BRIEF COMMUNICATIONS

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Genetic control of instrumental conditioning by striatopallidal neuron–specific S1P receptor Gpr6

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Instrumental conditioning allows animals to learn about the consequences of their own actions, but the underpinning molecular mechanisms remain elusive. Here we show that the sphingosine-1-phosphate (S1P) receptor Gpr6 is selectively expressed in the striatopallidal neurons in the striatum. Gpr6-deficient mice showed reduced striatal cyclic AMP production *in vitro* and selective alterations in instrumental conditioning *in vivo*. Thus, Gpr6 is the first striatopallidal neuron–specific genetic regulator of instrumental conditioning in a mammal.

To successfully adapt to complex environments, animals require two fundamental learning mechanisms: Pavlovian conditioning, which encodes predictive associations between external events, and instrumental conditioning, which encodes the consequences of the animal's own actions[1]. Dopamine signaling in the basal ganglia and cortex is known to have a critical role in instrumental conditioning[2,3]. The striatum is a major basal ganglia nucleus that receives converging inputs from both the cortex and the dopaminergic neurons in the substantia nigra. About 95% of the striatal neurons are medium spiny neurons (MSNs), which are subdivided into two mosaically distributed populations: the striatonigral MSNs (direct pathway) and the striatopallidal MSNs (indirect pathway)[4]. The direct and indirect pathway MSNs are postulated to antagonize each other to produce a balanced striatal output[5]. Despite an established role for the striatum in instrumental conditioning, the circuitry and molecular mechanisms underlying this learning process remain poorly understood.

To investigate the striatal-specific molecular mechanisms involved in behavioral control and learning, we developed the fluorescence-activated cell-sorting–array technique to purify MSN subtypes for differential gene-expression analyses[6]. We also used this technique to test candidate genes for their expression specificity in the MSN subtypes. One such gene, *G protein–coupled receptor 6* (*Gpr6*), is a striatal-specific, constitutively active G protein–coupled receptor[7–9]. Its constitutive activity can be further stimulated by its ligand, sphingosine-1-phosphate (S1P)[7,8]. Using purified MSN subtypes, we found that Gpr6 transcripts were highly enriched in the striatopallidal MSNs (**Fig. 1a**). This result was confirmed using transgenic mice that expressed GFP under the control of Gpr6 regulatory elements (**Fig. 1b**)[10] and using mice with a targeted Gpr6 null allele that replaced the endogenous *Gpr6* with an in-frame *LacZ* reporter gene (**Fig. 1c,d**; http://www.informatics.jax.org/external/ko/deltagen/356.html). Together, this converging evidence demonstrates that Gpr6 is selectively expressed in the striatopallidal MSNs.

