only within the four cells of the quiescent center, whereas expression in the nww mutant expanded to include a larger number of cells (Fig. 3, I and J).

Because WOX5 has been shown to be downstream of auxin signaling, we also analyzed pDR5-GFP expression to determine whether auxin signaling is altered in the nww mutant root (Fig. 3, K and L, and fig. S7). In WT roots, pDR5-GFP was observed in only the quiescent center and columella cells. In the rescued roots of the nww mutant, pDR5-GFP was misexpressed throughout the presumptive root cap. Although auxin signaling still occurs in the nww mutant, the altered pDR5-GFP expression pattern suggests that the roots are unable to respond to auxin in an appropriate manner. Thus, the NWW genes are required for appropriate development of the quiescent center and the distal root meristem.

Misexpression of NTT might be sufficient to transform other stem cells within the root meristem into a distal fate. To test this prediction, we created an inducible line by fusing NTT to the glucocorticoid receptor (p35S-NTT-GR) (16). In the wild type, columella cells are restricted to the distal region within the root meristem. In contrast, induction of NTT activity within mature root meristems caused ectopic production of columella cells (Fig. 4, A and B). The QC25 marker, normally expressed only in the quiescent center, was ectopically expressed in the proximal region when NTT activity was induced. Thus, NTT is both necessary and sufficient to pattern distal stem cell identity within the root meristem. The p35S-NTT-GR line can also mimic the root-inducing effects of exogenous auxin application. When WT seedlings were transferred to normal media after germinating on 10-μM 1-naphthylacetic acid (NAA), extra roots were produced. Similarly, extra roots formed after p35S-NTT-GR seeds were germinated in the presence of the dexamethasone inducer and then transferred to normal media (fig. S8). This is consistent with a role of the NWW genes in mediating an auxin signal for root initiation.

NTT misexpression can also change stem cell fate within the embryo. In WT embryos, the apical region gives rise to the shoot apical meristem and two groups of primordial cells known as the cotyledon initials. The ASI gene is strongly expressed in the cotyledon initials in transition-stage embryos (17). Misexpression of NTT under the control of the ASI promoter caused roots to form instead of cotyledons in the resultant seedlings (Fig. 4, C and D, and figs. S8, E to G). This suggests that NTT expression is sufficient to transform cotyledon primordia to a root meristem fate within the apical region of the embryo.

More widespread NTT misexpression in the protodermal layer of the apical cells of early globular-stage embryos using the AtML1 promoter (pML1-NTT) (18) resulted in embryos with asymmetrical structure, losing both the cotyledons and the shoot apical meristem (Fig. 4, E and F, and fig. S8). Taken together, these studies support a model in which NTT misexpression is sufficient to pattern basal stem cell identity within the embryo and distal stem cell identity in the root meristem (fig. S8K).

There is tremendous interest in identifying the major pathways that specify stem cells in both animal and plant systems. Identification of the NWW genes will help to explain the formation of stem cells and may ultimately allow for the manipulation of the root to enhance agricultural yield. Additionally, although many regulators have been found to pattern plant meristems, it is likely that additional intrinsic factors remain undiscovered due to genetic redundancy, as is the case with the NWW genes.

REFERENCES AND NOTES


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ADDITION THERAPY

Refining deep brain stimulation to emulate optogenetic treatment of synaptic pathology

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Circuit remodeling driven by pathological forms of synaptic plasticity underlies several psychiatric diseases, including addiction. Deep brain stimulation (DBS) has been applied to treat a number of neurological and psychiatric conditions, although its effects are transient and mediated by largely unknown mechanisms. Recently, optogenetic protocols that restore normal transmission at identified synapses in mice have provided proof of the idea that cocaine-adaptive behavior can be reversed in vivo. The most efficient protocol relies on the activation of metabotropic glutamate receptors, mGLRs, which depolarizes excitatory synaptic inputs onto dopamine D1 receptor medium-sized spiny neurons and normalizes drug-adaptive behavior. We discovered that acute low-frequency DBS, refined by selective blockade of dopamine D1 receptors, mimics optogenetic mGLR-dependent normalization of synaptic transmission. Consequently, there was a long-lasting abolishment of behavioral sensitization.

