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Calcium currents regulate dopamine autoreceptors

This scientific commentary refers to '$Ca_v1.3$ channels control D2 autoreceptor responses via NCS1 in substantia nigra dopamine neurons' by Dragicevic *et al.* (doi:10.1093/brain/awu131).

Appropriate activity of substantia nigra dopamine neurons is required for proper motor function, habit formation and motivation, and degeneration of these neurons in Parkinson's disease leads to disrupted control of voluntary movement. In this issue of *Brain*, Dragicevic *et al*. unite two previously separate lines of research on the regulation of substantia nigra dopamine neuron activity—one based on L-type calcium channels and the other on D2 autoreceptors—and suggest that these mechanisms converge in a previously unsuspected way in Parkinson's disease (Dragicevic *et al*., 2014).

One of the two lines of research stems from decades of work to define how the activity of midbrain dopamine neurons is controlled. These neurons alternate between a relatively slow, baseline, pacemaking activity (~4 Hz) that presumably supplies the striatum with tonic low levels of extracellular dopamine, and bursts of activity of variable duration and only slightly higher frequency (~15 Hz) (Grace *et al.*, 2007). The resulting 'bandwidth' is not large: in contrast, activities of cortical output neurons can range from silent states to firing at frequencies of 20 Hz or more. While questions such as how salient sensory stimuli cause bursting are active areas of research, the pacemaking activity—which is autonomous, occurring even in cultured substantia nigra neurons—is fairly well elucidated, albeit subject to continuing elaboration in papers such as the one being discussed here.

In contrast to most other tonically active CNS neurons, which depend on monovalent cation channels to generate spontaneous action potentials, depolarization of mature pacemaking substantia nigra dopamine neurons may involve the opening of L-type calcium $Ca_v1.3$ channels, together with hyperpolarization-activated, cyclic nucleotide-gated (HCN) sodium channels (Puopolo *et al.*, 2013). The large calcium conductance through $Ca_v1.3$ channels has been suggested to underlie the specific vulnerability of substantia nigra (as well as locus coeruleus and dorsal motor nucleus of the vagus) neurons to cell death in Parkinson's disease (Surmeier and Schumacker, 2013). These neurons, moreover, exhibit wide action potentials (>2 ms), giving rise to further calcium entry via voltage-activated channels in the interspike interval (Puopolo *et al.*, 2013). Substantia nigra dopamine neurons also lack significant calcium buffering by proteins such as parvalbumin and calbindin—the latter of which is more highly expressed in ventral tegmental area dopamine neurons, which are relatively spared in Parkinson's disease.

Studies by James Surmeier and collaborators demonstrate that the high intracellular calcium load in substantia nigra dopamine neurons causes mitochondrial and oxidative stress, and others have provided evidence that high calcium can exacerbate neurodegeneration through the accumulation of neurotoxic levels of cytosolic catecholamines (Mosharov *et al.*, 2009). Inhibition of L-type calcium channels with dihydropyridines protects substantia nigra pars compacta neurons against neurotoxins associated with Parkinson's disease in a variety of animal studies (Surmeier and Schumacker, 2013). These data suggest that inhibition of $Ca_v1.3$ channel activity may be neuroprotective for the remaining substantia nigra pars compacta neurons in patients with Parkinson's disease, and isradipine, a dihydropyridine L-type calcium channel blocker shown to be effective in mouse models of the disorder, is currently in a clinical trial as a Parkinson's disease therapy (Parkinson Study Group, 2013).

The second line of research extends from the study of dopamine receptor-mediated auto-inhibition of neuronal activity. In substantia nigra neurons, this is mediated by D2-type receptors, which activate G protein coupled potassium channels (GIRKs) that hyperpolarize neurons and block cell firing (Lacey *et al.*, 1987). The response of substantia nigra neurons to dopamine is highly regulated, with chronic loss of dopamine leading to receptor sensitization (Schultz and Ungerstedt, 1978), a phenomenon strongly implicated in Parkinson's disease and its animal models. Work by John Williams and collaborators has shown that somatodendritic dopamine release drives rapid D2 receptor-mediated hyperpolarization of neighbouring dopaminergic neurons (Beckstead *et al.*, 2004). It may be, therefore, that dopamine autoreceptor activation inhibits the voltage and activity-dependent calcium-mediated stress associated with Parkinson's disease, and it is further possible that this is another advantage of clinical treatment with L-DOPA and dopamine agonists, although this has not been directly addressed.