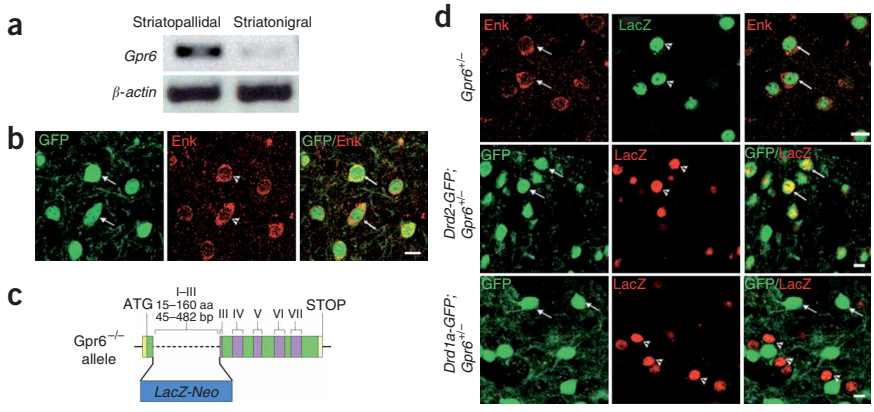

Figure 1 Gpr6 is enriched in the striatopallidal MSNs. (**a**) RT-PCR revealed that Gpr6 is enriched in the fluorescence-activated cell-sorting–purified striatopallidal MSNs relative to the purified striatonigral MSNs. β-actin expression was similar in the two MSN subpopulations. (**b**) GFP was selectively expressed in the striatopallidal MSNs in transgenic mice expressing GFP under the control of Gpr6 regulatory elements[10], as shown by colocalization (yellow, small arrows) of GFP (green, big arrows) and enkephalin-positive striatopallidal MSNs (red, arrowheads). (**c**) Representation of the *Gpr6*$^{-/-}$ allele in which *LacZ-Neo* (blue) replaces a portion of the *Gpr6* single exon, deleting amino acids (aa) 15–160, which contain the first three transmembrane domains. Nondeleted transmembrane domains IV–VII are also shown. (**d**) Colocalization (yellow, small arrows) of enkephalin-positive striatopallidal MSNs (red, big arrows) with *LacZ* (β-galactosidase)-positive MSNs (green, arrowheads) in the striatum of *Gpr6*$^{+/-}$ mice (top). LacZ expression (red, arrowheads) also colocalized (yellow, small arrows) with the majority of Drd2-GFP–positive[10] striatopallidal MSNs (green, big arrows) in the striatum of *Drd2-GFP; Gpr6*$^{+/-}$ double transgenic mice (middle). In contrast, LacZ-positive MSNs (red, arrowheads) did not colocalize with Drd1a-GFP–positive[10] striatonigral MSNs (green, big arrows) in *Drd1a-GFP; Gpr6*$^{+/-}$ mice (bottom). Scale bars, 10 μm.

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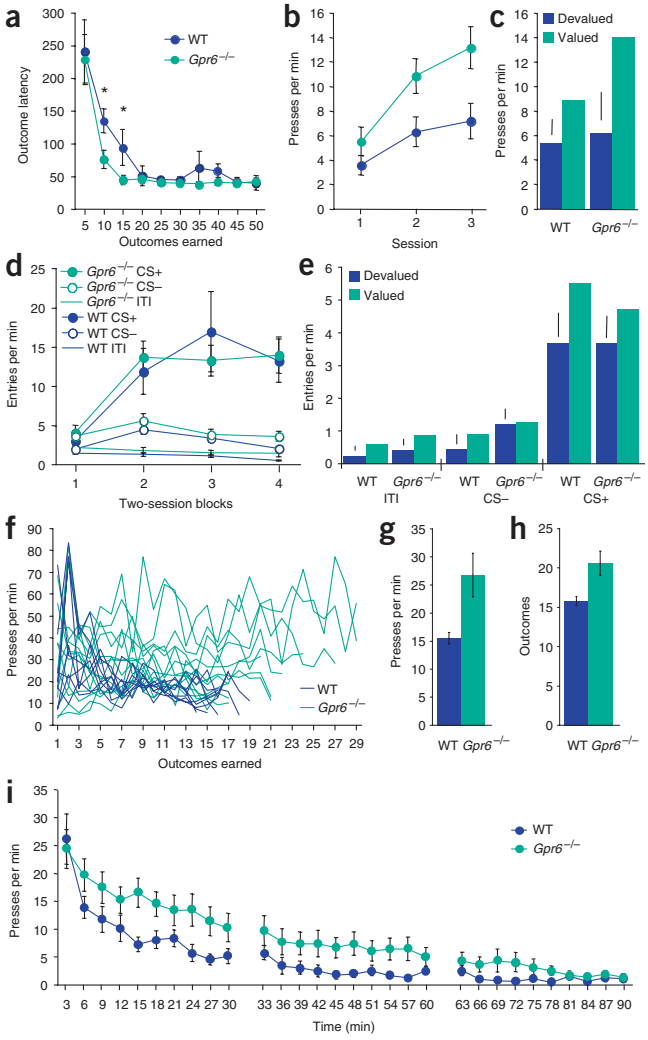

