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Role of G_q protein in behavioral effects of the hallucinogenic drug 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane

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SUMMARY

Extensive evidence suggests that 5-HT2 receptors may play a role in mental disorders including schizophrenia. In addition, several studies indicate that G_q-coupled 5-HT_{2A} receptors are likely targets for the initiation of events leading to the hallucinogenic behavior elicited by lysergic acid diethylamide (LSD), (±)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), and related drugs. However, 5-HT_{2A} receptors couple to other G proteins in addition to G_q protein. To evaluate the role of the G_q signaling pathway in DOI-induced behaviors, we utilized two behavioral models of 5-HT_{2A} receptor activation: induction of head-twitches by DOI, a common response to hallucinogenic drugs in rodents, and DOI elicited anxiolytic-like effects in the elevated plus maze. Experimental subjects were genetically modified mice [$G\alpha_q(-/-)$] in which the G_q alpha gene was eliminated. $G\alpha_q(-/-)$ mice exhibited a decrease in DOI-induced head-twitches, when compared to wild-type littermates. In addition, the DOI-induced increase in anxiolytic-like behavior was abolished in $G\alpha_q(-/-)$ mice. These results, combined with our finding that DOI-induced FOS expression in the medial prefrontal cortex was also eliminated in $G\alpha_q(-/-)$ mice, suggests a key role for G_q protein in hallucinogenic drug effects.

Keywords

Hallucinogens; 5-HT_{2A} receptors; G_q protein; Elevated plus maze; Head-twitch response; *c-fos*

INTRODUCTION

G-proteins transduce signals from a wide array of G-protein coupled receptors (GPCRs) initiating a plethora of second messenger cascades. Heterotrimeric G-proteins, consisting of three subunits, Gα, Gβ, Gγ, act as molecular switches between their active and inactive states in response to guanine nucleotides. The $G_{q/11}$ subfamily of G-proteins mediates the activation of phospholipase Cβ (PLCβ), which in turn catalyzes the hydrolysis of phosphatidylinositol-4,5-biphosphate (PIP_2) into inositol triphosphate and diacylglycerol. Inositol triphosphate promotes intracellular calcium mobilization, while diacylglycerol stimulates the activity of protein kinase C (Exton, 1996).

5-Hydroxytryptamine$_{2A}$ (5-HT)$_{2A}$ receptors are a subtype of the 5-HT2 subfamily of receptors, known to stimulate the PLC pathway via $G\alpha_q$ (Chang et al., 2000). 5HT_{2A} receptors are widely

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distributed in brain, including cortex, caudate nucleus, olfactory tubercle, nucleus accumbens, and hippocampus. They are believed to be involved in many peripheral, as well as, central nervous system functions such as smooth muscle contraction, platelet aggregation, cognition and mood, and in psychiatric disorders such as depression and schizophrenia (for review, see Barnes and Sharp, 1999). Furthermore, there is now vast evidence from biochemical, electrophysiological, and behavioral studies that hallucinogens, such as lysergic acid diethylamide (LSD), have a key site of action as agonists at 5-HT_{2A} receptors in the brain (for review, see Aghajanian and Marek, 1999;Marek and Aghajanian, 1996;Nichols, 2004). Cortical pyramidal neurons constitute the major 5-HT_{2A} receptor-expressing neurons, where the receptors are located presynaptically on glutamatergic nerve terminals as well as postsynaptically on pyramidal neurons. It has been suggested that the “positive” hallucination-like symptoms observed in acute schizophrenia may be related to a dysfunctional 5-HT_{2A} receptor signaling system in apical dendrites of pyramidal cells (Jakab and Goldman-Rakic, 1998). In addition, clozapine, the classical atypical antipsychotic drug, is a 5-HT_{2A} receptor antagonist (Meltzer et al., 1989). These data suggest that 5-HT_{2A} receptors are important mediators of the behavioral effects induced by hallucinogenic drugs and behavior abnormalities in psychiatric disorders.

The hallucinogen (±)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a highly selective 5-HT2 agonist (McClue et al., 1989), has been utilized to evaluate the role of 5-HT_{2A} receptors in many biochemical and behavioral responses in rodents (Darmani et al., 1990;Leslie et al., 1993;Mazzola-Pomietto et al., 1995). Acute administration of DOI elicits a 5-HT_{2A} receptor-dependent induction of the immediate early gene *c-fos* and its protein FOS in the rat cortex (Leslie et al., 1993;Scruggs et al., 2000;Tilakaratne and Friedman, 1996). In mouse behavioral assays, DOI has been shown to exhibit anxiolytic-like properties in anxiety paradigms such as the four plates test and the elevated plus maze (Nic Dhonnchadha et al., 2003;Onaivi et al., 1995). Moreover, activation of 5-HT_{2A} receptors by DOI evokes a head-twitch response in both rats and mice (Dursun and Handley, 1996;Kleven et al., 1997;Schreiber et al., 1995).

The precise signal transduction pathway involved in the *in vivo* effects of hallucinogenic drugs is unknown. The objective of the present study is to evaluate the role of G_q proteins in hallucinogen-induced *in vivo* effects. As a tool to assess the role of the G_q signaling pathway, we have made use of genetically modified mice in which the gene encoding for the α-subunit of G_q protein has been eliminated [$G\alpha_q(-/-)$].

