with female strangers. To assess pair-bond stability following D1 receptor upregulation, the authors used a resident-intruder behavioral test. If a pair-bonded female partner was the intruder in the test, then the resident males engaged in close affiliative behavior, but if the intruder was a female stranger, males instead became extremely aggressive and attacked the female intruder. When the investigators infused a D1 receptor antagonist into the nucleus accumbens to block the upregulated D1 receptors, this selective aggression was abolished: bonded male voles eagerly engaged in close contact with female strangers as if they had returned to their nonbonded bachelor status. This suggests that the increase in D1 receptor expression in the nucleus accumbens maintains monogamy by transforming the response to new females from affiliative interest to aggression.

Aragona and colleagues also show that nonmonogamous meadow voles have naturally high levels of D1 receptor expression in the nucleus accumbens, even before mating. Blocking D1 receptors in these voles increased their affiliative behavior toward both female partners and strangers but did not induce partner preference. The authors hypothesize that higher D1 receptor levels contribute to the generally asocial demeanor of meadow voles, but that other factors may also contribute to their lack of pair bonds. One caveat is that D1 receptors were blocked during a 6-hour cohabitation period that is not normally sufficient to induce partner preference in prairie voles; and the D1 receptors were not blocked during the test for expression of the partner preference. It also remains unclear whether a similar D1 versus D2 dichotomy regulates pair-bond formation in female prairie voles.

From an anatomical perspective, D1 and D2 receptors exist (at least partially) in distinct striatal projection neurons[9] that could underlie their differential effects in inducing and maintaining pair bonds. D2 receptors are expressed in neurons that project to the ventral pallidum, where vasopressin receptors could interact downstream in complex neural networks that help form partner preferences[10]. Differences in the signaling pathways activated by these receptors might also explain their opposing influences. D1 and D2 receptors couple to stimulatory and inhibitory G proteins, respectively, with opposite effects on intracellular cyclic AMP (cAMP) production, and preliminary studies by the authors suggests a role for cAMP signaling in the nucleus accumbens in pair-bond formation[11].

D1 and D2 receptors are also thought to make differential contributions to other forms of reward-related learning. Whereas D1 receptors are important for learning new associations, D2 receptors enhance the influence of previously learned associations on appetiteive behavior[12–14]. In this sense, these receptors seem to function somewhat differently in pair-bond formation in the prairie vole, because D2 rather than D1 receptor activation facilitates initial bonding during mating.

However, the prairie vole's fierce loyalty to a single partner is paralleled by the strong cravings of drug addicts and their avoidance of alternative rewards (including potential mates) to the point of personal devastation. In this regard, it is interesting that the study by Aragona and colleagues reveals a striking similarity between the roles of D1 and D2 receptors in pair-bond formation in voles and in cocaine-seeking behavior in rats[15]. In rats reinforced by cocaine self-administration, D2 receptor stimulation triggers relapse to cocaine seeking, reminiscent of the ties that bind prairie voles to their lifelong partners. Conversely, D1 receptor stimulation inhibits cocaine-seeking behavior, perhaps because animals are satisfied and have no desire for more cocaine, or potential suitors for that matter. The development of cocaine addiction may also be related to different changes in D1 and D2 responses, as rats become more sensitive to stimulation of D2 receptors and less so to that of D1 receptors (S.E. & D.W.S., *Soc. Neurosci Abstr.* **28**, 2002). Heightened D2 receptor activity could foster a 'pair bond' between user and drug, but without an upregulation in D1 receptor expression, drug users lack a physiological brake on reward-seeking behavior like that in their pair-bonded prairie vole counterparts. Thus, where dopamine receptor signaling is concerned, drug addiction could reflect a sort of pathological inverse of pair-bond formation in prairie voles.

Ultimately, our knowledge of naturally occurring pair bonds may shed light on the psychopathology of abnormal attachment and asocial behavior related to addiction or other mental illnesses. Our ability to unravel the neurobiological knot that ties together monogamous prairie voles, for better or for worse, could eventually help us to develop new treatments for some psychiatric disorders.

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Internalizing channels: a mechanism to control pain?

Diane Lipscombe & Jesica Raingo

The opioid receptor–like receptor inhibits the $Ca_v2.2$ calcium channel even without the receptor ligand, nociceptin. A new study finds that long-term exposure to nociceptin causes internalization of the receptor-channel complex.