Figure 2 Gpr6 deletion facilitates instrumental conditioning. (**a**) Latency to earn each outcome during instrumental acquisition averaged across five-outcome blocks in wild-type (WT, $n = 5$) and *Gpr6*$^{-/-}$ mice ($n = 9$). A group × block ANOVA revealed a main effect of group ($F_{1,12} = 4.96$, $P < 0.05$) and block ($F_{9,108} = 25.46$, $P < 0.001$), but did not show a group × block interaction ($F < 1$). Asterisk signifies significant simple effect of group ($P < 0.05$). (**b**) Lever presses per min in WT ($n = 15$) and *Gpr6*$^{-/-}$ mice ($n = 22$) in each of the last three sessions of initial variable-interval training. A group × session ANOVA resulted in main effects of group ($F_{1,35} = 5.16$, $P < 0.05$) and session ($F_{2,70} = 27.39$, $P < 0.001$), and a group × session interaction ($F_{2,70} = 3.47$, $P < 0.05$). (**c**) Rate of pressing in WT ($n = 15$) and *Gpr6*$^{-/-}$ mice ($n = 22$) during devalued and valued tests. A group × test ANOVA revealed a main effect of group ($F_{1,35} = 6.25$, $P < 0.05$) and test ($F_{1,35} = 16.71$, $P < 0.001$), but no group × response interaction ($F_{1,35} = 1.65$, $P = 0.2$). (**d**) Acquisition of conditioned approach in WT ($n = 10$) and *Gpr6*$^{-/-}$ mice ($n = 13$), plotted across two-session blocks for CS+, CS– and intertrial interval (ITI) periods. A group × period × block ANOVA found an effect of period ($F_{2,42} = 48.78$, $P < 0.001$) and block ($F_{3,63} = 12.09$, $P < 0.001$), as well as a period × block interaction ($F_{6,126} = 15.10$, $P < 0.001$). There was no effect of group ($F < 1$), nor were any of the interactions with group reliable (F values < 1). (**e**) Rate of conditioned approach behavior in WT ($n = 10$) and *Gpr6*$^{-/-}$ mice during devalued and valued tests. A group × period × test ANOVA found effects of period ($F_{2,42} = 27.74$, $P < 0.001$) and test ($F_{1,21} = 13.54$, $P < 0.001$) and an interaction between period and test ($F_{2,42} = 4.26$, $P < 0.05$); there was a significant effect of test for period CS+ ($F_{1,22} = 8.20$, $P < 0.01$), but not for period CS– ($F_{1,22} = 1.35$, $P > 0.25$). There was no effect of group, nor any interactions involving group (F's < 1). (**f**,**g**) Results of progressive-ratio testing. Rate of responding for successive outcomes plotted for individual WT ($n = 9$) and *Gpr6*$^{-/-}$ mice ($n = 12$) that terminate at the last outcome earned (**f**). *Gpr6*$^{-/-}$ mice responded at a significantly higher rate than did WT mice over the entire test session ($F_{1,19} = 7.21$, $P < 0.05$) (**g**). (**h**) *Gpr6*$^{-/-}$ mice also earned significantly more outcomes than did WT mice ($F_{1,19} = 8.28$, $P < 0.05$). (**i**) Rate of responding during omission training for WT ($n = 10$) and *Gpr6*$^{-/-}$ ($n = 13$) mice, plotted across 3-min bins. A group × block ANOVA resulted in a marginal group effect ($F_{1,21} = 4.23$, $P = 0.05$), a significant block effect ($F_{29,609} = 32.31$, $P < 0.001$) and a significant group × block interaction ($F_{29,609} = 1.55$, $P < 0.05$).

To study Gpr6 function in the brain, we analyzed the targeted Gpr6-null mutant mice (*Gpr6*$^{-/-}$). These mice showed normal breeding, body weight (**Supplementary Fig. 1** online), gross neuroanatomy and differentiation of the MSN subtypes and of their axon terminals (**Supplementary Fig. 2** online). These results are consistent with a prior extensive behavioral and pathological screening of *Gpr6*$^{-/-}$ mice that did not reveal any obvious phenotypes (http://www.informatics.jax.org/external/ko/deltagen/356.html).