METHODS

Animals

$G\alpha_q(-/-)$ mice were generated by mating heterozygous [$G\alpha_q(+/-)$] males and heterozygous females to obtain wild-type and knock-out littermates. Mice were kept on a C57BL/6x129/Sv background, and their genotype was determined by PCR of genomic DNA from tail samples as previously described (Offermanns et al., 1997). Although $G\alpha_q(-/-)$ mice exhibit signs of ataxia and motor incoordination, their peripheral and central nervous system morphology is largely undisturbed (Offermanns et al., 1997). Animals had free access to food and water, and were maintained on a 12:12 light/dark cycle. An equal number of male and female mice were used for experimental studies; the mice were 6–8 weeks of age at the time of testing. C57BL/6 mice (Harlan. Indianapolis, IN) were utilized for control experiments evaluating the role of 5-HT_{2A} receptors in the *in vivo* effects of DOI. All experiments were done in compliance with the guide *Principles of Laboratory Animal Care* (NIH publication No. 85-23) and the Vanderbilt University Animal Care and Use Committee.

FOS Immunohistochemistry

Mice were anesthetized with 150 mg/kg pentobarbital i.p. before transcardiac perfusion with 30 ml of 0.1 M phosphate-buffered saline (PBS) followed by 30 ml of 4% paraformaldehyde in 0.1M PBS. Brains were removed immediately, post fixed in paraformaldehyde overnight at 4°C, and then transferred to increasing concentrations of sucrose. Coronal sections were cut at 40μm thickness on a vibratome and collected into buffer containing 30% ethylene glycol, 30% glycerol, 10% 0.1M PBS, and 30% water. Immunohistochemistry was performed on free floating sections. Sections were preincubated for 30 min in 0.3% hydrogen peroxide, washed 3 times with PBS, and then incubated for one hour in 5% goat serum/2% Triton X-100 to block non-specific binding. Sections were incubated for 48h at 4°C in anti-FOS primary antibody (Oncogene Research Products) diluted in blocking solution 1:30,000. Following 3 washes in PBS, a Vectastain Elite ABC horseradish peroxidase kit (Vector Labs) was used for the secondary antibody and avidin–biotin complex steps. The colorimetric detection reaction produced 3-3′-diaminobenzidine tetrahydrochloride (DAB) as a brown chromagen product. Sections were then washed, mounted on slides, dehydrated through a series of solutions with increasing ethanol concentrations, treated with xylenes, and coverslipped with Mounting Medium (Richard-Allan Scientific. Kalamazoo, MI)

Analysis of FOS-Li positive nuclei

Brain sections containing medial prefrontal cortex were analyzed as described previously (Gresch et al., 2002). Briefly, bright field images were taken using Openlab 2.2.5 software (Improvision. Lexington, MA) with a Coolsnap cf camera (Photometrics. Tucson, AZ) mounted on a Zeiss Axioverts S100 microscope. All settings were kept constant throughout the image collection process. Analysis and quantification of images were performed using Image J 1.33u (Wayne Rasband, NIH) in mPFC sections that correspond to AP +1.34mm, ML +0.3mm, and DV −2.25mm relative to bregma (Franklin and Paxinos, 1997). An image of 600μm × 450 μm area was analyzed for the number of FOS-Li positive nuclei. Cells with brown black nuclei were considered positive FOS-Li. These were determined by using the particle count macro in Image J 1.33u where the pixel density threshold had been set four times above background levels.

Radioligand binding

Frontal cortex was dissected and homogenized in binding buffer (50mM Tris and 10mM $MgCl_2$, pH 7.4). The homogenate was centrifuged at 20,000g for 20 min at 4°C, and the pellet was resuspended in binding buffer. Protein concentration was determined with Bio-Rad protein assay dye reagent (Hercules, CA). Membrane preparations (200μg/sample) were incubated with [^{3}H]-ketanserin (10nM) for 30 min at 37°C. Nonspecific binding was determined with 10μM methysergide. Following incubation, free radioligand was separated from bound by vacuum filtration through Whatman GF/C glass filters (Brandel, Gaithersburg, MD). Filters were placed in vials and counted in a liquid scintillation counter.

Elevated Plus Maze

The elevated plus maze (Hamilton-Kinder. San Diego, CA) consisted of two open arms (37.5 × 5.0 × 0.25 cm) and two closed arms (37.5 × 5.0 × 15 cm) emanating from a central platform (5cm × 5cm) to form a plus shape. The maze was built from black Plexiglas, and equipped with infrared photobeams. The entire maze was elevated 45 cm above the floor. Light beam breaks were recorded and analyzed automatically by Motor Monitor software (Hamilton-Kinder).

Animals were transported to the experiment room, and following a habituation period of 15 min, they were injected i.p. with drug, and placed back into their home cages for 30 min after

DOI or 5 min after ethanol. Animals were individually placed into the central platform of the plus maze and allowed 5 min of free exploration. Time and percent time spent in the open arms were used as an index of anxiolytic-like effects, and total distance traveled was used as a measure of general activity. After each session, the plus maze was cleaned with 30% ethanol and allowed to completely air dry prior to placing the next animal for testing.

Head-twitch Response

The head-twitch response is a distinctive behavior characterized by a rapid, rotational movement of the head, ears, and neck. Mice were injected i.p and immediately transferred to a 3000 mL glass beaker lined with pinedust bedding for observation. Head-twitches were counted, in consecutive 5 min bins, for 30 minutes following drug administration by two observers (one of them blind to the treatment) with over 95% inter-rater reliability.