Deep brain stimulation (DBS) consists of passing electric current, typically in excess of 100 Hz, through electrodes surgically implanted into subcortical nuclei of the brain. DBS is currently an FDA-approved treatment for Parkinson’s disease, dystonia, and essential tremor (1, 2). Additional indications, such as depression, obsessive-compulsive disorders, and addiction have been considered (3). The mechanisms by which DBS produces its therapeutic effects remain largely unknown (4, 5), although recent studies suggest that it may have widespread effects on brain network activity (6, 7). In the context of addictive disorders, altered activity in areas projecting to the nucleus accumbens (NAc), such as the medial prefrontal cortex (mPFC), has been implicated in the effects of DBS (8). The effects of classical high-frequency DBS are transient. Symptoms

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typically reappear once stimulation is stopped, requiring lifelong continuous stimulation (9). Because pathological synaptic plasticity drives circuit dysfunction in many neurological and psychiatric disorders (10, 11), tailoring DBS to restore normal transmission may have long-lasting effects and thus represent a major therapeutic advance.

Addiction may be a condition ideally suited to test the potential of DBS to correct pathological synaptic function, because the disease is associated with behavioral changes (22) that are caused by drug-evoked synaptic plasticity of glutamatergic transmission in the mesolimbic dopamine system (13, 14). Specifically, in the NAc, cocaine exposure increases the strength of excitatory afferents onto dopamine D1 receptor-expressing medium-sized spiny neurons (DIR MSNs) (15, 16). This plasticity underlies behavioral changes associated with drugs of abuse, such as psychomotor sensitization (15, 17). Low-frequency optogenetic stimulation of the excitatory projections to the NAc is able to reverse cocaine-evoked plasticity and erase drug-adaptive behaviors (15, 18).

Locomotor sensitization is a straightforward behavioral paradigm used to model drug-adaptive behavior (19, 20). In rodents, repeated cocaine exposure induces progressively enhanced locomotor activation in response to a cocaine injection; after five injections, the locomotor response is typically fully sensitized, a state that persists for months after cocaine withdrawal (21). Locomotor sensitization is thus thought to underlie important aspects of vulnerability to drug addiction and relapse, specifically drug craving (19, 21, 22). The expression of locomotor sensitization is mediated by enhanced glutamatergic transmission in the NAc (23–25). After repeated cocaine exposure, glutamate projections selectively onto DIR MSNs of the NAc are strengthened (15, 26), which is driven by the insertion of AMPA receptors (27–29).

As expected, sequential injections of cocaine in mice (20 mg per kilogram of body weight (mg/kg), intraperitoneal (i.p.)) progressively enhanced the locomotor response, which plateaued after five sequential injections and was still elevated during the cocaine challenge test, given after 10 days of withdrawal (Fig. 1A). Classical high-frequency DBS (130 Hz, 90 μs) applied to the shell of the NAc (Fig. 1B and fig. S1) during the cocaine challenge suppressed the sensitization, but had no effect on the acute locomotor response to cocaine in saline controls (Fig. 1C). When DBS was applied to the NAc shell for 60 min leading up to the cocaine

**Fig. 1. Classical DBS transiently suppresses locomotor sensitization to cocaine and fails to depotentiate excitatory transmission onto DIR MSNs.**

