Balance of Go1α and Go2α expression regulates motor function via the striatal dopaminergic system

Jens Baron,* Ainhoa Bilbao,† Heide Hörttagl,‡ Lutz Birnbaumer,§ Sarah Leinzer,† Rainer Spanagel,¶ Gudrun Ahnert-Hilger*† and Irene Brunk*†

*Institute of Integrative Neuroanatomy, Charité-Universitätsmedizin, Berlin, Germany
†Medical Faculty, Behavioural Genetics Research Group, Institute of Psychopharmacology, Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany
‡Department of Pharmacology, Innsbruck Medical University, Innsbruck, Austria
§School of Medical Sciences, Catholic University of Argentina, Institute of Biomedical Research (BIOMED UCA-CONICET), Buenos Aires, Argentina
¶Medical Faculty, Institute of Psychopharmacology, Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany

Abstract
The heterotrimeric G-protein Go with its splice variants, Go1α and Go2α, seems to be involved in the regulation of motor function but isoform-specific effects are still unclear. We found that Go1α-/- knockouts performed worse on the rota-rod than Go2α-/- and wild-type (WT) mice. In Go1+2α-/- mice motor function was partially recovered. Furthermore, Go1+2α-/- mice showed an increased spontaneous motor activity. Compared to wild types or Go2α-/- mice, Go1+2α-/- mice developed increased behavioural sensitization following repetitive cocaine treatment, but failed to develop conditioned place preference. Analysis of dopamine concentration and expression of D1 and D2 receptors unravelled splice-variant-specific imbalances in the striatal dopaminergic system: In Go1α-/- mice dopamine concentration and vesicular monoamine uptake were increased compared to wild types. The expression of the D2 receptor was higher in Go1α-/- compared to wild type littermates, but unchanged in Go2α-/- mice. Deletion of both Go1α and Go2α re-established both dopamine and D2 receptor levels comparable to those in the wild-type. Cocaine treatment had no effect on the ratio of D1 receptor to D2 receptor in Go1+2α-/- mutants, but decreased this ratio in Go2α-/- mice. Finally, we observed that the deletion of Go1α led to a threefold higher striatal expression of Go2α. Taken together our data suggest that a balance in the expression of Go1α and Go2α sustains normal motor function. Deletion of either splice variant results in divergent behavioural and molecular alterations in the striatal dopaminergic system. Deletion of both splice variants partially restores the behavioural and molecular changes.

Keywords: behavioural sensitization, cocaine, conditioned place preference, D1-D2 receptor signalling, Goα splice variants, motor function.

projection targets. Medium spiny neurons (MSN) of the direct pathway are positively modulated by dopaminergic input via D1-receptors (D1R), MSN of the indirect pathway are negatively modulated via D2-receptors (D2R). The conceptualization of a movement promoting direct and a movement inhibiting indirect pathway is still common, though challenged by recent findings such as the finding that both pathways are concurrently active during movement (Keeler et al. 2014).

Besides the dopaminergic input, MSNs of both pathways inhibit each other with a stronger inhibitory connectivity from MSNs of the indirect pathway onto those of the direct pathway. This mutual inhibitory connectivity establishes a bistable competitive interaction between the two pathways, which can be shifted depending on dopamine concentrations in the striatum (Calabresi et al. 2014; Bahuguna et al. 2015). Because of the higher affinity of D2R, lower dopamine concentrations will suppress MSNs of the indirect pathway thereby resulting in preponderance of the direct pathway.

Dopaminergic neurotransmission and neuroplasticity within the cortico-basal ganglia-thalamic circuits is critical for drug-induced sensitization, reward processing and as such is relevant for the development of addictive behaviour (Spanagel and Weiss 1999; Lobo and Nestler 2011; Yager et al. 2018 International Society for Neurochemistry, J. Neurochem. (2018) 146, 374–389).

Materials and methods
Antibodies
The following antibodies were used: Monoclonal mouse antibodies raised against both Goz splice variants (Synaptic Systems Cat# 271 111, RRID:AB_2619996), specifically recognizing Go2α (Synaptic Systems Cat# 271 011; RRID:AB_2619995), synaptophysin (Synaptic Systems Cat# 101 011C5, RRID:AB_887823), synaptobrevin (Synaptic Systems Cat# 104 211, RRID:AB_887811) (Winter et al. 2005; Brunk et al. 2010) and specifically detecting Go1α (Santa Cruz Biotechnology, Santa Cruz, CA, USA; Cat# sc-13532, RRID:AB_2111645) (Brunk et al. 2008). Further antibodies include rabbit antibodies against Gsα (Santa Cruz Biotechnology Cat# sc-823, RRID:AB_2111645), Goα (Santa Cruz Biotechnology Cat# sc-385, RRID:AB_10160576), tyrosine hydroxylase (Merck KGaA, Darmstadt, Germany; Cat# AB152, RRID:AB_390204), dopamine plasma membrane transporter (DAT, Merck KGaA Cat# AB2231, RRID:AB_1586991) and mouse monoclonal antibodies against the D2R (Santa Cruz Biotechnology Cat# sc-3503, RRID:AB_668816), the D1R (Merck KGaA Cat# MAB5290, RRID:AB_2094841) and actin (Sigma-Aldrich, St. Louis, MO, USA Cat# A5060, RRID:AB_476738).

