transients in the processes and cell bodies of premyelinating and myelinating oligodendrocytes. Such localized Ca^{2+} activity declined during development. To link oligodendrocytes Ca^{2+} transients to axonal activity, action potentials were either blocked through tetrodotoxin (TTX) treatment or stimulated electrophysiologically. Inhibition of action potentials by TTX substantially reduced Ca^{2+} transient frequency while, conversely, stimulation increased oligodendrocyte Ca^{2+} transients. The stimulated increase in Ca^{2+} transients results in an increase in myelin sheath growth. To mechanistically link increases in intracellular Ca^{2+} to myelin sheath growth, Krasnow et al. buffered intracellular Ca^{2+} in oligodendrocytes and demonstrated that strong Ca^{2+} buffering was associated with myelin sheath shortening.

Previous studies have implicated axonal activity in modulating myelination[5,6] using approaches such as delaying eye opening or TTX treatment of the optic nerve[7]. Until these two studies, however, the mechanisms linking axonal activity and myelin sheath formation were largely missing. While together these papers provide a compelling case for the role of Ca^{2+} levels in modulating the elongation of individual myelin sheaths during development (Fig. 1), they also raise a host of additional questions. For example, it is unclear what is driving the increase in oligodendrocyte Ca^{2+} transients. One possibility is that release of neurotransmitters such as glutamate, either along the length of the axon or at synapses between oligodendrocyte precursor cells and unmyelinated axons[8], may be responsible, but this is currently unresolved. In addition, it is unclear how changes in oligodendrocyte and myelin intracellular Ca^{2+} mediate changes in myelin sheath growth or shrinkage. The ability to reduce sheath shrinkage induced by high levels of Ca^{2+} through inhibition of calpain strongly suggests a role for proteases that may disrupt microtubule and actin organization; however, the growth-promoting effects of intermediate levels of Ca^{2+} are more complex. There is evidence that partial disruption of the cytoskeleton promotes myelin sheath formation[9], and perhaps the rapid growth of myelin sheaths represents the 'sweet spot' of Ca^{2+} concentration.

In a more general sense, it would be interesting to define what level of myelination coordination occurs at the oligodendrocyte cell body, given that there is a short time window in which a cell generates its full complement of myelin sheaths[4]. The present manuscripts focus on developmental control of myelination. Whether myelin stability or formation in the adult CNS is regulated in a similar manner may have important implications for learning and memory[10–12]. Likewise, conditions such as multiple sclerosis and stroke result in loss of oligodendrocytes and myelin. Indeed, in some chronically demyelinated multiple sclerosis plaques, substantial numbers of oligodendrocyte precursor cells have been shown to associate with axons but fail to myelinate[13]. Could elevating intracellular Ca^{2+} levels in those cells to an appropriate level stimulate repair? Finally, while the current studies provide provocative insights into the control of myelin growth by Ca^{2+}, they have yet to address the role of astrocytes in this process[14]. Given the propensity of astrocytes to regulate Ca^{2+} levels, it seems highly likely that they are more than just bystanders in the control of myelin growth. ❐

Robert H. Miller
School of Medicine and Health Sciences, George Washington University, Washington, DC, USA.
e-mail: rhm3@gwu.edu

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Competing interests

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OREXIN

Stay alert, don't get hurt

Both nucleus accumbens and orexin play clear roles in motivated behavior, but the functions of orexin projections to accumbens are poorly understood. Blomeley et al. show that this pathway, via specific orexin excitation of dopamine D2 receptor–expressing neurons, can inhibit reward seeking and exploratory drive when danger is perceived.

Stephen V. Mahler

In a dangerous world, we must attain the necessities of life but also stay vigilant for potential harms. Indeed, navigating this balance is one of the most fundamental functions of the brain. Blomeley et al.[1] show that the decision to play it safe is mediated, in part, by a tiny cluster of a few thousand neurons expressing the neuropeptide orexin (also called hypocretin) that are exclusively present in a restricted region of the hypothalamus. These neurons play wide-ranging roles in so-called motivational activation, coordinating wide-ranging behavioral and physiological responses when key fitness-related situations are encountered[2].

Neurons expressing orexin, via their widespread projections throughout the brain, are essential both for highly motivated pursuit of natural and drug rewards and for responses to acute stressors. Blomeley et al.[1] add an important new piece to this puzzle regarding orexin's role in motivation. They show that orexin neurons also

mediate avoidance of risk, via a previously undescribed and remarkably selective projection to a subset of neurons in the nucleus accumbens. This lends new insight into how orexin neurons not only facilitate motivation to act, but also inhibit behavior in service of avoiding danger. In other words, these neurons promote an even wider range of survival strategies than previously expected.

In the life of a mouse, avoiding predation is a crucial evolved drive that must always be considered when deciding what to do and what not to do. The authors synthesize the known roles of orexin in coordinating specific adaptive behavioral programs[2] and the potential role of 'indirect pathway' striatal medium spiny neurons (MSNs) expressing the dopamine D2 receptor in inhibiting actions[3]. In dorsal striatum, D2- and D1-expressing MSNs are interspersed and, via their differential projections to globus pallidus interna (the D2-expressing indirect pathway) or substantia nigra (the D1-expressing direct pathway), inhibit or promote actions, respectively. In this way, dopamine can both promote (via excitatory D1 receptors) and inhibit (via inhibitory D2 receptors) activity of the MSN populations, thereby helping select amongst competing behavioral plans represented by these populations and their differential outputs to thalamocortical motor pathways.

