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Quantitative Changes in Gαolf Protein Levels, but not D1 Receptor, Alter Specifically Acute Responses to Psychostimulants

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Striatal dopamine D1 receptors (D1R) are coupled to adenylyl cyclase through Gαolf. Although this pathway is involved in important brain functions, the consequences of quantitative alterations of its components are not known. We explored the biochemical and behavioral responses to cocaine and D-amphetamine (D-amph) in mice with heterozygous mutations of genes encoding D1R and Gαolf (*Drd1a*+/− and *Gnal*+/−), which express decreased levels of the corresponding proteins in the striatum. Dopamine-stimulated cAMP production *in vitro* and phosphorylation of AMPA receptor GluR1 subunit in response to D-amph *in vivo* were decreased in *Gnal*+/−, but not *Drd1a*+/− mice. Acute locomotor responses to D1 agonist SKF81259, D-amph and cocaine were altered in *Gnal*+/− mice, and not in *Drd1a*+/− mice. This haploinsufficiency showed that Gαolf but not D1R protein levels are limiting for D1R-mediated biochemical and behavioral responses. *Gnal*+/− mice developed pronounced locomotor sensitization and conditioned locomotor responses after repeated injections of D-amph (2 mg/kg) or cocaine (20 mg/kg). They also developed normal D-amph-conditioned place preference. The D1R/cAMP pathway remained blunted in repeatedly treated *Gnal*+/− mice. In contrast, D-amph-induced ERK activation was normal in the striatum of these mice, possibly accounting for the normal development of long-lasting behavioral responses to psychostimulants. Our results clearly dissociate biochemical mechanisms involved in acute and delayed behavioral effects of psychostimulants. They identify striatal levels of Gαolf as a key factor for acute responses to psychostimulants and suggest that quantitative alterations of its expression may alter specific responses to drugs of abuse, or possibly other behavioral responses linked to dopamine function.
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INTRODUCTION

Psychostimulant drugs, such as cocaine and D-amphetamine (D-amph), are widely abused by humans. They produce intense short-term psychomotor activation and long-term behavioral alterations related to their powerful reinforcing properties. Repeated exposure to these drugs leads to the development of sensitized responses, conditioned positive association with drug-related cues and compulsive drug self-administration (Koob and Le Moal, 2001; Koob *et al*, 1998; Pierce and Kalivas, 1997; Robinson and Berridge, 2003; Vezina, 2004). In a low percentage of animals, this leads to a state similar to addiction in humans (Deroche-Gamonet *et al*, 2004). Interestingly, considerable variability in the individual reactions to drugs is observed in rodents as well as in humans, depending on both genetic and environmental factors (Nestler, 2000; Piazza and Le Moal, 1998). Although little is known about the genetic factors responsible for this variability, quantitative variation in the levels of expression of specific genes is a plausible mechanism for many pathological or phenotypical traits (Knight, 2005). In the present study, we address this question by exploring the functional consequences of quantitative variations of two major components of the signaling pathways that mediate the effects of drugs of abuse.

Acute stimulant effects of cocaine and D-amph result primarily from the enhancement of extracellular dopamine levels in limbic regions (Di Chiara and Imperato, 1988; Giros *et al*, 1996; Jones *et al*, 1998). This increase in

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extracellular dopamine is also essential for reinforcing properties of psychostimulants (for a review see Berke and Hyman (2000)). Dopamine acts on two types of G-protein-coupled receptors, the D1-type receptors (D1 and D5) and D2-type receptors (D2, D3, D4), which activate and inhibit adenylyl cyclase (AC), respectively. Although dopamine D1 receptors (D1R) signaling was consistently reported to be essential for acute responses to psychostimulants, its role in delayed responses is more controversial since divergent results were obtained in D1R antagonist-treated animals and in D1R knockout mice (Crawford *et al*, 1997; Karper *et al*, 2002; Xu *et al*, 2000). In the striatum D1R is coupled to AC through a specific G protein alpha subunit, Gαolf, whereas in other brain regions including the cerebral cortex, it is coupled through Gαs, the quasiubiquitous isoform (Corvol *et al*, 2001; Zhuang *et al*, 2000). Complete impairment of D1R signaling, resulting from homozygous null mutations of genes encoding for D1R or Gαolf proteins, *Drd1a* and *Gnal* genes respectively, has major consequences on behavioral responses to psychostimulants in mice (Corvol *et al*, 2001; Herve *et al*, 2001; Xu *et al*, 2000; Zhuang *et al*, 2000). In the present study, we examined the consequences of quantitative variations in D1R or Gαolf protein levels using heterozygous knockout mice which express reduced amounts of the corresponding protein in the striatum, as compared to wild-type mice (Corvol *et al*, 2001; Drago *et al*, 1994). We investigated biochemical and behavioral effects of acute and chronic administration of psychostimulants in *Drd1a* and *Gnal* heterozygous mice (*Drd1a*+/− and *Gnal*+/− mice, respectively). We show that *Gnal*+/− but not *Drd1a*+/− mice displayed decreased biochemical behavioral responses to acute injections of psychostimulants, whereas long-term responses to repeated psychostimulant administrations were preserved in *Gnal*+/− mice.

MATERIALS AND METHODS

Animals

Heterozygous mice with a disrupted *Drd1a* gene had a hybrid 129 and C57Bl/6 genetic background. They were produced by Drago and coll. (Laboratory of mammalian genes and development, NIH, Bethesda; Drago *et al* (1994)) and backcrossed in our laboratory for up to five generations with C57BL/6J mice (purchased from Charles River France, L'Arbresle, France) to obtain heterozygous mice (*Drd1a*+/− mice) and their control littermates (*Drd1a*+/+ mice). Similarly, mice with a disrupted *Gnal* gene were produced by Belluscio et coll. (Department of biochemistry and molecular biophysics, Columbia University, New York; Belluscio *et al* (1998)) and backcrossed in our laboratory for up to eight generations with C57BL/6J mice to obtain homozygous (*Gnal*−/−) and (*Gnal*+/−) heterozygous mice as well as their control littermates (*Gnal*+/+). For comparing heterozygous mutant and wild-type mice, 8-week-old male mice were used for biochemical experiments while both male (2/3) and female (1/3) of 8-week-old mice were used in behavioral experiments. As *Gnal*/- mice have an increased mortality rate after weaning (Belluscio *et al*, 1998), *Gnal*−/− mice and their control littermates (*Gnal*+/+ and *Gnal*+/−) were used 4 weeks after birth. Animals were kept in stable conditions of temperature (22°C) and humidity (60%) with a constant cycle of 12 h light and 12 h dark and had free access to food and water. All experiments on animals were performed in accordance with the guidelines of the French Agriculture and Forestry Ministry for handling animals (decree 87849, license 01499).

Antibodies and Drugs

The following phosphospecific antibodies were used for Western blotting: rabbit polyclonal antiphospho-GluR1 (Ser-845, 1 : 500, Upstate biotechnology, Mundolsheim, France), mouse monoclonal antiphospho-ERK1/2 (Thr183–Tyr185, 1 : 1000 Promega, Charbonnière, France). The following antibodies were used to detect the total amount of proteins: rabbit polyclonal anti-GluR1 (1 : 500, Upstate biotechnology, Mundolsheim, France), rabbit polyclonal anti-ERK1/2 (1 : 2000, Upstate biotechnology, Mundolsheim, France), mouse monoclonal anti-D1R (1 : 500), produced as described (Luedtke *et al*, 1999), rabbit polyclonal anti-adenylate cyclase V/VI (1 : 400, sc-590 (C-17), Santa Cruz Biotechnology, Heidelberg, Germany). Specific anti-Gαolf antibodies were affinity purified rabbit polyclonal antibodies directed against full-length Gαolf and immunoadsorbed on full-length Gαs as previously described (Corvol *et al*, 2001). D-Amph, cocaine hydrochloride, SKF81259 and quinpirole were purchased from Sigma-Aldrich, St Quentin Fallavier, France) and dissolved in 0.9% NaCl. Animals received a 20 ml/kg volume intraperitoneal (i.p.) of the appropriate diluted drug or saline solution.