(A) Schematic of experiment: Cocaine locomotor activity is monitored for 60 min, immediately after the injection of cocaine or saline. (B) Coronal section depicting bilateral electrode placement in the anterior portion of the NAc shell. Scale bar, 250 μM. (C) After five daily cocaine injections, a robust locomotor sensitization is observed that persists for 10 days after withdrawal; 130-Hz DBS applied during the cocaine challenge on day 15 reduced the locomotor sensitized response [controls: saline/cocaine = 10/10; 130-Hz DBS: saline/cocaine = 8/8]. Repeated measures analysis of variance (ANOVA) with post-hoc t test: cocaine control versus cocaine 130-Hz DBS, t = 2.27, P = 0.035. (D) 130-Hz DBS applied immediately before cocaine challenge [time (T) = 0 hours] also suppressed the sensitized response to cocaine (controls: saline/cocaine = 9/14, T = 0 hours; 130-Hz DBS: saline/cocaine = 5/9). Repeated measures ANOVA with post-hoc t test: cocaine control versus cocaine 130-Hz DBS, t = 2.19, P = 0.041. (E) 130-Hz DBS did not have an effect on the sensitized response to cocaine when applied 4 hours (T = 4 hours, 130-Hz DBS: saline/cocaine = 6/8) and (F) 24 hours (T = 24 hours, 130-Hz DBS: saline/cocaine = 5/8) before cocaine challenge. (G) Cocaine treatment significantly increased the AMPA/NMDA ratio (controls: saline/cocaine = 8/7; 130-Hz DBS: saline/cocaine = 7/7). Two-way ANOVA with post-hoc t test, saline versus cocaine control, t = 2.34, P = 0.036, saline 130-Hz DBS versus cocaine 130-Hz DBS, t = 2.23, P = 0.049. (H) Cocaine treatment also significantly increased the rectification index (controls: saline/cocaine = 7/6; 130-Hz DBS: saline/cocaine = 7/7). Two-way ANOVA with post-hoc t test, saline versus cocaine control, t = 2.39, P = 0.034; saline 130-Hz DBS versus cocaine 130-Hz DBS, t = 2.21, P = 0.050) measured in DIR MSNs: 130-Hz DBS had no effect on either parameter. Representative traces of control animals (left) and animals treated with 130-Hz DBS (right) are shown. Saline-treated animals are shown in black and cocaine-treated animals in red. Scale bars are 20 pA and 20 ms. All plots are means with SEM, *P < 0.05, **P < 0.01.
challenge, locomotor sensitization was still suppressed (Fig. 1D and fig. S2, A and B). This suppression was not observed when DBS was applied to the mPFC (fig. S2C) or the core of the NAc (fig. S2D). However, when the interval between DBS offset and cocaine challenge was extended to 4 hours (Fig. 1E) or 24 hours (Fig. 1F), the sensitized response was not reduced, relative to unstimulated controls. High-frequency DBS had no effect on general ambulatory activity, as measured by an open-field task (fig. S3A). Classical high-frequency DBS thus has only a transient effect on behavioral sensitization, probably because this manipulation does not affect cocaine-evoked synaptic plasticity.

As in previous studies (15), cocaine led to a long-lasting strengthening of excitatory transmission onto D1R MSNs (Fig. 1G), which were identified by using BAC transgenic mice expressing a reporter protein (td-Tomato) under the control of a drd1a promoter. This potentiation was determined by measuring an increase of the ratio of AMPA receptor (AMPA) excitatory post-synaptic potentials (EPSCs) over N-methyl-D-aspartate receptor (NMDAR) EPSCs (the AMPA/NMDA ratio). In addition, we observed an inward rectification of AMPAR currents (Fig. 1H).

This inward rectification is indicative of GluA2-lacking AMPARs. Their insertion also contributes to increases in synaptic strength (30–32). Viral insertion of GluA2-lacking AMPARs into the NAc is indeed sufficient to induce sensitization (33). Neither of these indices of cocaine-evoked plasticity was affected by the 130-Hz DBS applied 24 hours before the ex vivo recordings (Fig. 1, G and H).

Why was high-frequency DBS unable to restore normal synaptic transmission in the NAc? To reverse cocaine-evoked potentiation, one would have to unmask a D1R-dependent LTD (LTP) protocol for use in vivo. Using the insight gained from these ex vivo LTD experiments, we sought to design a rational DBS protocol for use in vivo. Using a two-injection protocol for use in vivo. Using the insight gained from these ex vivo LTD experiments, we sought to design a rational DBS protocol for use in vivo. Using the insight gained from these ex vivo LTD experiments, we sought to design a rational DBS protocol for use in vivo.
sensitization paradigm, we determined that 12-Hz in vivo optogenetic stimulation (Fig. 2, E and F, and fig. S4B) applied bilaterally to the NAc 24 hours before the cocaine challenge abolished the sensitized locomotor response (Fig. 2G). Neither 12-Hz DBS alone nor SCH23390 (0.3 mg/kg, i.p.) administered alone affected sensitization, but when given in combination, sensitization was abolished when challenged 24 hours later (Fig. 2H). To confirm that local blockade of D1Rs is necessary for the effects of 12-Hz DBS, we infused SCH23390 (0.15 μg in 300 nl) bilaterally into the NAc shell. This infusion, in combination with 12-Hz DBS, was sufficient to abolish sensitization, confirming the important role of the antagonism of D1Rs specifically in the NAc (Fig. 2I). None of the above interventions affected spontaneous locomotor activity (fig. S3, B and C).

