



Published in final edited form as:

CNS Neurosci Ther. 2014 July ; 20(7): 679–684. doi:10.1111/cns.12261.

Positive allosteric modulation of GABA_B receptors ameliorates sensorimotor gating in rodent models

Roberto Frau^{1,2}, Valentina Bini^{1,2}, Giuliano Pillolla¹, Pari Malherbe³, Alessandra Pardu¹, Andrew W. Thomas⁴, Paola Devoto^{1,2}, and Marco Bortolato^{2,5,6}

¹“Guy Everett” Laboratory, Dept. of Biomedical Sciences, University of Cagliari, Italy

²Tourette Syndrome Center, University of Cagliari, Italy

³F. Hoffmann-La Roche AG, pRED, Pharma Research & Early Development, DTA Neuroscience, Grenzacherstrasse 124, Basel, Switzerland, CH4070

⁴F. Hoffmann-La Roche AG, pRED, Pharma Research & Early Development, Small Molecule Research, Grenzacherstrasse 124, Basel, Switzerland, CH4070

⁵Dept. of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence (KS), USA

⁶Consortium for Translational Research on Aggression and Drug Abuse (ConTRADA), University of Kansas, Lawrence (KS), USA

Abstract

Background—Converging evidence points to the involvement of γ -amino-butyric acid B receptors (GABA_BRs) in the regulation of information processing. We previously showed that GABA_BR agonists exhibit antipsychotic-like properties in rodent models of sensorimotor gating deficits, as measured by the prepulse inhibition (PPI) of the acoustic startle reflex. The therapeutic potential of these agents, however, is limited by their neuromuscular side effects; thus, in the present study we analyzed whether *rac*-BHFF, a potent GABA_BR positive allosteric modulator (PAM), could counter spontaneous and pharmacologically induced PPI deficits across various rodent models.

Methods—We tested the antipsychotic effects of *rac*-BHFF on the PPI deficits caused by the *N*-methyl-D-aspartate glutamate receptor antagonist dizocilpine, in Sprague-Dawley rats and C57BL/6 mice. Furthermore, we verified whether *rac*-BHFF ameliorated the spontaneous PPI impairments in DBA/2J mice.

Results—*rac*-BHFF dose-dependently countered the PPI deficits across all three models, in a fashion akin to the GABA_BR agonist baclofen and the atypical antipsychotic clozapine; in contrast with these compounds, however, *rac*-BHFF did not affect startle magnitude.

Corresponding author: Marco Bortolato, MD PhD, Dept. of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, 1251 Wescoe Hall Dr., Rm 5040, Lawrence, KS 66044, Tel.:785-864-1936; Fax: 785-864-5219, bortolato@ku.edu.
Roberto Frau and Valentina Bini contributed equally to this work

No conflicts of interest were declared by any authors. None of the institutions had any further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflicts of interest: Pari Malherbe and Andrew W Thomas are employees of Hoffmann-La Roche.

Conclusions—The present data further support the implication of GABA_BRs in the modulation of sensorimotor gating, and point to their PAMs as a novel promising tool for antipsychotic treatment, with fewer side effects than GABA_BR agonists.

Keywords

GABA_B receptors; prepulse inhibition of the startle; sensorimotor gating; schizophrenia; NMDA receptors; DBA/2J mice

Introduction

Ample evidence shows that γ -amino-butyric acid (GABA), the main inhibitory neurotransmitter in the CNS, is implicated in schizophrenia pathogenesis [1-3]. In particular, several clinical investigations have documented deficits in the expression of the metabotropic GABA_B receptors (GABA_BRs) in the cortex and hippocampus of schizophrenia patients [4-6]. The mechanisms supporting the involvement of GABA_BRs in psychotic disorders remain elusive; however, recent findings suggest that the dysfunction of this receptors in the cortex leads to alterations of glutamate signaling and excitatory/inhibitory imbalances [7-8], which contribute to the aberrant information processing and cognitive deficits in schizophrenia [9-10].

In keeping with this background, our group and others showed that the prototypical GABA_BR agonist baclofen (BAC) countered the disruption of prepulse inhibition (PPI) of the acoustic startle reflex produced by the blockade of N-methyl-D-aspartate glutamate receptors (NMDARs) in rats [11-12] and mice [13]. This endophenotype is widely regarded as a heuristic index of sensorimotor gating, the cognitive function that enables pre-attentive information filtering and governs the detection of perceptual salience [14]; notably, PPI deficits are featured in schizophrenia and related neuropsychiatric disorders [15-16].

In subsequent studies, we found that BAC rescued the marked deficits in sensorimotor gating present in DBA/2J mice [17], a strain that features antipsychotic-sensitive PPI deficits [18].