Drugs

Drugs were administered i.p. in an injection volume of 10 ml/kg. All drugs were diluted in 0.9% saline solution. Ethanol concentration, 15% (w/v), was obtained by diluting absolute ethanol (AAPER. Shelbyville, KY) with saline. DOI was purchased from Sigma-Aldrich, and MDL 100907 was a gift from Marion Merrell Dow (Cincinnati, Ohio).

Statistical Analyses

All data are presented as mean ± S.E.M. The effects of DOI and ethanol in the elevated plus maze, and the effects of DOI in the head-twitch response test were compared by two-way analysis of variance (ANOVA) followed by Bonferroni post-hoc tests. The effects of MDL100907 were analyzed by a one-way ANOVA followed by Tukey's multiple comparison tests.

RESULTS

Role of 5-HT_{2A} receptors in DOI-induced behaviors

Consistent with earlier reports (Nic Dhonnchadha et al., 2003), DOI significantly increased open arm activity in the elevated plus maze. As illustrated in fig 1A, pretreatment with the highly selective 5-HT_{2A} receptor antagonist MDL 100907 abolished the anxiolytic-like effects of DOI on elevated plus maze behavior (i.e., MDL-DOI does not differ from Sal-Sal). In an additional experiment, we showed that MDL-DOI does not differ from MDL-Sal, confirming that the effects are not mediated by some independent effect of MDL100907. As illustrated in fig 1B, DOI induced a robust head-twitch response in mice, which was also completely blocked by pretreatment with the selective 5-HT_{2A} receptor antagonist MDL100907.

Characterization of DOI-induced FOS expression in mice

The induction of FOS by DOI is a reliable marker for 5-HT_{2A} receptor activation. The effect of DOI on FOS expression has not been previously evaluated in C57BL/6 mice. We therefore performed control pharmacological experiments to establish the potency of DOI to induce FOS expression in the medial prefrontal cortex (mPFC). DOI (0, 0.3, 3, 10 mg/kg, i.p. 3 hours pre-test) elicits a dose-dependent induction of FOS-Li nuclei in the mPFC (fig. 2A) with an EC_{50} value of 5.6 mg/kg. Pretreatment with the 5-HT_{2A} receptor antagonist MDL 100907 blocked the ability of DOI (5mg/kg) to induce FOS-Li expression in the mPFC (fig. 2B). In an additional experiment, we found that treatment of mice with MDL 100907 alone had no effect on FOS-like positive cells in the mPFC (15.4±5 for Sal-Sal vs 21.0±2 for MDL-Sal; n=6).

$G\alpha_q$ is required for the anxiolytic-like effect of DOI in the elevated plus maze

The anxiolytic-like effect of DOI was compared in the elevated plus maze using wild-type and $G\alpha_q(-/-)$ mice. Although DOI elicited an increase in both time spent in the open arms ($p<0.01$) (fig. 3A) and percent open arm time ($p<0.01$) (fig. 3B) in wild-type littermates, this effect of DOI was abolished in mice deficient for $G\alpha_q$. DOI treatment did not alter percent open arm entries in either genotype. As a positive control, ethanol (1.5 g/kg, i.p. 5 min pre-test) was shown to significantly increase the time spent in the open arms (fig. 3A), as well as percent open arm time (fig. 3B), in both wild-type and $G\alpha_q$ knockout mice. These effects were independent of activity changes; there was no difference in total distance traveled between $G\alpha_q(-/-)$ and wild-type littermates following any of the treatment conditions (fig. 3C).

As an additional control, we compared [^{3}H]-ketanserin binding in wild-type and $G\alpha_q(-/-)$ mice to determine whether altered 5-HT_{2A} receptor expression could explain the behavioral difference. There was no difference in binding of a maximum concentration of [^{3}H]-ketanserin (10nM) between $G\alpha_q(-/-)$ and wild-type littermates (500±40 fmole/mg protein for $G\alpha_q(-/-)$ vs 486±64 fmole/mg protein for WT, n=6; $p>0.05$)

DOI-induced head-twitch response is decreased in $G\alpha_q$ knockouts

Intraperitoneal administration of DOI elicited a dose-dependent head-twitch response in wild-type mice which peaked at 10 minutes (fig. 4A). This response was significantly blunted in mice deficient for $G\alpha_q$ ($p<0.05$ for 1.0 mg/kg DOI. $p<0.001$ for 2.5 mg/kg DOI) (fig. 4B). The percent decrease was comparable for the two doses of DOI (40% for 1mg/kg vs 35% for 2.5 mg/kg). Thus the head-twitch response appears to be less sensitive to a loss of $G\alpha_q$ than is the elevated plus maze. This conclusion is supported in heterozygous $G\alpha_q(+/-)$ mice, in which $G\alpha_q$ protein is reduced by 50% (Offermanns et al., 1997). The DOI-induced head-twitch response was reproduced in $G\alpha_q(+/-)$ mice; however, DOI effects in the elevated plus maze were completely eliminated in $G\alpha_q(+/-)$ knockouts (data not shown). In an additional experiment, we determined that MDL 100907 blocks the DOI-induced head-twitch response in $G\alpha_q(-/-)$ mice (44±14 head-twitches for Sal-DOI vs 4±1.3 head-twitches for MDL-DOI; n=6), confirming that DOI is acting through 5-HT_{2A} receptors to elicit head twitch behavior in $G_q(-/-)$ mice.