In their new study, Dragicevic *et al*. connect these two lines of research by demonstrating that L-type calcium channels can promote D2 receptor function in juvenile substantia nigra pars compacta dopamine neurons (in contrast to the above D2

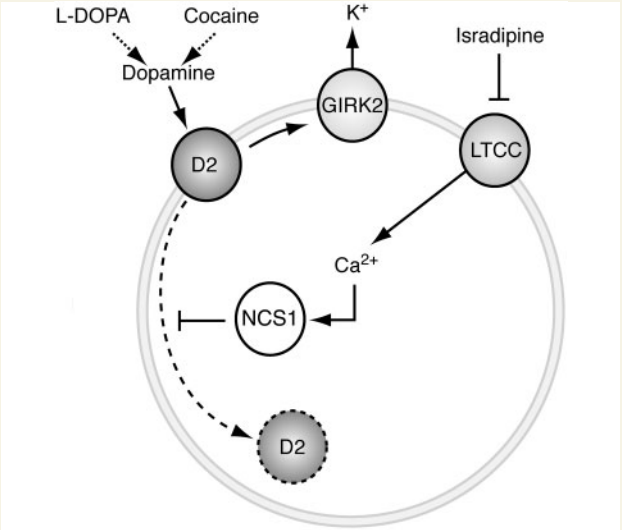

Figure 1 Proposed mechanism by which L-type calcium channels stabilize autoreceptor function in dopamine neurons. Dopamine D2 receptors are prototypical autoreceptors that control the firing rate of pacemaking substantia nigra dopamine neurons via activation of inhibitory GIRK channels. In response to stimulation by dopamine or indirect ligands such as cocaine, the inhibitory effect on dopamine neuron firing is reduced due to internalization of D2 receptors. This D2-desensitization is prevented by the neuronal calcium sensor protein NCS1, which is regulated by calcium supplied via $Ca_v1.3$ L-type calcium channels active during autonomous firing of dopamine neurons. The L-type calcium channel (LTCC) blocker isradipine, which is currently being evaluated as a neuroprotective therapy for Parkinson's disease, facilitates desensitization of the D2 receptor and disrupts the ability of dopamine to inhibit neuronal activity.

receptor-mediated inhibition of calcium currents). They show that this occurs after *in vivo* high dopamine states (induced by L-DOPA or cocaine) via $Ca_v1.3$-mediated interactions between the D2 receptor and a neuronal calcium sensor protein known as NCS1. In response to increased intracellular calcium, NCS1 prevents the internalization of plasma membrane D2 receptors, thus blocking receptor desensitization. Blocking L-type calcium channels consistently prevented the development of non-desensitized responses in these juvenile neurons (Fig. 1).

Thus, the high calcium load in substantia nigra neurons might provide a protective stress response, in which mature neurons do not undergo autoreceptor desensitization: this might block further calcium-related damage that could otherwise lead to Parkinson's disease. Remarkably, the authors report that NCS1 expression was increased in surviving dopamine neurons in the brains of patients with Parkinson's disease, suggesting that D2 receptors may indeed have been driven to a non-desensitized state; while this clearly did not prevent the disease, it may have slowed its progression. An important point to note, however, is that these patients had almost certainly been treated with L-DOPA and/or dopamine agonists, which may have contributed to the increased NCS1 expression and reduced autoreceptor desensitization.

The introduction of this novel pathway—calcium entry → NCS1 activation → inhibition of D2 receptor desensitization—raises numerous questions. One raised by Dragicevic *et al*. themselves is that dihydropyridines, by exacerbating D2 receptor desensitization, could (perversely) lead to excitotoxicity, although there is so far no evidence for this possibility. Another issue to explore is the importance of this response in mature substantia nigra neurons; while the authors report that desensitization of dopamine responses occurred selectively in juvenile substantia nigra pars compacta neurons, it is not clear if it also occurs in adult cells. Indeed, Dragicevic *et al*. studied a knockout line that did not express the $Ca_v1.3$ channel during development, and in contrast to the expected autoreceptor desensitization, they detected non-desensitized receptors in substantia nigra dopamine neurons in both juvenile and adult mice. Further questions are raised by studies from Mark Brodie and colleagues (Nimitvilai *et al.*, 2012) demonstrating that desensitization of D2 receptors in ventral tegmental area neurons is enhanced by calcium signalling, in a manner that is independent of L-type calcium channels—what underlies these apparent differences? Irrespective of how these issues are eventually resolved, Dragicevic *et al*. have introduced a new pathway: and, as seems to be the usual pattern in our field, they have revealed that brain activity is more complex and interactive than we thought. This may make our job of deciphering neural function more challenging, but it is precisely these complexities that provide us with the ability to recognize these patterns at all.

