ARTICLE

Several behavioral traits relevant for alcoholism are controlled by ɣ2 subunit containing GABA$_A$ receptors on dopamine neurons in mice

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The risk factors for developing alcohol addiction include impulsivity, high sensitivity to the rewarding action of ethanol, and low sensitivity to its sedative and intoxicating effects. Genetic variation in GABA$_A$ receptor subunits, including the ɣ2 subunit (Gabrg2), affects the risk for developing alcoholism. Alcohol directly potentiates GABA$_A$ receptors and activates the mesolimbic dopamine system. Here, we deleted Gabrg2 selectively in dopamine cells of adult mice. The deletion resulted in elevated firing of dopamine neurons and made them less sensitive to drugs acting at GABA$_A$ receptors. At the behavioral level, the deletion increased exploratory behavior and augmented both correct and incorrect responding in the go/no-go task, a test often used to assay the response inhibition component of impulsivity. In addition, conditioned place preference to alcohol, but not to cocaine or morphine, was increased. Ethanol-induced locomotor activation was enhanced in the mice lacking Gabrg2 on dopaminergic cells, whereas the sedative effect of alcohol was reduced. Finally, the alcohol drinking, but not the alcohol preference, at a high concentration was increased in the mutant mice. In summary, deletion of Gabrg2 on dopamine cells induced several behavioral traits associated with high risk of developing alcoholism. The findings suggest that mice lacking Gabrg2 on dopaminergic cells could be used as models for individuals at high risk for developing alcoholism and that GABA$_A$ receptors on dopamine cells are protective against the development of excessive alcohol drinking.

INTRODUCTION

Excessive intake of alcohol causes considerable morbidity and mortality [1]. Whereas many people drink alcohol occasionally, only a minority escalate their intake and develop alcohol addiction, here equated with alcoholism. The heritability of alcoholism is 50–70% [1]. This genetic susceptibility to a large extent operate through intermediate characteristics, such as impulsivity [2], high sensitivity to the rewarding effects of alcohol [3, 4], and low sensitivity to the sedative and intoxicating effects [5, 6].

Human gene association studies have identified strong linkage between variations in genes encoding GABA$_A$ receptor (GABA$_A$R) subunits and alcoholism [7]. For example, variants of the α2 subunit (GABRA2) and the ɣ2 subunit (GABRG2) genes have been associated with increased risk of alcohol abuse [8, 9]. GABA$_A$Rs are one of the best studied direct targets of ethanol. Many functional features of the GABA$_A$Rs, including ethanol sensitivity, depend on their subunit composition [7, 10–12].

Ethanol induces dopamine release in the ventral striatum of both rodents [13] and humans [14, 15]. In rodents, it has been shown that alcohol activates dopaminergic cells both in vitro [16, 17] and in vivo [16] and that the activation of dopamine cells at

least partially mediate the reinforcing effects of alcohol [1, 18]. Whereas the exact mechanisms behind the activation of dopamine cells are not clear, it most likely involves the modulation of GABAergic input onto dopaminergic cells [1, 19, 20]. Collectively, these findings suggest that the subunit composition of GABA$_A$Rs on neurons of the dopaminergic reward system might be critical for shaping the response to alcohol and underpin the individual variability in the risk of developing alcoholism. Here, we generated mice selectively lacking Gabrg2 subunits on dopamine cells. We then examined their dopamine cells with emphasis on GABA responsiveness and their behavior with emphasis on phenotypes associated with risk for alcoholism.

MATERIALS AND METHODS

Animals

All experiments followed international and national guidelines and were approved by local animal care and use committees. The mouse line with floxed Gabrg2 was acquired from Jackson Laboratories (stock no: 016830). The DATCreER line has been described previously [21]. The animals were single-housed for a

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Several behavioral traits relevant for alcoholism are contro…
A Stojakovic et al.

1549

minimum of 24 h prior to the experiments and kept in a pathogen-free facility on a regular 12-h light/dark cycle. Only male mice were used. All mice were >10 weeks old at the onset of the behavioral or electrophysiological experiments, and the typical age was 10–20 weeks. Food and water were provided ad libitum, and all experiments were performed during the light phase, unless otherwise noted. The Cre-recombinase activity was induced by administering tamoxifen dissolved in sunflower seed oil/alcohol mixture 10:1. The mixture was intraperitoneally (i.p.) injected at 1 mg per mouse twice a day for 5 days to both Cre-positive and Cre-negative mice. Experiments were initiated at earliest 3 weeks after tamoxifen administration. The mice had a mixed 129x1/SvJ × C57BL/6J background (~50% of each). At the start of the behavioral experiments, there was no difference between the genotypes in body weight (Fig. S4A).

Electrophysiology

The electrophysiological experiments were performed using previously published protocols [22]. Briefly, mice were anesthetized with urethane and neuronal activity was extracellularly recorded. DA-like cells were identified based on electrophysiological criteria [23, 24] and localization in the ventral tegmental area and the substantia nigra (Figs. S1, S2). The responses of DA-like neurons to bicuculline and muscimol were tested by local, iontophoretic application of the drugs. All technical details are described in the Supplemental Methods.

Drugs

Ethanol was dissolved in 0.9% saline and injected i.p. in a volume of 0.1 ml per 10 g of body weight. Control mice received a similar volume of saline. Mice were injected with the dose of either 1 g/kg (for ethanol-induced locomotion), 2 g/kg (for conditioned place preference (CPP)), or 3.6 g/kg (for loss of righting reflex). Cocaine hydrochloride (APL, Kungens Kurva, Sweden) was dissolved in 0.9% saline and administered in a single i.p. injection at a dose of 15 mg/kg. Morphine hydrochloride (Biophausia AB, Stockholm, Sweden) was injected i.p. at 10 mg/kg.

Behavioral studies

Open-field test. Locomotor activity was assessed by placing individual mice in an open-field arena (45 × 45 cm), and recording their activity for 15 min with a video camera placed from the top of the arena. The software EthoVision (Noldus) was used for data collection and processing, which provided measures of the total distance traveled and the time mice spent in center of the arena.