Secondary antibodies conjugated to horseradish peroxidase include horse anti-mouse (Vector Laboratories, Burlingame, CA, USA; Cat# PI-2000; RRID:AB_236177) and goat anti-rabbit (Vector Laboratories Cat# PI-1000; RRID:AB_236198).

G-protein deletion mutants
Go1+/2α/- (NIE/Birnb Goalpha 129SV/C57BJ/6) and Go1α/-/Go2αα-/- (NIE/Birnb Go1alpha 129SV/C57BJ/6) or Go2αα-/- (NIE/Birnb Go2alpha 129SV/C57BJ/6) splice variant-specific mutants were
obtained from the lab of Lutz Birmbaum, bred in the local animal facility (no RRID available) and genotyped as described (Jiang et al. 1998; Dhingra et al. 2002). For our studies, mutant and WT littermates were obtained by interbreeding of heterozygous parents. All experimental procedures were approved by the Committee on Animal Care and Use and were carried out in accordance with the local Animal Welfare Act taking into account the impairments of the Animal Care and Use committee (Regierungspräsidium Karlsruhe, Karlsruhe, Germany). The experimental flow for both tests is given in Fig. 1. No randomization was performed; animals were arbitrarily assigned to the rota-rod and to the motor sensitization/CPP experiments. As a pre-defined inclusion criterion, only animals older than 18 days were used.

**Behavioural studies**

Male WT and mutant mice (minimum 8 weeks old) were maintained on a light–dark cycle (12–12 h) under controlled temperature (21 ± 2°C) and humidity (50 ± 5%) conditions. The studies were not pre-registered. For all studies, mice were single housed and received *ad libitum* access to food and water. Behavioural testing was performed during the light phase of the light/dark cycle. All experiments were conducted in accordance with the EU guidelines for the care and use of laboratory animals and were approved by the local animal care committee (Regierungspräsidium Karlsruhe, Karlsruhe, Germany). The experimental flow for both tests is given in Fig. 1. No randomization was performed; animals were arbitrarily assigned to the rota-rod and to the motor sensitization/CPP experiments. As a pre-defined inclusion criterion, only animals older than 18 days were used.

**Rota-rod performance test**

WT and mutant mice, originating from the Go1a-/-, Go2a-/- and Go1+2a-/- line (20–24 days old), were set on a rotating rod (Rozas et al. 1997). The body weights for WT were 10–11 g, for Go2a 11–12 g and for both Go1a and Go1+2a 6–7 g.

The rotating rod (home-made Charité) accelerates in speed from three to 50 rpm during a time course of 3 min. The performance in this test depends on motor activity, balance and coordination. Mice underwent one training round before being subjected to the final experiment. All mice managed to cling at least to the rod and this behaviour was included in the measurement until the end of the 3 min rotation or before the mice faa (see below). No animals were excluded. For the exact evaluation of the performance, mice were videotaped (digital-camcorder by Panasonic, Kadoma, Osaka, Japan). Three different parameters were analysed: The time mice spent on the rod while it is rotating, the time mice performed a first spin by clinging to the rotating rod and the overall number of spins mice performed on the rotating rod until they fall down or the test ended (Fig. 1).

**Cocaine-induced behavioural sensitization and development of conditioned place preference (CPP)**

The procedure of cocaine-induced CPP was adapted from our original description (Abarca et al. 2002; Brunk et al. 2008; Bilbao et al. 2014). The CPP paradigm consisted of three different phases: pre-conditioning, conditioning and drug-free test. The weight of the mice was as follows: Go1+2a 27.65 ± 2.18 g; WT 33.63 ± 0.95 g. For the pre-conditioning, the mice were injected with saline and immediately placed in the conditioning boxes for 20 min and allowed to explore the apparatus. During conditioning

![Fig. 1 Flowchart of rot rod or behavioural sensitization/ conditioned place preference (CPP) experiments.](image-url)

(a) Rota-rod  
Training  
Experiment  
1  
2  
3 min  
3  
50 rpm  
(b) Behavioural test with cocaine  
Pre-conditioning  
C1  
S  
C2  
S  
C3  
S  
C4  
S  
Drug-free  
CPP  
C5  
Biochemical analysis  

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phase, mice were treated for 8 days with daily alternating injections of cocaine (Cocaine hydrochloride, Sigma-Aldrich Chemie GmbH, Munich, Germany, 10 mg/kg, i.p.) or saline. The CPP apparatus consisted of a box with two equally sized compartments interconnected by a rectangular corridor. The compartments were differentiated by the motifs painted on the walls (dots or stripes), the colour (different shade of grey tones, light or dark) and texture (smooth or rough) of the floor (Panlab, Harvard Apparatus, Spain, boxes LE895 76-0728). All sessions were monitored by a video-tracking system (Ethovision 2.0, Noldus License number: EVPR20-99120008), which enabled us to determine locomotion and spatial placement of each mouse every 0.2 s across the whole session. Then, mice were confined into the corresponding compartment immediately after the injection for 30 min. For the expression or drug-free test, the mice were allowed to explore the whole apparatus without any treatment on day 9. The CPP score calculated represents the difference between the time spent (seconds) in the cocaine or saline-paired floor during the test day. During the CPP procedure, the effects of repeated cocaine injections on locomotion were assessed by comparing the distance travelled during the first and fourth drug-paired and the first non-drug-paired trials, and the expression of behaviour sensitization was assessed 11 days after the last cocaine injection. Mice were killed immediately after the end of the test (day 20) Their brains were collected, frozen on dry ice and stored at −80°C for further biochemical analyses (Fig. 1).