Cortical FOS expression is reduced in $G\alpha_q(-/-)$ mice

Based on the previous dose response data, an EC_{50} dose of DOI (5 mg/kg) was utilized. DOI markedly increased the number of FOS-Li positive nuclei in the medial prefrontal cortex of wild-type mice ($p<0.001$). This increase was abolished in $G\alpha_q(-/-)$ mice (fig. 5).

DISCUSSION

5-HT_{2A} receptors are known to be a key site of action for hallucinogenic drug action in both humans and laboratory animals. There is abundant evidence from biochemical, electrophysiological, and behavioral studies in rats that hallucinogens, including LSD and DOI, are potent partial agonists at 5-HT_{2A} receptors in the central nervous system (Aghajanian and Marek, 1999;Sanders-Bush et al., 1988). Furthermore, correlations between human hallucinogenic potency and 5-HT_{2A} receptor binding affinity are consistent with the hypothesis that classical hallucinogens exert their hallucinogenic effects through 5-HT_{2A} receptors (Glennon et al., 1984). This conclusion was confirmed recently in genetically modified mice lacking the 5-HT_{2A} receptor (Gonzalez-Maeso et al., 2007). In addition, our studies with DOI in mice suggest a major role for the 5-HT_{2A} receptor in the elevated plus maze and head-twitch response (present study), as well as in drug discrimination (Smith et al., 2003).

5-HT_{2A} receptors are known to couple to multiple G-proteins including G_q, G_{11}, and G_{13} to mediate a wide array of second messenger signaling pathways (Berg et al., 1998;Kurrasch-Orbaugh et al., 2003;Robertson et al., 2003); however, the classical pathway associated with 5-HT_{2A} receptor signaling is stimulation of PLCβ;via $G\alpha_q$ protein. In the present study, we evaluated the role of the $G\alpha_q$ protein in several behavioral and biochemical assays, including the elevated plus maze, head-twitch response, 5-HT_{2A} receptor binding, and *c-fos* immunohistochemistry.

The current studies demonstrate that activation of the G_q signaling pathway is required for the mediation of the anxiolytic-like effects of DOI in the elevated plus maze. DOI significantly increased time spent in the open arms, as well as percent open arm time in wild-type animals; however, these effects were absent in $G\alpha_q$ knockout mice. Baseline performance on the maze and total exploratory activity did not differ between $G\alpha_q$ knockout mice and wild-type controls suggesting that deletion of the α subunit of G_q does not produce alterations in overall anxiety-related behavior. As an additional, positive control to determine whether the absence of DOI effects in $G\alpha_q$ knockouts was due to non-specific, ectopic effects or developmental abnormalities affecting anxiety-related behavior, we evaluated ethanol, known to exert its anxiolytic-like effects through the ionotropic GABA-A receptor system (Durcan and Lister, 1988;Prunell et al., 1994). The ability of ethanol treatment to significantly increase open arm activity in the elevated plus maze for wild-type mice was reproduced in $G\alpha_q$ knockouts, suggesting that the behavioral deficits in $G\alpha_q(-/-)$ mice are specifically related to the loss of the α-subunit of the G_q protein. Additional experiments using conditional knockouts or knockins are needed to entirely rule out developmental issues.

As with the elevated plus maze, the DOI-induced head-twitch response was blunted in $G\alpha_q(-/-)$ mice; however, unlike the elevated plus maze results, DOI still produced a significant head-twitch response in the knockout mice. Thus it appears that the G_q signaling pathway is not the sole mediator of the 5-HT_{2A} receptor dependent behavioral effects of DOI. G_q and G_{11} proteins are close structural and functional analogs that substitute for each other in the intracellular signaling cascade. Although these two proteins coexist, G_q expression exceeds G_{11} throughout the brain (Milligan, 1993), including cortical and midbrain areas that mediate these behaviors. Compensatory changes, for instance, in G_{11} or in other components of the signaling complex, such as RGS proteins, may differ within the relevant brain sites, leading to the differential sensitivity of the two behaviors. Alternatively, some other signaling pathway, e.g., G_{13} activation of PLD (McGrew et al., 2002;Kurrasch-Orbaugh et al., 2003) or $G_{i/o}$ activation of the *erg* signaling pathway (Gonzales Maeso et al., 2007), may contribute to the head-twitch behavior. How this differential behavioral sensitivity in G_q null mice relates to human hallucinogenic drug experience is not known. Recent studies of psilocybin in humans suggest that anxiety is a significant symptom (Griffiths et al., 2006), although presumably unrelated to the unique psychedelic experience. The head-twitch response is a stereotypical behavior that is a well accepted behavioral model of 5-HT_{2A} receptor activation in rodents (Dursun and Handley, 1996;Kleven et al., 1997;Schreiber et al., 1995). Although head-twitch behavior is difficult to relate to the human hallucinogenic experience, recent evidence that the head-twitch behavior is unique to 5-HT_{2A} receptor agonists with hallucinogenic properties (Gonzalez-Maseo et al., 2007) suggests it is a useful model. The introceptive cues, responsible for drug discrimination in rodents, are believed to model the subjective effects of drugs in humans. This is based largely on extensive studies showing that discriminative stimuli of psychoactive drugs in rats closely parallel the subjective effects reported by humans (Altman et al., 1976;Barry, 1974). Additional behavioral studies characterizing the hallucinogen-induced drug discriminative cue in $G\alpha_q(-/-)$ mice are planned.

