

nals³ that operate via the adrenal gland (which produces endogenous cardioactive steroids) and the heart (which makes atrial natriuretic factor)⁴.

An insufficient blood supply to the kidneys is answered by increased release of the pressure hormone angiotensin II from the kidneys. Furthermore, blood pressure must be regulated to respond to the physiological requirements in healthy individuals. This is accomplished by the release of additional pressure hormones from the adrenal glands (epinephrine, serotonin or endogenous cardioactive steroids) or from vascular endothelial cells (endothelin-1).

Unfortunately, a long-term rise in blood pressure causes the heart, vascular tissue and kidneys to adapt to the situation. This tissue remodeling, which proceeds by altered gene expression, may lead within days or weeks to defective organ function.

Wirth *et al.*¹ asked how blood pressure is increased by pressurizing hormones in arterial smooth muscle cells. They investigated how docking of hormones to G protein-coupled hormone receptors (GPCRs) leads to the phosphorylation of myosin light chain (MLC), a trigger for the contraction of arterial smooth muscle cells.

GPCRs form complexes with GTP-hydrolyzing G proteins in caveolae of smooth muscle cells. There, the complexes transduce the amplified signal to contract through calcium-dependent and calcium-independent pathways^{5,6}. The calcium-dependent pathway starts with G_q-G₁₁, induces calcium release from intracellu-

lar stores and activates MLC phosphorylation, thereby stimulating contraction.

The calcium-independent pathway blocks the degradation of phospho-MLC and thereby the relaxation of smooth muscle contraction. This pathway uses G_{α12}-G_{α13} as a signal amplifier and operates through leukemia-associated guanine nucleotide exchange factor (LARG), Rho and the protein kinase ROCK—ultimately blocking the dephosphorylation of phospho-MLC and preventing relaxation. This pathway also affects gene transcription and induces the remodeling of vascular tissue^{7,8} (Fig. 1).

The authors asked which G protein is used to regulate vascular tone under normal and salt-induced hypertensive conditions¹. They generated mice in which the α subunits of G_q-G₁₁ or G₁₂-G₁₃ could be selectively switched off in smooth muscle cells and found that none of the pressure hormones tested used exclusively G₁₂-G₁₃ to activate the GPCR signaling cascade. When the G₁₂-G₁₃ signaling pathway was switched off, basal blood pressure remained unchanged. Hence the pathway governed by G_q-G₁₁ is responsible for the maintenance of basal blood pressure.

Treatment of mice with DOCA-salt resulted in a rise of arterial blood pressure, but this did not happen in mice in which the signaling cascade starting with G_q-G₁₁ or G₁₂-G₁₃ had been knocked out. The authors next knocked out the gene encoding LARG, which is part of the G₁₂-G₁₃ pathway¹. They found that mice deficient in LARG were unable to respond to DOCA-salt treatment. The findings suggest

that the GPCR-G₁₂-G₁₃-LARG signaling pathway is key for generating salt-induced hypertension—and that the lack of salt-induced hypertension in G_q-G₁₁ knockout mice is probably due to the loss of the regulation of basal blood pressure (Fig. 1).

The findings not only illuminate how hormones binding GPCRs may regulate blood pressure, but also may provide insight into how the cardiac glycosides may work in vasoconstriction. These hormones spike in response to sodium and bind to a sodium-potassium ATPase to regulate increases in smooth muscle contraction and arterial hypertension⁴. A signal may be sent from the sodium-potassium ATPase via LARG and Rho to block smooth muscle relaxation; this makes sense, as Rho is a member of the Ras superfamily, and Ras is part of the sodium-potassium-ATPase signaling complex⁴ (Fig. 1).

It is now possible to search for antihypertensive drugs that specifically interrupt the G₁₂-G₁₃-LARG pathway.

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The movers and shakers of deep brain stimulation

Wael Asaad & Emad Eskandar

Deep brain stimulation is increasingly used in the treatment of Parkinson's disease, essential tremor and other disorders, yet its mechanism of action remains unknown. New findings suggest that at least some of its action involves the release of adenosine, dampening tremors (pages 75–80).