Elevated plus maze test. The protocol used was based on previously published studies [25, 26]. The test was performed during the dark phase, ~1–2 h after lights were switched off. The apparatus consisted of two open (25 × 5 × 0.5 cm) and two closed arms (25 × 5 × 16 cm) that were perpendicularly connected with central platform (5 × 5 × 0.5 cm) [27]. The apparatus was elevated 50 cm above the ground. In order to decrease the number of animals falling down from the apparatus, open arms had a low raised lip (0.5 cm) around the edges. The test was performed in a brightly illuminated room. The mice were placed on the central platform facing the open arm and their behavior was recorded for 5 min. Video files were analyzed by EthoVision tracking system and the percentage of time mice spent in the open arm was quantified.

Forced swim test. The forced swim test [28] was conducted in glass cylindrical beakers (30 cm H × 20 cm diameter) with water temperature set at 25 ± 0.5 °C. The mice were placed in the beakers and videotaped by a camera set in front of beakers for 6 min. Subsequently, they were taken out and dried with paper towels before being placed back in their original cages. Water was replaced between each session and temperature adjusted.

Recorded video files were analyzed by EthoVision software with immobility threshold set to 20%. Results were expressed as percentage of time mice spent immobile.

Reciprocal social interaction test. This test was used as an indicator of social exploration and the protocol was adopted from previous studies [29]. The mice were allowed to freely interact in novel arena and the time they spent interacting was measured. Interaction time for each mouse in the pair is directly impacted by the behavior of the partner animal. Prior to the experiment, mice were single-housed for at least 2 days. At the day of experiment, male mice of similar age and weight (one control and one transgenic) were placed in a larger cage that was brightly illuminated and unfamiliar to the subjects. A camera was mounted above the cage and interaction was videotaped for 10 min. Recorded video files were manually scored for the time mice individually spent in active social behavior, such as following, sniffing, and climbing on or under the other mouse. Aggressive behaviors were not considered as social behavior.

Sucrose preference test. Sucrose preference was assessed as described before [30]. Prior experiment, mice were single-housed for at least 3 days and habituated to the two bottles (50 ml Falcon tubes with nibbles) containing water for 2 days. In the next 2 days, both bottles were replaced with 1% (w/v) sucrose dissolved in water. On the fourth day, mice were offered one bottle of water and one of sucrose and preference was measured over the course of next 4 days. Bottles were switched daily to exclude possible side bias. Consumed fluid was measured daily. Preference for sucrose was calculated by the following formula: $100 \times (vol_{sucrose} /(vol_{water} + vol_{sucrose}))$ and averaged across the last 4 days.

Go/no-go test. The go/no-go test was performed based on protocols previously used by us [31]. Details of the procedure can be found in the Supplemental Methods. Briefly, water-deprived (1.5 ml per day) mice were trained under continuous reinforcement schedule and were rewarded (10 µl water sweetened with 0.01% (w/v) saccharin) by poking their noses into the active port (with cue-light on). Animals were trained until they reached the criterion of 60 rewarded responses in 45 min. Subsequently, animals underwent two training phases during which they learned to respond to a "go" signal (cue-light in the nose-poke port). Beginning of each trial was signaled by a stimulus light located above the nose-poke port. At the same time, pre-cue period ranging from 9 to 24 s was initiated, after which the "go" signal was presented. Correct "go" response (a "hit") resulted in delivery of 10 µl of saccharin solution. Trials in which animals failed to respond to a "go" signal (a "miss") were punished by 5 s time-out period during which the house light was lit, and after which the 10 s inter-trial interval followed (with the house light off).

Next, animals had to discriminate between "go" (cue-light in the nose-poke port presented for 5 s) and "no-go" signals (cue-light and a continuous 65 dB 2.9 kHz tone presented for 5 s). Responding to the "go" signal and refraining from responding during "no-go" signal presentation (a "correct rejection") was rewarded. Conversely, a nose-poke during the "no-go" signal (a "false alarm") resulted in immediate trial termination and transition into 5 s time-out period, followed by 10 s ITI. Animals were tested for 10 consecutive sessions (each comprising 60 trials), during which "go" and "no-go" signals were presented randomly. A single session contained 30 "go" and 30 "no-go" trials.

Conditioned place preference test. We used a biased place conditioning procedure and 3-chambered Panlab Spatial Place Preference Boxes (Harvard Apparatus). On day 1, during a 15-min pretest, the individual mouse was allowed to move freely between the chambers of the box. Time spent in each compartment was recorded by camera mounted above the apparatus and analyzed

Several behavioral traits relevant for alcoholism are contro…
A Stojakovic et al.