To establish a causal link between cocaine-evoked synaptic plasticity and the abolition of fully established locomotor sensitization, we evaluated
the effects of LTD on synaptic transmission by returning to the five-injection sensitization protocol. Mice underwent 5 days of cocaine treatment, and electrophysiological recordings were performed after 10 days of withdrawal (Fig. 3A and fig. S5B). In control animals, cocaine treatment led to an increase in the AMPA/NMDA ratio and rectification index (Fig. 3, B and C, and fig. S4C).

12-Hz DBS, when applied in combination with the D1R antagonist, normalized these parameters, but 12-Hz DBS or D1R antagonist applied separately failed to do so (Fig. 3, B and C, and fig. S5, C and D). Accordingly, in the five-injection sensitization protocol, 12-Hz DBS alone (Fig. 3D) or SCH23390 alone (Fig. 3E) had no effect on sensitization, whereas SCH23390 or SCH39166 in combination with DBS significantly reduced the sensitization, without affecting the acute response to cocaine (Fig. 3F and fig. S6). There was a trend toward a decrease in the RI with SCH23390, which could be due to an endogenous activation of mGluR signaling. In the case of 12-Hz DBS alone, the strong mGluR activation may partially overcome inhibition by DIR signaling. However, these effects were not significant, and there was no effect of either intervention on locomotor sensitization.

These results were comparable in magnitude to the effects of 12-Hz optogenetic stimulation on the sensitized response to cocaine and cocaine-evoked plasticity in the same five-injection protocol (fig. S6, A to D). Furthermore, we confirmed that the sensitized locomotor response to cocaine was still present 2 weeks after cocaine exposure (Fig. S6G), and we demonstrated that the sensitized locomotor response was still suppressed when DBS in combination with SCH23390 was given 1 week before the challenge. Cocaine-evoked plasticity was also still normalized at this time point (Fig. 3, I and J). These results strengthen the observation that the acute intervention has long-lasting effects on both cocaine-evoked plasticity and consequent locomotor sensitization.

There are two general classes of LTD available in MSNs of the NAc. mGluR-dependent LTD is induced at frequencies between 10 and 15 Hz, whereas a second form depends on NMDAR activation and is induced by stimulation at lower frequencies (~1 Hz) (38–40). Given that DBS was delivered at 12 Hz, a mGluR-dependent mechanism seems likely. In a final series of experiments, we confirmed the crucial role of mGluR1 in the effects of DBS in combination with SCH23390. Mice pretreated with the selective mGluR1 antagonist A-841720 (0.1 mg/kg, i.p.) were not sensitive to the effects of SCH23390 in combination with 12-Hz DBS, in reversing both locomotor sensitization (Fig. 4A) and cocaine-evoked plasticity (Fig. 4B). Conversely, pretreatment with NMDAR antagonist MK-801 (0.2 mg/kg, i.p.) had no effect on the efficacy of SCH23390 in combination with 12-Hz DBS (Fig. 4, C and D). Again, no intervention tested affected acute locomotor activity (fig. S2D). To further highlight the crucial role of the mGluR1 receptor in the depotentiation mechanism induced by DBS, we first showed that the activation of mGluR1 by dihydroxyphenylglycine (DHPG) in presence of the mGluR5 antagonist MPEP (see

![Fig. 4. Optogenetically inspired DBS exerts its effects via an mGluR-dependent mechanism.](http://science.sciencemag.org/)
methods) induced a LTD of excitatory transmission onto DIR MSNs (Fig. 4E). Consistent with previous studies, the magnitude of this LTD was enhanced in cocaine-treated as compared to saline-treated animals (41, 42). However, in cocaine-treated animals that underwent SCH23390 exposure in combination with 12-Hz DBS 24 hours before being killed, this enhanced mGluR1 LTD was occluded, suggesting a shared mechanism between DBS and D1HGP-induced LTD. Finally, cocaine exposure occludes the ability of high-frequency stimulation (HFS) to induce a LTP in DIR MSNs (25). SCH23390 in combination with 12-Hz DBS rescued HFS LTP in cocaine-treated animals, further suggesting that DBS induces a depotentiation in vivo (Fig. 4F).