Subcellular fractionation
To obtain synaptic vesicles (SVs), mouse brains were homogenized (Wheaton, Potter-Elveljem, clearance 0.1–0.15 mm) in ice-cold buffer (320 mM sucrose, 4 mM HEPES/KOH, pH 7.4, 1 mM Phenylenmethylsulfonyl fluoride (PMSF), and 1 μM protease inhibitor cocktail (P, Sigma-Aldrich)) and centrifuged for 10 min at 4°C and 1300 g (Beckman rotor TLA-100.4). The resulting supernatant 1 was centrifuged again at 14 000 g for 15 min, yielding a synaptosome-containing pellet (P2). The synaptosomes were osmotically shocked by diluting them 1:10 in lysis buffer (10 mM HEPES/KOH, pH 7.4, 1 mM PMSF and 1 μM protease inhibitors and mechanically disrupted by three strokes at 2000 rpm (Potter-Elveljem, clearance 0.1–0.15 mm). SVs were collected by two sequential centrifugation steps at 29 000 g (20 min) and the resulting supernatant then centrifuged at 350 000 g (30 min) (Huttner et al. 1983; Becher et al. 1999).

Immunoblot analysis
Samples from brain fractions were analysed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and western blot techniques using the ECL detection system (GE Healthcare, Munich, Germany). For signal quantification, ECL-processed films were scanned and protein bands were densitometrically measured using the Labmage 1D program (KAPELAN, Halle, Germany) ensuring that signals were in the linear range of the ECL detection system. Comparative quantification of protein samples from WT mice and deletion mutants was performed in the same gel using actin, synaptophysin or synaptoprevin as internal controls.

HPLC analysis
Littermates of the Go1α+/− and Go1+2α−/− mice were decapitated and their brains removed at the age of P20 and P15 respectively. After removal, the brains were immediately frozen on dry ice and kept at −80°C until use. The striatum was dissected on a cold plate (−15°C). The samples were then weighed and stored at −80°C again until they were used for homogenization. After adding 10–20 volumes of deionized water, frozen samples were homogenized by ultrasonication at 4°C. An equal volume of 0.2 N perchloric acid was added to the homogenates, which were then centrifuged for 10 min at 25 000 g and 4°C. Monoamines were determined in the supernatant using high performance liquid chromatography with electrochemical detection as given. Briefly, Purospher™ RP-18 (5 μm) LiChroCART™ 125-4 (Merck 150142) was used with a flow rate of 1 mL/min. Serotonin was analysed using a mobile phase of 0.1 M sodium acetate, 0.1 mM EDTA, 0.35 mM heptane sulphonic acid, 5% methanol at a pH of 4.5. Dopamine was analysed using a mobile phase of 0.1 M sodium phosphate, 1 mM EDTA, 0.75 mM octane sulphonic acid, 5% methanol adjusted to pH 5. The standards for DA and 5-HT were purchased from Sigma (Hortnagl et al. 1993).

Monoamine oxidase activity assay
The monoamine oxidase activity (type A and B) was determined with the Amplex™ Red Monoamine oxidase assay Kit by Molecular Probes, Invitrogen, Germany.

Monoamine uptake
Monoamine uptake by synaptic vesicles derived from wt, Go1α+/− and Go1+2α−/− mice was performed using 5-Hydroxy-[3H] tryptamine trifluoroacetate (specific activity, 4.33 TBq/mmol) purchased from Amersham Biosciences. Briefly SVs were resuspended in potassium glutamate (KG) buffer (150 mM KG; 20 mM 1,4-piperazinediethanesulphonic acid; 4 mM EGTA; 2.9 mM MgCl2, equivalent to 1 mM free Mg2+ and 2 mM ATP, adjusted to pH 7.0 with KOH). Uptake was started by adding KG-ATP buffer, supplemented with 1 mM ascorbic acid and 40 nM [3H]serotonin or [3H]dopamine supplemented with or without reserpine (6 μM) to determine non-specific uptake. Incubation (10 min at 25°C) was stopped by addition of 500 μL of ice-cold KG buffer followed by rapid centrifugation at 440 000 g for 10 min at 4°C. The pellets were lysed in 0.4% Triton X-100 to determine radioactivity by liquid scintillation counting and protein content using the bicinchoninic acid method (Brunk et al. 2008).

Data analysis
Samples were run in triplicates for biochemical experiments and experiments were repeated at least four times. The behavioural data were analysed by Student’s t test (Excel) and one- or two-way ANOVAS, with a repeated measures factor when necessary, followed by Newman–Keuls’s post hoc tests, when appropriate (Graph Pad Prism). For the rota-rod data no assessment of normality has been performed. For biochemical data, the Kruskal–Wallis test and the Mann–Whitney U-test, (IBM SPSS Statistics) for independent samples were applied; both tests are valid also for data without normal distribution. All values are given as mean ± SEM or SD and statistical significance was set at p < 0.05. No sample calculation was performed to predetermine the sample size. No test for outliers was conducted on the data.
Results