1550
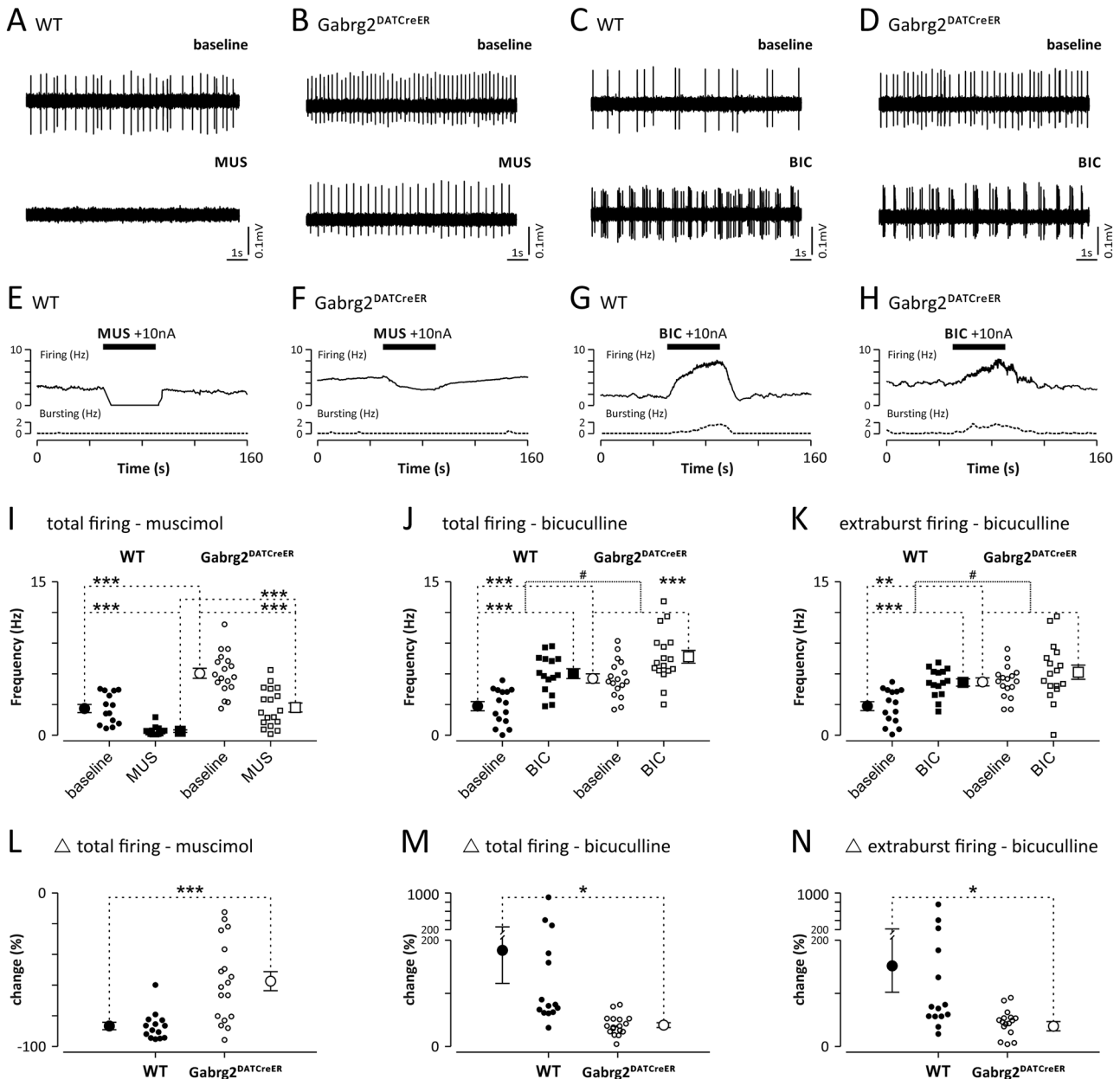
Fig. 1 DA-like neurons of Gabrg2^{DATCreER} mice have impaired reactions to GABA$_A$ receptor agonist and antagonist. **a–d** show raw traces of extracellularly recorded firing of four representative DA-like neurons from WT **a**, **c** and Gabrg2^{DATCreER} mice **b**, **d**. Top traces show firing during baseline conditions. Bottom traces show alternation of firing of the same neurons induced by application of the GABA$_A$ receptor agonist muscimol (MUS) or the GABA$_A$ receptor antagonist bicuculline (BIC). **e–h** show firing and bursting rate histograms of the example neurons shown on panels **a–d**, respectively. Time of drug application is marked with a black bar. Note that muscimol application induced a complete cessation of DA-like neuron firing in WT animals **a**, **e** and only partial reduction of DA-like neuron firing in Gabrg2^{DATCreER} mice. Also increase of DA-like neuron firing induced by application of bicuculline was significantly larger in WT **c**, **g** comparing to Gabrg2^{DATCreER} mice **d**, **h** animal. On **i–k** baseline and drug altered firing of all recorded DA-like neurons in WT (•—baseline, ■—drug) and Gabrg2^{DATCreER} (○—baseline, □—drug) mice, are shown. Note that under baseline conditions, total and extraburst firing rates were significantly higher in mutant comparing to control animals. **l–n** show the relative change in firing induced by muscimol **l**, and bicuculline **m**, **n**. Vertical lines with whiskers indicate the mean value and SEM. * or $^{\#}$ indicates Bonferroni corrected t tests that resulted in $p < 0.05$, ** indicates $p < 0.01$ and *** indicates $p < 0.001$

by EthoVision software. Each mouse had to cross the corridor, entering the opposing chamber a minimum of five times to be included in the experiment. On day 2, in the morning session, mice were treated with saline (i.p.) and confined in the vehicle-assigned chamber for 15 min. Similarly, 4 h after the first injection, mice were injected i.p. with either ethanol, cocaine, or morphine and confined for 15 min in the compartment least preferred during the pretest. This training procedure was conducted for 4 consecutive days, until day 6, when the CPP was assessed by

allowing the mice to freely explore all compartments of the box for 15 min. The preference score was calculated by subtracting the time the mouse spent in the drug-paired chamber during the post-test from that of the pretest.

Alcohol-induced locomotion. During 3 consecutive days, mice were placed in one of the chambers of a Panlab Spatial Place Preference Box (20 × 18 cm, Harvard Apparatus) for 15 min before receiving a saline injection (10 µl/g i.p.). After injection, they were

Several behavioral traits relevant for alcoholism are contro…
A Stojakovic et al.

1551

put back in the chamber for another 15 min. On the fourth day, saline was replaced with an equal volume of ethanol (1 g/kg). Distance moved before and after injection was analyzed with EthoVision software. Difference in locomotion was calculated as distance moved after ethanol-injection day 4 minus distance moved after saline-injection day 3.

Loss of righting reflex. In order to assess the sedative effect of ethanol, mice were injected (i.p.) with 3.6 g/kg of ethanol (as 20% solution dissolved in saline). The time elapsed for mice to become ataxic was measured and then mice were turned on their back. Duration of LORR was measured in minutes. Mice were considered to regain their righting reflex if they were able to right themselves within 60 s [32].

Ethanol drinking. Mice were single-housed for 1 week before starting the experiment and offered two bottles of water in order to acclimatize. Ethanol preference was assessed by introducing one bottle filled with ethanol and another with water. Mice were offered escalating concentrations of ethanol (in tap water) ranging from 3 to 21%. Each ethanol concentration was offered vs. water for 2 days. Fluid intake was measured and positions of the bottles were switched every second day. Body weight was measured every 4 days. Ethanol consumption (g/kg × day) and total fluid consumption were measured [32].