Deep brain stimulation (DBS) has been used successfully in the treatment of neurologic illnesses such as Parkinson's disease, tremor and dystonia, and it is currently being explored as a treatment for major depression and obsessive-compulsive disorder. Yet,

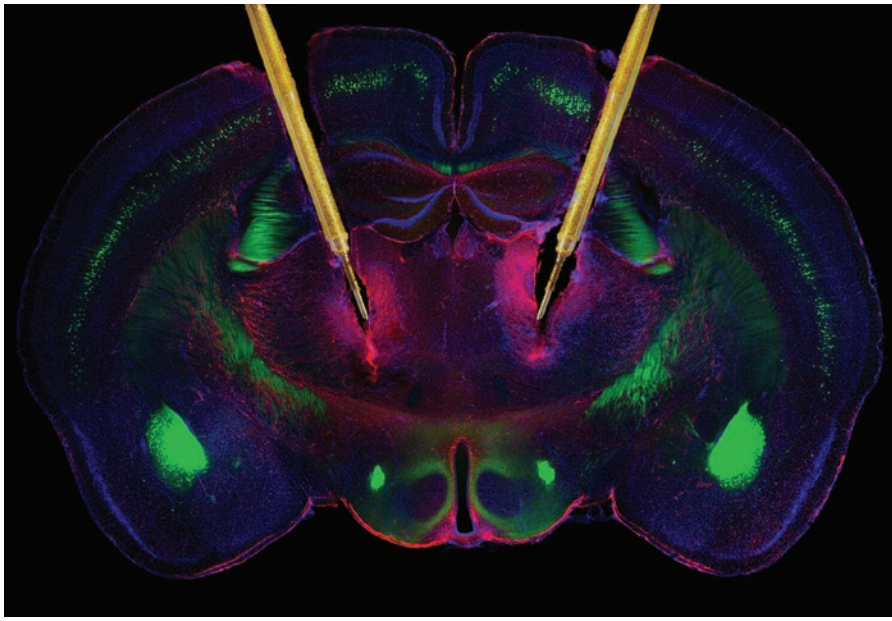
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empirical efficacy aside, little is known of the mechanisms by which the electrical stimulation of deep brain structures alleviates these conditions. In fact, there is not even widespread agreement as to whether such stimulation facilitates, impedes or 'overwrites' information passing through the stimulated nuclei¹.

Nevertheless, the reversible and adjustable nature of DBS has made it an attractive, if rather blunt, tool for treating an increasingly large number of problems—when the only

surgical tool available is a hammer, every disorder starts to look like a nail. Hence, it is of immense importance to understand the mechanistic basis of DBS in order to improve, revise or expand its application in a more rational fashion. Toward this end, Bekar *et al.*² show that, at least in the case of tremor, adenosine may have a major role in mediating the therapeutic efficacy of DBS.

Adenosine, a neuromodulator found throughout the brain, exerts its postsynaptic effect through G protein-coupled receptors^{3,4}.



Xiaoning Han and Takahiro Takano

Figure 1 Really deep. Two electrodes planted into the thalamus of a transgenic mouse expressing yellow fluorescent protein (in axons) under the Thy1 promoter. Astrocytes were stained against glial fibrillary acidic protein (red). Note the intense gliosis around the electrode tips. Bekar *et al.*² implicate adenosine in the effects of deep brain stimulation, a treatment with a mysterious mechanistic basis.

In particular, the A1 class of receptors tends to decrease the activity of adenylyl cyclase and open potassium channels, thus hyperpolarizing neurons and rendering them less active. This ability to dampen neural activity is at the heart of the findings by Bekar *et al.*², demonstrating that adenosine is a product of DBS and inhibits tremor.

Tremor is an involuntary, rhythmic movement that can occur in isolation, for example in benign or 'essential' tremor, or as part of a more elaborate movement disorder, such as Parkinson's disease. Benign tremor is a 5–8-Hz oscillation that is most pronounced during purposeful movement. It generally affects the hands or feet, but can involve other parts of the body such as the head. The tremor of Parkinson's disease, in contrast, is usually slower—closer to the 4–5-Hz range—and is most prominent at rest; it lessens with movement. Furthermore, whereas the cerebellum is believed to have a central role in the etiology of benign tremor, it is probably only indirectly involved in parkinsonian tremor.

Despite these differences, DBS of the thalamic cerebellar relay nucleus (the ventral intermediate nucleus) has been successfully applied to the treatment of both benign and Parkinson's disease tremors^{5,6}.