Motor function of WT, Go2α−/−, Go1α−/− and Go1+2α−/− mice

To investigate the specific role of Goα and its splice variants, Go1α and Go2α, and their interrelation regarding motor function we first compared motor performance on the rotarod of all deletion mutants and their WT littermates. In the rotarod test, three different parameters were recorded: (i) total time spent on the rod, (ii) the time mice were running on the rod when the mice were running on the rod during the 3 min course. There was no difference regarding any of the three parameters for Go2α−/− mice when compared to the WT mice (Fig. 2a–c), indicating an unaltered spontaneous motor activity of Go2α−/− mutants as described previously (Brunk et al. 2008). Performance of Go1α−/− and Go1+2α−/− mice was significantly worse regarding all three parameters. The average time WT and Go2α−/− mice stayed on the rotating rod was longer than for Go1α−/− and Go1+2α−/− mice, whereby Go1α−/− mice spent significantly less time on the rod when compared to Go1+2α−/− animals (Fig. 2a). The time until the first spin occurred was significantly shorter in Go1α−/− and Go1+2α−/− mice compared to both WT and Go2α−/− mutants. Again Go1α−/− mice spent significantly less time on the rod before a first spin occurred compared to Go1+2α−/− animals (Fig. 2b). Finally, both Go1α−/− and Go1+2α−/− genotypes showed a significant increase in the number of spins compared to WT animals and Go1α−/− again performed worst (Fig. 2c).

Taken together our results reveal that motor performance of both Goα1 mutants lacking Go1α is affected. Surprisingly, however, Go1α−/− mice with the Go2α isoform present show a stronger deficit than the Go1+2α−/− mutants. Thus, the additional elimination of the Go2α splice variant partially restores the motor deficits when compared with the lack of Go1α alone.

Cocaine-induced behaviours in WT and Go1+2α−/− mice

The data obtained from the rotarod test revealed deficits in motor function only in Go1α−/− and Go1+2α−/− but not in Go2α−/− mice. As reported previously motor deficits in Go2α−/− mice become obvious when the motor system is challenged by either cocaine (Brunk et al. 2008) or amphetamine (Brunk et al. 2010). Therefore, we next analysed the impact of the deletion of both the Go1α and Go2α splice variant on motor response to psychostimulant treatment. As Go1α−/− mice have a very short life time (mean life span 24 days) we could only test the Go1+2α−/− mice and their WT littermates.

Mice were injected daily with cocaine (10 mg/kg, i.p.) or saline for eight alternating days to test the development of CPP and behavioural sensitization simultaneously (Fig. 3). In the Go1+2α−/− mutants basal motor activity determined after injection of saline (Fig. 3a, saline) was significantly higher compared to WT littermates confirming hyperactivity previously described (Jiang et al. 1998).
After the first, acute administration, cocaine induced a higher increase in locomotor activity in the mutant mice compared to WT animals (Fig. 3a Coc-1). Following repeated injections, the locomotor response to cocaine was further increased only in the mutants indicative of development of behavioural sensitization (Fig. 3a Coc-4). In the WT, behavioural sensitization did not develop to a significant degree (because of the mixed 129SvxC57BL/6J background) and was less pronounced than in our previous studies (Brunk et al. 2008). Furthermore, in contrast to the WT, after a drug-free period of 11 days, Go1+2α/- mice further increased their sensitized response to cocaine as the mice exhibited a more robust response than WT mice (Fig. 3a, Coc-5). Deletion of only Go2a mice exhibited a more robust response than WT mice further increased their sensitized response to cocaine as the mice exhibited a more robust response than WT mice (Fig. 3a, Coc-5). Deletion of only Go2α had been shown to attenuate development of motor sensitization induced by cocaine (Brunk et al. 2008) and amphetamine (Brunk et al. 2010). The enhancement of motor sensitization in the Go1+2α/- mice indicates the importance of a balanced expression of both splice variants for adequate motor function.

Repeated exposures to the cocaine-associated compartment induced a CPP in WT mice, whereas Go1+2α/- mice failed to develop cocaine preference (Fig. 3b). As we demonstrated previously, deletion of Go2α alone did not impair the development of cocaine-induced CPP (Brunk et al. 2008), indicating that the absence of CPP in Go1+2α/- mice can be attributed to the deletion of Go1α.

Monoamine content and expression of Goα splice variants in striata of WT, Go2α/-, Go1α/- and Go1+2α/- mice

In our previous analysis of Go2α/- mice, we found that striatal dopamine levels are decreased in comparison to WT animals and this is accompanied by a reduced monoamine uptake by synaptic vesicles (Brunk et al. 2008). Therefore, we investigated if altered motor function in Go1α- and Go1+2α deletion mutants is also associated with changes in striatal dopamine levels. Indeed, HPLC analyses revealed increased dopamine as well as serotonin levels in the striatum of Go1α/- mice compared to the corresponding WT. In contrast, in the striata of Go1+2α/- mice (lacking both isoforms) dopamine and serotonin levels remained comparable (Fig. 4a).

Furthermore, we found an increased vesicular uptake of monoamines in striatal SV preparations of Go1α/- mice (59% total uptake) when compared with WT littermates (41% total uptake). This difference was attenuated when analysing littermate pairs of WT (45.0% total uptake) and Go1+2α/- mice (55.0% total uptake) (Fig. 4b).