Statistics
For the behavioral analysis, two-tailed Student's *t* test was used when comparing one variable in two groups. For comparing multiple sessions in two groups, two-way repeated measures analysis of variance (ANOVA) was used. Analysis of ethanol consumption was done by two-way ANOVA followed by Sidak's multiple comparisons test. For the electrophysiology, two-way ANOVA followed by Bonferroni multiple comparisons test was used.

RESULTS
We generated mice lacking Gabrg2 in dopamine cells (Gabrg2^{DATCreER} mice) by crossing mice in which critical parts of the gene encoding Gabrg2 were floxed [33], with mice carrying an inducible Cre under the control of the dopamine transporter promoter [21]. This Cre-line has been used in several previous studies and it has been shown to mediate efficient and selective recombination of dopaminergic cells using several different techniques [21, 34–36]. The mutations were induced by tamoxifen administration in mice of at least 7 weeks of age in order to minimize the risk for the development of compensatory mechanisms [21]. Mice with selective deletions induced by Cre were compared to littermates homozygous for the floxed Gabrg2 allele but without Cre (called WT (wild type) in the following text), which were also treated with tamoxifen.
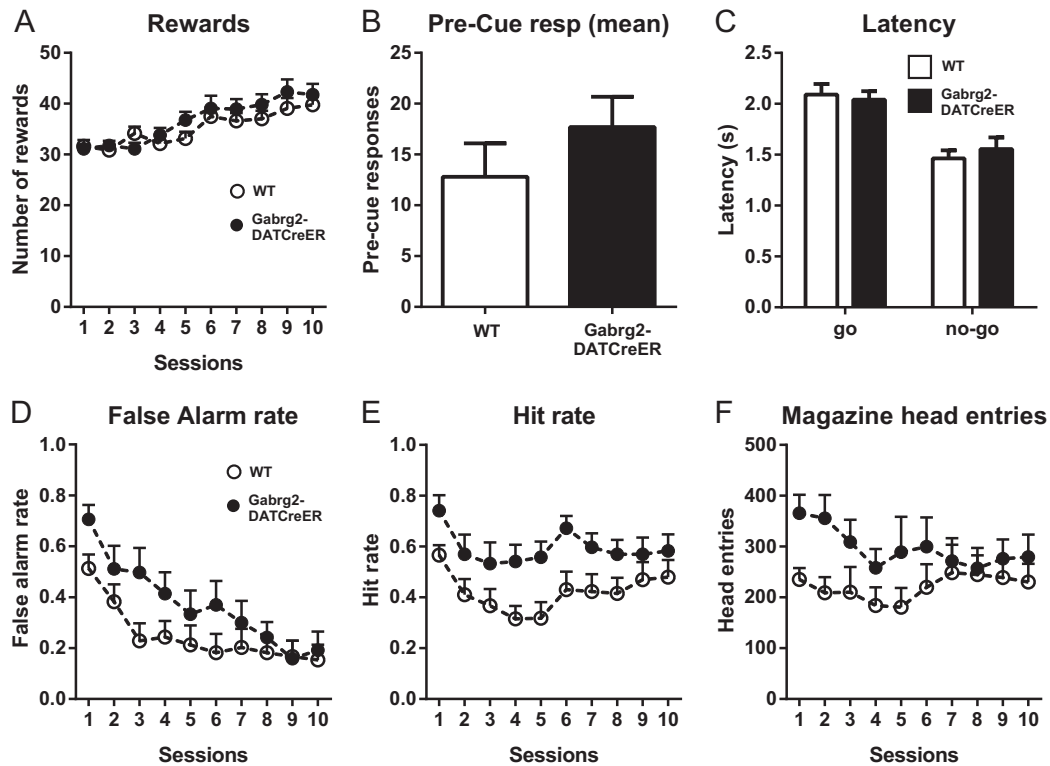
First, we used in vivo electrophysiology to investigate the consequences of the Gabrg2 deletion in the dopamine cells. We recorded spontaneous activity of 15 neurons with the electrophysiological characteristics of dopamine neurons (DA-like neurons) from four WT mice and 18 DA-like neurons from five Gabrg2^{DATCreER} mice. The DA-like neurons in Gabrg2^{DATCreER} mice had significantly higher total (5.6 ± 0.4 Hz vs. 2.9 ± 0.4 Hz; $p <$ 0.001, unpaired Student's *t* test) and extraburst (5.3 ± 0.4 Hz vs. 2.8 ± 0.5 Hz; $p <$ 0.001, unpaired Student's *t* test) firing rates compared to cells in the WT mice. Intensity of the baseline bursting and the burst parameters tended to be higher in the mutant animals but the differences did not reach statistical significance (Fig. S3).

