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Maintaining Your Youthful Spontaneity: Microcircuit Homeostasis in the Embryonic Spinal Cord

Many developing networks generate spontaneous network activity (SNA) that plays an important role in setting up functional circuitry, but how the proper level and pattern of SNA is itself maintained has not been clear. In this issue of *Neuron*, Gonzalez-Islas and Wenner show that SNA in the intact embryo regulates itself through a set of adaptive homeostatic plasticity mechanisms.

A hallmark of young neuronal circuits is the presence of robust spontaneous activity. Two well-studied examples are the waves of activity that sweep across the mammalian retina prior to eye opening (Feller, 1999; Torborg and Feller, 2005) and local circuit activity in the embryonic spinal cord that drives bursts of action potentials in motoneurons (Marder and Rehm, 2005). The widespread nature of such spontaneous network activity (or SNA) during embryonic and early postnatal development strongly suggests that it is important for some aspects of circuit formation, but like most things that might seem obvious, the role of SNA in setting up functional circuitry has been hotly debated. Is SNA an epiphenomenon—an unavoidable consequence of crude early wiring—or is it designed into the system as an essential element in the generation of functional connectivity? And if such activity is necessary, is it merely permissive, or does the pattern of activity somehow instruct the pattern of connectivity? Lately, the pendulum has swung back toward the latter view (Torborg and Feller, 2005; Marder and Rehm, 2005), but many issues about the mechanisms that generate and maintain SNA are unresolved. A report in this issue of *Neuron* (Gonzalez-Islas and Wenner, 2006) brings an interesting perspective to bear on this issue, by demonstrating that SNA is actively maintained in the intact embryonic spinal cord through a complex set of homeostatic mechanisms.

Early wiring of neural circuitry is likely achieved through an interplay between molecular guidance cues and activity-dependent processes. While some features of circuit development appear to be independent of SNA, others are clearly disrupted by manipulations that block or modulate it. In the visual system, blocking or modulating retinal waves during an early critical period severely disrupts retinotopic projections from thalamus to colliculus and cortex (Cang et al., 2005; McLaughlin et al., 2003). The role of SNA in generating proper motor function is less extensively studied, but recent experiments suggest that it is required for the guidance of motoneuron axons (Hanson and Landmesser, 2004). SNA in the spinal cord generates spontaneous limb movements, and it has been suggested that these movements are necessary for proper neuromuscular development. But not any old activity will do—SNA needs to have certain characteristics, which likely depend on the particular plasticity mechanism that must be engaged for development of a given circuit. The correlated bursts of activity in groups of nearby neurons that characterize retinal SNA, for example, are exactly what one would like in order to use a "fire together, wire together" plasticity rule to refine retinotopic projections to higher structures—because when nearby neurons fire together they will cooperate to depolarize any postsynaptic neuron onto which their axons converge, and these inputs will then be potentiated. Further, to be effective such activity should occur in a spatially restricted "bump," and move around the retina in the right temporal pattern to sequentially activate all the possible topographically arranged groups of neurons. Presumably, there are similar constraints on the properties of SNA in other developing networks.

The requirement for precise spatiotemporal patterns of SNA raises the question of how such activity is generated and maintained. If developing systems were not plastic and undergoing dramatic changes during the time that SNA is required, the problem would be relatively trivial, as the activity pattern could be hard-wired into the network. But given the state of flux of developing systems—with changes in neuronal size and intrinsic excitability, synapse formation and elimination, and synaptic strength all generating major potential perturbations to network activity—a more dynamic approach to generating SNA is required. Recent work on circuit homeostasis has suggested that many systems—ranging from the neuromuscular junction, to invertebrate central pattern generators, to cortical microcircuits (Davis and Bezprozvanny, 2001; Turrigiano and Nelson, 2004)—have plasticity mechanisms in place that allow the system to detect how active it is and self-adjust to keep this activity relatively constant. This homeostatic plasticity has been suggested to serve many functions, including preventing epilepsy, balancing excitation and inhibition, and stabilizing Hebbian plasticity rules. In this report, Gonzalez-Islas and Wenner suggest that homeostatic plasticity may play an additional important role during early development, to generate and maintain the proper level and pattern of SNA.

Previous work in an in vitro spinal cord preparation had shown that when perturbed pharmacologically (by blocking one neurotransmitter system), SNA levels recover through compensatory changes in other neurotransmitter systems (Chub and O'Donovan, 1998). A major advance in the Gonzalez-Islas and Wenner study was to block activity in ovo (in the egg), to test for the presence of compensatory plasticity in the intact animal. Using chick embryos, they were able to block activity by injecting the sodium channel blocker lidocain; this blocked all spiking activity within the embryo, as confirmed by observing the cessation of limb movements normally driven by SNA. After 2 days of this in vivo manipulation, spinal cords with attached muscles were isolated, so that extracellular nerve recordings could be used to confirm the identity of spinal cord neurons.

