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Formula for Unsilencing Plasticity: Spike with GABA

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A complication for developmental plasticity mechanisms like spike-timing-dependent plasticity (STDP) is that immature postsynaptic neurons may lack sufficient input to fire action potentials. In this issue, van Rheede et al. (2015) report an activity-dependent mechanism that converts non-spiking cells into spiking neurons, priming them for further plasticity.

During development, connectivity of neural circuits is refined through plasticity mechanisms that rely on the patterned firing of action potentials. A prominent example is the phenomenon of STDP in which the relative order of presynaptic input activation and the firing of a back-propagating action potential in the postsynaptic neuron determines the direction of synaptic change (Feldman, 2012). An underlying requirement for STDP to drive circuit refinement in response to sensory stimulation is that temporally correlated inputs must converge to trigger an action potential in the postsynaptic cell. A fundamental complication for this model is that immature neurons may lack sufficient intrinsic excitability, input number, or synaptic strength needed to fire an action potential.

It has long been understood that the key operation by which information travels through neural networks is the all-or-nothing action potential or "spike." The code of information processing in neural circuits is thus defined by individual neurons' ability to generate spikes and thereby relay information from presynaptic inputs to postsynaptic outputs. In this issue of *Neuron*, van Rheede et al. (2015) utilize the optic tectum of the *Xenopus laevis* tadpole to investigate a novel activity-dependent mechanism by which non-spiking silent tectal neurons are converted into circuit-contributing spiking neurons. They present a novel form of developmental plasticity in which experience can transform a neuron from being on the functional sidelines to being "in the game" of information flow.

The visual system has long served as a model to study activity-dependent circuit formation (Huberman et al., 2008). In particular, the retinotectal system of the *Xenopus laevis* tadpole, owing to its amenability to in vivo whole-cell recording and anatomical labeling, has provided important insights into the physiological and morphological plasticity that occurs during development. The *Xenopus* retinotectal synapse was also one of the very first sites where STDP was demonstrated (Zhang et al., 1998). The relative timing of inputs and postsynaptic spiking has been shown to affect both the complex receptive field properties of tectal neurons (Vislay-Meltzer et al., 2006; Mu and Poo, 2006) as well as the structural remodeling of retina axons (Munz et al., 2014) in the developing visual system. In these cases, the generation of action potentials by postsynaptic neurons in response to visual stimuli is a necessary condition for plasticity to occur.

In the current study, van Rheede et al. (2015) recorded from neurons in the optic tecta of early developmental stage *Xenopus* tadpoles to investigate whether immature neurons that receive visually driven synaptic inputs are able to fire action potentials in response to such stimuli and whether sensory experience can alter the firing properties of such neurons. Their first critical finding was that in the developing *Xenopus* optic tectum one encounters two populations of neurons receiving visual synaptic input: those that show spiking activity in response to visual stimuli (white square mapping, full-field flash, and moving bar stimuli) and those in which an action potential cannot be evoked by visual stimulation, referred to as "visually spiking" and "visually non-spiking," respectively.

What could account for the difference in response properties of these two groups of cells? The authors examined two key physiological properties, known to change over development, that contribute to the generation of spikes in response to visual stimulation: strength of synaptic inputs and intrinsic membrane excitability of the neurons (Pratt and Aizenman, 2007; Hamodi and Pratt, 2014). They found that while visually spiking and non-spiking cells showed little difference in intrinsic membrane properties, the strength of visually evoked excitatory synaptic inputs in visually spiking neurons was nearly 5-fold greater than that recorded in the non-spiking cells.