In Vivo Protein Phosphorylation: Analysis by Immunoblotting

After having been habituated to injection by saline i.p. administration during the 3 days preceding the experiment, mutated heterozygous mice or their control littermates were injected with saline or D-amph (10 mg/kg). At 15 min after treatment, the animals were decapitated and their head was immediately frozen in liquid nitrogen (12 s) (Pascoli *et al*, 2005). The frozen heads were then sliced with a cryostat (210-μm-thick) and five microdisks (1.4-mm diameter) were punched bilaterally from the dorsal striatum and stored at −80°C. Micropunchs were rapidly homogenized by sonication in a 1% SDS (w/v) solution containing 1 mM sodium orthovanadate (preheated at 100°C) and placed at 100°C for 5 min. Equal amounts of micropunch lysates (100 μg) were separated by SDS–polyacrylamide gel electrophoresis (10%) before electrophoretic transfer onto nitrocellulose membrane (Hybond Pure, Amersham, Orsay, France). Membranes were blocked 1 h at room temperature in Tris buffered saline (TBS, 100 mM NaCl, 10 mM Tris, pH 7.5) + 0.05% Tween 20 (TBS–tween) or 5% skimmed milk. Membranes were then incubated overnight at 4°C with phosphospecific primary antibodies, rinsed three times in TBS–tween, blocked 30 min at room temperature in TBS–tween + 5% skimmed milk and blotted with horseradish peroxydase-conjugated anti-rabbit or anti-mouse secondary antibodies (Amersham, diluted 1 : 4000). The immunoreactive band was visualized by enhanced chemiluminescent detection (ECL, Amersham). The corresponding nonphosphorylated protein was detected after stripping in buffer

containing 100 mM glycine pH 2.5, 200 mM NaCl, 0.1% Tween 20 (v/v) and 0.1% (v/v) β-mercaptoethanol for 45 min at room temperature, followed by extensive washing in TBS before reblocking and reprobing.

D1R and Gαolf Immunoblotting and AC Assay

Animals were killed by decapitation and their brains were rapidly dissected out, frozen in dry ice and sectioned into 500-μm coronal slices at −10°C with a freezing microtome. Tissue microdisks were punched out from the caudate-putamen using a stainless steel cylinder (2.2-mm diameter) and stored at −80°C before homogenization. Western blotting with antibodies recognizing specifically Gαolf or D1R was performed as described above with the exception that samples for D1R detection were not boiled before loading, but incubated at 37°C during 30 min (Luedtke *et al*, 1999).

Mice of various genotypes were killed and their brains were sectioned in 300 μm slices in ice cold Ca^{2+}-free artificial cerebrospinal fluid (125 mM NaCl, 2.4 mM KCl, 1.9 mM $MgCl_2$, 0.5 mM KH_2PO_4, 0.5 mM Na_2SO_4) using a refrigerated Vibroslice apparatus. Microdisks were punched out from the caudate-putamen and homogenized in Tris-maleate buffer (2 mM, pH 7.2) containing 2 mM EGTA and 300 mM sucrose, in a Potter-Elvehjem homogenizer. AC activity was measured at 30°C by the conversion of α-^{32}P-ATP into cyclic ^{32}P-cAMP for 7 min as previously described (Corvol *et al*, 2001), and normalized to the protein concentration measured by the bicinchoninic acid method. The same method was used for Mn^{2+}-stimulated AC activity except for the Mg^{2+}-free incubation buffer which was replaced by various concentrations of $MnCl_2$. AC activity was expressed as cAMP formed in pmol/min/mg protein.

Locomotor Responses

Habituation. Locomotor responses were evaluated using a circular corridor with four infrared beams placed at every 90° (Imetronic, Pessac, France). Locomotor activity was counted when animals interrupted two successive beams and, thus, had traveled 1/4 of the circular corridor. Mice were individually placed in the corridor and locomotion was recorded in a low luminosity environment avoiding stress. Mice were first habituated to the test environment and locomotor activity was measured during 45 min for three consecutive days (days −2, −1 and 0): in each session the spontaneous activity was recorded for 15 min, mice received a saline injection and their activity was recorded for an additional 30 min period.

Acute Locomotor Response

The acute locomotor effects of cocaine (0, 10, 20 and 30 mg/kg), D-amph (2 mg/kg), SKF81259 (2 mg/kg) or quinpirole (2 mg/kg) were evaluated at day 1 as follows: spontaneous activity during 15 min, locomotor activity after administration of saline or drug during 30 (cocaine) or 60 (D-amph) min. Locomotor activity was measured at 5 min intervals and cumulative counts during the first 30 min after drug were taken for data analysis.

Context-dependent sensitization and conditioned locomotor response. For context-dependent sensitization to cocaine or D-amph sensitization, mice were treated with cocaine (20 mg/kg, i.p.), D-amph (2 mg/kg) or saline once daily for 5 consecutive days in the apparatus used for measuring locomotor activity (day 1 to day 5). Animals were left in the apparatus during 60 and 30 min for D-amph and cocaine, respectively, and cumulative counts during the first 30 min after drug were taken for data analysis. Association between context and drug effect was evaluated by the drug conditioned locomotor activity: 1 day after the end of cocaine, D-amph or saline treatments (day 6), all the mice received a saline injection in the drug-associated environment (actimeter) and the locomotor activity was registered at 5 min intervals. Cumulative counts during the first 30 min after saline were taken for data analysis. Persistence of sensitization in repeatedly cocaine- or D-amph-treated mice was evaluated by measuring their locomotor responses to a challenge injection of cocaine (20 mg/kg) or D-amph (2 mg/kg) after 9 days of withdrawal (day 14). Locomotor activity was recorded and analyzed as described above.

Groups of mice treated with saline or D-amph during 5 days were killed 7 days after the last injection and were used for measuring the levels of Gαolf or the AC activity in response to D1R activation in the striatum. Seven days after the last injection, other groups of similarly pretreated mice were injected with saline or D-amph (10 mg/kg) and killed 15 min later. The phosphorylation of GluR1 at Ser-845 was measured in their striatum as described above.

Conditioned Place Preference

The place preference apparatus consisted of two different compartments that were distinguished by different patterns on floors and walls separated by a central neutral area. The place preference protocol was performed in three different phases.

Preconditioning (habituation). Mice were placed in the middle of the neutral central area and allowed to explore both compartments (day 1). The time spent in each compartment was measured during 18 min. The various groups of mice did not display any significant preference for one compartment (unbiased procedure).

Conditioning. Mice were treated for 6 consecutive days with alternate injections of drugs (D-amph, 2 mg/kg) or saline in order to pair one compartment with drug administration and the other one with saline during conditioning phase. After injection, mice were confined to a given compartment for a period of 25 min. D-Amph was administrated on days 2, 4 and 6 and vehicle on days 3, 5 and 7. Control mice received saline every day.

Postconditioning (test). This phase was conducted the following day (day 8) exactly as the preconditioning phase, that is, with free access to both compartments. The time spent in each compartment was measured during 18 min. A score value was measured for each mouse as the difference between the times spent in the drug paired compartment during the postconditioning and preconditioning phases.

Statistical Analyses

For immunoblots, the relevant immunoreactive bands were quantified with laser scanning densitometry using Scion Image software (Frederick, Maryland). To allow comparison between different autoradiographic films, the density of bands was expressed as a percentage of the average of controls (saline treated or wild-type littermates). Statistical analysis was performed with the Student's *t*-test when two groups were compared. For most biochemical and behavioral experiments, two-way ANOVA analysis followed by paired Bonferroni's multiple comparison post-test. Statistical analyses were performed using Prism 3.02 software (GraphPad Software, San Diego, USA).

RESULTS

Dopamine-Stimulated AC Activity is Decreased in *Gnal* +/− but not in *Drd1a* +/− Heterozygous Mice

Dopamine-stimulated AC activity requires both D1R and Gαolf as indicated by its complete absence in D1R (Friedman *et al*, 1997) and Gαolf knockout mice (Corvol *et al*, 2001). To investigate the consequences of quantitative changes in D1R and Gαolf protein levels on D1R signaling, we used *Gnal* +/− and *Drd1a* +/− mice. D1R protein levels were measured by immunoblotting of striatal extracts with an anti-D1R antibody (Luedtke *et al*, 1999) (Figure 1a and b). D1R protein levels remained unchanged in *Gnal* +/− (Figure 1a) in agreement with previous findings using autoradiographic ligand binding assays (Corvol *et al*, 2001; Drago *et al*, 1994). In the striatum of *Drd1a* +/− mice the D1R immunoreactive band was markedly decreased (Figure 1b). This large decrease in total protein immunoreactivity may not reflect the number of functional receptor at the membrane, but was in agreement with previous quantitative ligand binding experiments showing a 60% decrease of Bmax (Drago *et al*, 1994). In addition no immunoreactive protein was observed in samples from *Drd1a*−/− mice, confirming the specificity of the D1R antibody (Figure 1b).