These findings collectively highlight GABA_BR as a highly promising target for antipsychotic treatment. Indeed, Daskalakis and George [19] hypothesized that GABA_BR activation may be the mechanism underlying the unique ability of the antipsychotic agent clozapine (CLO) in reducing the severity of negative symptoms in schizophrenia. While BAC monotherapy does not elicit significant antipsychotic effects [20], preliminary studies have documented its therapeutic effectiveness as an adjunctive treatment [21-22]. The therapeutic employment of BAC in schizophrenia is limited by several factors, including its poor ability to cross the blood-brain barrier, its short latency of action [23], as well as its potentially severe side effects, including muscle flaccidity, sedation, loss of reflexes, and, at higher dosages, bradycardia, respiratory depression, hypothermia and coma [24]. A new class of GABA_BR positive allosteric modulators (PAMs) has been recently developed [25] to harness the therapeutic potential of GABA_BR activation without eliciting the side effects of BAC. The mechanism of these compounds is based on the enhancement of the effects of

GABA on GABA_BRs [26-27]. One of the most potent drugs in this family, *rac*-BHFF [(*R,S*)-5,7-di-*tert*-butyl-3-hydroxy-3-trifluoromethyl-3*H*-benzofuran-2-one] [25], has been shown to produce behavioral effects without altering motor coordination and nervous reflexes, and with a much better safety index than GABA_BR agonists [25; 28-31]. Here we show that *rac*-BHFF elicited antipsychotic-like effects on the pharmacologically induced and spontaneous gating deficits in rodent models, as assessed through the paradigm of the prepulse inhibition (PPI) of the acoustic startle reflex; although these effects were comparable to those of BAC and CLO, *rac*-BHFF did not induce the same alterations of startle reflex associated with these drugs.

Materials and methods

Animals

We used behaviorally-naïve male Sprague-Dawley rats weighing between 250-300 g, and male DBA/2J and C57BL/6J between 18-24 g. Animals were housed 4/cage in a room maintained at a temperature of 22°C and kept under an artificial 12/12-h light/dark cycle. Animals were given *ad libitum* access to food and water and handled for 5 min daily to minimize experimental stress. All experimental procedures were approved by the ethical committee of the University of Cagliari and carried out in strict accordance with the guidelines for experimental animals care [European Economic Community (86/609; DL 27/01/92, number 110)].

Drugs

rac-BHFF (Hoffmann-La Roche, Basel, Switzerland) was suspended in a mixture containing Cremophor EL, 1,2-propanediol and distilled water (4:1:30 ratio) and administered intragastrically (per os, PO) at an injection volume of 10 ml/kg. The NMDAR antagonist dizocilpine (DIZ; Sigma-Aldrich, Milan, Italy) was dissolved in 0.9% saline and administered subcutaneously (SC). BAC (Tocris Cookson, Bristol, UK) was dissolved in saline and administered intraperitoneally (IP). CLO (Sigma-Aldrich) was dissolved in a single drop of 1 N HCl and diluted with saline; the pH was adjusted to 7 with NaHCO₃. Parenteral injections were administered in injection volumes of 1 ml/kg for rats and 10ml/kg for mice.

Experimental procedures

Acoustic startle reflex and PPI were measured in 4 sound-attenuated chambers (Med Associates, St Albans, USA) with fan ventilation. Each chamber consisted of a Plexiglas cylinder (9 cm diameter for rats and 3.2 cm diameter for mice) mounted on a piezoelectric accelerometric platform and connected to an analogue-digital converter. Background noise and acoustic bursts were conveyed by two separate speakers, properly spaced from the cylinder so as to produce a variation of sound within 1 dB across it. Both speakers and startle cylinders were connected to a main PC computer, which detected and analyzed all chamber variables by means of specific software. Before every testing session, acoustic stimuli and mechanical responses were calibrated via specific devices supplied by Med Associates.

The experimental procedure was based on the protocols described in Frau *et al* [32]. Briefly, three days before the experiment, animals went through a brief baseline startle session. Animals were exposed to 70 dB background white noise for a 5 min acclimation, followed by presentation to a randomized sequence of twelve 40 ms acoustic pulses of 115 dB, interposed with three trials in which an 82 dB prepulse preceded the 115 dB pulse by 100 ms. Subsequently, treatment groups were established so that average startle responses and % PPI (calculated as: $100 - [(mean\ startle\ amplitude\ for\ prepulse + pulse\ trials / mean\ startle\ amplitude\ for\ pulse\ alone\ trials) \times 100]$) were equivalent across groups. On the testing day, each animal was placed in the cylinder for a 5-min acclimation to 70 dB background white noise, which continued for the remainder of the entire session. The session consisted of three consecutive blocks of trials. Unlike the first and the third block, during which animals were presented with only five pulse-alone trials of 115 dB, the second block displayed a pseudorandom sequence of 50 trials, including 12 pulse-alone trials, 30 trials of pulse preceded by 74, 78 or 86 dB prepulses (ten for each level of prepulse loudness) and eight no stimulus trials, where only background noise was delivered. Intertrial intervals (ITI) were selected randomly between 10 and 15 s. The duration of pulses and prepulses were 40 and 20 ms, respectively. Prepulse-pulse delay amounted to 100 ms.

