Dopamine: should I stay or should I go now?

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In *C. elegans*, dopamine signaling regulates locomotion behavior. Chase and colleagues report that this signaling occurs through extrasynaptic and antagonistically acting receptors coexpressed in motor neurons. These results provide surprising insights into the G-protein pathways mediating this antagonism, with implications for dopamine signaling across species.

In 1986, John White and colleagues published the complete reconstruction of *Caenorhabditis elegans* nervous system connectivity[1]. For years *C. elegans* biologists have pored over this wiring diagram of the worm, hoping that rabbinical study of its pages would reveal how the nervous system generates behavior. In this issue, Chase *et al.*[2] demonstrate that those late nights with a yad and a candle were doomed to failure and could never reveal the motivations or even explain the movement of a worm.

First, the authors show that the neurotransmitter dopamine can have opposite effects on locomotion, instructing the worm to stay or to go, depending on the type of receptor activated. Second, the authors report that the dopamine inputs are not synaptic but rather humoral. Because control of locomotion is nonsynaptic, a complete map of the synaptic connectivity of the worm nervous system will never fully explain its behavior. Third, they find that antagonistic receptor types are coexpressed on the motor neurons that control locomotion, suggesting that the antagonism is battled out within a single cell, rather than between opposing circuits.

Conflicting actions of dopamine have long been known in vertebrates, and Chase *et al.* are able to demonstrate the effects of antagonistic signaling pathways on behavior in a physiological context. Intriguingly, the G-proteins mediating this antagonism differ from those previously implicated in dopamine signaling. Dopamine receptors can be divided into D1-like and D2-like subclasses. The antagonistic actions of these two classes have been thought to occur through activation of $G\alpha_s$ and $G\alpha_i$ signaling pathways. However, Chase *et al.* show that in the nematode, D1 and D2 antagonism appears to be mediated through $G\alpha_q$ and $G\alpha_o$. This finding, along with recent results in mice, might force a re-evaluation of the dogma in the dopamine signaling field.

Dopamine modulates a worm's response to food. A worm swims actively without food, but slows when it encounters the edge of a bacterial lawn. Mutants lacking dopamine do not slow when they encounter food[3], and worms exposed to exogenous dopamine are paralyzed and stop moving on or off food. However, it was unclear where dopamine acts in the network between the sensory neuron and the motor nervous system. Five dopamine receptors in the worm have been identified by

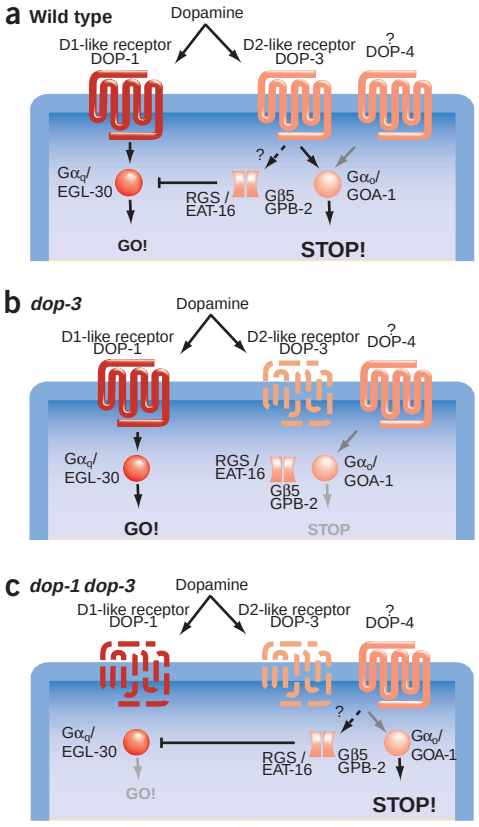

Figure 1 Dopamine regulation in a cholinergic motor neuron in *C. elegans*. (**a**) In the wild type, dopamine activation of DOP-3 predominates, and the worm stops swimming. (**b**) In the *dop-3* mutant, activation of DOP-1 predominates and the worm continues to swim. (**c**) *dop-1 dop-3* double mutants respond to dopamine and stop swimming. Thus, there must be another dopamine receptor, which we have called DOP-4. It could act in another cell, or in the cholinergic motor neuron as shown.

