

Cocaine effects on mouse incentive-learning and human addiction are linked to $\alpha 2$ subunit-containing GABA_A receptors

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Because GABA_A receptors containing $\alpha 2$ subunits are highly represented in areas of the brain, such as nucleus accumbens (NAcc), frontal cortex, and amygdala, regions intimately involved in signaling motivation and reward, we hypothesized that manipulations of this receptor subtype would influence processing of rewards. Voltage-clamp recordings from NAcc medium spiny neurons of mice with $\alpha 2$ gene deletion showed reduced synaptic GABA_A receptor-mediated responses. Behaviorally, the deletion abolished cocaine's ability to potentiate behaviors conditioned to rewards (conditioned reinforcement), and to support behavioral sensitization. In mice with a point mutation in the benzodiazepine binding pocket of $\alpha 2$ -GABA_A receptors ($\alpha 2$ H101R), GABAergic neurotransmission in medium spiny neurons was identical to that of WT (i.e., the mutation was silent), but importantly, receptor function was now facilitated by the atypical benzodiazepine Ro 15-4513 (ethyl 8-amido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo [1,5-a] [1,4] benzodiazepine-3-carboxylate). In $\alpha 2$ H101R, but not WT mice, Ro 15-4513 administered directly into the NAcc-stimulated locomotor activity, and when given systemically and repeatedly, induced behavioral sensitization. These data indicate that activation of $\alpha 2$ -GABA_A receptors (most likely in NAcc) is both necessary and sufficient for behavioral sensitization. Consistent with a role of these receptors in addiction, we found specific markers and haplotypes of the *GABRA2* gene to be associated with human cocaine addiction.

GABRA2 | behavioral sensitization | nucleus accumbens | mutant mouse | human genetics

The nucleus accumbens (NAcc) is centrally implicated in the actions of drugs of abuse and in mediating the effects of environmental cues conditioned to drug reward in motivating drug-seeking behavior (1). GABAergic medium spiny neurons (MSNs) are the major neuronal type within the accumbens, their activity being regulated by dopaminergic and glutamatergic inputs. Local GABAergic interneurons and collateral projections (2) between neighboring MSNs may serve to sharpen competition between MSNs for access to the major output pathways. Intra-accumbal GABAergic systems may thus play an important role in regulating incentive motivational outputs, leading to compulsive behaviors, such as drug abuse. We therefore predicted that manipulation of GABAergic transmission within the NAcc will alter the ability of the NAcc to respond to psychostimulant drugs and to incentives, such as reward-predictive cues.

GABA mediates “fast” neural inhibition by activating ionotropic GABA_A receptors, leading to the transmembrane flux of chloride and bicarbonate ions and a decrease in neuronal input resistance. The majority of these oligomeric protein complexes are made up of subunits from major subunit families, α , β , and γ . Given the established role of the NAcc in mediating the

behavioral effects of both reward-associated cues, and of cocaine, determining the GABA_A receptor subtypes that mediate the signal generated in this brain region by abused drugs may offer an approach to designing treatment strategies and identifying genetic risk for cocaine addiction. GABA_A receptors containing $\alpha 2$ subunits are densely expressed in NAcc (3) but are absent, or only modestly represented, in the pallidal and ventral tegmental area output targets, respectively (4). Thus, this subtype of receptor may play an important role in these processes.

To test this hypothesis, we used mice with genetic manipulations of the *Gabra2* gene. Gene deletion ($\alpha 2^{-/-}$) reduced responses mediated by synaptic GABA_A receptors in the MSNs of NAcc, and abolished cocaine's ability to enhance behaviors conditioned to rewards (conditioned reinforcement) and to support behavioral sensitization. As gene-deletion experiments may be complicated by compensation, we additionally used mice in which their $\alpha 2$ subunit was genetically engineered ($\alpha 2$ H101R) to selectively change the pharmacology of $\alpha 2$ -GABA_A receptors, but not their physiological function. Importantly, the mutation did not influence NAcc GABAergic transmission, but selectively made $\alpha 2$ -GABA_A receptors sensitive to the GABA-enhancing effects of Ro 15-4513, an atypical benzodiazepine. Remarkably, infusion of Ro 15-4513 into the NAcc of $\alpha 2$ H101R but not WT mice enhanced locomotor activity, and in common with cocaine, repeated systemic treatment resulted in maintained sensitization. $\alpha 2$ receptors are also located in other brain areas involved in addictive behavior; however, by selecting behaviors known to be mediated by NAcc core, and by using local infusions of drugs into NAcc, we were able to provide some anatomical specificity to our findings. Importantly, we report that haplotypes of the *GABRA2* gene are associated with cocaine addiction in humans.

Results

Expression of GABA_A Subunits in $\alpha 2^{-/-}$ Mice. RT-PCR analysis of expression of other GABA_A receptor subunits, including $\alpha 1$, $\alpha 3$,

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$\alpha 4$, $\alpha 5$, $\alpha 6$, $\beta 1$, $\beta 2$, and $\gamma 1$ and $\gamma 2$ in punches obtained from NAcc, showed unchanged expression in the knockout mice (Fig. S1).