The authors now show a mechanism by which orexin neurons control MSN populations in nucleus accumbens, a key aspect of the ventral striatum. Specifically, they find that orexin projections to accumbens selectively target and excite D2-expressing MSNs, thereby inhibiting risky behaviors such as venturing into the potentially dangerous center of an open field or crossing, in pursuit of reward, a portion of a chamber scented with the odor of a predator (Fig. 1). Orexin, through actions at orexin 1 and/or 2 (OX1/2) receptors, excites D2-expressing accumbens MSNs, while it does not excite D1 expressing MSNs. They also show that orexin robustly excites neuropeptide Y interneurons in accumbens (these neurons also contain somatostatin and nitric oxide synthase, and they are sometimes called persistent and low-threshold spike neurons), which strongly inhibit MSNs[4]. Potentially, these neurons could inhibit D1 MSNs while orexin directly excites D2-expressing ones, decreasing the likelihood of making a risky move. If so, this means orexin can inhibit risky action plans by stimulating D2-expressing MSNs while simultaneously (indirectly) inhibiting D1 MSNs that would facilitate such actions, a possibility that should be tested directly in future studies. The authors demonstrate

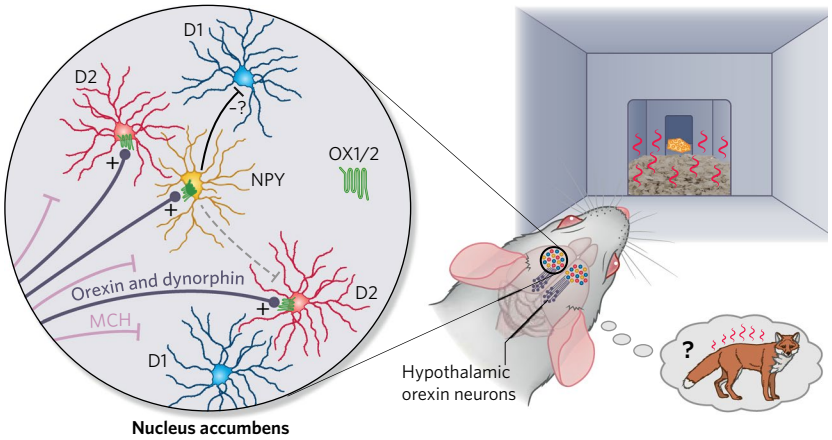

Fig. 1 | An orexinergic input to nucleus accumbens that promotes risk avoidance. Hypothalamic orexin (but not melanocortin concentrating hormone, MCH) neuron projections to nucleus accumbens excite D2-expressing indirect-pathway MSNs, which may inhibit specific action plans in favor of others. Here this pathway promotes avoidance of risky situations such as crossing a predator-scented chamber to attain food. D1-expressing accumbens MSNs are not similarly excited by orexin, though neuropeptide Y (NPY) interneurons are, showing the remarkable anatomical and functional specificity of this pathway. Credit: Debbie Maizels/Nature Publishing Group.

the specific anatomical characteristics and behavioral functions of the orexin→D2 MSN pathway using a combination of slice electrophysiology, opto- and chemogenetic manipulations, and rabies-assisted tracing of monosynaptic connectivity between orexin and D2 neurons. In contrast, the parallel projection from an interspersed hypothalamic population of neurons expressing melanocortin concentrating hormone to striatum does not similarly target or excite D2-expressing accumbens MSNs, showing marked specificity in the connectivity between these important motivation-related hypothalamic and striatal populations.

Surprisingly little was previously known about the functions of orexin projections to accumbens, despite the presence of orexin receptors there and the presence of dense orexin-expressing axons in the dorsomedial accumbens shell[5,6]. Several reports show a role for this pathway in motivation, hedonics and arousal[7,8], but none have examined how it affects functionally opposed MSN subpopulations. The present report is likely to make a large impact on those studying striatal MSN circuits or the motivational functions of either accumbens or orexin. However, it is hard to understand how these findings relate to prior reports showing that almost 80% of accumbens neurons are excited by orexin[9], while D2 neurons there represent less than half of the MSN population[10]. In addition, dopamine plays overlapping roles with orexin in appetitive motivation[2], and washing dopamine onto ex vivo accumbens shell brain slices excites many of the same accumbens cells (presumably D1-expressing MSNs) that are excited by orexin B peptide (here shown to be D2 MSNs)[9]. This implies additional complexity in the physiological and behavioral functions of orexin inputs to accumbens core and/or shell that is still obscure and that should be further investigated in light of these intriguing new data.

This report also highlights several other areas in which more research is sorely needed to understand this complex circuit. First, the impact of circadian oscillations in orexin signaling is untested, as all experiments here were performed in the resting (light) phase of the day, when nocturnal rodents spend most of their time sleeping and when central orexin neuron levels are low[11]. An examination of how this newly discovered pathway functions in the context of greater basal orexin levels in the active phase is therefore needed.