These data complement the behavioural experiments indicating that lack of Go1α results in changes in striatal monoamine levels and vesicular uptake. Removal of both splice variants appears to restore partially the WT situation. These results suggest that imbalance in the expression of Goα isoforms leads to opposite effects depending on the splice variant deleted.
To test if the deletion of one splice variant induces an increased expression of the other splice variant, we next analysed the striatal amounts of the respective splice variants using the recently characterized Goα splice variant-specific antibodies (Brunk et al. 2010). Go1α-/- mice served as negative controls. Expression of Go2α was found to be increased three-fold in the striatum of Go1α-/- mice compared to WT littermates, whereas the expression of Go1α was not altered in the striatum of Go2α-/- mice (Fig. 5). This increased amounts of Go2α in the striatum of Go1α-/- mice could not be resolved previously when using synaptosomes from whole brain (Brunk et al. 2008). The striatal imbalance in Goα splice variant expression is more severe in Go1α-/- mutants compared to Go2α-/- mutants since besides the lack of Go1α expression of Go2α is increased. An increase in Go2α, as found in the Go1α mutants, may explain their pronounced poor performance in the rota-rod test (compared to the absence of both splice variants).

**Striatal expression of dopamine receptors, D1R and D2R, in WT, Go1α-/- and Go1α+2α-/- mice**

Previous findings indicated that most central nervous D2R are coupled to Go1α (Jiang et al. 2001; Brunk et al. 2010). Cocaine-induced motor-sensitization and CPP are based on...
changes in D1R and D2R signalling in various brain areas (ventral tegmental area, nucleus accumbens and medial prefrontal cortex) (Steketee and Kalivas 2011). Thus, deletion of Go1α, the main coupling Gα-subunit of D2R, may cause the lack of cocaine-induced CPP observed in Go1+/2α-/- mutants observed here (see Fig. 3).

Analysis of striatal expression of the D1R revealed no difference between WT animals and Go1α-/- mutants. In contrast, expression of the D2R was higher in the Go1α-/- mutants compared to their WT littermates (Fig. 6a). In Go1+/2α-/- mutants, striatal expression of both D1R and D2R was not altered in comparison to WT mice, both without (Fig. 6b) or after (Fig. 6c) repetitive application of cocaine. Taken together the ratio of D1R and D2R expression is lowered in striata of Go1α-/- mice (45.0 ± 5.7% of WT) and resembles the situation in cocaine treated Go2α-/- mice (41.2 ± 15.5% of WT). In Go1α-/- mice the expression of D2R in the striatum was increased (Fig. 6a). However, an increase in the striatal D2R content was also observed in cocaine treated Go2α-/- mutants in the presence of Go1α (Brunk et al. 2008). Although other possibilities cannot be excluded, the data support the idea that both Gα subunits may couple to D2R but may differentially respond to cocaine. In this line, no change in the D2R amount was seen in Go1+/2α-/- mutants, irrespective of cocaine treatment (Fig. 6b and c). The D1R/D2R ratio was not altered significantly in striata of untreated and cocaine treated Go1+/2α-/- mutants compared to WT animals, though the analyses tended to result in a higher ratio in the mutants (Fig. 6d, Table 1). These findings are in line with development of motor sensitization after repetitive cocaine treatment in Go1+/2α-/- mice and with lack of motor sensitization in Go2α-/- mice, but they do not account for higher motor activity of Go1+/2α-/- mutants compared to their WT littermates.

The amounts of D1R, Gsα, TH and VMAT2 did not differ in striata of all mutants tested here. There was also no difference in the striatal expression of calbindin, TH, VMAT2 and Gsα of monoamine oxidase activity (Table 2) in between the different genotypes. Amounts of Gof1z were increased in Go1z-/- mutants in contrast to the decreased expression in Go2α-/- mutants (Table 3; (Brunk et al. 2008)). Gsα, Gof1z, TH and VMAT2 were comparably expressed in striata of WT mice and of Go1+/2α-/- mutants after repetitive treatment with cocaine (Table 3).

**Discussion**

This study reveals that Go1α and Go2α, the two major Gα splice variants, affect the dopaminergic system in a complementary manner. Motor function tests revealed that Go1α-/- mice perform considerably worse in comparison to both WT and Go1+/2α-/- mice lacking both splice variants. Furthermore, in Go1α-/- mice dopamine concentration and vesicular monoamine uptake was higher compared to WT littermates (this paper), whereas in Go2α-/- mice dopamine uptake and content was reduced (Brunk et al. 2008). As both Gα splice variants reside on secretory vesicles (Takamori et al. 2002) they may fine tune antagonistically vesicular monoamine content. Besides the dopamine content, the expression of the D2R receptor was higher compared to WT littermates or Go2α-/- mice. Deletion of both Go1α and Go2α re-established both dopamine levels and D2R amounts comparable to those in the wt. As we found no evidence for changes in the leading enzymes for dopamine synthesis of degradation it is not clear yet whether the dopamine imbalances are owing to the changes at the vesicular level, the receptor level or both.

**Distinct opposing effects of Go1α and Go2α on basal motor function**

Gox comprises about 1% of total membrane protein content of the brain with Go1α expression exceeding that of Go2α in mature neurons (Jiang and Bajpayee 2009). In mouse brain, we have previously measured an overall ratio of 2:1 for Go1α expression over Go2α (Brunk et al. 2008). Here we show that the two splice variants exert opposing effects on the striatal dopaminergic system. Unexpectedly, when tested...
on a rota-rod, motor function was not affected in Go2α-/- mutants but was severely impaired in Go1α-/- mutants. Moreover, motor activity was improved in mutants deficient of both splice variants when compared with Go1α-/- mice.