Electrophysiology in MSNs of $\alpha 2^{-/-}$ Mice. Deletion of the $\alpha 2$ subunit produced an $\approx 33\%$ decrease in miniature inhibitory postsynaptic current (mIPSC) amplitude and a clear prolongation of the mIPSC decay (Fig. 1A and B and Table S1). The frequency of mIPSCs did not differ from that recorded for WT neurons (Table S1).

Cocaine Effects in $\alpha 2^{-/-}$ Mice. $\alpha 2^{-/-}$ mice displayed a stimulant response to cocaine, indistinguishable from that of WT mice (Fig. 2A). However, in contrast to WT, $\alpha 2^{-/-}$ mice did not develop behavioral sensitization when given cocaine repeatedly at 2- to 3-day intervals (Fig. 2B), so that even though the WTs showed an enhanced response to cocaine following repeated treatments, the $\alpha 2^{-/-}$ mice did not (Fig. 2C). Because the stimulant response of $\alpha 2^{-/-}$ mice to cocaine was normal, this deficit in sensitization was not attributable to altered drug sensitivity. Behavioral sensitization depends on the test context, and one possibility is that the $\alpha 2^{-/-}$ mice fail to form conditioned associations between the cocaine effect and the test environment. However, in a test of place-preference conditioning, the $\alpha 2^{-/-}$ mice were unimpaired in forming drug-environment associations (Fig. S2).

Nevertheless, even if $\alpha 2^{-/-}$ mice are capable of forming environment-cocaine associations, and the acute response to cocaine is unaltered, the ability of cocaine to facilitate the conditioned response (5) may be attenuated in $\alpha 2^{-/-}$ mice. We tested this possibility using a measure of conditioned reinforcement known to be facilitated by psychomotor stimulants (6). Conditioned reinforcers (i.e., discrete environmental cues conditioned to a primary reward) are important in maintaining motivation to seek drug rewards when the drug itself is not immediately available, and thus contribute both to maintenance of drug habits and to relapse in abstaining addicts (7). Mildly food-deprived mice were first trained to associate delivery of food pellets with illumination of two cue lights flashing at 1 Hz and simultaneous presentation of a 2.9-kHz tone for 10 s. $\alpha 2^{-/-}$ and WT mice learned this association equally readily (Fig. 2D) again showing that the deletion has little effect on conditioning. Next, two levers were introduced into the apparatus, operation of one lever giving rise to cue presentation (but no food deliv-

ery); operation of the alternative lever had no programmed consequences. Both genotypes readily learned to operate the lever to obtain access to the cues (Fig. 2E), indicating that they had acquired conditioned reinforcing properties. However, although cocaine facilitated lever-pressing for the conditioned reinforcer in a dose-dependent fashion in the WT mice, it failed to do so in the $\alpha 2^{-/-}$ mice (Fig. 2F), indicating a loss of cocaine's ability to facilitate conditioned reinforcement.

$\alpha 2H101R$ Mutant Mice. GABA_A receptors containing $\alpha 1$, $\alpha 2$, $\alpha 3$, or $\alpha 5$ subunits possess binding sites for classical benzodiazepines, such as diazepam, whereas those containing $\alpha 4$ or $\alpha 6$ subunits do not. $\alpha 4$ and $\alpha 6$ subunits differ from benzodiazepine-sensitive subunits in possessing an arginine (R) instead of a histidine (H) residue in the benzodiazepine binding pocket (8). This single amino acid difference also changes the pharmacology of agents like Ro 15-4513 that normally act at the benzodiazepine binding site as negative allosteric modulators of GABAergic inhibition. Thus, whereas the atypical benzodiazepine derivative, Ro 15-4513, acts as an "inverse agonist" (i.e., decreases GABA-evoked currents) at "H" ($\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ -, and $\alpha 5$ -containing) GABA_A receptors, it acts as an "agonist" (i.e., facilitates GABA-evoked currents) at "R" ($\alpha 4$ - and $\alpha 6$ -containing) receptors (9, 10). Importantly, the point mutation does not alter the sensitivity of the receptor to its natural ligand, GABA. As selective ligands for $\alpha 2$ -containing receptors are not available, we used this knowledge to differentially facilitate transmission at $\alpha 2$ -containing receptors, using knock-in mice (11, 12) in which the normal H at position 101 within the benzodiazepine binding pocket of the $\alpha 2$ protein was replaced by R ($\alpha 2H101R$ mice). In such mutant mice, Ro 15-4513 will continue to act as an inverse agonist at $\alpha 1$ -, $\alpha 3$ -, and $\alpha 5$ -containing receptors, and as an agonist at $\alpha 4$ - and $\alpha 6$ -containing receptors, so that the only difference between WT and mutant mice will be the direction of Ro 15-4513s action at $\alpha 2$ -containing receptors.