Gαolf protein levels were diminished in the striatum of *Gnal* +/− mice by 60% compared to their wild-type littermates (Figure 1c, for a review see Corvol *et al* (2001)) while they increased by 15% in the striatum of *Drd1a* +/− mice (Figure 1d). This slight increase in Gαolf levels in the striatum of *Drd1a* +/− mice is in agreement with Gαolf levels regulation by D1R receptor usage (Herve *et al*, 2001). As previously reported (Corvol *et al*, 2001), both basal and dopamine-stimulated AC activities were decreased by 44±5 and 38±9%, respectively, in the striatum of Gαolf +/− mice, although the reduction in basal activity did not reach statistical significance in these experiments (Figure 1e). The dopamine-stimulated cAMP production was significantly decreased by 30±7% (delta, Figure 1e). However, because of the decrease in basal activity, the dopamine-stimulated/basal ratio was similar in *Gnal* +/− and *Gnal* +/+ mice (2.5±0.1 *vs* 2.8±0.1 respectively, $p > 0.05$, Student's *t*-test). By contrast, basal and dopamine-stimulated AC activities were not significantly changed in the striatum of *Drd1a* +/− mice despite the decrease of D1R concentration (Figure 1f).

To determine whether the deficit in basal AC activity in *Gnal* +/− mice was due to a change in intrinsic AC activity, we measured the Mn^{2+}-stimulated AC activity, a classical index of total cyclase activity (Limbird and MacMillan, 1981). The Mn^{2+}-stimulated AC activity was slightly decreased in *Gnal* +/− (−30%, Figure 1g and h). To assess whether this was indicative of an alteration of the expression of AC protein, we used immunoblotting with antibodies recognizing type 5 AC, the major isoform expressed in the striatum. The AC levels were identical in wild-type and *Gnal* +/− mice (Figure 1i). Altogether these results show that the mutation of Gαolf did not alter the levels of AC5 and that the decreased basal and Mn^{2+}-sensitive cyclase activity reflect the decrease in stimulatory G protein, as even the Mn^{2+}-stimulated AC remains sensitive to the presence of G protein (Bender and Neer, 1983; Scholich *et al*, 1997).

D-Amph-Induced GluR1 Phosphorylation is Decreased in Gαolf but not D1R Heterozygous Mice

We investigated the cAMP-controlled signal transduction pathway *in vivo* in the striatum of *Drd1a* +/− and *Gnal* +/− mice. As the increased cAMP levels resulting from D1R stimulation activate cAMP-dependent protein kinase (PKA), we measured the phosphorylation state of one of its major substrates in striatal neurons. The GluR1 subunit of AMPA-type glutamate receptors is an important substrate for PKA, which phosphorylates Ser-845 and increases thereby the AMPA receptor conductance (Roche *et al*, 1996). Acute injection of psychostimulants induces GluR1 phosphorylation at Ser-845 in the striatum *in vivo* via D1R activation (Snyder *et al*, 2000; Valjent *et al*, 2005). GluR1 protein expression measured in Western blots was not significantly different form wild-type mice in the striatum of *Gnal* +/− and *Drd1a* +/− mice (*Gnal* +/− mice: 103±5%, $n = 15$; *Drd1a* +/− mice: 98±5%, $n = 10$; % of wild-type mice± SEM). We studied the phosphorylation of GluR1 at Ser-845 as an index of the activation of the PKA pathway *in vivo*, using a rapid brain freezing method to avoid protein dephosphorylation. As previously reported (Snyder *et al*, 2000; Valjent *et al*, 2005), GluR1 phosphorylation was dramatically increased in the striatum, 15 min after an injection of D-amph (10 mg/kg) (Figures 2a and b). The increase in GluR1 phosphorylation was dependent on D1R coupling to AC since it was abolished in D1R or Gαolf knockout mice as well as in mice pretreated with the D1R antagonist, SCH 23390 (Valjent *et al*, 2005), and data not shown. In the striatum of *Gnal* +/− mice, the D-amph-induced GluR1 phosphorylation was dramatically decreased (Figure 2a). In contrast, in the striatum of *Drd1a* +/− mice, D-amph-induced GluR1 phosphorylation was not significantly altered (Figure 2b). These results strongly suggest that the cAMP signaling linked to D1R is deficient *in vivo* in the striatum of *Gnal* +/− mice, whereas it remains largely unaffected in *Drd1a* +/− mice.

Acute Locomotor Responses to D1 Agonist, D-Amph and Cocaine are Markedly Decreased in *Gnal* +/− but not in *Drd1a* +/− Mice

Given the different effects of *Gnal* and *Drd1a* heterozygosis on acute biochemical responses to psychostimulants, we