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Glycine receptor antibodies in PERM: a new channelopathy

This scientific commentary refers to 'Glycine receptor antibodies in PERM and related syndromes: characteristics, clinical features and outcomes', by Carvajal-González *et al.* (doi:10.1093/brain/awu142).

Ligand- or voltage-gated ion channels can be affected by mutation or autoimmune attack, leading to so-called channelopathies. A number of CNS disorders, such as limbic encephalitis and certain forms of epilepsy, have been shown to associate with specific serum autoantibodies against ion channels or related proteins (Irani and Lang, 2008). In 2008, the glycine receptor (GlyR) was first recognized as a possible target for autoantibodies in a patient with progressive encephalomyelitis, rigidity and myoclonus (PERM) (Hutchinson *et al.*, 2008). The GlyR is a member of a superfamily of ligand-gated ion channels that also includes *N*-methyl-D-aspartic acid (NMDA) receptors and nicotinic acetylcholine receptors. GlyRs are present throughout the brain, but are most abundant in the spinal cord and brainstem. The GlyR is also the target of the alkaloid strychnine, which causes generalized muscle spasms and cramps, muscle stiffness and tightness, agitation, heightened awareness and responsiveness, stimulation-evoked seizures, myoclonus, respiratory failure, and sometimes death. For comparison, the symptoms of PERM include muscle spasms, cramps, myoclonus, stimulus-evoked startle and respiratory failure. In this issue of *Brain,* Carvajal-González *et al.* (2014) report the presence of antibodies against the GlyR in a relatively large cohort of patients with PERM, and describe the characteristics and clinical features of these patients. Using cellular assays, the authors present strong evidence that GlyR antibodies are the causative agents in this disorder.

The main clinical significance of this paper is that it demonstrates that PERM is a treatable autoimmune disease. There is considerable symptom overlap between PERM, stiff person syndrome and neuromyotonia. Moreover, antibodies against glutamic acid decarboxylase and the voltage-gated potassium channel complex have been detected in both PERM and stiff person syndrome. In the current study, Carvajal-González *et al.* prospectively identified 52 patients with GlyR antibodies, and classified 33 of these as PERM, two as stiff person syndrome and five as limbic encephalitis or epileptic encephalopathy. Patients with PERM were initially identified by the presence of GlyR antibodies, but the final classification was based on Meinck and Thomson (2002) and Espay and Chen (2006), in which PERM is defined on the basis of brainstem involvement in addition to the axial or limb rigidity typical of stiff person syndrome. Notably, autonomic disturbances were marked in many patients, and respiratory failures may have contributed to two of the four hospital deaths during the study. Another clinically important observation was the association of thymomas and lymphomas with PERM, as stiff person syndrome is more often associated with breast and lung cancer. The role of amphiphysin and gephyrin autoantibodies, previously detected in stiff person syndrome, remains to be characterized in PERM.

How do pathogenic immunoglobulins such as anti-GlyR antibodies gain access to the brain? Antibodies typically have only a limited ability to cross the blood–brain barrier. However, there is a large body of evidence indicating that pathogenic autoantibodies can enter the CNS (for a review see Martinez-Martinez *et al.*, 2013). The mechanisms by which antibodies manage to cross the blood–brain barrier under normal conditions are still unclear, but the blood–brain barrier is known to become 10-times more permeable following local inflammatory reactions (Cutler *et al.*, 1970). Some CNS autoimmune channelopathies occur only when the integrity of the blood–brain barrier is disrupted and an increased number of antibodies and/or lymphocytes gain access to the brain (Martinez-Martinez *et al.*, 2013). On the basis of the ratio of GlyR antibodies to total immunoglobulins in serum and CSF, Carvajal-González *et al.* concluded that there was intrathecal synthesis of GlyR antibodies in three of six patients for whom matching serum and CSF samples were available. However, this was not true of all patients, thus we must assume that there was substantial antibody access to the brain.

Most patients in the study benefited substantially from immunotherapy. This suggests that the autoantibodies cause only limited neuronal cell death and instead affect GlyR functions