This allowed them to assess the effects of in vivo activity deprivation on synaptic inputs onto a relatively homogenous population of motoneurons, an important consideration given the complexity of spinal cord circuitry. They found that previous activity blockade increased the amplitude of both AMPAergic and GABAergic synaptic inputs onto motoneurons and increased the frequency of SNA episodes. Because GABA is depolarizing at this age, both of these synaptic changes are in the right direction to increase cord excitability. Reducing either AMPAergic or GABAergic synaptic transmission pharmacologically reversed the effect of activity blockade, suggesting that compensatory changes in synaptic transmission within the cord are causally involved in the increase in SNA frequency. These data nicely demonstrate that SNA is actively adjusted through an activity-dependent mechanism and suggest that this plasticity is homeostatic in nature—that is, designed to maintain a relatively constant level and pattern of SNA. One important open question is what aspect of activity is necessary for this homeostatic adjustment, since in ovo lidocain presumably blocks not just spinal circuits but activity throughout the embryo, including muscle activation (through silencing of the motoneurons). A second important question is the site of regulation within the cord: here they examine changes in transmission onto spinal motoneurons, but what role interneuronal networks might play in homeostatic regulation of SNA is not clear. More selective means of manipulating in vivo activity, and of dissecting changes in intra-cord circuitry, will be an important next step: recent advances in the ability to genetically manipulate spinal circuits suggest that such studies will not be long in coming (Kiehn and Kullander, 2004; Lanuza et al., 2004).

In several respects, the changes they observe are reminiscent of a form of homeostatic plasticity characterized first in cultured central neurons and later in visual cortex in response to altered sensory drive, in which synaptic strengths are scaled up or down in the right direction to stabilize firing rates (Turrigiano and Nelson, 2004). Taken together with previous work these in vivo results suggest that homeostatic synaptic scaling of AMPAergic and GABAergic transmission likely play a highly conserved and important role in the development of central networks. However, there is a novel feature of this phenomenon in spinal cord that raises some interesting questions about how excitation and inhibition are balanced during homeostatic plasticity. In cortical circuits probed after GABAergic inputs have become inhibitory (i.e., the chloride reversal potential is below threshold), excitation and inhibition onto principle neurons are regulated in opposite directions, and the balance between them can be dynamically adjusted by ongoing activity. Here, Gonzalez-Islas and Wenner observe that at a time when GABAergic inputs are excitatory they are scaled in the *same* direction as AMPAergic inputs. While this makes sense from a homeostatic point of view, it raises the question of how the direction of plasticity is coupled to the postsynaptic effect of the input. GABAergic scaling in cortical neurons is accomplished (at least in part) through changes in postsynaptic GABAA receptor accumulation (Turrigiano and Nelson, 2004). Assuming a similar mechanism contributes to GABAergic scaling in chick embryonic spinal cord, the plasticity mechanism that couples a change in activity to changes in synaptic GABAA receptor number must change sign when GABA becomes depolarizing. How this might take place is unclear. One possibility is that developmental changes in GABAAR subunit composition (which roughly coincide with changes in chloride reversal potential) alter the coupling between activity and receptor insertion/removal mechanisms. Because at inhibitory synapses onto some cortical neurons GABAAR subunit composition varies by synapse class, this also suggests a way to differentially regulate different classes of inhibitory input during homeostatic plasticity, as has been observed in visual cortical circuits in response to visual deprivation (Maffei et al., 2004).

GABAergic inputs depress after a bout of SNA, and slowly recover. This recovery rate is one of the determinants of the SNA interepisode interval, since the recovered GABAergic inputs help to trigger the next burst of activity. A second novel feature of the GABAergic plasticity they observe here is that, although depression is comparable between control and activity-reduced preparations, the recovery from depression of GABAergic inputs occurs faster. This should combine with the increased synaptic drive to reduce the interepisode interval, suggesting that network features that control SNA frequency are actively adjusted in a homeostatic manner. This observation brings us back to the point that what likely matters in many developing networks is less the overall level of activity than the spatiotemporal pattern of that activity (Torborg and Feller, 2005). How homeostatic plasticity mechanisms are tuned within complex biological networks to regulate the temporal features of network activity is a fascinating and largely unexplored problem (Soto-Trevino et al., 2001). The demonstration that spinal networks in the intact embryo use homeostatic plasticity mechanisms to maintain SNA opens up exciting possibilities for understanding the role of this plasticity in the generation of functional circuitry during embryonic development.