The three different behavioural tests (rota-rod performance test, cocaine-induced sensitization and CPP) used in this study address different aspects of the role of dopamine in the striatum. The rota-rod performance test examines motor coordination, overall activity as well as muscle strength. In this test both Go1α-/- and Go1+2α-/- mutants perform worse compared to the Go2α-/- mice and the corresponding WT littermates. The poor performance of the Go1α-/- and the Go1+2α-/- mice compared to both WT and Go2α-/- can be attributed not only to deficits in motor control (Jiang et al. 1998) but also to their overall weakness. Indeed, in the Go1+2α-/- mutants a weight reduction by about 40% has been observed earlier (Jiang et al. 1998). Surprisingly, however, the Go1α-/- mutants perform even worse compared to the Go1+2α-/- mice despite the fact that the postnatal weight development is comparable in both genotypes.

![Fig 6 Striatal expression of DR in WT, Go1α-/- and Go1+2α-/- mice.](image)

(a) D1R was comparably expressed in striata of WT and Go1α-/- mice, expression of D2R was increased in deletion mutants compared to WT littermates (relative OD D1R: WT 0.14 ± 0.05; Go1α-/- 0.09 ± 0.02; relative OD D2R WT 0.03 ± 0.01 Go1α-/- 0.07 ± 0.01 p < 0.05). (b) D1R and D2R were expressed in comparable amounts in striata of WT and Go1+2α-/- mice (relative OD D1R: WT 0.10 ± 0.05; Go1+2α-/- 0.16 ± 0.07; relative OD D2R: WT 0.39 ± 0.10; Go1+2α-/- 0.29 ± 0.06). (c) D1R and D2R amounts were comparable in striata of cocaine-treated WT and Go1+2α-/- mice (relative OD D1R: WT 0.22 ± 0.05; Go1+2α-/- 0.28 ± 0.06; relative OD D2R: WT 0.13 ± 0.03; Go1+2α-/- 0.10 ± 0.02). (d) The ratio of D1R to D2R expression was lower in Go1α-/- striata compared to WT (45.0 ± 5.7% of WT), resembling the situation in Go2α-/- mice after repetitive treatment with cocaine (41.2 ± 15.5% of WT) (p < 0.05). There was no difference between untreated WT mice and Go2α-/- or Go1+2α-/- mutants (214.7 ± 94.6% of WT) as well as between cocaine treated WT mice and Go1+2α-/- mutants (135.1 ± 41.2% of WT). Values are obtained from four animals per mouse line and genotype and are expressed as percent of the respective WT and as means ± SD (Kruskal–Wallis test and the Mann–Whitney U-test) using synaptophysin or synaptobrevin as internal controls.
Table 1: Relations of D1R and D2R expression in striata of Go2a-/-, Go1x-/-, Go1+2x-/- mice compared to WT littermates

<table>
<thead>
<tr>
<th>Parameter of interest</th>
<th>Go2a-/-</th>
<th>Go1x-/-</th>
<th>Go1+2x-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1R (OD ko/OD wt)</td>
<td>0.765 ± 0.095</td>
<td>0.772 ± 0.284</td>
<td>1.603 ± 0.691</td>
</tr>
<tr>
<td>D2R (OD ko/OD wt)</td>
<td>0.986 ± 0.096</td>
<td>1.777 ± 0.745*</td>
<td>0.763 ± 0.127</td>
</tr>
</tbody>
</table>

Expression levels of the indicated proteins were determined by western blot analysis of four animals from each genotype. D1R and D2R expression and D1R/D2R ratio was set 1 for WT animals.

Although Go1+2x-/- mutants are impaired with respect to their body weight and muscle strength, motor performance appears to be functional besides the effects directly influenced by strength.

Opposing effects of Go1x and Go2x on the striatal dopaminergic system

Consistent with these divergent effects on motor functions, striatal dopamine concentration as well as the D2R expression were differentially affected in Go1x-/- vs Go2x-/- mutants with the former showing increased and the latter decreased levels, respectively, compared to their WT littermates. Recently it has been shown that the two splice variants of D2R differentially affect the dopaminergic system. D2RL mediates post-synaptic effects including motor activity following cocaine treatment, whereas D2RS pre-synaptically modulates dopamine synthesis (Radl et al. 2018). The comparable increase in total D2R amounts observed in Go1x-/- and Go2x-/- following cocaine treatment could be therefore because of differential Gox-splice variant-specific changes in the expression of the D2R splice variants.