Electrophysiology in MSNs of $\alpha 2H101R$ Mice. In coronal brain slices prepared from WT and $\alpha 2H101R$ mice, a comparison of mIPSCs from NAcc core neurons revealed no significant difference in their properties (Table S1), confirming that basal GABAergic transmission is unaltered by the mutation. Ro 15-4513 enhances

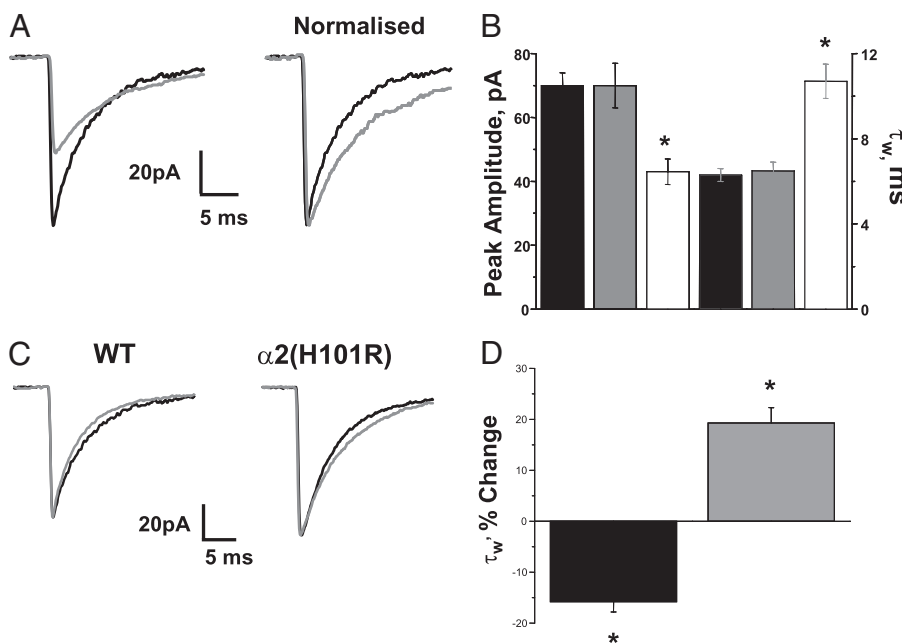


Fig. 1. Receptors incorporating the $\alpha 2$ subunit are expressed at GABAergic synapses in NAcc MSNs. (A) Representative ensemble averages of GABA_A receptor mIPSCs recorded from neurons of the NAcc of WT (WT) (black) and $\alpha 2^{-/-}$ (gray) mice. These events are shown normalized to the WT amplitude on the right to aid comparison of the decay kinetics. (B) Bar chart comparing the average peak amplitude (Left y axis) and decay time constant τ_w (Right y axis) of GABA_A receptor mediated mIPSCs of neurons from WT (black), $\alpha 2H101R$ (gray), and $\alpha 2^{-/-}$ (white) mice. (C) Representative ensemble averages of GABA_A receptor-mediated mIPSCs recorded from exemplar neurons of WT [i.e., four of eight neurons showing decrease decay time (see text)] and $\alpha 2H101R$ mice in the absence (black) and presence (gray) of 10- μ M Ro15-4513. (D) Effects of 10- μ M Ro15-4513 on the decay kinetics (i.e., τ_w , expressed as % of change) of mIPSCs of WT (black) and $\alpha 2H101R$ (gray) neurons of the NAcc. Data were obtained from 4 to 19 (WT), 8 to 11 ($\alpha 2H101R$), and 37 ($\alpha 2^{-/-}$) neurons. Error bars indicate SEM. *, $P < 0.01$ vs. WT or control (unpaired Student's *t* test and one-way RMA, respectively).