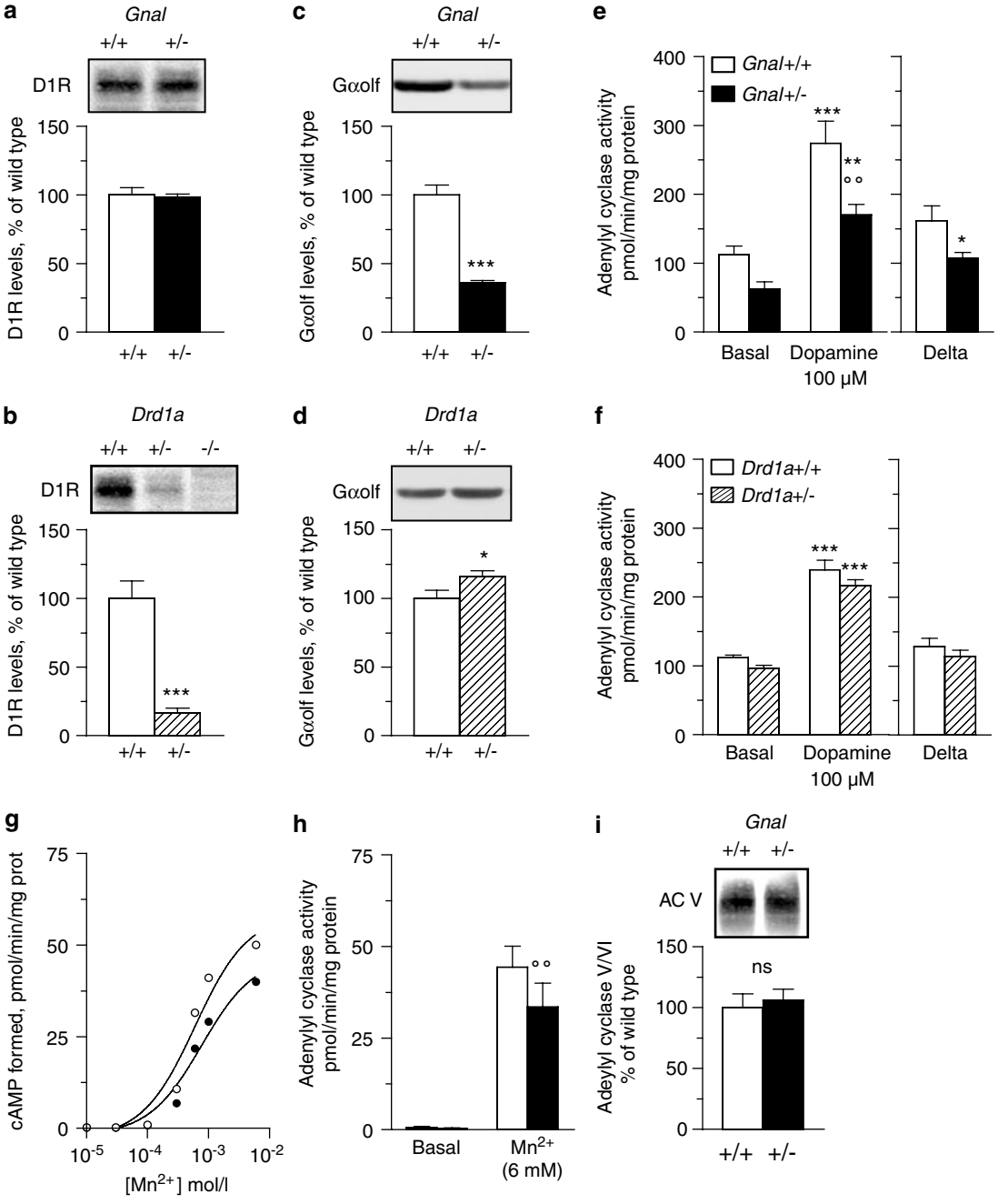

Figure 1 D1R, Gαolf levels and AC activity in the striatum of *Gnal* +/− and *Drd1a* +/− mice. (a–d) D1R (a, b) and Gαolf (c, d) protein levels were measured by immunoblotting in striatal extracts of *Gnal* +/− ($n = 5$, a, c) and *Drd1a* +/− ($n = 6$, b, d) mice and expressed as percentages of the levels found in the striatum of their wild-type littermates ($n = 5$–6). Student's *t*-test: $^{*}p < 0.05$, $^{***}p < 0.001$. (e, f) AC activity was measured in striatal membranes of *Gnal* +/− (e) and *Drd1a* +/− (f) mice and their wild-type littermates in basal condition and with 100 µM dopamine ($n = 5$–6 per group). Two-way ANOVA: *Gnal* mice, interaction between genotype and treatment $F_{(1,16)} = 1.9$, NS; genotype $F_{(1,16)} = 15.4$, $p = 0.001$; treatment $F_{(1,16)} = 47.5$, $p < 0.001$; *Drd1a* mice, interaction between genotype and treatment $F_{(3,20)} = 0.4$, NS; genotype $F_{(1,20)} = 4.1$, NS; treatment $F_{(3,20)} = 202.0$, $p < 0.001$). Bonferroni post-test: $^{**}p < 0.01$, $^{***}p < 0.001$ as compared to saline, $^{\circ\circ}p < 0.01$ as compared to wild type. Difference between cAMP formed in basal condition and in the presence of 100 µM dopamine (delta) was calculated (right panel); Student's t-test: $^{*}p < 0.05$. (g, h) AC activity was measured in striatal membranes from *Gnal* +/− and *Gnal* +/+ mice with increasing concentrations of Mn^{2+}. A representative dose–response curve is presented for each genotype (g) as well as comparison of basal activity and maximal response to Mn^{2+} (h). Two-way ANOVA with matched values by row: interaction between genotype and treatment, $F_{(1,2)} = 170.7$, $p < 0.01$, effect of genotype, $F_{(1,2)} = 188.3$, $p < 0.01$, effect of treatment, $F_{(1,2)} = 39.2$, $p < 0.05$. Bonferroni post-test: $^{\circ\circ}p < 0.01$ as compared to wild type. (i) AC protein levels were measured by immunoblotting in striatal extracts from *Gnal* +/− ($n = 6$) and *Gnal* +/+ mice ($n = 8$); Student's *t*-test: $p > 0.05$. Data are means ± SEM.

investigated their consequences on behavioral responses involving D1R signaling. In contrast with D1R or Gαolf knockout mice that display severe phenotypes including decreased weight, postnatal mortality after birth or weaning, and spontaneous hyperlocomotor activity (Belluscio *et al*, 1998; Drago *et al*, 1994; Zhuang *et al*, 2000), *Drd1a* and *Gnal*

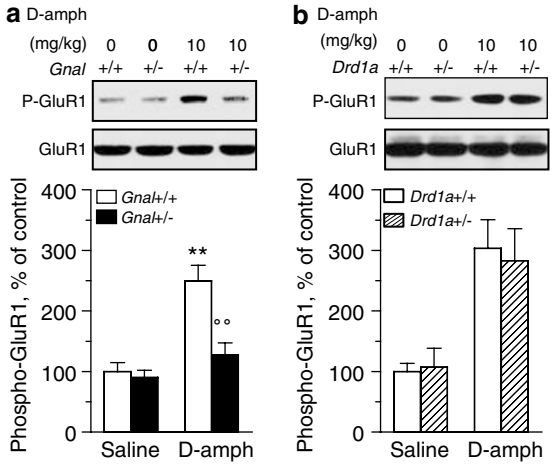

Figure 2 *In vivo* D-amph-induced GluR1 phosphorylation in the striatum of *Gnal* +/− and *Drd1a* +/− mice. At 15 min after injection of saline (0) or D-amph (10 mg/kg), phosphorylation of GluR1 at Ser-845 was quantified in the striatum by immunoblotting of striatal extracts of *Gnal* +/− mice (a) and *Drd1a* +/− mice (b) and their respective wild-type littermates. A representative immunoblot is shown (upper panel). Data, expressed as a percentage of the corresponding wild-type mice treated with saline, are means ± SEM ($n = 7$–10 per group). Two-way ANOVA: *Gnal* mice, interaction between treatment and genotype $F_{(1,29)} = 8.9$ $p < 0.01$, effect of genotype $F_{(1,29)} = 12.2$ $p = 0.001$, effect of treatment $F_{(1,29)} = 24.5$ $p < 0.001$; *Drd1a* mice, interaction between treatment and genotype $F_{(1,37)} = 0.1$, NS, effect of genotype $F_{(1,37)} = 0.3$, NS, effect of treatment $F_{(1,37)} = 23.0$ $p < 0.001$. Bonferroni post-test: $^{**}p < 0.01$ as compared to saline, $^{\circ\circ}p < 0.01$ as compared to wild type.

Table 1 Weight and Spontaneous Locomotion of *Gnal*+/− and *Drd1a*+/− Mice

	***Drd1a* mice**		***Gnal* mice**	
	+/+	**+/−**	**+/+**	**+/−**
Weight (g)	25.1 ± 5.7	25.2 ± 4.9	25.2 ± 3.7	24.9 ± 4.8
Locomotion				
Day 1	144 ± 49	128 ± 38	139 ± 61	138 ± 52
Day 2	60 ± 41	51 ± 24	64 ± 36	71 ± 38
Day 3	62 ± 35	57 ± 30	64 ± 51	59 ± 30

Weight and locomotion were measured in *Gnal*+/− and *Drd1a*+/− mice and their wild-type littermates ($n = 30$ per group) 8 weeks after birth. The difference between genotypes was not significant for weight (one-way ANOVA, $p > 0.05$, $F_{(3,116)} = 0.03$). Locomotion was measured during 30 min following a saline injection and expressed as $\frac{1}{4}$ turns performed in the circular corridor of the apparatus measuring locomotor activity.
Differences between groups were significant for time (days 1–3), but not significant for genotype or interaction between time and genotype (two-way ANOVA; for time: $F_{(2,348)} = 135$, $p < 0.01$; for genotype: $F_{(3,348)} = 1.34$, NS; interaction: $F_{(3,348)} = 1.34$, NS).
Data are means ± SD.

heterozygous mice were apparently similar to wild-type mice. Both *Drd1a* +/− and *Gnal* +/− mice grew normally, had a normal body weight (Table 1) and were fertile (data not shown). They displayed normal spontaneous activity as well as habituation in locomotor activity boxes (Table 1). However, the hyperlocomotion induced by the selective D1R agonist SKF81259 (2 mg/kg) was dramatically decreased in *Gnal* +/− mice (Figure 3a) and only slightly reduced in *Drd1a* +/− mice (Figure 3b). These changes in the behavioral responses to D1R agonist were consistent with those observed in AC activity in the striatum. In contrast, the blockade of locomotion induced by a D2R-specific agonist, quinpirole (2 mg/kg), was normal in both genotypes (Figure 3a and b), in agreement with normal D2R binding found in the striatum of *Gnal* +/− (Herve *et al*, 2001) and D1R knockout mice (Smith *et al*, 1998).

We then investigated the acute responses to psychostimulant drugs in the two lines of heterozygous mice. *Gnal* +/− mice displayed decreased acute hyperlocomotor responses to D-amph (Figure 3c) as previously reported (Herve *et al*, 2001), whereas these responses were normal in *Drd1a* +/− mice (Figure 3d). Acute locomotor responses to cocaine were also decreased in *Gnal* +/− mice (Figure 3e). At 10 and 20 mg/kg, cocaine induced virtually no hyperlocomotion in *Gnal* +/− mice, whereas at 30 mg/kg the response was decreased by about half. In contrast, the locomotor effects of cocaine were similar in *Drd1a* +/− and wild-type mice (Figure 3f), as previously reported (Drago *et al*, 1996). These results demonstrate that the decrease in Gαolf levels has dramatic consequences on the locomotor effects of cocaine, whereas a similar decrease in D1R has no detectable consequence.

A Strong Locomotor Sensitization to Psychostimulants is Present in *Gnal* +/− Mice

As acute locomotor responses to psychostimulants were dramatically altered in *Gnal* +/− mice, it was interesting to determine whether these decreased responses could still undergo a sensitization following repeated treatments. We used a protocol in which the drug was repeatedly administered in the actimeter during 5 consecutive days (day 1–5) and a challenge was performed after 9 days withdrawal (Day 14, see Materials and methods). Using this procedure, administration of 2 mg/kg D-amph or 20 mg/kg cocaine led to a persistent sensitization of locomotor responses in wild-type mice (Figure 4a and b). In *Gnal* +/− mice a steady and progressive increase in locomotor responses to D-amph was observed and the locomotion scores were similar in the mutant mice and wild-type controls at day 5, and remained so after at day 14 (Figure 4a). In *Gnal* +/− mice which received five injections of cocaine, a progressive increase in the locomotor responses was also observed, but these responses remained significantly lower than in wild-type mice (Figure 4b). Remarkably, however, when mice were tested after 9 days of abstinence, the difference between wild-type and *Gnal* +/− mice disappeared (Figure 4b). If one takes into account the ratio between the acute (day 1) and sensitized responses (day 14), the sensitization of locomotor response to D-amph and cocaine was much more pronounced in *Gnal* +/− mice than in their wild-type controls (Figure 4c and d). Altogether, these results show that *Gnal* +/− mice displayed sensitized responses to D-amph and cocaine despite marked decreased acute responses to these drugs.