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A New Peptide Input to Learning and Addiction

In this issue of *Neuron*, Borgland et al. report that the arousal-associated peptide orexin enhances LTP-like changes in glutamatergic excitability of ventral tegmental dopamine neurons. This parallels a similar effect of corticotropin-releasing factor and suggests a form of neuroadaptation that increases the likelihood of addiction relapse.

Borgland and colleagues (Borgland et al., 2006) report that orexin A input to the ventral tegmental area (VTA) (1) potentiates, by a PKC/PLC pathway, NMDA receptor-mediated excitatory postsynaptic currents in dopamine neurons; (2) modulates NMDA receptor composition; (3) translocates NMDA receptors to the synapse; (4) causes AMPA receptor-mediated synaptic plasticity; (5) facilitates cocaine-induced potentiation of excitatory input to VTA dopamine neurons; and (6) plays a critical role in cocaine-induced psychomotor sensitization. These findings parallel similar findings with CRF, made earlier by the Bonci group (Ungless et al., 2003). The two findings link potential mechanisms of learning and memory to mechanisms of addiction, adding an important dimension to addiction research.

It is widely suspected that drug-induced neuroadaptations (and not just those of the simple memory trace for the drug experience) differentiate the addicted from the nonaddicted brain. While eating and sexual habits can become compulsive to the point of risk for diabetes, cardiovascular disease, or loss of public office, addiction is seen to involve even more compulsive habits and to require even stronger explanations and more complex mechanisms. From this perspective, it is not enough that addictive drugs activate brain reward circuitry more strongly or more immediately; rather, addictive drugs are seen to alter the brain in ways that make the drug more habit-forming than "natural" rewards and more habit-forming for the addict than for the nonaddict. Addictive drugs do, of course, alter the brain, and many of the alterations are long-lasting.

The findings of the Bonci group advance the field of addiction research in three ways. First, rather than a mechanism primarily involved in pharmacological responses to ingested cocaine, they have identified a neuroadaptation (sensitization of mesolimbic dopaminergic neurons to glutamatergic input) that links drug reward circuitry to the arousal messenger orexin and, in earlier studies (Ungless et al., 2003), to the stress messenger corticotropin-releasing factor (CRF). Stress and arousal are phenomena that can trigger drug-seeking in abstinent subjects (Shalev et al., 2002); they can get drug-seeking started and not just maintain it once it is under way. Here we have clues, perhaps, as to how early experience can predispose some individuals to addiction by sensitizing the brain to emotional events.

Second, these studies begin to bring peptide neurotransmitters into the discussion of neuroplasticity in addiction. While opioid peptide neurotransmitters have long been studied because they act at the same receptors as addictive opiates, studies of neuroadaptations to drugs of abuse have focused largely on the "primary" neurotransmitters dopamine, glutamate, and GABA.

Third, these studies take an important step toward relating the neuroadaptations of addiction to mechanisms associated with the cellular basis of learning and memory (see Kelley, 2004). Orexin in the new study and CRF in the earlier work each modulate glutamatergic activation of midbrain dopamine neurons in much the same way as is seen in long-term potentiation. The very similar characteristics of orexin-induced and CRF-induced sensitization of the activation of dopaminergic neurons by glutamate suggest a common or at least strongly overlapping mechanism. Changes in synaptic efficacy such as those induced by orexin and CRF are not only likely to underlie learned stress and arousal responses to the environment, they are also likely, perhaps in other parts of the brain, to help consolidate the specific memories (the "remembered high") associated with the drug experience.

Intraventricular (Boutrel et al., 2005) or VTA (Harris et al., 2005) infusions of orexin are known to reinstate drug-seeking in rodents, as do intraventricular (Shaham et al., 1997) or VTA (Wang et al., 2005) infusions of CRF. Ventral tegmental injections of an orexin antagonist block the development of heroin-conditioned place preferences (Narita et al., 2006). Inasmuch as arousal or stress can trigger drug-seeking in drug-free animals, the neuroadaptations discovered by the Bonci group are important not only for how rewarding the drug is after an animal starts taking it, but, perhaps more importantly, for how likely the animal is to initiate drug-seeking during periods of abstinence. Because it is during periods of abstinence, not periods of intoxication, that addicts seek treatment, the peptide signaling pathways for orexin and CRF may prove to be fruitful targets in the search for addiction medications.

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