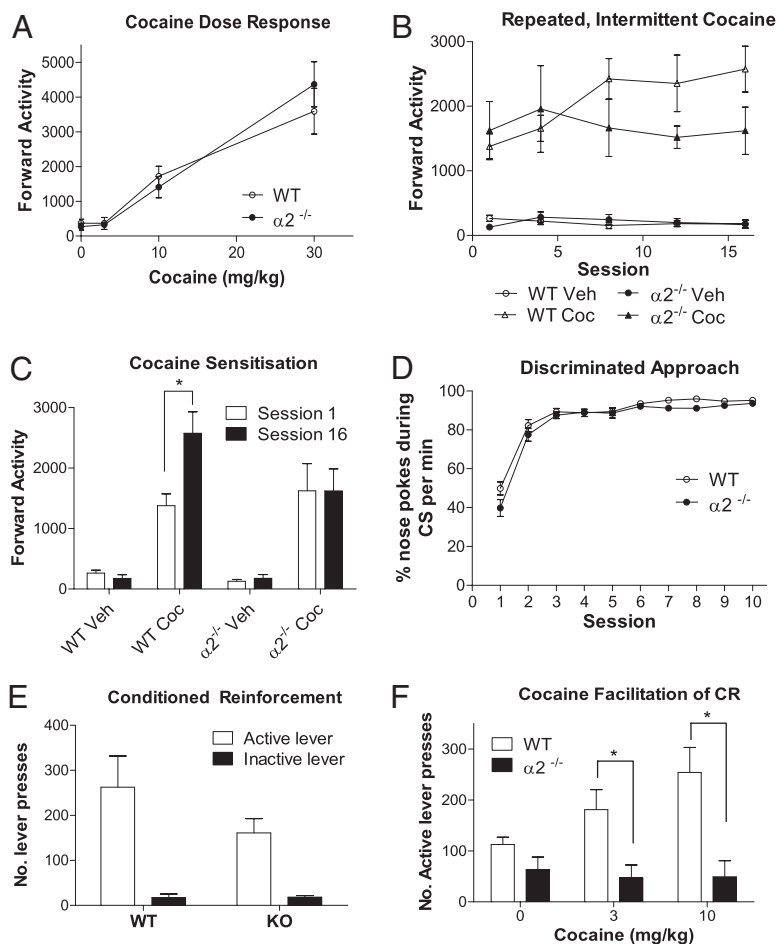


Fig. 2. Effects of cocaine on conditioned behavior of $\alpha 2^{-/-}$ mice. (A) Effects of acute cocaine on locomotor activity during 60 min in WT ($n = 8$) and $\alpha 2^{-/-}$ mice ($n = 8$). Cocaine increased activity equally in both genotypes (dose by genotype interaction; $F_{(3,39)} = 0.729$, NS). (B) Behavioral sensitization to cocaine (10 mg/kg) in WT ($n = 9$) but not in $\alpha 2^{-/-}$ ($n = 7$) mice compared with control groups of WT ($n = 9$) and $\alpha 2^{-/-}$ ($n = 9$) treated with saline. Data show mean (\pm SEM) number of beam breaks. Analysis was carried out for all 16 sessions but, for clarity of presentation, data from every fourth session are displayed. Locomotor activity increased over sessions for WT mice but not for $\alpha 2^{-/-}$ mice (Session by genotype by drug interaction; $F_{(15,450)} = 3.096$, $P < 0.05$, $\epsilon = 0.291$). (C) Locomotor activity on sessions 1 and 16 of repeated intermittent cocaine (session by genotype by drug interaction, $F_{(1,30)} = -0.650$, $P < 0.05$). Asterisk denotes significance between sessions 1 and 16 (paired samples t test; $P < 0.005$). (D) Acquisition of Pavlovian conditioning in WT ($n = 10$) and $\alpha 2^{-/-}$ ($n = 10$) mice. Data show percentage of conditioned stimulus (light + tone) presentations eliciting nose pokes into the food delivery magazine over 15 sessions. No difference was evident between genotypes (session by genotype interaction; $F_{(9,162)} = 1.401$, NS; effect of genotype; $F_{(1,18)} = 3.753$, NS). (E) Active and inactive lever presses in tests of conditioned reinforcement in WT ($n = 5$) and $\alpha 2^{-/-}$ ($n = 6$) mice. Significant effect of lever ($F_{(1,9)} = 28.562$, $P < 0.001$) demonstrates conditioned reinforcement, which did not differ between genotypes (lever by genotype interaction; $F_{(1,9)} = 1.968$, NS). (F) Cocaine facilitation of conditioned reinforcement in WT ($n = 5$) and $\alpha 2^{-/-}$ ($n = 6$) mice. Responding on the active lever for CS presentation differs between genotypes (dose by genotype interaction, $F_{(2,18)} = 8.816$, $P < 0.05$). WT mice showed an increase in responding (main effect of dose, $F_{(3,12)} = 3.284$, $P = 0.05$), whereas $\alpha 2^{-/-}$ mice showed a decrease (main effect of dose, $F_{(3,15)} = 19.564$, $P < 0.001$) after administration of increasing doses of cocaine. Asterisk indicates significant difference between WT and $\alpha 2^{-/-}$ lever-pressing rates using independent samples t test (3 mg/kg; $t(9) = 3.093$, $P < 0.05$; 10 mg/kg; $t(9) = 3.087$, $P < 0.05$).

GABA-evoked responses by the mutated $\alpha 2$ -containing receptor expressed in heterologous cell systems (10). Correspondingly, Ro15-4513 (10 μ M) significantly prolonged the mIPSC decay in all accumbal neurons ($n = 8$) tested from $\alpha 2$ H101R mice (Figs. 1 C and D), while it accelerated the mIPSC decay phase in four out of eight WT accumbal cells tested (Kolmogorov-Smirnov test, $P < 0.01$), and either had no effect (two cells, Kolmogorov-Smirnov test, $P > 0.01$), or prolonged the decay time constant $\tau_w \sim 20\%$ (two cells, Kolmogorov-Smirnov test, $P < 0.01$) in others. The expression in some WT spiny neurons of synaptic receptors incorporating the $\alpha 4$ subunit provides a parsimonious explanation for the heterogeneous effects of Ro 15-4513. These observations reinforce the $\alpha 2^{-/-}$ studies in confirming that a significant proportion of synaptic GABA_A receptors in the NAcc core incorporate the $\alpha 2$ subunit, and reveal that Ro 15-4513 facilitates the function of the mutated receptor.