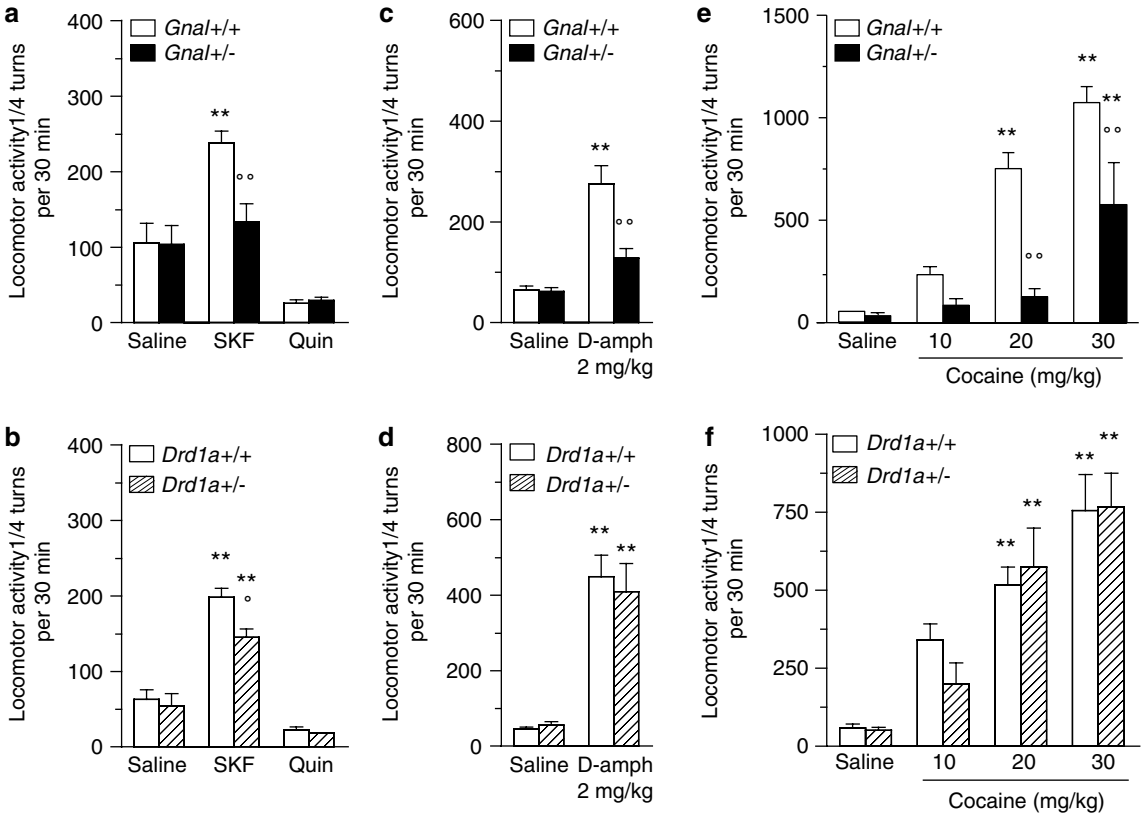

Figure 3 Drug-induced locomotor activity in *Gnal* +/− and *Drd1a* +/− mice. Locomotor activity of *Gnal* +/− (a, c, e) and *Drd1a* +/− (b, d, f) mice and their wild-type littermates were measured after injection of saline, SKF81259 (SKF, 2 mg/kg), or quinpirole (Quin, 2 mg/kg) (a, b), D-amph (2 mg/kg, c, d) and cocaine (10, 20 or 30 mg/kg, e, f). Locomotor activity is expressed as the number of $\frac{1}{4}$ turns performed by the animals in the circular corridor during the 30 min following injection. Data are means ± SEM ($n = 10$ per group). Two-way ANOVA: (a and b) interaction between genotype and treatment with dopamine receptor agonists was significant only for *Gnal* mice ($F_{(2,54)} = 5.1$, $p < 0.01$); effect of genotype: *Gnal* mice $F_{(2,54)} = 4.8$, $p < 0.05$; *Drd1a* mice $F_{(2,54)} = 6.1$, $p < 0.05$; effect of treatment: *Gnal* mice $F_{(2,54)} = 34.2$, $p < 0.001$; *Drd1a* mice $F_{(2,54)} = 104.1$, $p < 0.001$. (c and d) interaction between genotype and D-amph treatment was significant only for *Gnal* mice ($F_{(1,36)} = 11.7$, $p < 0.01$); effect of genotype: *Gnal* mice $F_{(1,36)} = 12.7$, $p < 0.001$; *Drd1a* mice $F_{(1,36)} = 0.1$, NS; effect of treatment: *Gnal* mice $F_{(1,36)} = 43.0$, $p < 0.001$; *Drd1a* mice $F_{(1,36)} = 64.5$, $p < 0.001$. (e and f) interaction between genotype and multiple doses of cocaine was significant only for *Gnal* mice $F_{(3,74)} = 32.0$, $p < 0.001$; *Gnal* mice: effect of genotype $F_{(1,74)} = 118.5$, $p < 0.001$; effect of cocaine $F_{(3,74)} = 86.24$, $p < 0.001$; *Drd1a* mice: effect of genotype $F_{(1,72)} = 0.73$, NS; effect of cocaine $F_{(3,72)} = 30.0$, $p < 0.01$. (a–f) Bonferroni post-tests: $^{*}p < 0.05$, $^{**}p < 0.01$ as compared to saline, $^{\circ}p < 0.05$, $^{\circ\circ}p < 0.01$ as compared to wild type.

Context-Associated Conditioned Effects of Cocaine and D-Amph are Preserved in *Gnal* +/− Mice

Repeated injections of cocaine and D-amph in the same novel environment lead to a conditioned association between the environmental context and the locomotor effects (Brabant *et al*, 2003). This effect was clearly demonstrated in wild-type mice by the pronounced locomotor activity observed after saline injection in the cocaine or D-amph-associated context as compared to that observed in saline-associated context (Figure 5a and b). In *Gnal* +/− mice, significant conditioned responses were detected in the environment associated with cocaine or D-amph. In addition, the magnitude of these responses was similar to that in wild-type controls (Figure 5a and b), showing that reduced levels of Gαolf did not affect this context-dependent response.

Rewarding Properties of D-Amph are Normal in Gαolf +/− Mice

As the acute locomotor effects of D-amph were dramatically decreased in *Gnal* +/− mice, whereas the repeated injections led to a normalization of the locomotor responses, we examined another response requiring repeated drug injections. We compared the ability of *Gnal* +/− mice and wild-type littermates to develop conditioned place preference (CPP) to D-amph. This test explores drug environment associations independently of locomotor responses but in relation to the rewarding properties of the drug. No CPP was observed when the two compartments were associated with saline injection (Figure 6a and b). Both *Gnal* +/− and *Gnal* +/+ mice spent significantly more time in the drug paired side after conditioning with D-amph than before (Figure 6a), and there was no significant difference between *Gnal* +/− and wild-type mice on the place preference score (Figure 6b). These results indicated that D-amph rewarding properties were preserved in *Gnal* +/− mice despite the reduction in acute locomotor effects.

Persistence of an Altered Gαolf/cAMP/PKA Signaling Pathway in Chronically D-Amph-Treated Gαolf Heterozygous Mice

The results reported above demonstrated an apparent recovery of locomotor responses to psychostimulant drugs