We next examined changes in the striatal MSN subtype–specific gene expression in *Gpr6*$^{-/-}$ mice. We found a dose-dependent reduction of Gpr6 expression in *Gpr6*$^{+/-}$ and *Gpr6*$^{-/-}$ mice, confirming that the latter were indeed null mutants (**Supplementary Fig. 3** online). Furthermore, expression of two striatopallidal-specific genes (*Drd2* and *Adora2a*) and one striatonigral-specific gene (*Tac1*) was moderately (<30%), but significantly, reduced in *Gpr6*$^{-/-}$ mice (**Supplementary Fig. 3**). The change in *Tac1* expression suggests that adaptive changes have occurred in the striatonigral MSNs of these mutant mice.

Because Gpr6 can constitutively stimulate adenylyl cyclase activity to generate cAMP in transfected cells, and because such activity can be further enhanced by its ligand, S1P[7,8], we hypothesized that *Gpr6*$^{-/-}$ mice may have deficits in striatal cAMP production. Using membranes prepared from the mutant and wild-type striatum, we found that the basal cAMP production *in vitro* was significantly impaired in the *Gpr6*$^{-/-}$ mutant mice compared with production in the wild-type littermates (**Supplementary Fig. 4** online, 55% reduction, Student's *t*-test, $P < 0.05$, $n = 3$ per genotype). Our initial study also indicated that S1P (0.1 μM and 1 μM) can stimulate cAMP production in the wild-type striata, but not in the *Gpr6*$^{-/-}$ striata (**Supplementary Fig. 4**). Finally, forskolin-induced cAMP production was intact in both genotypes (>30-fold induction with forskolin compared with the basal production without forskolin; **Supplementary Fig. 4**), suggesting that there was similar overall striatal adenylyl cyclase activity. These results suggest that Gpr6 is a critical determinant for the striatal basal cAMP production, at least *in vitro*.

We next examined the behavioral consequences of Gpr6 deficiency in striatal-mediated motor and learning behaviors. *Gpr6*$^{-/-}$ mice showed normal locomotor behavior and motor learning in the Rotarod test (**Supplementary Fig. 5** online). These results confirmed a prior study that demonstrated normal motor and emotional behaviors in *Gpr6*$^{-/-}$ mice (http://www.informatics.jax.org/external/ko/deltagen/356.html).

Given the considerable evidence of striatal involvement in instrumental conditioning[3], we next examined *Gpr6*$^{-/-}$ mice on a series of well-established reward learning assays (see **Supplementary Methods** online)[1,3]. Hungry mice were placed in an operant box and trained to bar press for sugar pellets. Relative to their wild-type littermates, *Gpr6*$^{-/-}$ mice were significantly faster in acquiring the bar-press response ($F_{1,12} = 4.96$, $P < 0.05$; **Fig. 2a**), and reached a significantly higher rate of asymptotic performance after more extensive training ($F_{1,35} = 5.16$, $P < 0.05$; **Fig. 2b**). This enhancement in instrumental

performance would be anticipated if *Gpr6*$^{-/-}$ mice tend to overestimate the incentive value of reward. To evaluate this account, we assessed whether *Gpr6*$^{-/-}$ mice appropriately suppress their instrumental performance to meet a reduction in reward value established through specific satiety. Prior to each of two tests, mice were given 1 h of *ad lib* access to either the sucrose reward (devalued test) or their maintenance diet (valued test). Both *Gpr6*$^{-/-}$ and wild-type mice showed normal sensitivity to reward devaluation, responding significantly less in the devalued test than in the valued test ($F_{1,35} = 16.71$, $P < 0.001$; **Fig. 2c**). However, although they did not differ from wild-type mice during the devalued test ($F < 1$), the *Gpr6*$^{-/-}$ mice responded, as they did in training, at a significantly higher rate than the wild-type mice did during the valued test ($F_{1,43} = 4.41$, $P < 0.05$).