Behavior of $\alpha 2$ H101R Mice. In keeping with its altered action at NAcc $\alpha 2$ -GABA_A receptors, local bilateral infusion of Ro 15-4513 (15 or 45 nM in 0.5 μ L) into the NAcc of $\alpha 2$ H101R, but not WT mice, gave rise to an increase in locomotor activity (Fig. 3A).

Behavioral Sensitization to Ro 15-4513. A stimulant effect was also seen when Ro 15-4513 was given i.p. in the H101R mutant, whereas it had no effect in WT mice. Moreover, the stimulant effect in the mutants increased with repeated treatment (Fig. 3B), indicating behavioral sensitization that persisted for at least 28 days following the final treatment (Fig. 3C). Because the action of Ro 15-4513 at all other GABA_A receptors would not differ between WT and $\alpha 2$ H101R mutant mice, we deduce that

facilitation of GABAergic transmission at $\alpha 2$ -containing receptors, as shown in the electrophysiological experiments, resulted in a form of behavioral sensitization that resembles that induced by drugs of abuse.

Dopamine Turnover Following Ro 15-4513. The locomotor stimulant properties of psychostimulants, such as cocaine, depend on their ability to increase dopamine turnover in the NAcc, cocaine-sensitized animals showing facilitated cocaine-induced dopamine release (13). Administration of Ro15-4513 to $\alpha 2$ H101R-mutant mice did not increase dopamine turnover, measured as the ratio of dopamine to its metabolite, DOPAC, in NAcc (Fig. 3D and Fig. S3). Thus, consistent with a location downstream from dopamine synapses, stimulant effects resulting from activation of GABA_A $\alpha 2$ -containing receptors may not depend upon activation of dopamine release. That a benzodiazepine, midazolam, was able acutely to potentiate the stimulant effects of cocaine in WT, but not $\alpha 2$ H101R mice (14), further supports an intimate involvement of $\alpha 2$ -containing GABA_A receptors in expression of cocaine's effects.

Variations in the GABRA2 Gene in Human Cocaine Addicts. We hypothesized that in human populations, variations in this gene might also confer susceptibility to cocaine addiction. We analyzed a sample from São Paulo, Brazil, which consisted predominantly of individuals of Caucasian origin, with some admixture of African and Native American ancestry. Adjustment for ethnicity was performed using ADMIXMAP (15) (*SI Text*) and key findings were verified by parallel analyses in Caucasians, the main ethnic group, defined according to both self-reported

European ancestry ($n = 876$) and >70% European ancestry ($n = 823$) in ADMIXMAP (Table S2).

Nine haplotype-tagging SNPs tagging 80% of the Hapmap SNPs in the gene and the promoter region at an average $r^2 > 0.9$ were genotyped (Table S2 and Fig. 4A), revealing a linkage disequilibrium block structure with two distinct blocks of (i) 5' SNPs rs894269, rs2119767, rs9291283, and (ii) the remaining downstream markers (Fig. 4A).

We tested both blocks and found that the promoter block is associated with cocaine addiction. Specifically, we identified a protective haplotype (TTG; 10.5% in cases, 14.4% in controls, $P = 1.5 \times 10^{-3}$, $P = 0.005$ after permutation) (Fig. 4B). We did not find an association with the downstream haplotype block. An odds ratio of ~ 0.7 (95% confidence interval or CI 0.56–0.87) for cocaine addiction for the protective haplotype compared with all other haplotypes, indicates that individuals carrying this form are about one-third less likely to develop cocaine addiction. Analyses in the Caucasian subsamples confirmed a similar association in both groups (odds ratios 0.68 and 0.69, respectively, 95% CI of 0.48–0.84), whereas other haplotypes did not (Table S2).

SNP rs894269 has the strongest association in the complete sample (Table S3) as well as both Caucasian subsamples. Its T allele reduces the risk by a factor of about 1.5 (odds ratio ~ 0.68), suggesting a major contribution of SNP rs894269 to the observed protective effect on cocaine addiction. Bioinformatic sequence analysis revealed that rs894269 lies in an ultraconserved region ~ 9 kb from the transcription start point, where the C allele interrupts an evolutionarily conserved *cis*-enhancer region predicted by ESPERR analysis (16), while the T allele conserves it.