The accurate perception of painful stimuli is essential for survival, but constant pain can render life unbearable. All nociceptive stimuli, useful and unwanted, use similar signaling pathways, which complicates efforts to design drugs that prevent continuous pain without affecting normal sensory processing. The human body produces its own analgesic endorphins that act on pathways involved in mediating chronic but not acute pain. By studying the molecules and pathways targeted by endogenous analgesics, Altier *et al.* in this issue have uncovered unexpected mechanisms that might help curtail persistent, unwanted pain[1].

The opioid receptor–like receptor (ORL1), also known as the orphanin FQ receptor, is G protein coupled and expressed at high levels in the dorsal horn of the spinal cord as well as in the brain[2]. Its endogenous ligand, nociceptin,

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is selectively enhanced during chronic inflammation and is thought to downregulate chronic but not acute pain[3,4]. $Ca_V2.2$ N-type channels are key targets of the ORL1 receptor in dorsal root ganglia[5]. $Ca_V2.2$ channels in primary sensory afferents of the dorsal horn serve as the regulatory gates controlling calcium entry and subsequent transmitter release. They are prime targets for analgesics, including those that act through G protein–coupled receptors[6].

ORL1 receptors form a unique partnership with $Ca_V2.2$ channels in a subset of sensory neurons, which is not observed with any other G protein–coupled receptor[1,5]. The $Ca_V2.2$–ORL1 complex exerts strong inhibitory control over $Ca_V2.2$ channel activity without requiring receptor activation by nociceptin[5] (agonist-independent inhibition; **Fig. 1**). This inhibitory effect is relieved by strong membrane depolarization, a characteristic feature of Gβγ-dependent modulation of $Ca_V2.2$ channels in many neurons[7]. Although several G protein–coupled receptors inhibit $Ca_V2.2$ channel gating in sensory neurons, these effects all depend on agonist activation[7]. The agonist-independent inhibition of $Ca_V2.2$ channels[5] is observed in small-diameter sensory neurons—presumptive nociceptors—and is mediated by interactions between the C terminus of $Ca_V2.2$ and ORL1. What then is the role of nociceptin, the endogenous ligand of the ORL1 receptor, if inhibition of $Ca_V2.2$ channels is independent of its presence?

Altier *et al.* used a combination of biochemical, imaging and electrophysiological experiments on both native and cloned channels to show that chronic exposure to nociceptin triggers internalization of this same receptor-channel complex, thereby downregulating channel activity[1] (**Fig. 1**; agonist-dependent inhibition). Agonist-dependent internalization of G protein–coupled receptors is extensively documented[8,9] and is a feature of ORL1 and μ-opioid receptors[10]. This new report[1] and another described in more detail below[11] show that G protein–coupled receptors in sensory neurons also have a profound effect on surface expression of $Ca_V2.2$ channels. Agonist- and activity-dependent internalization of postsynaptic receptors underlies several forms of synaptic plasticity[12]. Similarly, nociceptin-dependent changes in presynaptic $Ca_V2.2$ channel density in primary sensory afferent terminals might induce long-term downregulation of synaptic strength in the pain pathway during chronic peripheral nerve injury or inflammation.

The near simultaneous publication of two papers reporting agonist- and G protein–dependent internalization of $Ca_V2.2$ calcium channels[1,11] suggests that this may emerge as a general mechanism for regulating presynaptic calcium channels and synaptic efficacy. Although G protein activation was required to drive the internalization of $Ca_V2.2$ channels in sensory neurons in both studies, the two pathways are distinct. ORL1- mediated internalization of $Ca_V2.2$ in a subset of sensory neurons depends on prolonged exposure to the agonist and on protein kinase C activation, and—implied, though not proven—the internalized protein complex seems destined for lysosomal degradation[1]. $GABA_B$ receptor activation, on the other hand, causes rapid, transient internalization of $Ca_V2.2$ channels in sensory neurons by disrupting a scaffolding protein complex that normally tethers the channel to the plasma membrane[11]. This latter process depends on tyrosine kinase activation, is rapidly reversible and causes channels to be sequestered into clathrin-coated vesicles presumably destined for recycling.