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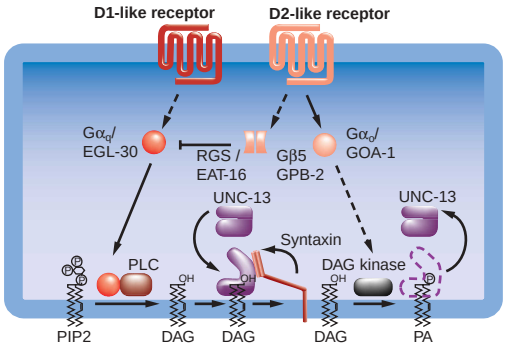

Figure 2 Model for $G\alpha_q$ and $G\alpha_o$ pathways in the nematode. $G\alpha_q$ stimulates PLC to convert PIP2 into DAG. DAG recruits UNC-13, which stabilizes syntaxin in the open state. Open syntaxin can prime synaptic vesicles for exocytosis by forming a SNARE complex (not shown). The $G\alpha_q$ pathway is antagonized by the $G\alpha_o$ pathway. The D2 dopamine receptor acts either by activating the RGS protein, which inhibits $G\alpha_q$, or by activating $G\alpha_o$, which stimulates DAG kinase. DAG kinase depletes DAG, UNC-13 is inactivated, and synaptic strength is weakened. Dotted lines indicate pathways for which the genetic and physical data are incomplete.

molecular criteria: two in the D1 class and three in the D2 class of receptors. Chase *et al.* now report that mutations in two of these genes affect locomotion.

Mutants null for *dop-3*, which encodes a D2-like receptor behaved like mutants lacking dopamine synthesis: that is, they did not slow down when they encountered food, and they were largely resistant to the paralyzing effects of exogenous dopamine exposure (**Fig. 1**). Knocking out *dop-1* caused no obvious defects on its own, but it suppressed the defect of a *dop-3* mutant: the *dop-3 dop-1* double mutant paused when encountering food and was paralyzed by exogenous dopamine just like the wild type. Analogous effects on slowing in response to food and paralysis by exogenous dopamine suggest that exogenous and endogenous dopamine act on the same signaling pathways. Because the *dop-3 dop-1* phenotype does not resemble that of a dopamine synthesis mutant, the other three dopamine receptors must make additional contributions to the locomotory response to dopamine. Because mutations affecting all these receptors are not available, further research will be required to fully characterize the multiple actions of dopamine on locomotion. In the meantime, Chase *et al.* have determined the cellular focus of DOP-1 and DOP-3 receptors.

The cellular site of action of these receptors is complicated by the known synaptic connections formed by neurons that make and release dopamine. Most dopamine neurons are found in the head ganglia of the worm, where the decisions to stay or go are presumably made. These neurons act redundantly to control pausing on food[3], suggesting a target in the head for the effects of dopamine on locomotion. However, *dop-3* dopamine receptors are not expressed in the command interneurons in the head, but rather in the cholinergic motor neurons of the ventral nerve cord. Chase *et al.* expressed the DOP-3 receptor in *dop-3* mutants specifically in these motor neurons, so that they were the only cells in the animal expressing DOP-3. The mosaic animals were rescued from the *dop-3* defect and were paralyzed by exogenously applied dopamine, indicating that dopamine acts on these distant motor neurons to control locomotion. However, because there are no synaptic inputs on the cholinergic motor neurons from any dopamine-expressing neurons, dopamine must function extrasynaptically as a hormone. Although dopamine is unlikely to function as a hormone in the mammalian brain, it does act in a paracrine fashion[4].