Additional associations of several genetic variations further downstream in the *GABRA2* gene with cocaine addiction were also found, as reported in studies of alcohol dependence (17, 18). Specifically, we observed significant association with three markers comprising a haplotype (rs279871, rs279845, and rs279836) defined by Edenberg et al. (18) (Table S3). Alcoholism was an exclusion criterion in our sample and alcohol consumption data were available in cases (although not controls). Alcohol consumption showed no association with *GABRA2* genotype (regression models with unit consumption: beta 0.004 for TT genotype of rs894269 vs. others; $P = 0.92$).

Discussion

Overall, our observations indicate a central role for GABAergic systems employing $\alpha 2$ -GABA_A receptors in cocaine-induced behavioral plasticity and addiction across species. Although human studies indicate an association of *GABRA2* with human addiction, the mouse studies pinpoint a possible mechanism. The mouse studies implicate $\alpha 2$ -receptors in a neural circuitry downstream of dopaminergic pathways, and demonstrate that their activation is a prerequisite for dopaminergic systems to achieve their effects in facilitating conditioned behavior. Because deletion of $\alpha 2$ subunits prevented the ability of cocaine to facilitate conditioned reinforcement, we hypothesize that the importance of the system lies in mediating cocaine's ability to facilitate behaviors elicited by environmental cues associated with rewards (including drug reward).

Drug addiction is frequently conceptualized in terms of maladaptive associative learning, allowing environmental cues associated

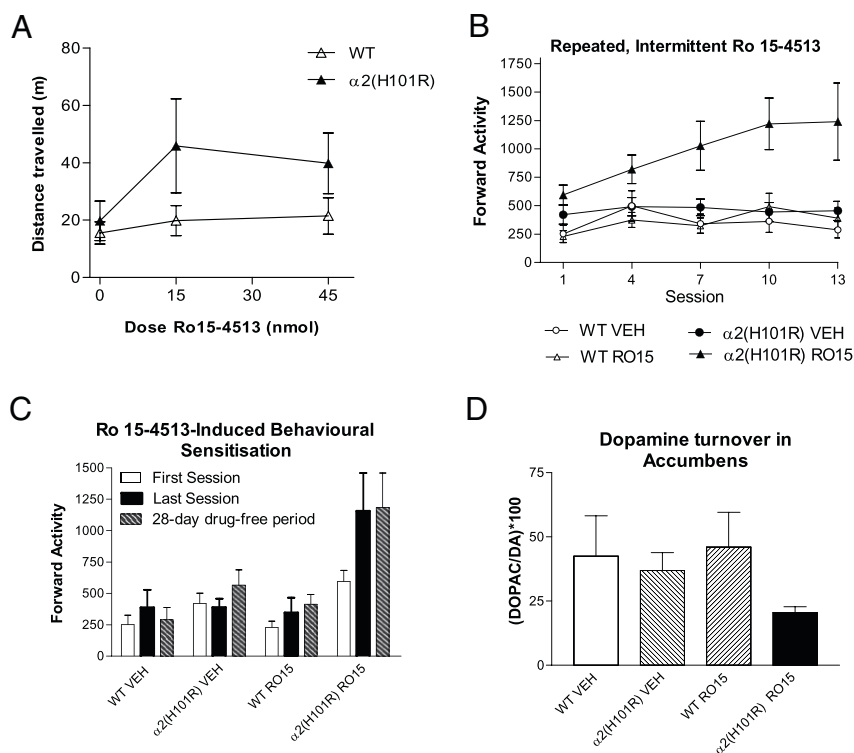


Fig. 3. Effects of Ro 15-4513 in $\alpha 2$ H101R mice. (A) Bilateral infusion of Ro 15-4513 (15 and 45 nmol in 0.5 μ L) into NAcc increased locomotor activity during 30 min after infusion in mutant mice ($n = 7$) but not in WT mice ($n = 9$) (Ro 15-4513 \times genotype interaction, $F_{2,32} = 3.98$; $P = 0.029$). Bars indicate SEM. (B) Repeated, intermittent Ro 15-4513 (10 mg/kg, i.p.) treatment increased locomotion in $\alpha 2$ H101R but not WT (WT) mice (Ro 15-4513 \times genotype interaction, $F_{(1,60)} = 7.17$, $P < 0.05$). The Ro 15-4513-induced stimulant effect in $\alpha 2$ H101R mice increased over sessions (main effect of session, $F_{(13,390)} = 3.13$, $P < 0.05$, $\epsilon = 0.206$). For clarity, data are presented for every third day. (C) Ro 15-4513-induced behavioral sensitization (relative to Session 1) in $\alpha 2$ H101R mice was still apparent after a 28-day drug-free interval (greater Ro 15-4513-induced activity levels at 28-days [$t(14) = -2.65$, $P < 0.05$). There were no differences between the final day of sensitization and day 28. (D) Dopamine turnover in the NAcc of WT and $\alpha 2$ H101R mice treated with vehicle or 10 mg/kg Ro 15-4513. There were no significant main effects of treatment or genotype, or interaction terms, on the DOPAC/dopamine ratio, or on levels of dopamine or DOPAC (SI Text).