Another unanswered question brought up by these data regards the role of dynorphin, an endogenous opioid peptide that is expressed in nearly 100% of orexin neurons[12]. Dynorphin, via actions at kappa opioid receptors in accumbens and elsewhere, is well-known to promote aversion, and upregulation of kappa signaling in accumbens is associated with depression-like phenotypes[13]. Kappa receptors modulate activity of both D1 and D2 neurons in accumbens[14], and dynorphin is co-released with orexin, at least in the ventral tegmental area[15]. This begs the question of how accumbens dynorphin and orexin co-release might interact to influence activity of these MSN subpopulations,

modulating neural activity and risk avoidance or other motivated behaviors.

These data also hint that orexin modulation of accumbens MSN subpopulations may be more complex than suggested. The authors show that chemogenetic inhibition of D2 MSNs strongly inhibits risk avoidance, as does (to a lesser extent) blocking orexin signaling at the OX1 receptor with intra-accumbens SB334867. However, when D2 neurons were inhibited while orexin was locally antagonized, only partial inhibition of risk avoidance was observed, equivalent to effects of blocking orexin alone. This implies that a more complex interaction between orexin and D2 neuron activity may exist than is characterized here. Potentially, this finding could result from inhibition of aversion-specific functions of accumbens orexin that compete with the peptide's newly discovered role in risk avoidance. However, orexin in accumbens promotes seeking of drug and natural rewards, as well as activity more generally[8], and inhibiting such effects would be expected to facilitate the behaviors here defined as risk avoidance: namely, hesitation to explore or to retrieve a reward. It is possible that this puzzling finding instead involves OX2 receptors, which are more robustly expressed than OX1 in accumbens and which were presumably not blocked by SB334867 in behavioral experiments (though both OX1 and OX2 were blocked in physiology experiments, where sedative effects of OX2 antagonism were not a concern). Complex interactions with dynorphin co-release from orexin neurons could also be relevant here, since dynorphin activity would be expected to remain intact after pharmacological orexin blockade. Alternatively, this finding could result from a complex and poorly characterized interaction of orexin effects on D1 and D2 MSNs, as well as neuropeptide Y interneurons or other interneurons that control MSNs. The present findings will likely spur interest in further exploring these possibilities or other potential mechanisms by which orexin neurons control ventral striatal MSN populations.

Like most important papers, this one poses as many questions as it answers. It is likely that unraveling the complex interactions between hypothalamic orexin and ventral striatal MSN subpopulations will keep researchers interested in the motivation and motor functions of hypothalamic–striatal interactions busy for years to come. ❐

Stephen V. Mahler
Department of Neurobiology & Behavior, University of California, Irvine, Irvine, CA, USA.
e-mail: mahlers@uci.edu

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Competing interests

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NEUROTRANSMITTER RELEASE

Munc13 marks the spot

Super-resolution optical imaging of presynaptic terminals shows that a protein essential to all known forms of neurotransmitter release is clustered in small assemblies that likely correspond to release sites for synaptic vesicle fusion.

Timothy A. Ryan

Bernard Katz was awarded the 1970 Nobel Prize in Physiology or Medicine in part for demonstrating that chemical synaptic transmission consists of a neurotransmitter-filled synaptic vesicle fusing with the plasma membrane at nerve endings and releasing its content to diffuse onto the opposing postsynaptic cell[1]. For fast-acting neurotransmitters, the sequence of events is rapid: mere hundreds of microseconds elapse between the arrival of a presynaptic action potential and the opening of postsynaptic ligand-gated ion channels. The question of what defines a release site in the active zone in molecular terms has loomed large since Katz provided his quantitative description of release as boiling down to two parameters: the number of sites that could have a release event (N) and the probability that at least one would release (P). In this issue, Sakamoto et al.[2] deploy a combination of sensitive optical approaches to demonstrate that a release site consists of a nanoassembly of Munc13 proteins and, furthermore, that Munc13 can self-assemble into clusters at the plasma membrane even in non-neuronal cells.

Much effort has gone into discovering and understanding the molecules that control and execute neurotransmitter release. Ten years after Bernard Katz refined the nature of synaptic transmission, Sydney Brenner began a forward genetic screen that became one of the most successful ever (certainly for neurobiology), identifying components critical for nervous system function in the nematode *Caenorhabditis elegans*. This work culminated in the publication of a landmark opus identifying 77 genes that would foreshadow a 'greatest hits' parade in synaptic biology[3]. One of these genes, *unc-13*, was later shown to be essential in all known forms of neurotransmitter release in worms, flies and rodents. As more information was obtained about the protein encoded by *unc-13*, Brenner and colleagues noted that it was likely to be particularly important as it had protein modules (C1 and C2 domains) that were membrane interactors and could be modulated by important effectors such as Ca^{2+} ions and phorbol esters, which mimic the second messenger diacylglycerol[3]. Genetic ablation of *unc-13* (or the mammalian homolog *Munc13*)