With these two populations physiologically defined, they attempted to see if it would be possible to convert the visually non-spiking cells into spiking cells by presenting visual conditioning stimuli. A brief 15 min stimulation period consisting of repeated moving bars or naturalistic scenes was found to cause a dramatic increase in excitatory, glutamatergic input sufficient to allow for subsequent spiking behavior in response to visual stimulation. It has long been appreciated that the process of retinotectal synaptic maturation involves an increase in the ratio of AMPAR to NMDAR responses as NMDA-only "silent synapses" become functionally effective through the trafficking of AMPARs to the synapse (Wu et al., 1996). Attempts to measure the AMPA/NMDA ratio of the visually evoked excitatory inputs in the current study also suggested that after conversion, this ratio was significantly increased, pointing to a postsynaptic locus for the conversion. This finding suggests an interesting

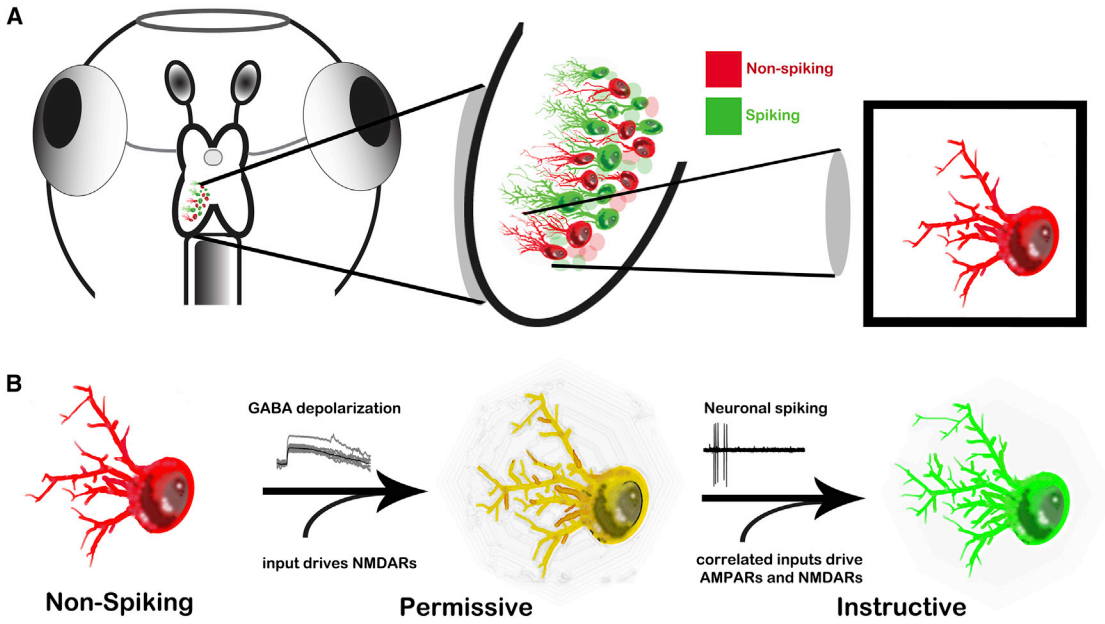

Figure 1. Model for the Two-Phase Conversion of Non-spiking Tectal Cells to Spiking Neurons Primed for Instructive Plasticity
(A) Schematic of a *Xenopus laevis* tadpole showing a magnification of the optic tectum that contains both spiking (green) and non-spiking (red) neurons as well as a further magnification of a single non-spiking neuron.
(B) Two-phase conversion model showing that GABAergic depolarization together with excitatory synaptic input activates NMDARs, which converts a non-spiking neuron to a spiking neuron (yellow) via synaptic AMPAR trafficking. The ability to generate a back-propagating action potential is permissive for STDP. This spiking neuron can now engage instructive plasticity mechanisms that rely on correlated inputs to drive spiking via AMPARs, leading to NMDAR-dependent associative plasticity. Example traces taken from Figures 4 and 7 of van Rheede et al. (2015).

alternative role for synapse ''AMPA-fication'' in circuit development, in which, by contrast to the input specificity of associative forms of plasticity like long-term potentiation, this phenomenon may mediate a general recruitment of the entire neuron into the functional circuit through the activity-dependent transformation of the cell from visually non-spiking to spiking, a kind of ''neuronal unsilencing.''