We used insight obtained from optogenetic in vivo manipulations to propose a novel DBS protocol, which efficiently abolishes behavioral sensitization to cocaine through the reversal of cocaine-evoked potentiation of excitatory transmission onto DIR MSNs. Classical high-frequency DBS does not alter cocaine-evoked plasticity and has only transient effects on locomotor sensitization; its behavioral effects are mediated through a mechanism that remains elusive. Low-frequency DBS applied on its own fails to affect drug-evoked plasticity, most likely because it causes release from dopamine terminals, due to the nonspecific nature of electrical stimulation. Only the combination of acute low-frequency DBS with a DIR antagonist (optogenetically inspired DBS) then enables the induction of the mGluR1 LTD necessary for the depotentiation of synapses on DIR MSNs, most likely formed by the projections from the mPFC (28), and abolishment of the drug-adaptive behavior. Given that SCH23396 is a U.S. Food and Drug Administration–approved DIR antagonist (43), translational studies in humans may be feasible.

Our results demonstrate the potential of novel DBS protocols inspired by optogenetic manipulations of synaptic pathology. Using DBS to correct synaptic pathology and restore normal behavior may have applications in other neuropsychiatric disorders. Given the obstacles to the rapid translation of optogenetic interventions to humans (44), these findings may lead to a full realization of the potential of novel DBS protocols.

GENOMIC VARIATION

Impact of regulatory variation from RNA to protein

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The phenotypic consequences of expression quantitative trait loci (eQTLs) are presumably due to effects on their protein expression levels. Yet the impact of genetic variation, including eQTLs, on protein levels remains poorly understood. To address this, we mapped genetic variants that are associated with eQTLs, ribosome occupancy (rQTLs), or protein abundance (pQTLs). We found that most QTLs are associated with transcript expression levels, with consequent effects on ribosome and protein levels. However, eQTLs tend to have significantly reduced effect sizes on protein levels, which suggests that their potential impact on downstream phenotypes is often attenuated or buffered. Additionally, we identified a class of cis QTLs that affect protein abundance with little or no effect on messenger RNA or ribosome levels, which suggests that they may arise from differences in posttranslational regulation.

To understand the links between genetic and phenotypic variation, it may be essential to first understand how genetic variation impacts the regulation of gene expression. Previous studies have evaluated the association between variation and transcript expression in humans (J–3). Yet protein abundances are more direct determinants of cellular functions (4), and the impact of genetic differences on the multistage process of gene expression through transcription and translation to steady-state protein levels has not been fully characterized. Studies in model organisms have shown that variations in mRNA and protein expression levels are often uncorrelated (5–9). Comparative studies (9–13) have suggested that protein expression evolves under greater evolutionary constraint than transcript levels (14) and have provided evidence consistent with buffering of protein expression with respect to variation introduced at the transcript level. Yet, in contrast to comparative work, there are few reports of quantitative trait loci (QTLs) associated with protein levels (pQTLs) in humans (15–17).

Here, we present a unified analysis of the association of genetic variation with transcript
Refining deep brain stimulation to emulate optogenetic treatment of synaptic pathology
Meaghan Creed, Vincent Jean Pascoli and Christian Lüscher
(February 5, 2015)

Editor's Summary

Reversing cocaine-evoked behavior in mice

Therapeutic optogenetic protocols are highly effective at reversing symptoms in animal models of neuropsychiatric disease. However, translating these protocols into the clinic is challenging because we have not yet made the technical leap required to perform effective optogenetic stimulation in primates. Creed et al. tested whether it would be possible to circumvent these challenges by avoiding the problem altogether. They adjusted an existing therapeutic approach—deep brain stimulation—to mimic an effective optogenetic stimulation protocol to treat a mouse model of cocaine addiction. Science, this issue p. 659

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