Data analysis

Normality and homoscedasticity of data were verified by Kolmogorov-Smirnov and Bartlett's tests. Data were compared across groups by one-way or two-way ANOVAs, as appropriate. As no interaction between prepulse levels and treatment were found in the statistical analysis, %PPI values were collapsed across prepulse intensity to represent average %PPI. *Post-hoc* analyses were performed using Tukey's test with Spjøtvoll-Stoline correction. Significance threshold was set at 0.05.

Results

Effects of rac-BHFF on the PPI deficits induced by DIZ in Sprague Dawley rats

In the first experiment, we investigated whether *rac*-BHFF pretreatment could prevent the PPI disruption induced by the NMDAR antagonist DIZ in Sprague Dawley rats. Animals were pretreated with either *rac*-BHFF (25-50 mg/kg, PO) or vehicle (control) and sixty minutes later were given an injection of either DIZ (0.1 mg/kg, SC) or saline (control). Animals were subjected to PPI testing five min after the last treatment.

No significant differences in startle amplitude were found between pretreatment-treatment groups (Fig. 1A) [Interaction: $F_{2,47}=0.10$; NS]. Conversely, we found a significant pretreatment \times treatment interaction (Fig. 1B) [Interaction: $F_{2,47}=4.62$, $P<0.05$] between groups. *Post-hoc* analyses revealed that the highest dose of *rac*-BHFF significantly reversed DIZ-induced PPI deficits ($P<0.05$; Tukey's test).

In contrast, BAC (5 mg/kg, IP) produced a significant reduction in startle amplitude [Main effect: $F_{1,37}=9.44$; $P<0.01$] (Fig. 1A); furthermore, it significantly prevented the PPI impairments caused by DIZ [Interaction: $F_{1,37}=4.89$; $P<0.05$; $P<0.05$ for vehicle-DIZ vs BAC-DIZ comparisons] (Fig. 1B). As expected, CLO (5 mg/kg, IP) elicited similar effects,

with a general reduction of startle magnitude [Main effect: $F_{1,37}=7.17$; $P<0.05$] (Fig. 1A) and a significant reversal of DIZ-mediated PPI deficits [Interaction: $F_{1,37}=5.27$; $P<0.05$; $P<0.05$ for vehicle-DIZ vs CLO-DIZ comparisons] (Fig. 1B).

Effects of *rac*-BHFF on the PPI deficits induced by DIZ in C57BL/6J mice

Next, we studied whether *rac*-BHFF (6.25-12.5 mg/kg, PO) exhibited antipsychotic-like effects in C57BL/6J mice treated with DIZ (0.3mg/kg, IP). In parallel with our results on rats, no differences were found between groups for startle amplitude (Fig. 2A) [Interaction: $F_{2,59} = 1.99$, NS]; however, we detected a marked pretreatment \times treatment interaction (Fig. 2B) [$F_{2,52}=5.32$, $P<0.01$]. Specifically, the PPI-disruptive effects of DIZ ($P<0.01$) were prevented by *rac*-BHFF treatment at a dose of 12.5mg/kg ($P<0.001$; Tukey's test). As previously shown [17], BAC (5 mg/kg, IP) did not affect startle amplitude in C57BL/6J mice (Fig.2A); notably, the PPI analysis indicated main effects for both pre-treatment (BAC) [$F_{1,41}=7.00$; $P<0.05$] and treatment (DIZ) [$F_{1,41}=8.13$; $P<0.01$], but no significant interactions [$F_{1,41}=2.32$; NS] (Fig. 2B). Conversely, CLO (5 mg/kg, IP) reduced startle magnitude [Main effect: $F_{1,41}=7.84$; $P<0.01$] (Fig.2A) and significantly countered the PPI disruption induced by DIZ [Interaction: $F_{1,41}=4.81$; $P<0.05$; $P<0.05$ for vehicle-DIZ vs CLO-DIZ comparisons] (Fig. 2B).

Effects of *rac*-BHFF on the spontaneous PPI deficit displayed by DBA/2J strain of mice

In the last experiment we evaluated the intrinsic effects of *rac*-BHFF on the spontaneous low PPI baseline displayed by DBA/2J mice. Animals were treated with VEH or *rac*-BHFF (6.25-12.5 mg/kg, PO) and subjected to PPI sessions sixty minutes later. The GABA_BR PAM did not elicit any changes in startle amplitude at any dose (Fig. 3A) [$F_{2,35}=0.99$, NS]. In contrast, low PPI in DBA mice was countered by *rac*-BHFF [$F_{2,35}=4.63$ $P<0.05$], specifically at the dose of 12.5 mg/kg ($P<0.05$; Tukey's test) (Fig. 3B). BAC and CLO failed to affect startle amplitude, but significantly countered DIZ-induced PPI deficits [BAC: $F_{1,20}=10.28$; $P<0.01$; CLO: $F_{1,20}=12.36$; $P<0.01$] (Fig. 3B).