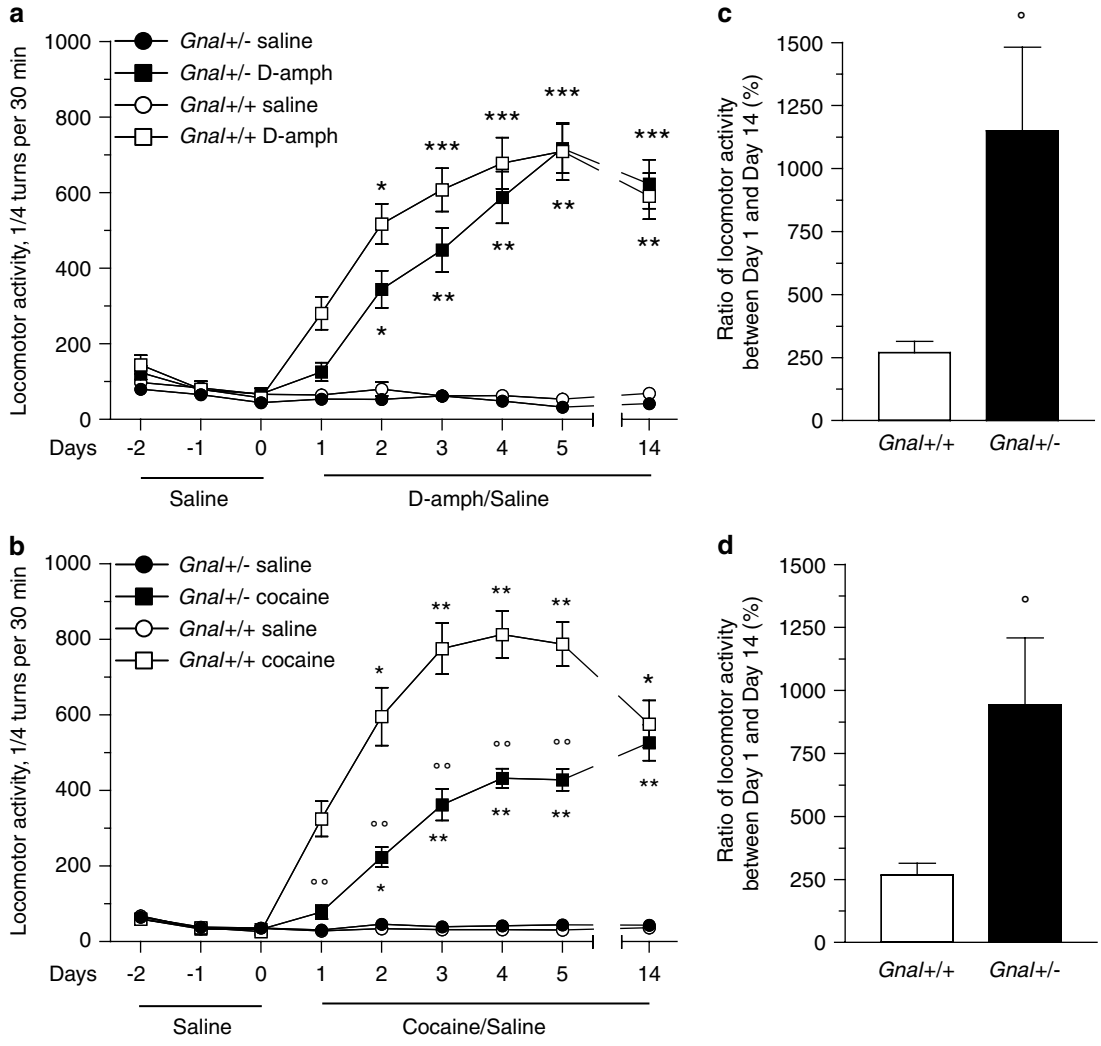

Figure 4 Locomotor sensitization induced by D-amph or cocaine in *Gnal* + /− mice. (a, b) After 3 days of habituation to saline injections (days −2, −1, 0, a, b), *Gnal* + /− mice and their wild-type littermates were treated daily with D-amph (2 mg/kg, a), cocaine (20 mg/kg, b) or saline (as indicated, a, b) for 5 consecutive days (days 1–5) in the actimeter. A challenge with D-amph (2 mg/kg, a), cocaine (20 mg/kg, b) or saline was performed after a 9 days withdrawal period (day 14). Locomotor activity was expressed as the number of $\frac{1}{4}$ turns in the circular corridor during 30 min after injection. Data are means ± SEM ($n = 16$ per group). Locomotor activities were significantly different along time and for genotype for mice treated with repeated D-amph treatment. Statistical analysis: (a) D-amph: two-way ANOVA, interaction between genotype and time: $F_{(8,245)} = 1.27$, NS; for genotype: $F_{(1,245)} = 5.9$, $p < 0.05$; for time: $F_{(8,245)} = 45.2$, $p < 0.001$; (b) cocaine: two-way ANOVA, interaction between genotype and time: $F_{(8,258)} = 11.0$, $p < 0.001$; for genotype: $F_{(1,258)} = 110.7$, $p < 0.001$; for time: $F_{(8,258)} = 84.0$, $p < 0.001$); Dunnett's test to compare locomotor activity on days 2–14 to that on day 1 for each drug and genotype: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$); Bonferoni test to compare locomotor activity for each day between *Gnal* + / + and *Gnal* + /− mice ($^{\circ\circ}p < 0.01$). (c, d) Locomotor activity ratios between the first day of drug injection (day 1) and the day of challenge (day 14) are presented for mice treated by D-amph (c) and cocaine (d) ($^{\circ}p < 0.05$, Student's *t*-test).

following repeated injections. A possible explanation for this recovery could be that repeated drug injections upregulated the Gαolf/cAMP/PKA signaling pathway in the striatum and compensated for the reduction of signaling seen in untreated *Gnal* + /− mice. We measured Gαolf protein levels, AC activity and PKA-dependent phosphorylation of GluR1 receptor in mice which had received a chronic treatment with D-amph (ie the treatment used for sensitization experiments, Figure 7). Gαolf levels, dopamine-sensitive AC and D-amph-induced GluR1 phosphorylation remained markedly decreased in the striatum of D-amph-sensitized *Gnal* + /− mice as compared to wild-type mice (Figure 7a, b and c). Thus, the impairment of the PKA pathway in *Gnal* + /− mice was unchanged by repeated injections of D-amph and no improvement of this pathway could account for the functional recovery of the behavioral responses. These results are in agreement with previous studies showing that sensitizing treatment does not increase the D1R-dependent stimulation of cAMP pathway (Crawford *et al*, 2004).

ERK Phosphorylation in Response to D-Amph in the Striatum is Preserved in *Gnal* + /− Mice

As the PKA pathway appeared permanently altered in *Gnal* + /− mice, the existence of normal or even increased sensitization suggested that additional D1/Gαolf-dependent signaling pathways were involved in the induction of these

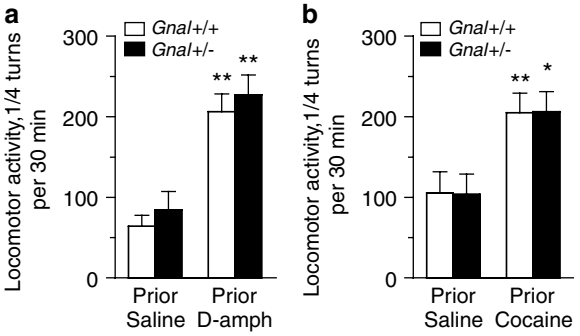

Figure 5 Conditioned locomotor responses in *Gnal* + /− mice sensitized to D-amph and cocaine. *Gnal* + /− mice and their wild-type littermates received daily injections of saline (prior saline), 2 mg/kg D-amph (a, prior D-amph) or 20 mg/kg cocaine (b, prior cocaine) in the actimeter for 5 consecutive days (data presented in Figure 4). The following day (day 6), the mice received a saline injection in the actimeter and their locomotor activity was recorded during 30 min. Data are means ± SEM ($n = 10$ per group). (a) D-Amph: two-way ANOVA, interaction between treatment and genotype $F_{(1,36)} = 0.5$, NS; genotype $F_{(1,36)} = 0.7$, NS; treatment $F_{(1,36)} = 53.5$, $p < 0.001$. (b) cocaine: D-amph: two-way ANOVA, interaction between treatment and genotype $F_{(1,36)} = 0.0$, NS; genotype $F_{(1,36)} = 0.0$, NS; treatment $F_{(1,44)} = 16.2$, $p < 0.001$. Bonferroni post-test: $^{*}p < 0.05$, $^{**}p < 0.01$ compared to prior saline.