The onset of DBS dampens tremor nearly immediately. When the stimulation is turned off, the tremor resumes without delay. The stimulation itself consists of a continuous train of relatively high-frequency (>130 Hz)

pulses delivered through wires implanted in the target brain nucleus and powered by a small pulse generator implanted under the clavicle. The system is open loop, meaning that stimulation is delivered without feedback modulation. Moreover, the effects of electrical stimulation are complex, influencing cell bodies, dendrites and axons in potentially different ways and probably causing the release of a myriad of neurotransmitters and neuromodulators. Bekar *et al.*² illuminate one path through this thicket of possible mechanisms by studying the effects of DBS in mouse brain slices and then in an *in vivo* mouse model of tremor (**Fig. 1**).

First, in thalamic slices, the authors showed that high-frequency stimulation (HFS, the experimental analog of therapeutic DBS) impedes synaptic transmission via adenosine². Specifically, HFS transiently reduced the amplitude of excitatory postsynaptic potentials both in the stimulated (homosynaptic) pathway and, more prominently, in separate converging (heterosynaptic) pathways. Complementing this electrophysiological effect, HFS resulted in a calcium-independent (nonvesicular) release of ATP into the extracellular space; this ATP was then metabolized to adenosine by an ecto-ATPase. Importantly, application of an adenosine (A1) receptor antagonist or an ecto-ATPase inhibitor blocked the HFS-induced reduction in excitatory postsynaptic potential amplitude.

Next, Bekar, *et al.*² tested the effect of A1 antagonists on a mouse model of tremor. They administered the MAO-A inhibitor harmaline systemically, resulting in the development of a generalized tremor that, although higher in frequency and evident at rest as well as during movement, is believed to mimic key aspects of human essential tremor. Local application of adenosine into the thalamus decreased the magnitude of the tremor with an efficacy comparable to that of HFS of the thalamus. Conversely, infusion of an adenosine A1 receptor antagonist augmented the tremor. In addition, in the presence of this antagonist, low electric currents produced side effects that otherwise were seen at only higher stimulation levels. Unfortunately, although it would have been interesting to see whether DBS could alleviate tremor in A1-knockout mice, these mice were prone to seizures, preventing the adequate testing of HFS at therapeutic levels.

As with any good experiment, unanswered questions remain. Specifically, how does adenosine activation of thalamic A1 receptors result in decreased tremor without apparently affecting normal movement? Is the neural activity that produces tremor at a certain frequency that is more susceptible to adenosine-derived inhibition? Or is there some more global influence on the metrics of movement—of which tremor is only the most obvious example? Answers to such questions will help refine our attempts at therapy.

Of course, as the authors themselves note, the therapeutic efficacy of DBS probably depends on multiple mechanisms (for instance, they show that the γ -aminobutyric acid agonist muscimol also reduces tremor in harmaline-treated mice²). These mechanisms are likely to include the accumulation of neuroactive substances, such as adenosine, as well as circuit-level effects, such as, perhaps, the activation of inhibitory pathways to dampen oscillatory neuronal activity. To further complicate the matter, DBS using uniform electrical parameters may act via distinct mechanisms when applied to different parts of the brain and may result in long-term plastic changes that are difficult to study acutely.

Within the next several years, experiments such as those of Nedergaard and colleagues² and the incorporation of new techniques will undoubtedly further elucidate those mechanisms. For example, the substitution of optical for electrical stimulation may allow the precise targeting of specific cell types, rather than generic geometric brain volumes, to more carefully control the target of DBS (Aravanis, A.M., Meltzer, L.A., Zhang, F.,

Mogri, M.Z., Wang, L.P. *et al.*, personal communication). Moreover, there is increasing interest in developing closed-loop stimulators that are responsive to electrical or neurochemical changes. These approaches may provide not only experimental utility, but also

significant therapeutic benefit. Ultimately, the expansion of our toolbox for repairing the brain will depend on such advances.

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Short-circuit recovery from spinal injury

Dennis J Stelzner

Propriospinal neurons, whose axons never leave the spinal cord, aid in recovery after spinal cord injury—even when all axons from the brain have been damaged (pages 69–74).