Discussion

The results of this study showed that *rac*-BHFF, a potent GABA_BR PAM, dose-dependently reversed DIZ-induced PPI deficits in both SD rats and C57BL/6J mice, and rescued the PPI impairments displayed by DBA/2J mice. Overall, these effects were akin to those elicited by the GABA_BR agonist BAC and the atypical antipsychotic CLO, and substantially confirm previous findings by our group and others [11-13; 18] on the therapeutic potential of GABA_BR activators on sensorimotor gating deficits induced by NMDAR blockade. Notably, in contrast with BAC and CLO, *rac*-BHFF did not significantly reduce the magnitude of startle reflex, irrespective of the animal model and dose. It is also worth mentioning that the same doses of *rac*-BHFF that elicited antipsychotic-like effects in our models also failed to affect locomotor responses or other spontaneous behavioral manifestations in the home cage [unpublished observations]. Taken together, our findings complement previous preclinical data on the beneficial effects of GABA_BR PAMs [33-34] and highlight this class of compound as a novel putative avenue for antipsychotic therapy with fewer side effects than GABA_BR antagonists.

One of the key problems in the potential employment of BAC as an add-on treatment lies in the exacerbation of locomotor impairments and sedative effects caused by dopamine D₂ receptor antagonism; indeed, in preliminary studies we observed that the association of BAC and antipsychotic drugs, such as CLO and haloperidol, was not suitable for behavioral studies in rodents, in view of the serious impairments in neuromuscular coordination produced by such combinations. From this perspective, GABA_BR PAMs may afford a safer and tolerable alternative as antipsychotic adjunctive therapies for schizophrenia or related disorders. Future clinical and preclinical studies are warranted to evaluate this interesting perspective and validate the potential usefulness of *rac*-BHFF and similar agents in the treatment of psychoses.

The PPI of the acoustic response refers to the reduction in the response amplitude to a sudden and intense startling stimulus [pulse], when it is immediately preceded by a weaker non-startling pre-stimulus [35]. This phenomenon is widely regarded as a dependable index of sensorimotor gating integrity, and is typically impaired in schizophrenia [17]. In rodents, DIZ and other NMDAR antagonists produce marked PPI deficits, which are sensitive to CLO and other atypical, but not typical, antipsychotics [36-38]. The effects of NMDAR antagonists on sensorimotor gating are in line with the well-known psychotomimetic effects of these drugs [39] and other alterations of informational processing [40].

Although the mechanisms by which DIZ and other NMDAR blockers impair PPI remain unclear, several studies point to a key role of the prefrontal cortex (PFC) and hippocampal regions in these phenomena [12; 41]. These areas are characterized by a large density of pre- and postsynaptic GABA_BRs, which finely regulate basal glutamatergic and dopaminergic functions [42]. Accordingly, disturbances in GABA_BR expression or function may affect informational salience by altering the inhibitory/excitatory balance of several neurotransmitter systems in corticolimbic regions.

It has been reported that MK-801 and PCP stimulate cortical glutamate release in PFC and hippocampus [43]. In this scenario, *rac*-BHFF could counteract the disinhibition of neuronal activity produced by exaggerated NMDAR stimulation in these areas or, alternatively, modulate distinct forebrain pathways under the control of non-NMDA glutamatergic receptors, such as AMPA and Kainate [44]. Alternatively, *rac*-BHFF may counter DIZ-mediated PPI-deficits by acting on the pallidotegmental nucleus (PTn). This predominantly GABAergic area exhibits high levels of GABA_BRs and acts as an interface between the brainstem and forebrain regions implicated in PPI regulation regions [45]. Accordingly, DIZ has been recently shown to induce PPI deficits through alterations of the giant neurons of this region; notably, GABA_BR activation reversed these impairments by stabilizing the hyperactivation of these nuclei [13].

rac-BHFF also significantly ameliorated the PPI deficits on DBA/2J mice, a strain characterized by spontaneous gating impairments sensitive to antipsychotics [19], as well as other phenotypes reminiscent of schizophrenia symptoms, such as poor exploration as well as high aggression and social avoidance [46-49].

The antipsychotic-like actions of *rac*-BHFF in this murine strain may be related to their reduced expression of GABA_BRs and NMDARs across the cortex and hippocampus, in comparison with C57BL/6J mice [18; 50].

Several limitations in the present study should be acknowledged. First, our analysis was limited to the behavioral analysis of startle reflex and PPI, but did not include the testing of other paradigms with great relevance to the negative and cognitive symptoms of schizophrenia-spectrum disorders, such as object recognition and social interaction test [51-52]. Second, we did not evaluate the effects of *rac*-BHFF in animal models with high translational validity with respect to schizophrenia, such as DISC1- and neuregulin1-deficient mice, or rodents subjected to chronic administration of DIZ or other NMDAR antagonists, which may have better simulated the neurobiological impairments associated with schizophrenia [53-58]. Third, our research did not encompass testing of GABA_B negative allosteric modulators or antagonists, which may be essential for a full definition of the role of these receptors in schizophrenia-related endophenotypes.

Although further investigations are needed to address these limitations, our findings extend and support for the role of GABA_BRs in the pathophysiology of psychiatric disorders associated with sensorimotor gating disturbances, and point to PAMs of these targets as interesting therapeutic tools to treat cognitive deficits and negative symptoms in schizophrenia.