These studies are certain to stimulate new investigations of the signaling pathways that regulate the internalization of voltage-gated calcium channels. Progress should be rapid, given the vast literature on the internalization of other membrane proteins, particularly G protein–coupled receptors and postsynaptic glutamate receptors[12,13]. This work raises several interesting questions, however, that relate specifically to $Ca_V2.2$ channels in sensory neurons. As ORL1 receptors are expressed only in a subset of sensory neurons[1], do all $Ca_V2.2$ channels have the same potential to associate with ORL1 receptors? Two distinct C-terminal splice isoforms of $Ca_V2.2$ channels are expressed in nociceptors that generate different size currents[14]. Altier *et al.* have also shown that the C terminus of $Ca_V2.2$ interacts with the ORL1 receptor and is the minimum structure necessary for membrane targeting and internalization (**Fig. 1**). It would be interesting to know if ORL1 preferentially associates with specific $Ca_V2.2$ splice isoforms found in nociceptors.

The kinetics and dynamics of the ORL1-mediated and the $GABA_B$ receptor–mediated internalization of $Ca_V2.2$ channels differ. ORL1 internalization depends on prolonged exposure to nociceptin and, once internalized, the ORL1-$Ca_V2.2$ channel complex seems destined for lysosomal degradation; on the other hand, $Ca_V2.2$ channel internalization after $GABA_B$ receptor activation is a rapid process that is reversible within minutes, consistent with the recycling of channels to the plasma membrane[11]. But what factors control the postendocytotic sorting of $Ca_V2.2$ channels? Is regulated ubiquitination necessary for $Ca_V2.2$ internalization as in other systems, and do other regulatory proteins such as β-arrestins contribute to $Ca_V2.2$ channel endocytosis and recycling? Alternative splicing is known to influence the sorting and fate of internalized μ-opioid receptors[15].

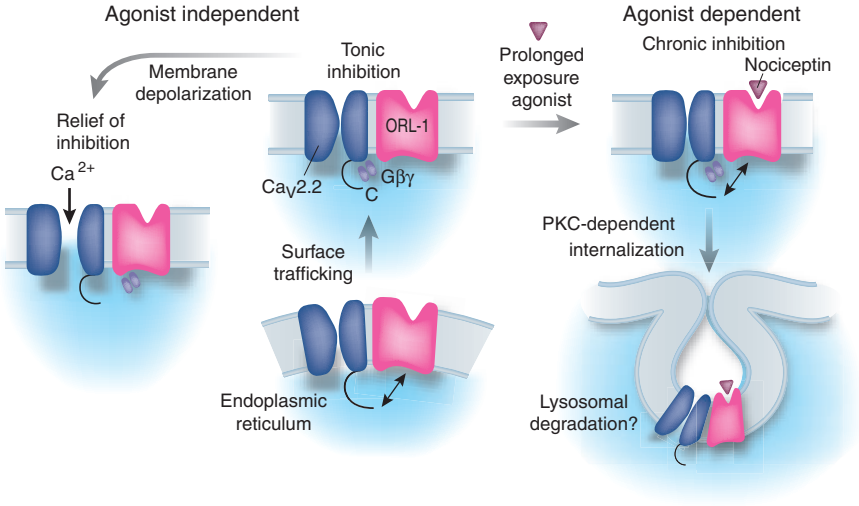

Figure 1 Regulation of $Ca_V2.2$ N-type calcium channels by the ORL1 receptor. Association of the ORL1 receptor with the $Ca_V2.2$ channel requires interaction with the C terminus of $Ca_V2.2$. This interaction is thought to promote the targeting of the ORL1 receptor–$Ca_V2.2$ channel complex to the plasma membrane. The ORL1 receptor exerts agonist-independent tonic inhibition of $Ca_V2.2$ channels when they are expressed on the plasma membrane. This inhibition depends on G protein activation and on the binding of Gβγ to the $Ca_V2.2$ subunit. Inhibition of $Ca_V2.2$ channels can be relieved by strong membrane depolarization. Prolonged exposure to the agonist, nociceptin, induces cointernalization of the ORL1 receptor and the $Ca_V2.2$ channel. Internalization depends on interaction with the C terminus of $Ca_V2.2$ and on protein kinase C activation. Agonist-dependent internalization leads to chronic inhibition of the $Ca_V2.2$ N-type current by removing $Ca_V2.2$ from the plasma membrane. Whether the internalized receptor-channel complex is destined for lysosomal degradation remains to be established.