We investigated the generality of this behavioral enhancement using an appetitive Pavlovian conditioning procedure in which one auditory cue (either a white noise or a clicker) was paired with the delivery of sucrose pellets (CS+) and the other cue was not (CS–). Over the course of training, the mice learned to selectively approach the location of the sucrose delivery during the CS+, relative to the CS– and the intertrial interval (**Fig. 2d**). Notably, *Gpr6*$^{-/-}$ and wild-type mice showed similar rates of acquisition and similar levels of asymptotic performance, suggesting that the phenotype shown by *Gpr6*$^{-/-}$ in instrumental conditioning does not extend into other appetitively motivated tasks. Furthermore, in a Pavlovian devaluation test, *Gpr6*$^{-/-}$ mice had normal sensitivity to sucrose devaluation, suppressing their performance of the approach response after being sated on sucrose (that is, during the devalued test), relative to their performance after being sated on maintenance diet (that is, during the valued test) (**Fig. 2e**).

These findings suggest that Gpr6 does not have a critical role in processing the reward value of the sucrose. However, the increased instrumental acquisition and performance is also consistent with an enhanced motivation to initiate actions in the *Gpr6*$^{-/-}$ mice after their selection based on the action-outcome association. Goal-directed instrumental performance is usually highly sensitive to manipulations of action-outcome contingency. Typically, rodents learn to withhold a particular action if the action-outcome contingency is made progressively leaner (that is, if an increasing number of responses are required to earn the reward in progressive-ratio training or if the contingency is reversed using an omission schedule). If the *Gpr6* deletion results in elevated motivation to initiate responding based on the action-outcome association, then increases and decreases in performance should be asymmetrical; that is, the *Gpr6*$^{-/-}$ mice should be faster than wild-type mice to increase, but slower to reduce, their response after reductions in the strength of the action-outcome contingency. To test this prediction we next assessed the performance of *Gpr6*$^{-/-}$ mice in the progressive-ratio 5 (PR5) schedule and in omission training. Under the PR5 schedule, the mutants responded at significantly higher rates ($F_{1,19} = 7.21$, $P < 0.05$; **Fig. 2f,g**) and earned significantly more rewards ($F_{1,19} = 8.28$, $P < 0.05$; **Fig. 2h**) than wild-type mice did. We observed a similar pattern with omission training: that is, *Gpr6*$^{-/-}$ mice persisted in lever pressing relative to wild-type mice (**Fig. 2i**). These data confirm that Gpr6 controls instrumental performance, probably by regulating the transition from response selection to response initiation.

Our study demonstrates that Gpr6 is a critical striatopallidal MSN–specific genetic regulator of instrumental conditioning in mice. Our analyses also have direct implications for the circuitry and molecular mechanisms underlying instrumental conditioning. As Gpr6 is a striatopallidal MSN–specific gene and is ranked as the fifth most striatal-specific gene in a recent genome-wide survey of brain gene expression[9], our results suggest that the striatopallidal MSNs may be a critical striatal neuronal substrate that mediates instrumental performance. This idea is consistent with recent electrophysiological findings that striatopallidal MSNs show several forms of plasticity that are absent in the striatonigral MSNs[11,12]. Our study also reveals that Gpr6 is essential for striatal basal cAMP production *in vitro*, implicating Gpr6-mediated signaling in instrumental conditioning. Finally, pathological alterations in the striatopallidal MSNs are implicated in major neurological and psychiatric disorders and in addictions[5,11–14]. Because G protein–coupled receptors in general are potential drug targets, and because Gpr6 has an exquisite expression specificity to the striatopallidal MSNs, future pharmacological targeting of Gpr6 should be explored for the treatment of neuropsychiatric disorders.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

M.K.L. conducted all phases of the experimental work and data analyses, except for the instrumental conditioning studies, and contributed to writing the manuscript. Y.C. contributed to the instrumental conditioning studies. S.B.O. contributed to manuscript preparation and to the data analysis of the instrumental conditioning studies. X.W.Y. and B.W.B. were responsible for the overall design of the project, analyses of the results and writing of the manuscript.

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