A subset of the recorded neurons was subjected to iontophoretic application of the selective GABA$_A$R agonist muscimol (WT, $n =$ 14; Gabrg2DATCreER, $n = 18$) or the selective GABA$_A$R antagonist bicuculline (WT, $n = 15$; Gabrg2^{DATCreER}, $n = 17$). Both firing rates,
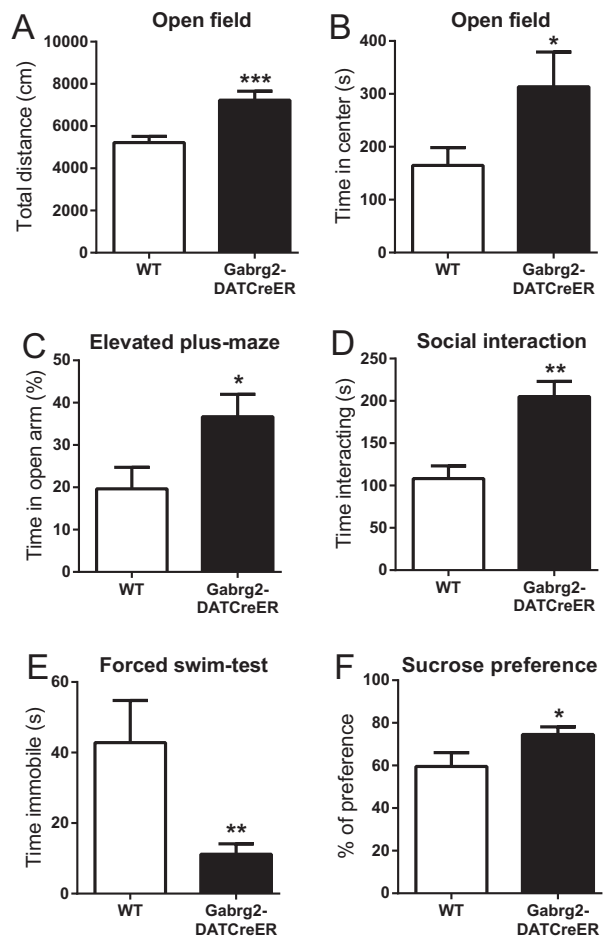
Fig. 2 Basic behavioral testing in Gabrg2^{DATCreER} mice. Behavior in the open-field test. **a** show total distance and **b** show the time spent in the center of the field ($n = 21$ and 16). Behavior in the elevated plus maze is shown in **c** ($n = 13$ and 15) and time spent interacting in the reciprocal social interaction test is shown in **d** ($n = 6$ and 6). **e** show the time spent immobile in the forced swim test ($n = 6$ and 13) and **f** show the sucrose preference in Gabrg2^{DATCreER} and WT mice ($n = 8$ and 14). Error bars represent SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, Student's *t* test

at baseline and after muscimol application, were significantly higher in Gabrg2^{DATCreER} mice (Fig. 1a, b, e, f, i; genotype × drug, $F_{1,30} = 4.031$, $p = 0.06$, genotype, $F_{1,30} = 30.37$, $p < 0.0001$, drug, $F_{1,30} = 101.0$, $p < 0.0001$) and significantly reduced effect of muscimol in the mutant mice was observed (Fig. 1l; $-87 ± 2.5\%$ vs. $-58 ± 6.2\%$; $p < 0.001$, unpaired Student's *t* test). In both groups of animals, iontophoretic application of bicuculline induced a significant increase in the total firing of DA-like neurons (Fig. 1c, d, g, h, j; genotype × drug, $F_{1,30} = 6.94$, $p = 0.01$; genotype, $F_{1,30} = 9.71$, $p = 0.004$; drug, $F_{1,30} = 173.0$, $p < 0.0001$), but the magnitude of the increase was significantly lower in Gabrg2^{DATCreER} mice compared to WT mice (Fig. 1m; $181 ± 63.0\%$ vs. $39 ± 4,6\%$; $p < 0.05$, unpaired Student's *t* test). Similarly, in both groups, the firing frequency during extraburst firing (tonic activity) was increased by application of bicuculline (Fig. 1c, d, k; genotype × drug, $F_{1,30} = 4.53$, $p = 0.04$, genotype, $F_{1,30} = 7.33$, $p = 0.011$, drug, $F_{1,30} = 25.45$, $p < 0.0001$), but the increase was significantly impaired in mutants (Fig. 1n; $146 ± 54.9\%$ vs. $21 ± 10.0\%$; $p < 0.05$, unpaired Student's *t* test). Neither the amplitude of observed changes, nor the baseline burst parameters differed between the WT mice and Gabrg2^{DATCreER} mice. The only exception was the intrabursts interspike interval, for which bicuculline induced

Several behavioral traits relevant for alcoholism are contro…
A Stojakovic et al.

1552

Fig. 3 Response inhibition testing of Gabrg2^DATCreER mice with the go/no-go task. The graphs show mean numbers of **a** rewards obtained during each session, **b** responses preceding signal presentation, **c** response latency for "go" and "no-go" trials, **d**, **e** number of responses per session during "no-go" and "go" signal presentation, **f** head entries into water receptacle per session. Data in **b** and **c** are averaged across all sessions. $n = 13$ and 12. Error bars represent SEM

opposite effects in mutants and controls (Fig. S3; genotype × drug, $F_{1,15} = 6.938$, $p = 0.005$, genotype, $F_{1,15} = 0.84$, $p = 0.37$, drug, $F_{1,15} = 1.52$, $p = 0.24$). In summary, the dopamine neurons of Gabrg2^DATCreER mice displayed increased activity and reduced sensitivity to drugs acting at GABA$_A$ receptors.

Before examining behaviors specifically related to alcohol, we subjected the mice to some basal behavioral tests measuring locomotor activity, exploratory behavior, and avoidance. In the open-field test, Gabrg2^DATCreER mice displayed increased locomotor activity (Fig. 2a) and spent more time in the center of the arena compared to WT mice (Fig. 2b). Furthermore, Gabrg2^DATCreER mice spent more time in the open arms of the elevated plus maze (Fig. 2c) without making significantly more entries (Fig. S4B). They also displayed a strongly increased social behavior in the reciprocal social interaction test (Fig. 2d). Gabrg2^DATCreER mice displayed a marked reduction of the time spent immobile in the forced swim test (Fig. 2e) and they also showed an increased sucrose preference (Fig. 2f). Collectively, these findings indicate that the mutant mice had an increased exploratory drive that was not inhibited by aversive features of the environment.