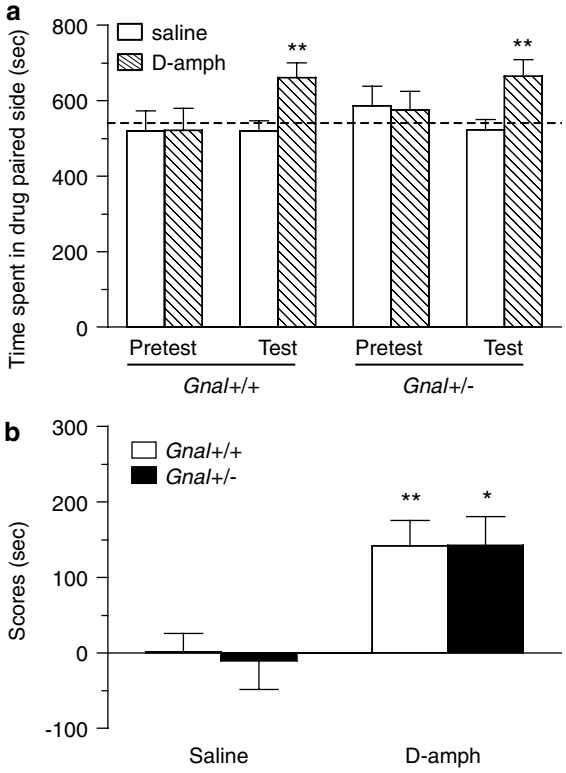

Figure 6 Conditioned place preference to D-amph in *Gnal* + /− mice. (a) *Gnal* + /− mice and their wild-type littermates received 2 mg/kg D-amph or saline alternatively in the two compartments (hatched bars) or received systematically saline in both compartments (white bars) as described in Materials and Methods ($n = 8$ per group). Time spent in the drug-associated compartment before conditioning (pretest) and after conditioning (test) is indicated. Data are means ± SEM ($n = 8$ per group); Paired *t*-test $^{**}p < 0.01$ as compared to saline group. (b) Scores were calculated as the difference between time spent in the D-amph paired compartment after and before conditioning. Data are means ± SEM ($n = 8$ per group). Two-way ANOVA: interaction between genotype and treatment $F_{(1,40)} = 0.0$, NS; genotype $F_{(1,40)} = 0.0$, NS; treatment $F_{(1,40)} = 18.7$, $p < 0.001$; followed by Bonferroni post-test: $^{*}p < 0.05$, $^{**}p < 0.01$ as compared to saline.

long-lasting effects. A good candidate was the mitogen-activated protein kinase (MAP kinase) extracellular signal-regulated kinase (ERK), which is activated in the striatum after acute injection of psychostimulants (Valjent *et al*, 2000, 2005). ERK activation is dependent on D1R activation since it is prevented by a D1R antagonist or in D1R knockout mice (Valjent *et al*, 2000, 2005). Importantly this pathway appears critical for the long-lasting effects of cocaine including CPP and locomotor sensitization (Valjent *et al*, 2000, 2005). We then explored ERK activation in the striatum of *Gnal* + /− mice 15 min after an injection of D-amph (10 mg/kg). D-Amph-induced ERK activation was not significantly different in the striatum of *Gnal* + /− and *Gnal* + /+ mice although Gαolf protein was required for its activation since it was abolished in *Gnal*−/− mice (Figure 8). These results showed that psychostimulant-induced ERK activation in the striatum was not affected by the partial reduction in Gαolf levels. The normal activation of the ERK pathway by D-amph in *Gnal* + /− and *Gnal* + /+ mice was consistent with the existence of sensitization and CPP in these heterozygous mutant mice.

DISCUSSION

Using heterozygous knockout mutant mice, we demonstrate that the decrease in the levels of two essential proteins in dopamine signaling, the D1 receptor and Gαolf, its associated G protein in the striatum, have strikingly different consequences on biochemical and behavioral responses to psychostimulant drugs in mice. Our results also show that different components of the D1R-dependent signaling pathways are limiting for the acute and chronic responses to psychostimulants.

Gαolf Levels Control the Efficacy of D1R-Activated cAMP Pathway in the Striatum

In *Drd1a* + /− mice the activation of cAMP pathway in response to dopamine *in vitro* or D-amph *in vivo* was not significantly altered, although the D1R protein levels were dramatically decreased as measured by ligand binding (Drago *et al*, 1994) and confirmed here by immunoblotting. The lack of functional alterations in *Drd1a* + /− mice is consistent with reports suggesting the presence of 'spare receptors' not coupled to AC in the striatum (Battaglia *et al*, 1986; Trovero *et al*, 1992).

By contrast, in *Gnal* + /− mice, decreased Gαolf levels were associated with decreased D1R-dependent activation of cAMP pathway as demonstrated *in vitro* by the reduction in D1R-activated cAMP production in striatal membranes and *in vivo* by decreased D-amph-induced GluR1 phosphorylation. The *Gnal* haploinsufficiency is particularly noteworthy since little is known about the relative importance of the various steps in G-protein-coupled receptors signaling

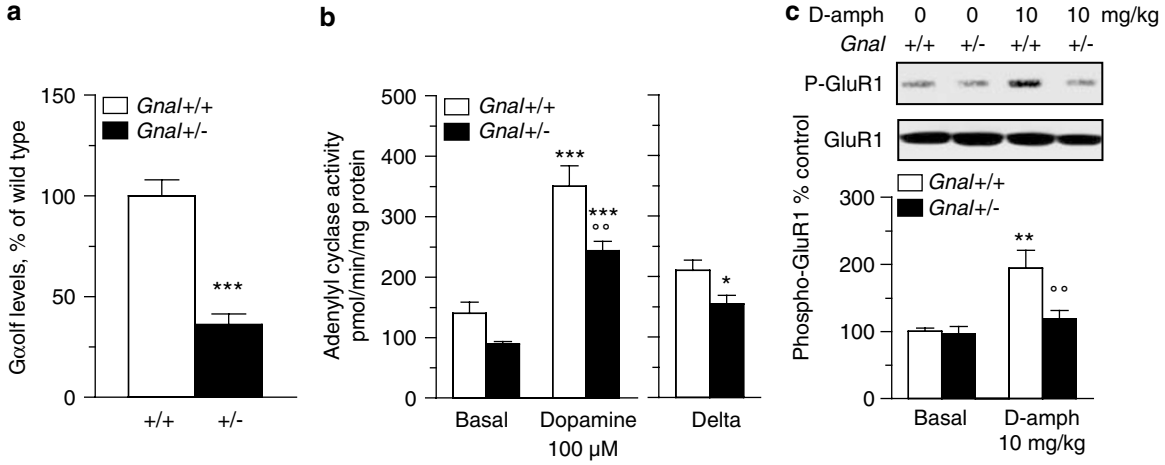

Figure 7 Gαolf levels and cAMP pathway in D-amph sensitized *Gnal* +/− mice. (a, b) *Gnal* +/− mice and their wild-type littermates received daily injections of D-amph (2 mg/kg) during 5 days. After a 7-day withdrawal, levels of Gαolf (a, $n=10$ per group) and AC activity in the presence or absence (basal) of dopamine (b, $n=4$ per group) were measured in striatal homogenates. (c) Sensitized mice ($n=9$ per group, as in Figure 4) were treated after 7 days of withdrawal by a challenge injection of D-amph (10 mg/kg) or saline and, 15 min later, phosphorylation of GluR1 on Ser-845 was analyzed and quantified in the striatum as in Figure 2. Data are means ± SEM. A, Student's *t*-test, ***$p<0.001$. (b) two-way ANOVA, interaction between genotype and treatment $F_{(1,12)}=0.21$, NS; genotype $F_{(1,12)}=14.0$, $p<0.01$; treatment $F_{(1,12)}=75.3$, $p<0.001$, Bonferroni post-test: ***$p<0.001$ as compared to basal condition, °°$p<0.01$ as compared to wild-type. Left panel represent the absolute difference in camp production between basal and 100 µM dopamine (delta), Student's *t*-test, *$p<0.05$. (c) two-way ANOVA, interaction between genotype and treatment $F_{(1,27)}=1.8$, NS; genotype $F_{(1,27)}=4.6$, $p<0.05$; treatment $F_{(1,27)}=10.0$, $p<0.01$. Bonferroni post-test: *$p<0.05$, **$p<0.01$ as compared to saline, °°$p<0.01$ as compared to wild-type mice.

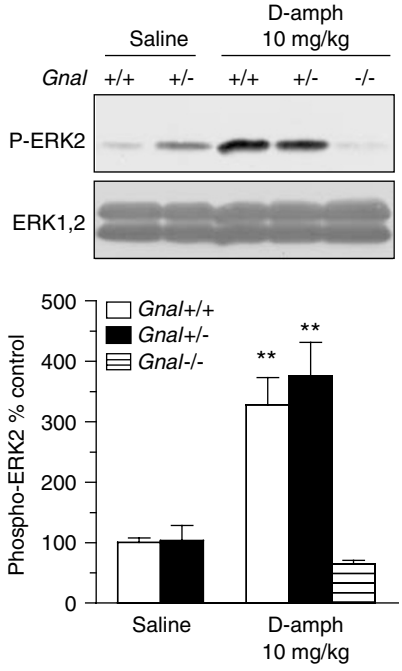

Figure 8 D-Amph-induced ERK phosphorylation in the striatum of *Gnal* mutant mice. *Gnal* +/+, *Gnal* +/− mice were injected with D-amph (10 mg/kg) or saline and 15 min later double phosphorylation of ERK1/2 (Thr183-Tyr185) and total ERK1/2 total was analyzed in the striatum by immunoblotting ($n=8$–10 per group). In three independent experiments, one *Gnal*−/− mice was also treatment with 10 mg/kg with their wild-type and heterozygous littermates. Representative immunoblots of phosphor-ERK1/2 (upper panel) and total ERK1/2 (lower panel) are shown. Phospho-ERK2 apparent immunoreactivity was quantified and expressed as the percentage of saline *Gnal* +/+ (control). ERK2 phosphorylation state was quantified for *Gnal*−/− mice but was not included in the analysis since no data were available for saline-treated *Gnal*−/− mice. Data are means ± SEM. Two-way ANOVA, interaction between genotype and treatment $F_{(1,31)}=0.3$, NS; genotype $F_{(1,31)}=0.4$, NS; treatment $F_{(1,31)}=38.3$, $p<0.001$. Bonferroni post-test: **$p<0.001$ as compared to saline.

in vivo. Careful studies of the β-adrenergic receptor/Gs pathway in cells in culture showed that Gs is not a limiting factor (Ostrom *et al*, 2000). In contrast, our results show that the levels of Gαolf protein, but not D1R, determine the amplitude of cAMP pathway response both *in vitro* and *in vivo* upon D1R activation in striatal neurons.