Acknowledgments

The present study was supported by National Institute of Health (NIH) grant R21HD070611 (to M.B.) and a Research Grant from the Tourette Syndrome Association (to MB). This study was also partially supported by sub-awards (to M.B.) from the NIH grants P20 GM103638 and UL1 TR000001 (formerly UL1RR033179), awarded to the University of Kansas and University of Kansas Medical Center. The authors are indebted to the EU COST Action CM1103 "Structure-based drug design for diagnosis and treatment of neurological diseases: dissecting and modulating complex function in the monoaminergic systems of the brain" for supporting their international collaboration. We are grateful to Sean Godar for his insightful comments on the manuscript, and Romina Pes for her valuable support in the execution of the experiments.

References

1. Benes FM, Berretta S. GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacol.* 2001; 25:1–27.
2. Blum BP, Mann JJ. The GABAergic system in schizophrenia. *Int J Neuropsychopharmacol.* 2002; 5:159–79. [PubMed: 12135541]
3. Wassef A, Baker J, Kochan LD. GABA and schizophrenia: a review of basic science and clinical studies. *J Clin Psychopharmacol.* 2003; 23:601–40. [PubMed: 14624191]
4. Mizukami K, Ishikawa M, Hidaka S, Iwakiri M, Sasaki M, Iritani S. Immunohistochemical localization of GABAB receptor in the entorhinal cortex and inferior temporal cortex of schizophrenic brain. *Prog Neuropsychopharmacol Biol Psychiatry.* 2002; 26:393–6. [PubMed: 11817519]
5. Ishikawa M, Mizukami K, Iwakiri M, Asada T. Immunohistochemical and immunoblot analysis of gamma-aminobutyric acid B receptor in the prefrontal cortex of subjects with schizophrenia and bipolar disorder. *Neurosci Lett.* 2005; 383:272–7. [PubMed: 15955420]
6. Fatemi SH, Folsom TD, Thuras PD. Deficits in GABA(B) receptor system in schizophrenia and mood disorders: a postmortem study. *Schizophr Res.* 2011; 128:37–43. [PubMed: 21303731]

7. Gandal MJ, Sisti J, Klook K, Ortinski PI, Leitman V, Liang Y, Thieu T, et al. GABAB-mediated rescue of altered excitatory-inhibitory balance, gamma synchrony and behavioral deficits following constitutive NMDAR-hypofunction. *Transl Psychiatry*. 2012; 2:e142. [PubMed: 22806213]
8. Hasan A, Wobrock T, Grefkes C, Labusga M, Levold K, Schneider-Axmann T, Falkai P, et al. Deficient inhibitory cortical networks in antipsychotic-naive subjects at risk of developing first-episode psychosis and first-episode schizophrenia patients: a cross-sectional study. *Biol Psychiatry*. 2012; 72:744–51. [PubMed: 22502988]
9. Kehrer C, Maziashvili N, Dugladze T, Gloveli T. Altered Excitatory-Inhibitory Balance in the NMDA-Hypofunction Model of Schizophrenia. *Front Mol Neurosci*. 2008; 8:1–7.
10. Yizhar O, Fenno LE, Prigge M, Schneider F, Davidson TJ, O'Shea DJ, Sohal VS, et al. Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature*. 2011; 477:171–8. [PubMed: 21796121]
11. Bortolato M, Frau R, Aru GN, Orrù M, Gessa GL. Baclofen reverses the reduction in prepulse inhibition of the acoustic startle response induced by dizocilpine, but not by apomorphine. *Psychopharmacology*. 2004; 171:322–30. [PubMed: 13680072]
12. Fejgin K, Pålsson E, Wass C, Finnerty N, Lowry J, Klamer D. Prefrontal GABA(B) receptor activation attenuates phencyclidine-induced impairments of prepulse inhibition: involvement of nitric oxide. *Neuropsychopharmacology*. 2009; 7:1673–84. [PubMed: 19145229]
13. Arai S, Takuma K, Mizoguchi H, Ibi D, Nagai T, Takahashi K, Kamei H, et al. Involvement of pallidotegmental neurons in methamphetamine- and MK-801-induced impairment of prepulse inhibition of the acoustic startle reflex in mice: reversal by GABAB receptor agonist baclofen. *Neuropsychopharmacology*. 2008; 33:3164–75. [PubMed: 18354384]
14. Vollenweider FX, Geyer MA. A systems model of altered consciousness: integrating natural and drug-induced psychoses. *Brain Res Bull*. 2001; 56:495–507. [PubMed: 11750795]
15. Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology*. 2001; 156:234–58. [PubMed: 11549226]
16. Koch M. Clinical relevance of animal models of schizophrenia. *Suppl Clin Neurophysiol*. 2013; 62:113–20. [PubMed: 24053035]
17. Bortolato M, Frau R, Orrù M, Piras AP, Fà M, Tuveri A, Puligheddu M, et al. Activation of GABA(B) receptors reverses spontaneous gating deficits in juvenile DBA/2J mice. *Psychopharmacology*. 2007; 194:361–9. [PubMed: 17604981]
18. Olivier B, Leahy C, Mullen T, Paylor R, Groppi VE, Sarnyai Z, Brunner D. The DBA/2J strain and prepulse inhibition of startle: a model system to test antipsychotics? *Psychopharmacology*. 2001; 156:284–90. [PubMed: 11549230]
19. Daskalakis ZJ, George TP. Clozapine, GABA(B), and the treatment of resistant schizophrenia. *Clin Pharmacol Ther*. 2009; 86:442–6. [PubMed: 19626000]
20. Gulmann NC, Bahr B, Andersen B, Eliassen HM. A double-blind trial of baclofen against placebo in the treatment of schizophrenia. *Acta Psychiatr Scand*. 1976; 54:287–93. [PubMed: 793304]
21. Frederiksen PK. Letter: Baclofen in the treatment of schizophrenia. *Lancet*. 1975; 1:702. [PubMed: 47138]
22. Schöpf J, Hucker H. Baclofen in the treatment of schizophrenia: a pilot study. *Pharmakopsychiatr Neuropsychopharmakol*. 1977; 10:89–91. [PubMed: 360234]
23. Bowery NG. GABAB receptor: a site of therapeutic benefit. *Curr Opin Pharmacol*. 2006; 6:37–43. [PubMed: 16361115]
24. Sullivan R, Hodgman MJ, Kao L, Tormoehlen LM. Baclofen overdose mimicking brain death. *Clin Toxicol (Phila)*. 2012; 50:141–4. [PubMed: 22292975]
25. Malherbe P, Masciadri R, Norcross RD, Knoflach F, Kratzeisen C, Zenner MT, Kolb Y, et al. Characterization of (R,S)-5,7-di-tert-butyl-3-hydroxy-3-trifluoromethyl-3H-benzofuran-2-one as a positive allosteric modulator of GABAB receptors. *Br J Pharmacol*. 2008; 154:797–811. [PubMed: 18536733]
26. Urwyler S, Mosbacher J, Lingenhoehl K, Heid J, Hofstetter K, Froestl W, Bettler B, et al. Positive allosteric modulation of native and recombinant gamma-aminobutyric acid(B) receptors by 2,6-Di-

- tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Mol Pharmacol*. 2001; 60:963–71. [PubMed: 11641424]
27. Urwyler S, Pozza MF, Lingenhoehl K, Mosbacher J, Lampert C, Froestl W, Koller M, et al. N,N'-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and structurally related compounds: novel allosteric enhancers of gamma-aminobutyric acidB receptor function. *J Pharmacol Exp Ther*. 2003; 307:322–30. [PubMed: 12954816]
 28. Carai MA, Colombo G, Froestl W, Gessa GL. In vivo effectiveness of CGP7930, a positive allosteric modulator of the GABAB receptor. *Eur J Pharmacol*. 2004; 504:213–6. [PubMed: 15541424]
 29. Cryan JF, Kelly PH, Chaperon F, Gentsch C, Mombereau C, Lingenhoehl K, Froestl W, et al. Behavioral characterization of the novel GABAB receptor-positive modulator GS39783 (N,N'-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine): anxiolytic-like activity without side effects associated with baclofen or benzodiazepines. *J Pharmacol Exp Ther*. 2004; 310:952–63. [PubMed: 15113848]
 30. Jacobson LH, Cryan JF. Evaluation of the anxiolytic-like profile of the GABAB receptor positive modulator CGP7930 in rodents. *Neuropharmacology*. 2008; 54:854–62. [PubMed: 18328507]
 31. Koek W, France CP, Cheng K, Rice KC. GABAB receptor-positive modulators: enhancement of GABAB receptor agonist effects in vivo. *J Pharmacol Exp Ther*. 2010; 335:163–71. [PubMed: 20628000]
 32. Frau R, Orrù M, Fà M, Casti A, Manunta M, Fais N, Mereu G, et al. Effects of topiramate on the prepulse inhibition of the acoustic startle in rats. *Neuropsychopharmacology*. 2007; 32:320–31. [PubMed: 16794573]
 33. Koek W, France CP, Cheng K, Rice KC. Effects of the GABAB receptor-positive modulators CGP7930 and rac-BHFF in baclofen- and γ -hydroxybutyrate-discriminating pigeons. *J Pharmacol Exp Ther*. 2012; 341:369–76. [PubMed: 22319197]
 34. Koek W, Cheng K, Rice KC. Discriminative stimulus effects of the GABAB receptor-positive modulator rac-BHFF: comparison with GABAB receptor agonists and drugs of abuse. *J Pharmacol Exp Ther*. 2013; 344:553–60. [PubMed: 23275067]
 35. Swerdlow NR, Geyer MA. Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophr Bull*. 1998; 24:285–301. [PubMed: 9613626]
 36. Bakshi VP, Swerdlow NR, Geyer MA. Clozapine antagonizes phencyclidine-induced deficits in sensorimotor gating of the startle response. *J Pharmacol Exp Ther*. 1994; 2:787–94. [PubMed: 7965797]
 37. Bakshi VP, Geyer MA. Antagonism of phencyclidine-induced deficits in prepulse inhibition by the putative atypical antipsychotic olanzapine. *Psychopharmacology*. 1995; 2:198–201. [PubMed: 8848537]
 38. Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology*. 2001; 156:117–54. [PubMed: 11549216]
 39. Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry*. 1991; 10:1301–8. [PubMed: 1654746]
 40. Umbricht D, Schmid L, Koller R, Vollenweider FX, Hell D, Javitt DC. Ketamine-induced deficits in auditory and visual context-dependent processing in healthy volunteers: implications for models of cognitive deficits in schizophrenia. *Arch Gen Psychiatry*. 2000; 57:1139–47. [PubMed: 11115327]
 41. Bakshi VP, Geyer MA. Multiple limbic regions mediate the disruption of prepulse inhibition produced in rats by the noncompetitive NMDA antagonist dizocilpine. *J Neurosci*. 1998; 18:8394–401. [PubMed: 9763482]
 42. Bowery NG. Historical perspective and emergence of the GABAB receptor. *Adv Pharmacol*. 2010; 58:1–18. [PubMed: 20655476]
 43. Roenker NL, Gudelsky GA, Ahlbrand R, Horn PS, Richtand NM. Evidence for involvement of nitric oxide and GABA(B) receptors in MK-801-stimulated release of glutamate in rat prefrontal cortex. *Neuropharmacology*. 2012; 63:575–81. [PubMed: 22579658]

44. Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci*. 1997; 17:2921–7. [PubMed: 9092613]
45. Takahashi K, Nagai T, Kamei H, Maeda K, Matsuya T, Arai S, Mizoguchi H, Yoneda Y, Nabeshima T, Takuma K, Yamada K. Neural circuits containing pallidotegmental GABAergic neurons are involved in the prepulse inhibition of the startle reflex in mice. *Biol Psychiatry*. 2007; 62:148–57. [PubMed: 17027927]
46. Elias PK, Elias MF, Eleftheriou BE. Emotionality, exploratory behavior, and locomotion in aging inbred strains of mice. *Gerontologia*. 1975; 1:46–55. [PubMed: 1140561]
47. Randt CT, Blizard DA, Friedman E. Early life undernutrition and aggression in two mouse strains. *Dev Psychobiol*. 1975; 3:275–9. [PubMed: 1241773]
48. Anisman H. Differential effects of scopolamine and D-amphetamine on avoidance: Strain interactions. *Pharmacol Biochem Behav*. 1975; 3:809–17. [PubMed: 1208621]
49. Stavnes KL, Sprott RL. Genetic analysis of active avoidance performance in mice. *Psychol Rep*. 1975; 36:515–21. [PubMed: 1144596]
50. Lu Y, Wehner JM. Enhancement of contextual fear-conditioning by putative (+/-)-alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor modulators and N-methyl-D-aspartate (NMDA) receptor antagonists in DBA/2J mice. *Brain Res*. 1997; 768:197–207. [PubMed: 9369316]
51. Rajagopal L, Massey BW, Huang M, Oyamada Y, Meltzer HY. The novel object recognition test in rodents in relation to cognitive impairment in schizophrenia. *Curr Pharm Des*. 2013 in press.
52. Jones CA, Watson DJ, Fone KC. Animal models of schizophrenia. *Br J Pharmacol*. 2011; 164:1162–94. [PubMed: 21449915]
53. Hikida T, Jaaro-Peled H, Seshadri S, Oishi K, Hookway C, Kong S, Wu D, et al. Dominant-negative DISC1 transgenic mice display schizophrenia-associated phenotypes detected by measures translatable to humans. *Proc Natl Acad Sci*. 2007; 104:14501–6. [PubMed: 17675407]
54. Karl T, Duffy L, Scimone A, Harvey RP, Schofield PR. Altered motor activity, exploration and anxiety in heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. *Genes Brain Behav*. 2007; 6:677–87. [PubMed: 17309661]
55. Kvajo M, McKellar H, Arguello PA, Drew LJ, Moore H, MacDermott AB, et al. A mutation in mouse Disc1 that models a schizophrenia risk allele leads to specific alterations in neuronal architecture and cognition. *Proc Natl Acad Sci*. 2008; 105:7076–81. [PubMed: 18458327]
56. Neill JC, Barnes S, Cook S, Grayson B, Idris NF, McLean SL, Snigdha S, et al. Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism. *Pharmacol Ther*. 2010; 128:419–32. [PubMed: 20705091]
57. Savage S, Kehr J, Olson L, Mattsson A. Impaired social interaction and enhanced sensitivity to phencyclidine-induced deficits in novel object recognition in rats with cortical cholinergic denervation. *Neuroscience*. 2011; 195:60–9. [PubMed: 21875653]
58. Meltzer HY, Rajagopal L, Huang M, Oyamada Y, Kwon S, Horiguchi M. Translating the N-methyl-D-aspartate receptor antagonist model of schizophrenia to treatments for cognitive impairment in schizophrenia. *Int J Neuropsychopharmacol*. 2013; 16:2181–94. [PubMed: 24099265]