The current studies also demonstrate that $G\alpha_q$ is required for DOI-induced FOS expression. Following DOI administration, wild-type mice exhibited a robust increase in the number of

FOS-Li positive nuclei in the mPFC; however this increase was abolished in $G\alpha_q(-/-)$ littermates. Since the DOI-induced FOS expression is mediated by activation of 5-HT_{2A} receptors, and 5-HT_{2A} receptors couple to G_q protein, a logical conclusion is that these two events are directly related. However, G_q protein is coupled to many neurotransmitter receptors, making it impossible to rule out the alternative interpretation that some other receptor's interaction with G_q is the relevant point of intervention leading to a disruption of the DOI-induced effects. Given that the 5-HT_{2A} receptor-G_q protein pathway is the likely signaling pathway, it is interesting that Mackowiak et al. (2002) reported that activation of phospholipase A_2 via 5-HT_{2A} receptors is engaged in the mechanism of DOI-induced FOS expression in the rat cortex. Since DOI-induced FOS expression is essentially eliminated in $G\alpha_q(-/-)$ mice, this suggests that phospholipase A_2 activation may be downstream of G_q activation in mice. Additional studies of the phospholipase C and phospholipase A_2 pathways in mice deficient for $G\alpha_q$ may enhance our understanding of the molecular mechanisms responsible for DOI's effects.

In conclusion, the present study provides evidence that activation of the G_q protein pathway, downstream of 5-HT_{2A} receptors, is a key signaling mechanism involved in the mediation of hallucinogen-induced behavioral effects. One of the most striking findings is the difference in sensitivity of two behaviors in $G\alpha_q$ null mice. DOI fails to elicit anxiolytic-like behavior in mutant mice, whereas the head-twitch response to DOI is reduced less than 50%, suggesting that other mechanisms are equally important for mediating this behavior. It is known that different brain sites mediate these behaviors (Graeff et al., 1993;Willins and Meltzer, 1997), and it is possible that 5-HT_{2A} receptors within these brain sites are differentially coupled to G protein signaling pathways or that different compensatory mechanisms exist. Importantly, our studies suggest that the G_q signaling pathway is required for the full expression of hallucinogen-induced behaviors.

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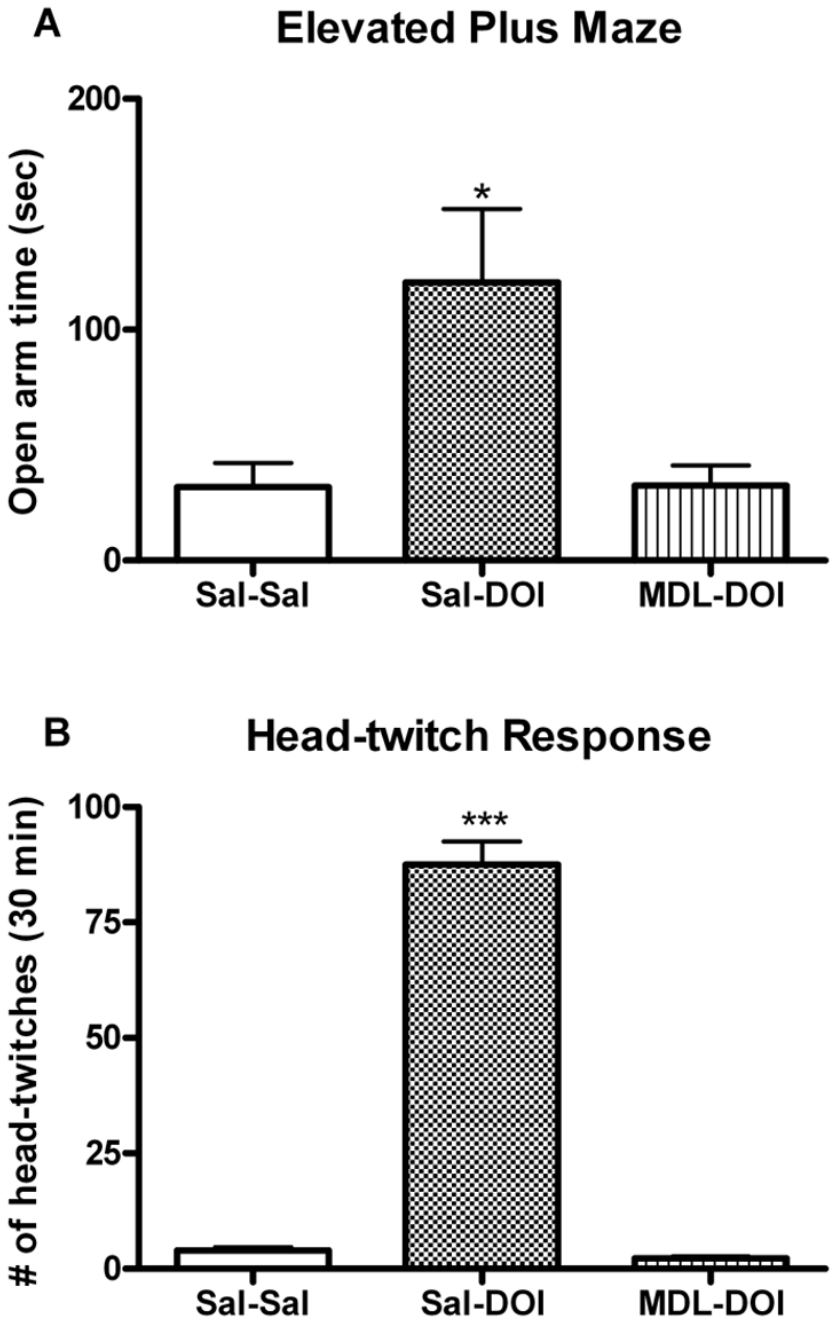

Fig 1.