To test if the increased exploratory behavior was associated with impaired response inhibition, we used the go/no-go task, which measures the response inhibition component of impulsivity. In this task, mice should either respond or withhold a response dependent on which cue is shown. Both Gabrg2^DATCreER and WT animals improved their performance over time and significantly increased the number of rewards obtained in subsequent sessions (Fig. 3a; session × genotype $F_{9,207} = 1.02$, $p = 0.43$, genotype $F_{1,23} = 1.03$, $p = 0.32$, session $F_{9,207} = 14.22$, $p < 0.0001$). The mutation did not significantly affect the rate of pre-cue responding (Fig. 3b) and had no effect on reaction times during "go" or "no-go" trials (Fig. 3c; trial type × genotype $F_{1,46} = 0.54$, $p = 0.46$, genotype $F_{1,46}$

$= 0.05$, $p = 0.82$, trial type $F_{1,46} = 33.46$, $p < 0.0001$). Initially, Gabrg2^DATCreER mice showed higher frequency of false alarms (responding to "no-go" signals), but they eventually reached the same level of performance as WT animals (Fig. 3d; session × genotype $F_{9,207} = 2.12$, $p = 0.03$, genotype $F_{1,23} = 1.94$, $p = 0.18$, session $F_{9,207} = 23.00$, $p < 0.0001$). Moreover, Gabrg2^DATCreER mice were generally more likely to correctly respond to "go" signals, as indicated by a higher level of hits (Fig. 3e; session × genotype $F_{9,207} = 0.62$, $p = 0.78$, genotype $F_{1,23} = 6.89$, $p = 0.02$, session $F_{9,207} = 4.25$, $p < 0.0001$). In addition, we noted that the mutant mice initially made more head entries into the receptacle where the liquid rewards were delivered (Fig. 3f; session × genotype $F_{9,207} = 2.05$, $p = 0.04$, genotype $F_{1,23} = 2.07$, $p = 0.16$, session $F_{9,207} = 1.91$, $p = 0.05$). Taken together, the results in this test showed that Gabrg2^DATCreER mice had heightened propensity to respond for both "go" and "no-go" signals and showed higher reward receptacle approach behavior.

We next investigated if the rewarding effects of ethanol (2 g/kg) was affected in the mutant mice. WT littermates of the Gabrg2^DATCreER mice displayed no CPP in response to ethanol (Fig. 4a). In contrast, Gabrg2^DATCreER mice displayed a clear CPP (Fig. 4a). The fact that the WT mice from the Gabrg2-colony did not show a CPP to ethanol is in line with previous reports confirming weak or no CPP in some mouse strains [37]. However, since tamoxifen has been shown to affect certain responses to ethanol [38, 39], we investigated if the pre-treatment with tamoxifen or the strain was responsible for the lack of CPP in the WT mice. The WT mice from the Gabrg2-colony did not display CPP to ethanol even if they were not pre-treated with tamoxifen (Fig. S4C). In contrast, C57BL/6J mice displayed robust ethanol-induced CPPs both when they were pre-treated with tamoxifen and when they received no pre-treatment (Fig. S4C). Both WT and

Several behavioral traits relevant for alcoholism are contro…
A Stojakovic et al.

1553
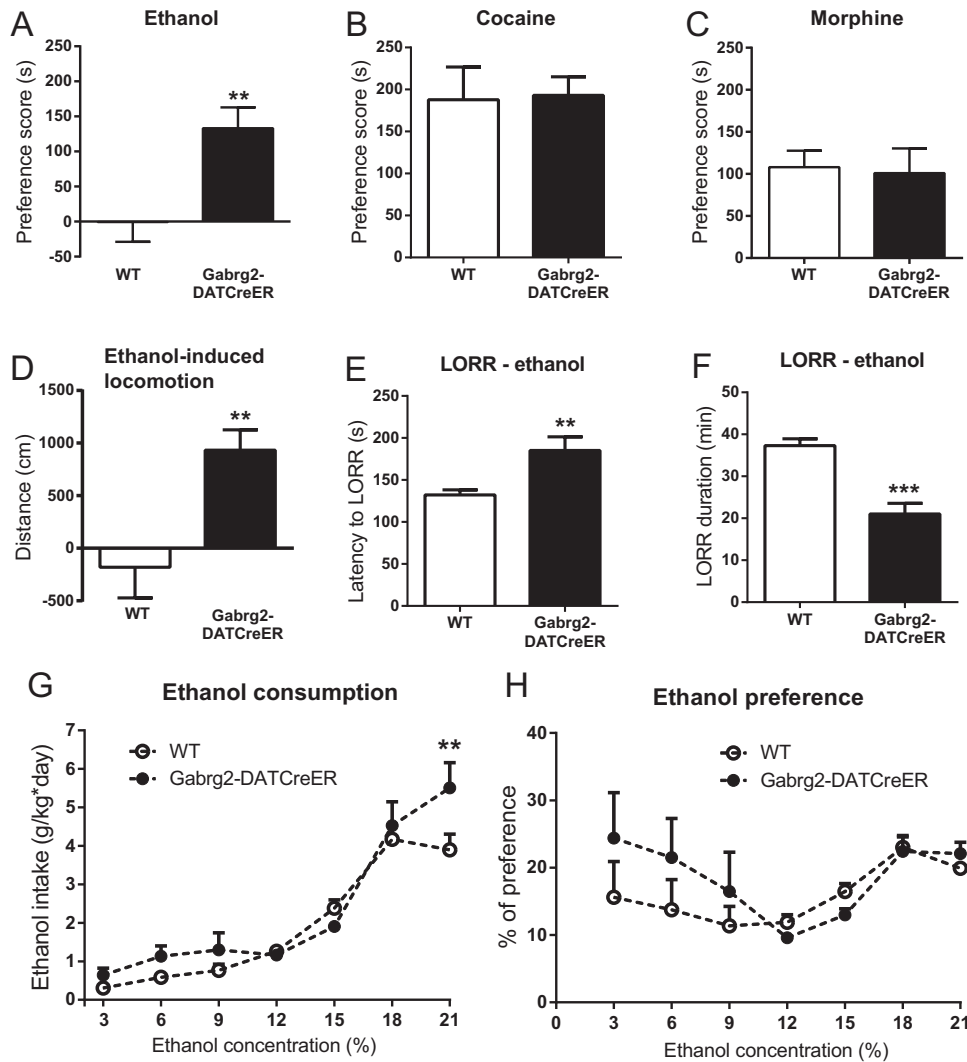
Fig. 4 Alcohol-related behaviors in Gabrg2^{DATCreER} mice. Conditioned place preference to ethanol in Gabrg2^{DATCreER} mice and their WT littermates (**a**; $n = 7$ and 12). Note that WT mice of this particular background did not form a place preference to ethanol. Place preference to cocaine (**b**; $n = 11$ and 8) and morphine (**c**; $n = 17$ and 14) in mutant and WT mice. Locomotor activity induced by ethanol (**d**; $n = 6$ and 7). Loss of righting reflex (LORR) in Gabrg2^{DATCreER} mice **e**, **f**. Latency to LORR is shown in **e** ($n = 10$ and 11) and duration of the loss is shown in **f** ($n = 9$ and 10). Ethanol consumption **g** and alcohol preference **h** of Gabrg2^{DATCreER} mice given increasing concentrations of ethanol in a two bottle-choice paradigm ($n = 8$ and 8). Preference scores were calculated by subtracting the time the mouse spent in the drug-paired chamber during the post-test from that of the pretest. Error bars represent SEM. *$p < 0.05$ **$p < 0.01$, ***$p < 0.001$. Student's t test in **a**–**e** and two-way ANOVA followed by Sidak's multiple comparisons test in **f** and **g**