In contrast, the presence of only Go2x, whose expression is increased in whole brain and striatum in Go1x-/- mutants, further deteriorates motor activity and coordination. This observation may be related to the fact that the striatal amount of D2R is increased and the ratio of D1R to D2R expression is reduced in Go1x-/- animals. So, in Go1x-/- mutants the indirect pathway via D2R signalling could be enforced leading to more severe deficits in motor control than the complete absence of both splice variants, which does not affect the ratio of D1R to D2R. Especially D2R is linked to Go proteins in the central nervous system (Jiang et al. 2001). Absence of Go2x has no impact on motor control in the rotarod performance test indicating that Go1x is more important for successful motor control under physiological conditions than Go2x. The additional involvement of Go2x becomes obvious after challenging the dopaminergic control of motor activity by treatment with psychostimulants (Brunk et al. 2008, 2010). In Go1+2x-/- mutants D2R signal transduction should be impaired because of the loss of Gox signalling. Interestingly these mice are still able to perform motor tasks and skills. This is reminiscent of the situation observed in D2R deletion mutants, which, nevertheless, are able to perform motor coordination tasks, although impaired in comparison to WT mice (Kelly et al. 1998; Clifford et al. 2000). In addition, the lack of D2R autoreceptors (corresponding to D2RS) (Radl et al. 2018) leads to locomotor...

Table 2: Key proteins of the monoaminergic system in brains of WT, Go1x-/- mice and Go1+2x-/- mice

<table>
<thead>
<tr>
<th>Expression of</th>
<th>Region</th>
<th>Go1x-/-</th>
<th>Go1+2x-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt</td>
<td>ko</td>
<td>wt</td>
</tr>
<tr>
<td>Calbindin</td>
<td>Striatum</td>
<td>0.923 ± 0.255</td>
<td>0.756 ± 0.092</td>
</tr>
<tr>
<td>D1R</td>
<td>Striatum</td>
<td>0.138 ± 0.053</td>
<td>0.092 ± 0.017</td>
</tr>
<tr>
<td>D2R</td>
<td>Striatum</td>
<td>0.033 ± 0.005</td>
<td>0.068 ± 0.006*</td>
</tr>
<tr>
<td>Golfx</td>
<td>Striatum</td>
<td>0.045 ± 0.018</td>
<td>0.131 ± 0.042*</td>
</tr>
<tr>
<td>Gsx</td>
<td>Striatum</td>
<td>0.056 ± 0.033</td>
<td>0.079 ± 0.034</td>
</tr>
<tr>
<td>TH</td>
<td>Striatum</td>
<td>0.718 ± 0.123</td>
<td>0.659 ± 0.135</td>
</tr>
<tr>
<td>VMAT2</td>
<td>Striatum</td>
<td>0.076 ± 0.016</td>
<td>0.041 ± 0.020</td>
</tr>
</tbody>
</table>

Expression levels of the indicated proteins were determined by western blot analysis using striata from four different animals of each genotype. Quantification is given as the mean of the relative optical densities ± SD, and was performed using synaptophycin or synaptobrevin as internal control. Overall activity of MAO was measured using the Amplex Red MAO Assay Kit with tyramine as substrate. $V_{max}$ (fluorescence intensity) and Km (min) values are expressed as means ± SD. Expression levels of D2R and Golfx were lower in striata of WT mice compared to Go1x-/- mutants. For all other parameters, no differences between WT mice and mutants could be observed ($p < 0.05$). For statistic Kruskal-Wallis test and the Mann-Whitney U-test were used.
Table 3 Influence of repetitive cocaine treatment on expression of proteins related to the monoaminergic system in striata of WT and Go1\(2\alpha\)/-/- mice

<table>
<thead>
<tr>
<th>Protein of interest</th>
<th>wt</th>
<th>ko</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1R</td>
<td>0.216 ± 0.053</td>
<td>0.278 ± 0.064</td>
</tr>
<tr>
<td>D2R</td>
<td>0.130 ± 0.025</td>
<td>0.097 ± 0.020</td>
</tr>
<tr>
<td>Gs(\alpha)</td>
<td>0.245 ± 0.136</td>
<td>0.385 ± 0.133</td>
</tr>
<tr>
<td>Golf(\alpha)</td>
<td>0.100 ± 0.065</td>
<td>0.107 ± 0.060</td>
</tr>
<tr>
<td>TH</td>
<td>0.450 ± 0.095</td>
<td>0.467 ± 0.125</td>
</tr>
<tr>
<td>VMAT2</td>
<td>0.404 ± 0.238</td>
<td>0.440 ± 0.226</td>
</tr>
</tbody>
</table>

Expression levels of the indicated proteins were determined by western blot analysis of brains from four animals of each genotype following cocaine treatment (see Fig. 2). No difference between WT mice and deletion mutants could be observed. Quantification is given as relative optical densities expressed as means ± SD using synaptophysin or synaptobrevin as internal control. For statistic Kruskal–Wallis test and the Mann–Whitney U-test were used.

hyperactivity. (Bello et al. 2011) This is reminiscent to the hyperactivity observed in the Go1\(2\alpha\)/-/- mice (see Fig. 2 this paper). As Go2\(\alpha\)/-/- mice do not show an increased but a reduced motor activity, the hyperactive phenotype in the Go1\(2\alpha\)/-/- mice may be mainly because of the loss of Go1\(\alpha\) (Brunk et al. 2008). When the D2R is ablated during adulthood motor control is severely disturbed. This indicates that reduced motor control as a result of deletion of D2R before birth can be partially overcome during postnatal development (Bello et al. 2016).