Effect of pretreatment with MDL100907 on DOI-induced behaviors. **A** Anxiolytic-like effects of DOI (2.5 mg/Kg, i.p. 30 min pre-test) are prevented by pretreatment with the 5-HT_{2A} receptor antagonist MDL100907 (0.25 mg/Kg, i.p. 50 min pre-test) **B** DOI-induced head-twitches counted during a 30 min observation period were inhibited by pretreatment with MDL100907 (0.25 mg/Kg, i.p. 20 min pre-test). Data shown as means ± S.E.M., n= 6 per group. (*)$p<0.05$, (***)$p<0.001$ relative to saline control group determined by a one-way ANOVA.

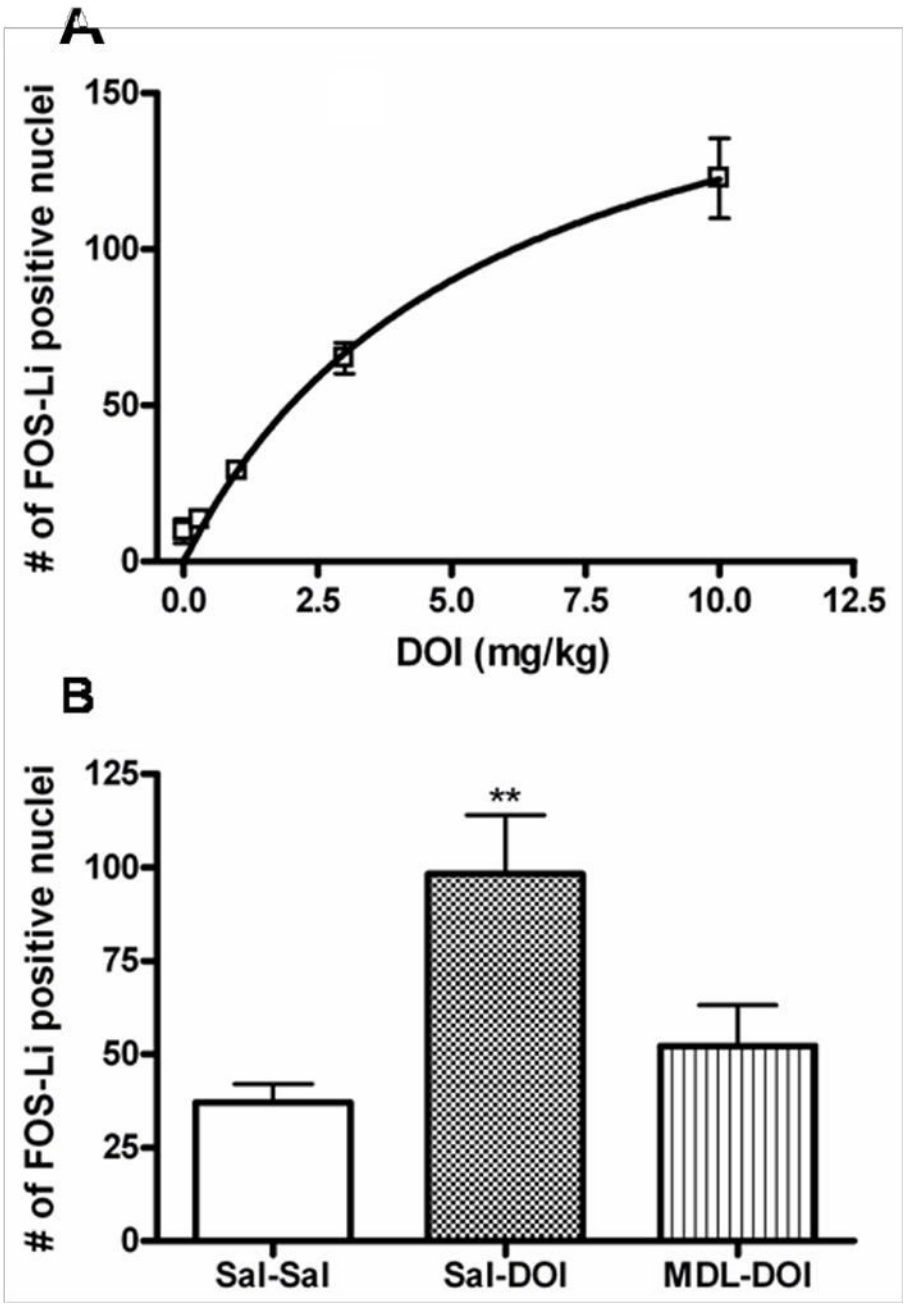

Fig 2.