Gabrg2^{DATCreER} mice developed robust preferences for cocaine (15 mg/kg; Fig. 4b) and morphine (10 mg/kg; Fig. 4c) without any obvious differences between genotypes. In order to determine if the stimulatory effects of ethanol were affected in Gabrg2^{DATCreER} mice, we next monitored the locomotor response induced by ethanol. Whereas, ethanol (1 g/kg) had no locomotor stimulating effect in WT mice, it induced a robust increase in locomotor activity in Gabrg2^{DATCreER} mice (Fig. 4d). To examine if the mutation affected the sedative effect of ethanol, we tested how rapidly a high dose of ethanol-induced loss of the righting reflex and how long such loss persisted. Latency to onset of the sedative effect was increased in Gabrg2^{DATCreER} mice (Fig. 4e; $t_{19} = 2.94$, $p = 0.008$) and the duration of sedation was decreased (Fig. 4f; $t_{17} = 5.30$, $p < 0.0001$). When we investigated alcohol drinking in a two bottle-choice test with increasing concentrations of ethanol, Gabrg2^{DATCreER} mice had a moderately increased ethanol consumption at a high concentration of ethanol (Fig. 4g; genotype ×

concentration, $F_{6,84} = 2.73$, $p = 0.018$, genotype, $F_{1,14} = 1.78$, $p = 0.20$; concentration, $F_{6,84} = 78.06$, $p < 0.0001$). Total fluid consumption was also increased by the mutation (genotype × concentration, $F_{6,84} = 2.97$, $p = 0.011$, genotype, $F_{1,14} = 2.00$, $p = 0.18$; concentration, $F_{6,84} = 20.32$, $p < 0.0001$) and consequently no genotype effect was seen on alcohol preference (Fig. 4h; genotype × concentration, $F_{6,84} = 1.47$, $p = 0.20$, genotype, $F_{1,14} = 0.49$, $p = 0.50$; concentration, $F_{6,84} = 4.59$, $p < 0.0004$).

DISCUSSION
Previous studies indicate that the effect of Gabrg2 deletion on GABA responsiveness varies considerably dependent on the cell type targeted. Gabrg2 was reported to be necessary for maintaining GABA$_A$R synapses in the hippocampus [33] and deletion of Gabrg2 reduces GABA responsiveness by 90% in hypothalamic neurons [40]. In contrast, deletion of Gabrg2 in the

Table 1. Summary of phenotypes in Gabrg2-DATCreER mice

Observed phenotype	Figure
Increased baseline firing frequency of dopamine (DA) cells	1
Attenuated decrease in firing of DA cells after a GABA$_A$ receptor agonist	1
Attenuated increase in firing of DA cells after a GABA$_A$ receptor antagonist	1
Increased locomotion in the open-field test	2A
Increased time spent in the center in the open-field test	2B
Increased time in the open arms in the elevated plus maze	2C
Increased time interacting in the reciprocal social interaction test	2D
Decreased time spent immobile in the forced swim test	2E
Increased sucrose preference	2F
Increased false alarm rate in the go/no-go task	3D
Increased hit rate in the go/no-go task	3E
More magazine head entries in the go/no-go task	3F
Increased conditioned place preference to ethanol	4A
Normal conditioned place preference to morphine and cocaine	4B, 4C
Increased ethanol-induced locomotion	4D
Attenuated loss of the righting reflex	4E, 4F
Increased ethanol consumption at high concentrations	4G
Normal alcohol preference	4H

adult neocortex did not block GABA$_A$R activity but only changed the characteristics of the GABA response [41]. Our findings indicate that Gabrg2 deletion in dopamine cells of adult mice leads to a substantial attenuation, but not a complete blockade, of GABA$_A$R function in these cells. Thus, dopamine neurons from Gabrg2^{DATCreER} mice displayed increased basal firing and an attenuated responsiveness to drugs acting at GABA$_A$Rs. The residual GABA responsiveness observed in dopamine cells of the mutants could reflect activation of GABA$_A$Rs not containing ɣ2 subunits and/or activation of GABA$_A$Rs on excitatory afferents to the dopaminergic cells. We used urethane anesthesia for the electrophysiological experiments. One advantage with urethane anesthesia, as compared to isoflurane anesthesia, is that it mimics the awake brain in the sense that firing of dopamine cells is brain state-dependent [42–44]. On the other hand, brain state-dependent differences can lead to increased variability in the parameters monitored. Brain state-dependent modulation can be a particularly important factor when investigating the potency of GABA$_A$ receptor antagonists. It has been suggested that the heterogeneity of DA activity of neurons observed under anesthesia, at least partially, may be due to cyclic changes in activity of GABAergic inputs to the ventral tegmental area and the substantia nigra [43]. However, in our study, all recorded neurons increased their firing (at least 20%) after local blockade of GABA$_A$ receptors.