Like the D1 and D2 receptors in vertebrates, DOP-1 and DOP-3 have antagonistic effects on behavior. This antagonism could occur in the same cells or in different cells in the behavioral circuitry. For the most part *dop-1* and *dop-3* are expressed in nonoverlapping cells of the head and tail ganglia. However, DOP-1 and DOP-3 receptors are coexpressed in the cholinergic motor neurons of the ventral nerve cord. To show that DOP-1 was antagonizing the DOP-3 receptor in the same cells, Chase *et al.* expressed DOP-1 in cholinergic motor neurons of a *dop-3 dop-1* double mutant, which caused the animals to be resistant to the paralyzing effects of exogenous dopamine (**Fig. 1b**). Thus, dopamine acts antagonistically on the actions of a single cell.

To define the downstream components of D2-class DOP-3 receptor signaling, the authors screened for mutants that were resistant to the paralyzing effects of dopamine. These screens identified components of the $G\alpha_o$ pathway known to act in locomotion: $G\alpha_o$ itself and $G\beta5$. Moreover, they identified the RGS protein, which antagonizes $G\alpha_q$. Previous studies have characterized the antagonistic pathways for $G\alpha_o$ and $G\alpha_q$ in the nematode (**Fig. 2**). Activation of the $G\alpha_q$ pathway leads to stimulation of synaptic vesicle exocytosis. $G\alpha_o$ antagonizes $G\alpha_q$ function, leading to reduced synaptic transmission[5–7]. Thus, if we were to put in all of the pieces of this puzzle where they seem to fit, dopamine would bind D1-like DOP-1 receptors and activate $G\alpha_q$, and also bind D2-like DOP-3 receptors, which would antagonize D1 function through activation of $G\alpha_o$.

The pathway put forward by Chase *et al.* conflicts with the canonical idea of dopamine function. A large body of work, starting with the classic work of Greengard and colleagues in the early 1970s, established that D1 receptors activate adenylyl cyclase via $G\alpha_s$, whereas D2 receptors inhibit adenylyl cyclase via $G\alpha_i$[8]. However, recent work in mice suggests that D1- and D2-class receptors may also activate $G\alpha_q$ and $G\alpha_o$ in the mammalian brain[9–11]. If true, these findings suggest that our concept of how dopamine acts in the brain must be revised. But is this model true? First, is $G\alpha_q$ the major pathway for D1 signaling in the nematode? A direct link has not yet been established. Moreover, the alternative possibility that D1 receptors act via $G\alpha_s$ has not been explored. A worm homolog of $G\alpha_s$ activates adenylyl cyclase in the motor neurons and could be responsible for D1 signaling[12,13]. Second, does dopamine activate $G\alpha_q$ signaling in the mammalian brain? It is necessary to assay dopamine responses in mutants lacking $G\alpha_q$ function. Unfortunately, $G\alpha_q$ family members are redundant in the mouse brain and double mutants are synthetically lethal[14]. Tissue-specific knockouts will be required to evaluate the role of $G\alpha_q$ proteins in dopamine responses in the mouse. Thus, further work is required in both the worm and mouse to sort out the signaling pathways.

Finally, there is a logical problem with having dopamine both stimulate and inhibit a cell; it seems unfair to jerk the cell around like that. However, antagonistic effects on the same cells can provide the cell with more sensitive regulation. First, altering the ratio of receptor expression levels in these competing pathways could result in opposite behavioral responses. Second, if the receptors have very different affinities for dopamine, simply altering dopamine secretion could reverse the response of the cell. Thus, a fixed circuit can respond in

very different ways to a stimulus—and that is why religious study of the wiring diagram of the worm will never lead to enlightenment.

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Ancient viral protein enrages astrocytes in multiple sclerosis

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Syncytin is a viral envelope protein encoded in the human genome. New work in this issue indicates that it is activated in multiple sclerosis astrocytes and microglia, contributing to the inflammation-induced myelin destruction that causes disease symptoms.