Quantitative analysis of FOS-Li positive nuclei in the mPFC of C57BL/6 mice. **A** Dose response of DOI-induced FOS expression in the mPFC, n=3. **B** DOI (2.5 mg/Kg, i.p.) induced FOS-Li expression is inhibited by pretreatment with MDL100907 (0.25mg/kg, i.p. 20 min pre-DOI). Data shown as means ± S.E.M., n= 6. (**) $p<0.01$ relative to saline (Sal-Sal) determined by a one-way ANOVA.

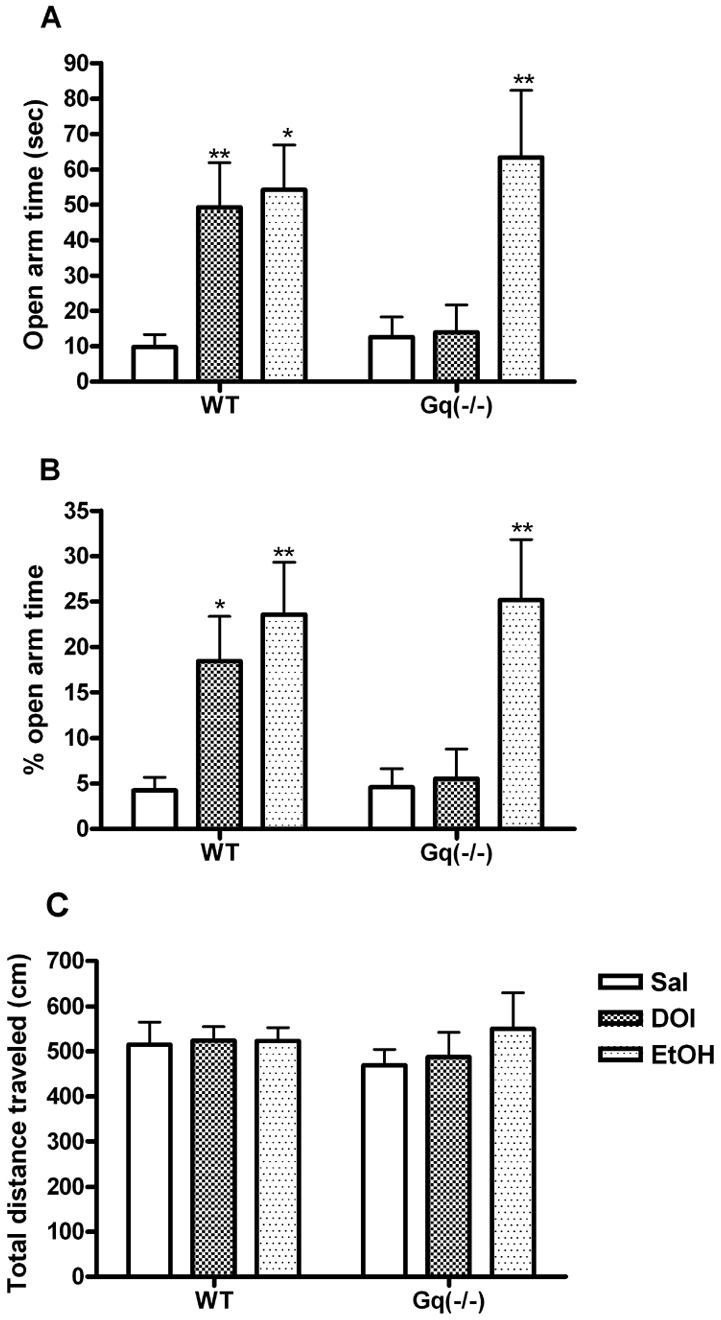

Fig 3.

Anxiolytic-like activity of DOI on the elevated plus maze is absent in mice deficient for $G\alpha_q$. **A** Time spent in the open arms, and **B** % time spent in the open arms, as a percentage of time spent in the open and close arms (center excluded) during the 5 min test session following administration of DOI (2.5 mg/kg, i.p. 30 min pre-test) or ethanol (1.5 g/kg, i.p. 5 min pre-test). **C** Exploratory activity measured in total distance traveled during the elevated plus maze test. Data shown as means ± S.E.M., n= 7–12 per group. (*) $p<0.05$, (**) $p<0.01$ relative to saline control group determined by a two-way ANOVA.