The investigators next delved into the mechanisms that promote this switch and showed that the NMDAR antagonist APV blocked conversion, indicating a requirement for NMDAR activation. Given that visual stimulation cannot evoke spiking in these neurons prior to conversion, the question arises of how postsynaptic depolarization to relieve the Mg^{2+} block and activate NMDARs can be achieved. Akerman's earlier work had shown that the high intracellular Cl^- concentration in immature neurons resulted in depolarizing GABAergic currents that could facilitate transmission through NMDARs (Akerman and Cline, 2006). Here again, utilizing painstaking gramicidin perforated patch recording, they observed that a sub-population of the non-spiking neurons exhibited a depolarizing GABAergic response. Impressively, they showed that only these non-spiking neurons in which GABA was depolarizing could be converted to spiking. Moreover, by blocking the Cl^- transporter NKCC1 with bumetanide to eliminate GABAergic depolarization, they were able to prevent visual stimulation-induced conversion.

Van Rheede and co-workers put forward the following model (Figure 1). Both non-spiking and spiking neurons initially co-exist in the developing optic tectum. Through brief exposure to permissive visual conditioning, non-spiking neurons with the help of depolarizing GABAergic currents can be converted to spike in response to visual stimuli. This occurs through the NMDAR-dependent enhancement of excitatory synaptic input by the addition of synaptic AMPARs, rather than through changes in intrinsic excitability of these neurons. Once cells become visually spiking, they are now competent to undergo instructive forms of associative plasticity like STDP that are considered to be important for activity-dependent circuit refinement.

This model brings new significance to the role of AMPAR trafficking in activity-dependent plasticity and raises many previously unanticipated questions. In a large population of non-spiking neurons, why is GABAergic depolarization, and hence the potential to become integrated into the circuit, only afforded to a few cells at a time? What may be the computational advantages of such gradual waves of integration of subsets of neurons into the functional circuit? Also, given that under the classic model for maturation of the Cl^- gradient in neurons GABA-A is initially depolarizing and gradually becomes hyperpolarizing as KCC2 expression is upregulated (Ben-Ari et al., 2007), what is the destiny of those visually non-spiking neurons in which Cl^- is already mature? What molecular signals determine whether a neuron will be primed for spiking, and are these signaling cascades distinct from those mediating similar phenomena such as homeostatic synaptic scaling (Turrigiano, 2012)? Is this permissive activation of neurons subject to its own developmental critical period, perhaps defined by KCC2 expression?

As development proceeds it is possible that all cells will eventually become visually

spiking. With only 27% of the neuronal population in the study showing conversion after 15 min exposure to the drifting bar and naturalistic visual stimuli, it will be important to determine whether longer duration or more appropriate stimuli might be more conducive to eventual maturation. Alternatively, it is intriguing to hypothesize that some of these differences in neuronal properties may reflect a diversity of distinct cell identities, rather than a developmental continuum of one single class of tectal cell. Perhaps some neurons may forever remain visually non-spiking in order to function as complex integrators, only firing in response to multimodal sensory inputs or to oscillatory activity within the tectum (Goddard et al., 2012). The continuing development and improvement of functional fluorescent indicators, including genetically encoded voltage indicators, should facilitate longitudinal studies of the maturation of synaptic and firing properties of entire circuits in small vertebrates like the *Xenopus* tadpole, which can help clarify these issues and avoid some of the caveats of cell-attached recordings such as diminished spike detection (Hochbaum et al., 2014).

In summary, van Rheede and colleagues have provided compelling new evidence for a novel activity-dependent mechanism that mediates the conversion of visually non-spiking cells into actively firing neurons. The finding is significant both at the cellular level, describing an initial step required to prime cells to undergo associative forms of plasticity like STDP that require the generation of a back-propagating action potential, as well as at the network level, where the ability to generate an output spike is a prerequisite for contributing to downstream network function. It will now be critical to determine whether similar phenomena can also be observed in the numerous other brain areas where GABAergic transmission is also initially depolarizing in developing neurons.

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