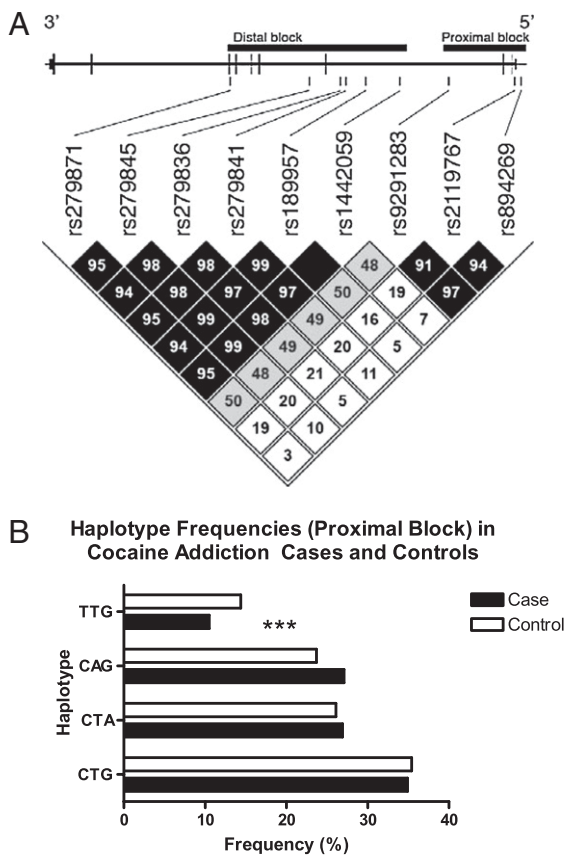


Fig. 4. Haplotypes of *GABRA2*. (A) Pair-wise scaled SNP linkage (D') of the 9 SNPs studied and an illustration of their position within the genomic sequence of the *GABRA2* gene, which is encoded on the reverse genomic strand (5' to 3' in relation to the gene). The SNPs all fall within introns or the 5' promoter region (thick vertical bars represent coding exons, and thin bars represent untranslated regions of the gene). These SNPs fall into two linkage disequilibrium (LD) blocks (black) consisting of six and three SNPs, respectively, with $D' > 90\%$ (the first three SNPs of the first block comprise the Edenberg three SNP haplotype). (B) Haplotype frequencies in cases and controls. Significantly different haplotypes are indicated by three stars ($P = 0.0014$).

with the drug to initiate drug taking by activating incentive processes or stimulus-response habits (1). Such cues come to activate incentive motivational pathways that depend upon interactions between basolateral amygdala and NAcc (19). Learning the cue-reward association depends on synaptic plasticity within the basolateral amygdala, and genetic manipulations of glutamatergic systems that impair or facilitate plasticity correspondingly impair and facilitate the development of conditioned reinforcement (20, 21). That $\alpha 2^{-/-}$ mice showed normal conditioned reinforcement indicates that learning processes within the basolateral amygdala were unaffected by the deletion, although $\alpha 2$ -GABA_A receptors are heavily expressed in this structure (3).

On the other hand, the ability of psychostimulants to facilitate conditioned reinforcement is attributable to their action in facilitating dopaminergic transmission within the NAcc (6, 19). The failure of cocaine to facilitate conditioned reinforcement in the $\alpha 2^{-/-}$ mice thus strongly suggests altered neuronal function within the accumbens. Within NAcc, dopamine acts on MSNs to modulate sensitivity to glutamatergic signals from basolateral amygdala and other corticolimbic areas (22). By mediating a phasic inhibitory input into MSNs, $\alpha 2$ -GABA_A receptors are likely to interact with such systems to determine MSN output.

During behavioral sensitization, the stimulant effects of addictive drugs increase with repeated administration. Sensitization is strongly influenced by the formation of associations between environmental cues and the drug effect (5, 23). Such learning depends upon neuronal plasticity in NAcc pathways (24), providing a simple model of drug incentive learning underlying the development of addictions (5, 25). The lack of sensitization to cocaine in the $\alpha 2^{-/-}$ mice is thus also consistent with a role of $\alpha 2$ -GABA_A receptors in pathways necessary for cocaine-induced facilitation of conditioned activity (9).

$\alpha 2$ -GABA_A receptors are not directly involved in signaling reward or in the animal's ability to form conditioned associations with rewarding events, as neither conditioned place preference nor conditioned reinforcement was impaired in the $\alpha 2^{-/-}$ mice. In agreement, data from primate self-administration studies (26, 27) suggest that compounds with some selectivity for $\alpha 2$ -GABA_A receptors may possess lower abuse potential than drugs with selectivity for $\alpha 1$ -receptors. Rather, $\alpha 2$ -receptors appear to mediate dopamine's ability to act as a "gain amplifier" so that, in their absence, facilitation of dopamine transmission by cocaine is no longer effective in strengthening behavior directed by conditioned cues.