Multiple sclerosis (MS) is a devastating disease that can cause repeated unpredictable bouts of motor disturbances, partial paralysis, sensory abnormalities and visual impairment. These symptoms result from an inflammatory process that selectively attacks and destroys oligodendrocytes, the cells that form the myelin sheaths around axons in the brain and spinal cord[1]. Several lines of evidence suggest a role for an autoimmune mechanism of disease pathogenesis. It has been suggested that exposure to a viral, bacterial or other pathogen may trigger the disease process, perhaps through a molecular mimicry mechanism where a protein in the pathogen is similar to protein(s) in myelin, eliciting an autoimmune response[2]. Current hypotheses mostly blame activated T lymphocytes and microglia/macrophages, which can produce cytotoxic cytokines and reactive oxygen molecules, for the destruction of oligodendrocytes in MS[2].

However, the findings of Antony *et al.* reported in this issue[3] suggest that normally docile astrocytes are among the executioners. In individuals with MS, the authors report, astrocytes express a protein called syncytin (**Fig. 1**), leading to their activation and the synthesis of reactive oxygen species. Syncytin is a viral envelope glycoprotein, but it does not come from any viral pathogen infecting MS patients. Instead, syncytin is produced from a human endogenous retrovirus (*HERV-W*, at the *ERVWE1* locus), a remnant of a virus that invaded humans during primate evolution, probably more than a million years ago. Approximately 8% of the human genome originated from retroviral genomes and, although most of the viral sequences are not expressed, some are, and the functions of these viral proteins in physiology and disease are of considerable interest.

Syncytin has apparently acquired important functions in humans because, unlike those of other *HERV-W* elements, which are defective, the open reading frame for syncytin is intact and has been preserved for thousands of years. Indeed, syncytin is highly expressed in the developing placenta, where it is important in trophoblast cell fusion and syncytium formation[4]. Syncytin is a 518-amino-acid membrane glycoprotein that may exert biological actions by binding to a receptor called ASCT2 (alanine, serine, cysteine transporter 2), which is both an amino acid transporter and a retrovirus receptor[5]. Viral envelope glycoproteins are known to affect immune responses, and syncytin has amino acid sequences that would be predicted to affect the activation of lymphocytes and macrophages[4].

Antony *et al.* found that levels of syncytin mRNA and protein were significantly higher in frontal cortex white matter tissue samples taken from MS patients as compared to samples taken from patients with Alzheimer disease or HIV encephalitis or from subjects without neurological disease. Syncytin expression was increased specifically in astrocytes and microglia associated with damaged oligodendrocytes, but not in the oligodendrocytes or neurons. When the authors exposed cultured human astrocytes or microglia to a phorbol ester to simulate immune activation, syncytin expression was increased. Overexpression of syncytin in astrocytes and macrophages was sufficient to cause the cells to produce high amounts of the proinflammatory cytokine interleukin-1β (IL-1β) and reactive oxygen radicals. The culture medium from astrocytes overexpressing syncytin was toxic to oligodendrocytes, and this toxicity was prevented by the antioxidant ferulic acid, by an anti-inflammatory drug and by inhibitors of nitric oxide production. Whether reactive oxygen intermediates are responsible for the observed toxicity or whether they are intermediates in the generation of additional cytolytic mediators remains to be determined.

To determine whether syncytin can cause demyelination *in vivo*, the authors injected a syncytin-producing viral vector into the corpus callosum of mice[3]. Astrocytes were infected and produced large amounts of syncytin, causing damage to oligodendrocytes and impaired sensorimotor function. When mice were administered ferulic acid, the astrocytes still produced syncytin, but oligodendrocyte damage did not occur and sensorimotor function was preserved. Thus, the *HERV-W* gene encoding syncytin is activated in astrocytes in MS, where it may induce the production of oxygen radicals that then damage adjacent oligodendrocytes, resulting in demyelination and associated symptoms.

Why is the expression of syncytin increased in astrocytes in MS? In placental cells, the production of syncytin is decreased in response to hypoxia, and this is associated with

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