Gαolf and not D1R Levels Control Acute Locomotor Effects of Psychostimulants

It is well established that pharmacological blockade of D1R or the targeted deletion of its gene impairs the acute hyperlocomotion induced by cocaine or D-amph (Drago *et al*, 1996; Smith *et al*, 1998; Valjent *et al*, 2000). The absence of acute locomotor responses to cocaine was also reported in Gαolf knockout mice (Zhuang *et al*, 2000). These observations demonstrate that acute responses to cocaine are highly dependent on D1R-linked signaling. Interestingly however, we show here that a partial decrease in D1R amounts did not significantly affect the acute locomotor response to D-amph or cocaine in *Drd1a* +/− mice. This is consistent with the unaltered biochemical responses of the PKA pathway in the striatum. By contrast, *Gnal* +/− mice displayed a clear reduction in acute locomotor response to psychostimulant drugs, in agreement with the decreased cAMP signaling responses *in vivo*. This effect may result entirely from the impaired signaling of D1R as suggested by the reduced locomotor response to D1R agonist. On the other hand, *Gnal* +/− mice display reduced cAMP production in response to an A_{2A} adenosine receptor ($A_{2A}R$) agonist and impaired locomotor activation by the adenosine receptor antagonist caffeine (Corvol *et al*, 2001; Herve *et al*, 2001), suggesting a possible contribution of $A_{2A}R$, which are also coupled to AC through Gαolf in the striatum (Corvol *et al*, 2001). However, the role of $A_{2A}R$ in the acute effects of D-amph and cocaine is still unclear,

depending on the approaches and experimental conditions. An attenuation or the absence of acute responses to cocaine or D-amph has been reported in various strains of $A_{2A}R$ knockout mice (Chen *et al*, 2000; Soria *et al*, 2005), whereas an $A_{2A}R$ antagonist enhances and agonist decreases the acute locomotor responses to cocaine or D-amph (Poleszak and Malec, 2002). Thus, it is probably safe to conclude that acute locomotor responses to psychostimulant drugs depend mostly on D1R signaling, with a possible contribution of $A_{2A}R$ signaling. At any rate our results indicate that Gαolf, and not D1R, is the limiting parameter for the functional responses which involve dopamine release *in vivo*.

Chronic Behavioral Responses to D-Amph and Cocaine are not Altered in *Gnal*+/− Mice

Pharmacological studies in rodents strongly suggest an important role of D1R stimulation in the chronic behavioral responses induced by D-amph and cocaine. Indeed, D1R antagonists impair both the expression and development of amphetamine-induced sensitization and the expression of cocaine-induced sensitization, although they fail to prevent its development (Mattingly *et al*, 1994; Ujike *et al*, 1989; Vezina, 1996; White *et al*, 1998). Similarly, both acquisition and expression of CPP with D-amph and cocaine were prevented by a D1R antagonist (Baker *et al*, 1998; Cervo and Samanin, 1995; Hiroi and White, 1991). Results in D1R knockout mice appear somewhat contradictory since locomotor sensitization induced by cocaine was strongly reduced, as expected, whereas locomotor sensitization to D-amph and cocaine-induced CPP were clearly obtained in these mice (Crawford *et al*, 1997; Karper *et al*, 2002; Xu *et al*, 2000). This apparent discrepancy may be related to the existence of compensatory mechanisms in D1R knockout mice (Karper *et al*, 2002; Stanwood *et al*, 2005). Our results show that the partial deficiency of D1R signaling does not prevent the development and the expression of locomotor sensitization to cocaine and D-amph. Moreover, because the acute locomotor response to these drugs was quasiabolished in *Gnal*+/− mice, the sensitized response appeared proportionally higher in these mice than in their control littermates (Figure 4). Similarly, CPP to D-amph was not altered in *Gnal*+/− mice. The contrast between altered responses to acute administration of psychostimulant and normal responses to repeated treatments in these mice suggests that different signaling pathways may be limiting for the two types of effects.

As the D1R/cAMP pathway does not seem to be limiting for the effects of repeated injections of psychostimulant drugs, the question arises as to what may be the nature of the critical steps. One interesting candidate is the ERK pathway which was normally activated by D-amph in *Gnal*+/− mice. ERK appears essential for long-lasting effects of drugs since its pharmacological inhibition blocks the induction of sensitization, conditioned locomotor response and conditioned place preference with only minor changes in acute responses (Valjent *et al*, 2000, 2005). It is important to point out that ERK activation was prevented following complete pharmacological or genetic blockade of D1R (Valjent *et al*, 2005) or in homozygous *Gnal*−/− mutant mice (present study), showing that its activation requires the D1R/Gαolf module. ERK activation by psychostimulants in the striatum depends on the 'coincident' activation of D1R and glutamate NMDA receptor (Valjent *et al*, 2000). The contribution of D1R in this interaction is, at least in part, to inhibit protein-phosphatase-1 through phosphorylation of dopamine- and cAMP-regulated phosphoprotein of $M_r = 32\,000$ (DARPP-32, Valjent *et al*, 2005). Therefore, the present results suggest that the degree of protein-phosphatase-1 inhibition achieved in *Gnal*+/− mice may be sufficient to allow normal D-amph-induced ERK phosphorylation. There may be also changes in the glutamate controlled pathways that compensate for the relative deficiency of the cAMP-dependent pathway. Finally, it should be kept in mind that the ERK cascade has strong intrinsic nonlinear properties, as demonstrated in other systems (Ferrell, 1996). This nonlinearity may account for a normal ERK activation in spite of a partial decrease in the activation of one of its upstream activators, the cAMP-dependent pathway.

Other mechanisms may participate in the normal development of long-term behavioral responses induced by psychostimulants in *Gnal*+/− mice. First of all, it should be emphasized that amphetamine and cocaine act on multiple targets and neurotransmitters. For example, the serotoninergic system may play an important role in psychostimulants rewarding properties and a serotonin 'switch' may occur in DAT KO mice accounting for their persistent sensibility to cocaine (Mateo *et al*, 2004). This kind of phenomenon may compensate the relative lack of D1R transmission in *Gnal*+/− mice and explain their preserved chronic responses to amphetamine and cocaine. However, it would not explain the totally preserved ERK activation in the striatum of these mice since serotoninergic drugs, such as fluoxetine, had a much less effective effect on ERK phosphorylation in our conditions (Valjent *et al*, 2004).

In addition, repeated exposure to psychostimulant drugs results in marked functional alterations in glutamate neurons of the prefrontal cortex projecting to the nucleus accumbens (Hotsenpiller *et al*, 2001; McFarland *et al*, 2003; Pierce *et al*, 1996; Pierce and Kalivas, 1997; Reid and Berger, 1996). Although Gαolf is very abundant in the striatum, it is not expressed in other regions such as the prefrontal cortex (Herve *et al*, 1993) where D1R are coupled to Gαs (Corvol *et al*, 2001). Functional effects of repeated administration of psychostimulants on cortico-striatal neurons may be essential for sensitized and conditioned responses to psychostimulants but are unlikely to be changed by reduced Gαolf levels. In agreement with this hypothesis, GluR1 and ERK phosphorylation induced by psychostimulants were unchanged in the prefrontal cortex of *Gnal*+/− mice (our unpublished observations), presumably because their activation depends in beta-adrenergic receptors rather than dopaminergic receptors in this structure (Pascoli *et al*, 2005).

In conclusion, our results in heterozygous mutant mice show that quantitative modifications of two key proteins in the striatal dopamine pathway, D1R and its cognate G protein Gαolf, lead to very different phenotypes. Quantitative genetic alterations of diverse components of the same signaling pathway have the potential to affect differently

various effects of drugs of abuse, or possibly other behavioral responses linked to dopamine function.

ACKNOWLEDGEMENTS

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