Both of our mouse models employed constitutive mutants, so that changes in receptor function will have occurred throughout the brain and during development. Nevertheless, it is highly unlikely that our findings reflect developmental changes as a result of the genetic manipulation. First, we found no evidence for altered expression of other GABA_A receptor subtypes in the $\alpha 2^{-/-}$ mice, indicating a lack of compensation in subunit expression, at least in the adult mouse, although we cannot exclude changes to other receptors/channels.

Second, the $\alpha 2H101R$ mouse provided persuasive evidence to further implicate $\alpha 2$ -GABA_A receptors in the absence of compensation. Importantly, as revealed here for the NAcc, the arginine to histidine manipulation does not affect GABAergic transmission mediated by the mutant receptor (28, 29). Therefore, in the absence of a benzodiazepine, the mutant receptor is physiologically identical to WT, providing no pressure for compensation. However, as the mutation selectively imparts a GABA facilitatory action to Ro15-4513, this drug was used to explore the role of $\alpha 2$ -GABA_A receptors. Importantly, Ro15-4513 enhanced locomotion and induced sensitization in $\alpha 2H101R$, but not WT mice.

The behavioral effects we observe are unlikely to depend on GABA directly regulating ventral tegmental area dopamine neurons, where the $\alpha 2$ subunit is absent (3, 30) or sparse (4), nor the ventral pallidum projection area of the MSNs, where the $\alpha 1$ subunit predominates (3, 4, 30). Instead, these behaviors are probably attributable to $\alpha 2$ -GABA_A receptors in the NAcc. Our electrophysiological studies revealed their expression within inhibitory synapses of this brain region. Furthermore, in $\alpha 2H101R$ mice, the acute stimulant effect of Ro 15-4513 was mimicked by direct administration of the drug into the NAcc.

Collectively, our findings indicate an important role for $\alpha 2$ -GABA_A receptors in modulating activity of GABAergic MSNs, thus contributing to the incentive effects of drugs of abuse. This mechanism provides a potential explanation for the association we observed between human cocaine addiction and haplotypes of the *GABRA2* gene encoding $\alpha 2$ subunits.

Drug-associated cues are well known to trigger drug seeking and to induce relapse in abstaining addicts, so that facilitation of their action by cocaine represents a double jeopardy for relapse. Our finding that $\alpha 2$ -GABA_A receptors are necessary for such facilitation points to a possible target for substances that might act to prevent "lapses" (i.e., single drug use) in abstaining addicts from developing into binge use of cocaine (i.e., return to addictive patterns of consumption).

Materials and Methods

Animals. $\alpha 2^{-/-}$ mice (31) and $\alpha 2H101R$ “knock-in” mice (11, 12) were generated as previously described. Mice were offspring of heterozygous pairings ($\alpha 2^{-/-}$ experiments) or F1 to F3 offspring of homozygous mutant or WT pairings bred at the Universities of Sussex or Dundee. In both cases, the background was mixed 50% C57BL/6J – 50% 129SvEv. All experiments were carried out under the United Kingdom Animal (Scientific Procedures) Act 1986, following ethical review by the University of Sussex Ethical Review Committee, or University of Dundee Ethical Review Committee.

Locomotor Activity. Locomotor activity, and behavioral sensitization to cocaine ($\alpha 2^{-/-}$) mice or Ro 15-4513 ($\alpha 2H101R$) mice were measured in circular runways, as previously described (32).

Conditioned Reinforcement. Mice were trained in operant boxes to press a lever to activate a stimulus previously paired with free food delivery. Rates of lever pressing following i.p. cocaine (0, 3, 10 mg/kg) were assessed.

Intra-Accumbens Administration of Ro 15-4513. Seven female $\alpha 2H101R$ and 9 WT littermates were implanted stereotaxically under isoflurane anesthesia with guide cannulae (26 ga.) aimed at NAcc (coordinates AP1.34; L^{+/−} 1.00; DV −3.20 in ref. 33), and locomotor activity measured after vehicle and Ro 15-4513 infusions. Location of cannulae was confirmed histologically.

Dopamine Turnover in the NAcc. WT and $\alpha 2H101R$ mice, males and females were dosed with vehicle or 10 mg/kg Ro 15-4513 (group size 7–12), killed 15

min later and NAcc dissected, immediately placed on dry ice, and stored at -80°C until assay by HPLC with electrochemical detection.

Electrophysiology. Coronal slices containing the NAcc were prepared from WT and $\alpha 2H101R$ mice [postnatal day 17 (P17) to P24]. Whole-cell patch clamp recordings were made at 35°C from neurons of the NAcc core. See *SI Materials and Methods* for further information.

Human Genetic Association Studies. Blood samples from 699 cocaine addiction patients were identified in outpatient inpatient units and 866 unpaid controls via the blood transfusion service of São Paulo, Brazil (34). Complete description of the methodology is provided in *SI Materials and Methods*. See also *Table S4*.

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