Many of the behavioral changes in Gabrg2^{DATCreER} mice (summarized in Table 1) fit well with the finding that their dopamine cells had an increased basal activity. Optogenetic activation of midbrain dopamine cells has been reported to result in effects similar to those seen here in Gabrg2^{DATCreER} mice in the forced swim test [45] and the social interaction test [46]. Furthermore, the deletion of NMDA receptors in dopaminergic cells induced changes in the opposite direction in many of the tests [22]. Even if results from basic behavioral tests such as the elevated plus maze and the forced swim test are difficult to interpret in general, our interpretation of the findings in this study is that that they,

together with the go/no-go test, indicate that the Gabrg2^{DATCreER} mice often display an increased activity and a high propensity to explore and interact. The mutant mice also seem to act and explore when the environment has aversive features or when withholding a response is rewarded. However, they eventually learned to withhold responses in the go/no-go task, so it is unclear if the phenotype in that task reflects heightened propensity for reward seeking, a deficit in certain aspects of behavioral inhibition or both.

We found that Gabrg2^{DATCreER} mice displayed an increased CPP and an augmented locomotor activation in response to ethanol. In contrast, cocaine and morphine-induced CPP were unaltered in the mutant mice. The cellular and molecular underpinnings of the changes in alcohol-induced behaviors are difficult to pin-point in an exact way since the mechanisms by which alcohol induces reinforcement are far from clear [1, 47, 18]). Alcohol has direct activating effects on dopamine cells [16, 17] and also activates local inhibitory GABAergic neurons that inhibit the dopaminergic cells [48, 49]. In addition, alcohol acts in the nucleus accumbens to induce dopamine release [50, 18]). The dopamine-enhancing effect of accumbal ethanol can be blocked by manipulations in the ventral tegmental area indicating an indirect activation or a disinhibition of the dopaminergic cells [18]. Unfortunately, the respective contributions of these different modes of action to the different behavioral effects of alcohol have not been firmly established. When interpreting the behavioral findings of this study, it should also be taken into account that alcohol can affect GABA signaling onto dopamine cells both by affecting GABA release through the mechanisms discussed above and by direct potentiation of GABA$_A$ receptors on dopamine cells. Consequently, in addition to reducing the general sensitivity to GABA, the removal of Gabrg2 most likely induced a difference in the direct ethanol sensitivity of the remaining receptors since the subunit composition of GABA$_A$Rs influence their responsiveness to ethanol. It is not clear which subunits that are most critical for the direct effect of ethanol [7, 51]. GABA$_A$ receptors containing ɣ2 subunits have been reported to be more sensitive to ethanol than those without [10, 52] but other studies indicate that extra-synaptic GABA$_A$Rs lacking ɣ2 subunits are most alcohol responsive [11, 12]. In our mutant mice, we do not know how the expression of other GABA$_A$R subunits was changed. The ɣ2 subunit can be replaced ɣ1, ɣ3, or δ subunits, but under normal conditions dopamine cells display no or very weak expression of these subunits [53, 54]. Finally, it should be noted that the Cre-line used also induces recombination in dopamine cells outside the ventral tegmental area. Thus, certain behavioral differences might be due to recombination in the substantia nigra or other structures with dopaminergic cells.

Irrespective of the exact mechanisms, our findings clearly demonstrate that the GABA-mediated inhibition of dopaminergic cells limits the reinforcing and stimulatory effects of alcohol. Removal of Gabrg2 on dopamine cells consequently exaggerates or unmasks ethanol-induced behaviors related to reinforcement and locomotor stimulation. Dopamine cells from mice selectively bred for high alcohol-induced locomotion and mice chronically treated with ethanol have dopaminergic cells that display reduced GABA responsiveness. Together with the findings presented here, this might indicate that different avenues leading to weakening of the inhibitory control of dopaminergic cells can increase the rewarding and stimulatory effects of alcohol and increase the risk of developing excessive alcohol intake.

In light of the strongly enhanced CPP in response to ethanol, it is perhaps surprising that the mice without Gabrg2 in the dopaminergic cells responded with normal CPPs to two different doses of morphine. This may suggest that GABA$_A$R signaling onto dopaminergic cells is limiting ethanol-induced but not morphine-induced reinforcement or that the subunit composition after Gabrg2 deletion in some way selectively decreases the sensitivity

Several behavioral traits relevant for alcoholism are contro…
A Stojakovic et al.

1555

of the receptors to ethanol. Morphine is assumed to activate dopamine cells by inhibition of GABAergic input [55, 56, 20]. Accordingly, one would predict that a complete deletion of GABA receptors on dopamine cells should abolish morphine CPP and a reduction in GABA$_A$R function should attenuate the CPP. However, we saw no signs of an attenuation in our experiments.

At high doses, alcohol has sedative rather than stimulating effects. Converging lines of evidence also indicate that aspects of dopamine transmission are suppressed at high doses of alcohol [57–60]. We found that GABA$_A$Rs on dopaminergic cells are powerful regulators of the sedative effect of alcohol. Together with studies showing that different substances increasing dopamine levels also reduce alcohol-induced sedation [59, 61, 62], this strongly suggests that activity of the dopamine system is a critical factor counteracting the sedative effects seen after alcohol drinking.

Our findings demonstrate that the deletion of Gabrg2 leads to several behavioral characteristics that, in humans, are known to be risk factors for developing alcoholism. These risk factors are to a large extent genetically determined. Whereas it is clear that variations in GABRG2 are associated with increased risk for alcoholism [8, 9], it has not been determined if these variations induce risk by operating through intermediate factors such as impulsivity or altered sensitivity to sedative or rewarding effects of ethanol. In studies on humans, it is notoriously difficult to pinpoint the neuronal groups in which a given allele is mediating the effect on specific behaviors. Since our findings establish that the subunit composition of GABA$_A$Rs on dopamine cells is critical for important responses to alcohol, they may suggest that genetic variability in GABA$_A$R subunits promotes alcoholism and associated risk behaviors partially through effects on dopamine cells. Finally, since GABRG2 is a risk-associated gene and the deletion induces such a broad range of risk-associated traits, the Gabrg2^{DATCreER} mice are an interesting model for the study of drinking in people at high risk for developing alcoholism.

ADDITIONAL INFORMATION
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