10.1038/nn.3172.

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New game for hunger neurons

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Hypothalamic neurons that express agouti-related protein have been thought to regulate appetite by counteracting the melanocortin signaling pathway. Evidence now indicates that these neurons can also modulate dopamine signaling.

A study by Dietrich *et al.*[1] in this issue of *Nature Neuroscience* adds a new dimension to the function of specific neurons in the hypothalamus that are traditionally associated with regulating feeding behavior and metabolism. Hypothalamic neurons are thought to promote the homeostasis of physiological systems involved in growth, reproduction, circadian rhythms, sleep, temperature, salt balance and energy expenditure, to name a few. Neurons in the arcuate region of the hypothalamus that make agouti-related protein (AgRP) have received particular attention over the last two decades because they modulate feeding behavior[2]. AgRP-expressing neurons also produce neuropeptide Y (NPY) and GABA, but they are now often referred to as AgRP neurons because they are the only neurons in the brain that produce AgRP, whereas NPY and GABA are expressed widely in the nervous system. The unique expression of AgRP allows selective genetic manipulation of these neurons in the mouse, which was essential for Dietrich *et al.*'s study[1].

Using two mouse models with ablated or compromised AgRP neurons, Dietrich *et al.*[1] expand the potential action of AgRP neurons from simply modulating pro-opiomelanocortin (POMC) neuron signaling to also modulating dopamine reward and motivational circuitry. Their models included mice lacking Sirt1, a deacetylase that serves as a metabolic sensor[3], selectively in AgRP neurons and mice in which AgRP neurons were ablated shortly after the mice were born. They report that, compared with littermate controls, mice from both models are hyperactive in a novel environment, display enhanced locomotor activity in response to cocaine and manifest increased preference for an environment that they associate with an injection of cocaine.

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Cocaine is known to enhance dopamine signaling by blocking the dopamine transporter. Thus, the authors explored the possibility that impaired AgRP neuron function affects dopamine signaling. They found that AgRP neurons send axonal projections to dopamine neurons in the ventral tegmental area (VTA) and that the amplitude, but not the frequency, of miniature inhibitory postsynaptic currents is reduced in both mouse models, suggesting reduced GABAergic input and, consequently, higher dopamine neuronal activity. The reduced inhibitory tone also facilitates induction of long-term potentiation in VTA neurons of the *Sirt1* knockout mice. Consistent with potential hyperactivity of VTA dopamine neurons, Dietrich *et al.*[1] observed elevated extracellular dopamine in the nucleus accumbens, a prominent target of VTA neurons and a brain region responsible for psychostimulant effects.

Notably, the regulation of the VTA by AgRP neurons appears to be mediated by GABA rather than neuropeptides. These results suggest that the activity of VTA dopamine neurons is inversely affected by AgRP neuron activity. Dietrich *et al.*[1] show that compromising AgRP neuron function decreases GABA signaling and enhances dopamine signaling, which should enhance dopamine-dependent processes, such as learning and motivation; conversely, activation of AgRP neurons should release more GABA and inhibit dopamine signaling. AgRP neurons are thought to be most active when animals are starved and least active when they are fat. Many experiments have shown that enhanced AgRP neuron activity promotes robust feeding, which fits with the view that reduced dopamine signaling, especially through dopamine D2-type receptors, promotes obesity[4]. However, as an animal becomes obese, enhanced dopamine signaling would be predicted to facilitate the motivation to explore and engage in various activities, which does not occur. This simplified analysis ignores the fact that we do not know how the activity of AgRP neurons changes under environmental conditions, as their activity has never been recorded *in vivo*. The overall effect of enhanced AgRP neuron activity could be complex, as AgRP neurons would simultaneously signal to many other brain regions[5], as well as to dopamine neurons (**Fig. 1**). Furthermore, other neuronal systems are affected by changes in energy balance.

The prevailing idea until now was that AgRP neurons promote feeding by counteracting signaling by neighboring POMC-producing neurons, thereby overcoming the anorexic

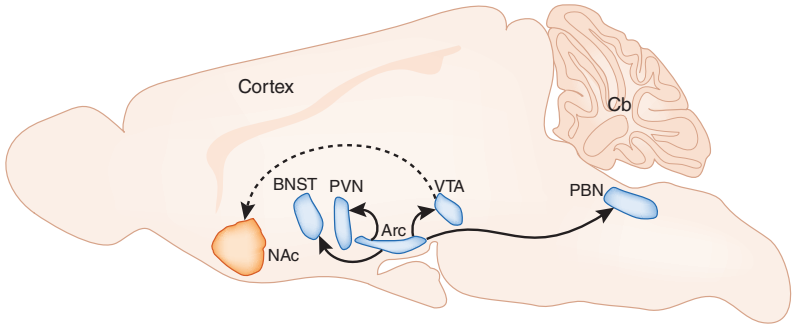

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Figure 1 Reduced activity of AgRP neurons facilitates VTA dopamine neuron activation of the nucleus accumbens. AgRP neurons in the arcuate region of the hypothalamus (Arc) produce AgRP, NPY and GABA and send their axonal projections to various brain regions, including the bed nucleus of the stria terminalis (BNST), paraventricular nucleus (PVN), parabrachial nucleus (PBN) and VTA. VTA neurons in turn project to the nucleus accumbens (NAc). Cb, cerebellum.