There are no proven therapies that enhance axonal regeneration and restore function after complete spinal cord injury in people. However, progress has been made in identifying factors in the spinal cord environment that inhibit regrowth of damaged axons and in developing strategies to neutralize these factors or increase the growth response of neurons to overcome them. These strategies include blocking myelin-inhibitory proteins—degrading chondroitin sulfate proteoglycans at the injury site that inhibit axonal growth, or modifying the regenerative response of damaged neurons¹.

These approaches, and others, have led to enhanced axonal regrowth and evidence of functional recovery in animal models. Yet, regeneration of a substantial number of long-tract axons from the brain to reconnect with spinal circuitry below the damaged spinal cord has been difficult to achieve and may require a combination of several therapeutic interventions.

In this issue of *Nature Medicine*, Courtine *et al.*² offer one potential avenue for intervention in the over half of all spinal cord-injured individuals whose spinal cord injury is incomplete. The findings indicate that even with spinal injury that damages all descending pathways from the brain, remaining axons from neurons at the level of injury (propriospinal neurons) are able to restore locomotor function.

It is already known that the same strategies that enhance regeneration can also improve recovery in animals with partial spinal

injury^{1,3}. In most instances, recovery seems to be related to the sprouting of branches of damaged axons or to other intact pathways remaining both above and below the region of spinal cord injury^{1,3,4}, rather than to actual axonal regeneration. Apparently, this reorganization of spared axons in response to injury takes over some of the functions of the damaged axons. Additionally, treadmill training can restore supporting and locomotor responses of the hindlimbs after complete spinal injury in cats and rodents and can aid recovery after incomplete spinal injury in humans. These studies show that the circuitry for stepping and other responses remains intact in the damaged spinal cord but is subthreshold until rehabilitation (training) restores its function, probably through activity-dependent mechanisms³.

Propriospinal axons, originating from neurons within the spinal cord and projecting intersegmentally to terminate at other spinal levels, are uniquely suited for injury-induced plasticity. They consistently show a greater growth response after spinal cord injury than long-tract axons do, and a population is often spared after spinal injury⁵. Moreover, propriospinal projections are coordinated with projections from the brain for spinal motor control and to interconnect the circuitry at different levels of the spinal cord.

Bareyre *et al.*⁴ made the seminal observation that when the dorsal half of the spinal cord is cut, damaged corticospinal tract axons destined for the lumbosacral spinal cord sprout onto propriospinal tract neurons above the lesion. Axons from propriospinal tract neurons passing ventral to this injury, and bridging the lesion, expanded their arborization among lumbar motoneurons. Thus, the propriospinal axons spontaneously formed a new functional intraspinal circuit that relayed input from the brain to its origi-

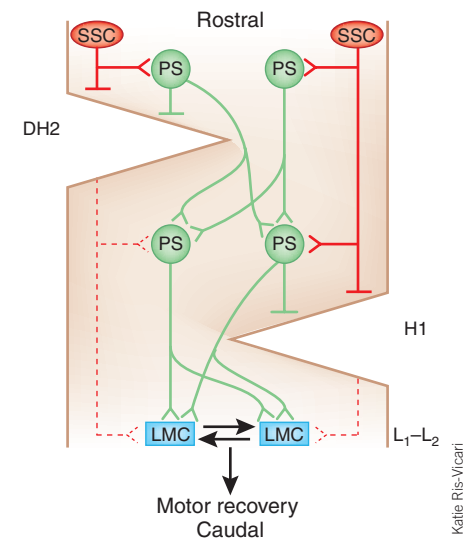


Figure 1 Courtine *et al.*² cut all long-tract axons descending from the brain (SSC) using opposite-side staggered lateral hemisections of the mouse spinal cord at the T7 and T12 segments. The only connections remaining that passed into and out of the gap between the lesions were short axons from propriospinal neurons that could relay motor information from the brain to lumbar motor circuits (LMC) involved in coordinated hindlimb stepping. Only if the time between making the initial more caudal hemisection at T12 (H1) and the second hemisection at T7 (DH2) was delayed by several weeks did coordinated stepping responses recover in both hindlimbs. The findings suggest that the remaining intact SSC connections present before the second lesion are necessary for this lesion-induced plasticity to occur in propriospinal (PS) connections.

nal spinal target. In this instance, however, pathways from the brain remaining in the ventral half of the spinal cord could have been responsible for, or could have contributed to, locomotor recovery.

Courtine *et al.*² generated more complete lesions, damaging all descending long-tract axons; they did so by making staggered hemi-

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