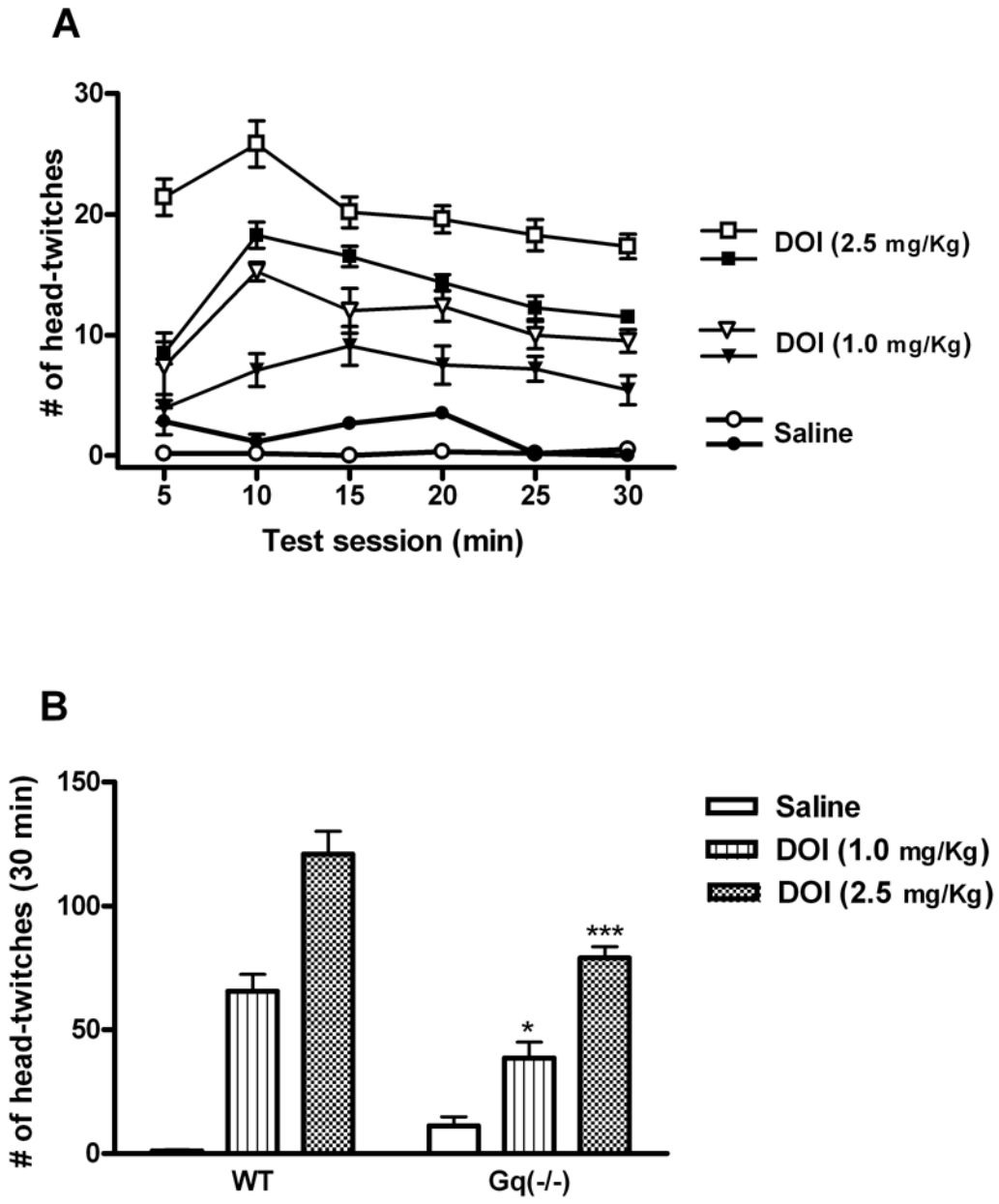

Fig 4.

DOI-induced head-twitch response is reduced in $G\alpha_q$ knockouts. Mice were injected with DOI (1.0 mg/Kg or 2.5 mg/Kg, i.p.) prior to a 30 min observation period. **A** Number of head twitches in 5 min bins (open symbols represent WT mice, closed symbols represent $G\alpha_q(-/-)$ littermates). Main effect of genotype, $p<0.0001$. **B** Each column represents the total number of head-twitches counted during 30-min test time. Data shown as means ± S.E.M., n= 6–7 per group. (*) $p<0.05$, (***) $p<0.001$ relative to respective wild-type control groups determined by a two-way ANOVA..

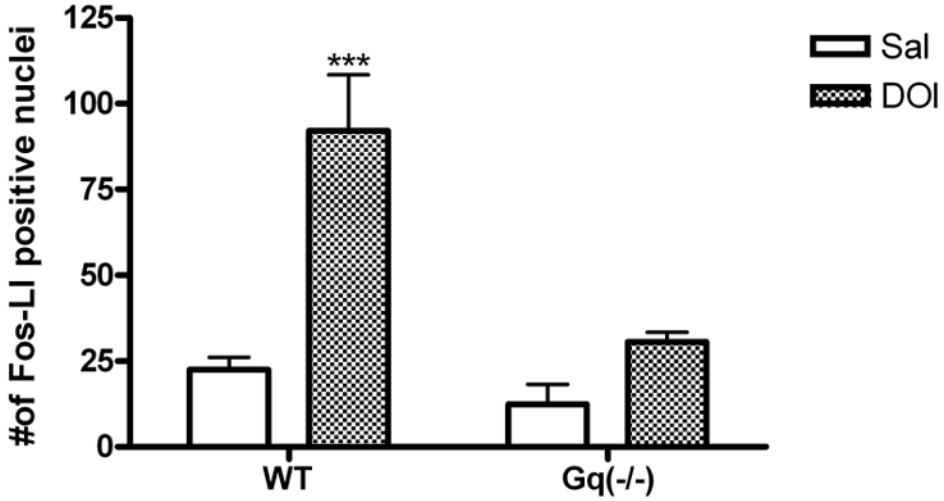

Fig 5.

Quantitative analysis of number of FOS-Li positive nuclei in the medial prefrontal cortex of wild-type and $G\alpha_q(-/-)$ mice. Values are numbers of FOS-Li positive cells (mean ± S.E.M. within area of analysis; n=6). DOI-induced FOS expression in the medial prefrontal cortex is abolished in $G\alpha_q(-/-)$ mice. (***) $p<0.001$ relative to saline control group determined by a two-way ANOVA.