The complex formed between ORL1 and the $Ca_V2.2$ channel exerts a uniquely strong inhibitory control over $Ca_V2.2$ channels in dorsal root ganglia. Only a subset of neurons express ORL1, suggesting that it is of special significance in the processing of nociceptive signals. Most notably, $Ca_V2.2$ channel activity is under constant inhibition from ORL1 even without receptor stimulation[1,5] (**Fig. 1**). However, because inhibition by ORL1 is voltage dependent, periods of intense neuronal activity would relieve Gβγ-dependent inhibition (**Fig. 1**). $Ca_V2.2$ channels associated with ORL1 could therefore serve as a high-pass filter, unaffected by low-frequency stimuli but recruited in response to strong, high-frequency stimuli, thus boosting their effective output. When the stimulus intensity is decreased, $Ca_V2.2$ channels that are associated with ORL1 would revert to their resting inhibited state. In this scheme, it is therefore attractive to speculate that when the hyperexcitable state associated with chronic pain continues unabated, prolonged exposure to nociceptin released during chronic inflammation disables $Ca_V2.2$ channels by triggering their internalization.

G protein–dependent internalization of $Ca_V2.2$ channels represents an important mechanism for regulating transmitter release from primary sensory neurons. $Ca_V2.2$ channels might be similarly regulated at synapses in other regions of the nervous system. The therapeutic implications of this newly described mechanism for controlling $Ca_V2.2$ channel expression may be hard to predict. Altier *et al.* point to potential advantages of agonists and antagonists of the ORL1 receptor. Morphine-induced internalization of the μ-opioid receptor has been implicated in morphine tolerance, but a clear link between these processes is not established[10]. If nociceptin normally acts through ORL1 receptors to promote the internalization of the $Ca_V2.2$ channel during chronic pain, agents that stimulate ORL1 receptor expression might in sensory neurons prove beneficial in alleviating the problem. Location, however, is critical. ORL1 receptor stimulation at supraspinal sites elicits hyperalgesia[4], and in other regions of the nervous system, ORL1 receptors are implicated in anxiety, learning, and memory and addiction.

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A functional genomics guide to the galaxy of neuronal cell types

Elva Diaz

Our understanding of the complexity of the mammalian brain is limited by our inability to identify and classify its many different cell types. A new study in this issue uses a functional genomics approach to uncover the molecular basis of neuronal identity.

The enormous range of functions of the central nervous system is reflected in the diverse shapes, projections, connections and firing patterns in the galaxy of its ~100 billion individual neurons. Despite the importance of this diversity in the development and function of neural circuits, we know relatively little about the molecules that contribute to differences among regions and cell types within the brain, or even about how many cell types might exist. But don't panic: in this issue, Sugino *et al.*[1] report an important step toward determining the molecular basis of neuronal diversity using a functional genomics approach.

How many neuronal types are there in the mammalian brain? This seems like a straightforward question that should have a simple answer, like 42, for instance[2]. Unfortunately, we do not know how to best classify neurons in the first place. Historically, methods have distinguished neurons by morphological criteria, electrophysiological properties and the expression of specific markers such as calcium-binding proteins. For example, a subset of the heterogeneous population of GABAergic neurons can be divided into three groups according to parvalbumin, somatostatin and calretinin expression[3,4]. However, although parvalbumin-immunoreactive neurons in the rat visual cortex share some common features—high GABA expression and fast, non-adapting action potentials—they are morphologically diverse and make synaptic connections typical of both basket and chandelier cells, two distinct morphological classes[4]. So, there is no guide to this galaxy yet, as no unifying theme to classify neuronal cell types has emerged.

Successful application of microarray technology in the subclassification of tumors[5] suggests that genome-wide approaches might be used to delineate neuronal cell types based on global gene expression. The ability to isolate distinct populations from transgenic mice expressing green fluorescent protein (GFP) variants under cell type–specific promoters[6], along with the ability to amplify minute quantities of RNA from small numbers of cells[7], has made such gene expression profiling feasible. Indeed, Sugino *et al.* took such an approach to define a molecular taxonomy of different neuronal classes in the adult mouse forebrain.

They did an exquisite and technically impressive analysis of gene expression in 12 distinct populations of adult mouse forebrain neurons[1]. The neuronal populations were derived from transgenic mice expressing GFP derivatives under the control of specific promoters or from mice injected with a retrograde fluorescent tracer. They included subpopulations of GABAergic and glutamatergic neurons—the two major classes of neurons in the brain—from the cingulate cortex, somatosensory cortex, hippocampus, amygdala and

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