Differing effects of Go1\(\alpha\) and Go2\(\alpha\) on psychostimulant-induced behaviour

Cocaine-induced behavioural sensitization could only be tested in Go1\(2\alpha\)/-/- mice. Go1\(\alpha\)/-/- mutants were not tested because of their reduced life span (mean 24 days). Similar to the observations on the rota-rod test, Go2\(\alpha\)/-/- mutants exhibited reduced cocaine-induced behavioural sensitization (Brunk et al. 2008), whereas Go1\(2\alpha\)/-/- mice strongly responded in this test (this paper). All three parameters addressed, basal motor activity, motor response to first cocaine injection and behavioural sensitization following repeated cocaine injections were increased in Go1\(2\alpha\)/-/- mutants compared to WT mice. Finally, development of CPP towards cocaine was almost completely abolished in Go1\(2\alpha\)/-/- mutants (this paper), whereas it was unaltered in the Go2\(\alpha\)/-/- mice (Brunk et al. 2008). The absence of both Go\(\alpha\) proteins boosts behavioural sensitisation, which is under these conditions probably mediated by D1R and Golf\(\alpha\). Golf\(\alpha\) is highly expressed in the striatum. Deletion of Golf\(\alpha\) abolishes cocaine-induced increase in motor activity, whereas the basal motor activity of the mutants is increased (Zhuang et al. 2000). Recently we found behavioural sensitization following repetitive cocaine administration in Go2\(\alpha\)/-/- mice to be decreased (Brunk et al. 2008). In this context Go\(\alpha\) proteins seem to have complementary effects with Go1\(\alpha\) down-regulating behavioural sensitization. On the other hand, the D2R also appears to be relevant for cocaine-induced behavioural sensitization as sensitization is diminished in D2R knockout mice (Welter et al. 2007). Thus, behavioural sensitisation may be sustained depending on either receptor equipment (D1R or D2R) or signal transducing G-proteins (Go, Gs or Golf). A partially restored balance of D1R and D2R expression in Go1\(2\alpha\)/-/- mice and the signal transduction by either Golf or Gs with both G-proteins present at amounts comparable to the WT situation may sustain the development of behavioural sensitization.

The CPP test addresses development of addiction but has to be distinguished from behavioural sensitisation according to our observations in the Go1\(2\alpha\)/-/- mutants. This is in accordance with our previous observation that behavioural sensitisation following cocaine or amphetamine treatment is reduced in Go2\(\alpha\)/-/- mice, whereas CPP is not changed (Brunk et al. 2008, 2010). This is in some respects contradictory to the literature, where sensitization and CPP are described to be interrelated, though not obligatory connected (Steketee and Kalivas 2011). The complete deletion of both Go\(\alpha\) splice variants almost abolishes CPP indicating that signal transduction via Go1\(\alpha\) is essential for the development of this behaviour. Go has been mainly linked to D2R activation (Jiang et al. 2001). The ability of GTP to promote a shift in the affinity of D2R for dopamine is absent in brains of Go1\(2\alpha\)/-/- mutants but unchanged in Go2\(\alpha\)/-/- mice indicating that central nervous D2R preferentially couples to Go1\(\alpha\) subunits (Jiang et al. 2001; Brunk et al. 2010). In this line, CPP is nearly abolished in D2R knock out animals (Welter et al. 2007). However, CPP is also decreased in animals lacking D1R (Abraham et al. 2016). Results obtained with constitutive Go1\(2\alpha\)/-/- mice suggest that CPP requires Go1\(\alpha\) to develop, probably using either D1R or D2R. Indeed, cocaine-induced sensitization and reinstatement is based on changes in D2R and D1R signalling in the ventral tegmental area (Steketee and Kalivas 2011).

Taken together our data indicate that a balance in the expression of Go1\(\alpha\) and Go2\(\alpha\) maintains normal motor function signalling via striatal dopamine receptors. Deletion of one of the splice variants leads to an imbalance affecting motor performance of the individual mutant. Balance is partially restored when both splice variants are deleted, because the expression of D1R and D2R is not affected in Go1\(2\alpha\)/-/- mice, and signalling can occur via Gs\(\alpha\) and Golf\(\alpha\), which are still present in the mutants. It has to be born in mind that the actual data were obtained with conventional mutants and therefore an impact on various cell types and brain regions besides dopaminergic neurons in the striatum could not be discriminated. This also implies that besides dopamine receptors other receptors like M2, opioid or...
somatostatin receptors that couple to Go proteins (Jiang and Bajpayee 2009) may be involved. So far, Go2α-specific effects have been described for somatostatin-receptor-mediated effects in pancreatic islets (Wang et al. 2011). In addition, there may be redundancy of G-protein signalling via different receptors. In this line it has been recently described that D1R differentially couple to either G0α or Gsα depending on agonist and brain region (Yano et al. 2018). Whether Go1α may be also linked to D1R under certain conditions is not known so far. The opposing effects of Go1α and Go2α shown here using global knockouts will pave the way for future studies using conditional knockouts for either Goα splice variant to improve our understanding how motor function is controlled.

Acknowledgement and conflict of interest disclosure

The authors are indebted to Marion Möbes, Birgit Metze and Antje Dräger for expert technical assistance. The Deutsche Forschungsgemeinschaft supported work in the author’s lab. Rainer Spanagel is an editor for Journal of Neurochemistry. The other authors declare no conflict of interest. The study was not pre-registered. All experiments were conducted in compliance with the ARRIVE guidelines.

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Manuscript Title: Balance of Go1α and Go2α expression regulates motor function via the striatal dopaminergic system
Corresponding Author: Gudrun Ahnert-Hilger

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Signature: _________________________________