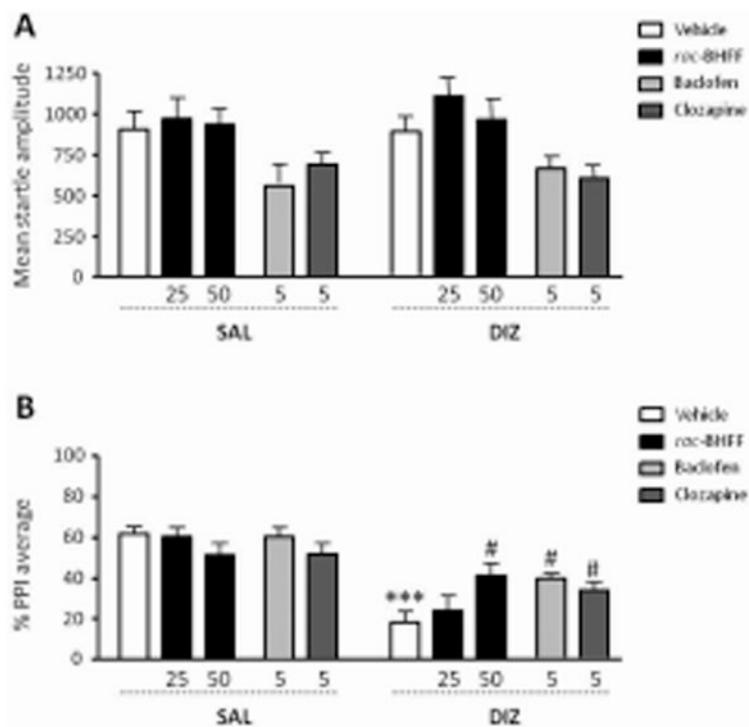


Figure 1. Effects of *rac*-BHFF on the mean startle amplitude (A) and prepulse inhibition deficits (B) induced by dizocilpine (DIZ, 0.1 mg/kg, SC) in Sprague Dawley rats, compared to baclofen and clozapine. All doses are given in milligrams per kilogram and are indicated below the horizontal axis. Values represent mean \pm SEM for each treatment. Percent prepulse inhibition (PPI) values were collapsed across all three prepulse intensities (4, 8, and 16 dB above 70 dB background noise). For all experimental groups, $n=8-12$. SAL, saline. ***, $P<0.001$, compared to VEH+SAL group; #, $P<0.05$, compared to VEH+DIZ group. For further details, see text.

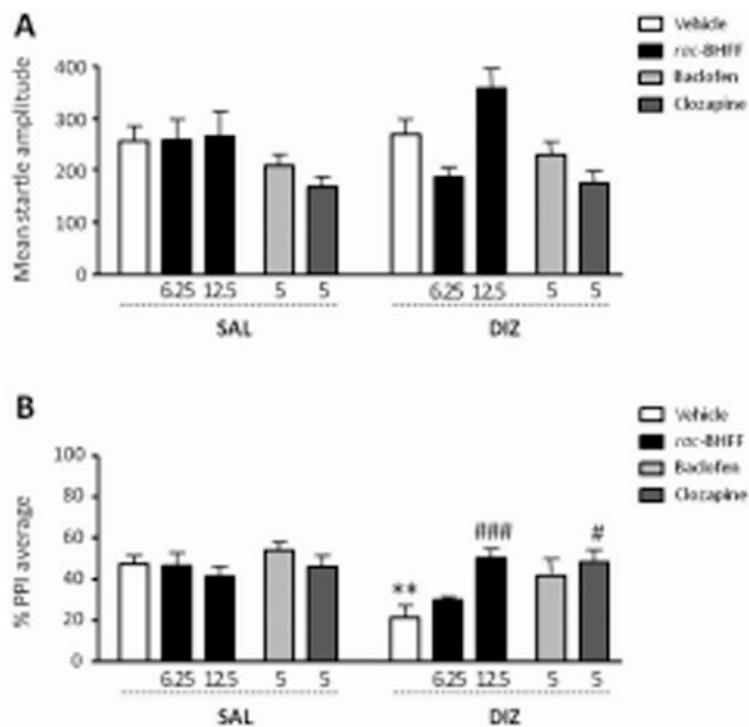


Figure 2. Effects of *rac*-BHFF on the mean startle amplitude (A) and spontaneously low prepulse inhibition deficits (B) induced by dizocilpine (DIZ, 0.3 mg/kg, IP) in C57BL/6J mice, compared to baclofen and clozapine. All doses are given in milligrams per kilogram and are indicated below the horizontal axis. Values represent mean \pm SEM for each treatment. Percent prepulse inhibition (PPI) values were collapsed across all three prepulse intensities (4, 8, and 16 dB above 70 dB background noise). For all experimental groups, $n=8-12$. SAL, saline. **, $P<0.01$, compared to VEH+SAL group; ###, $P<0.001$, #, $P<0.05$, compared to VEH+DIZ group. For further details, see text.

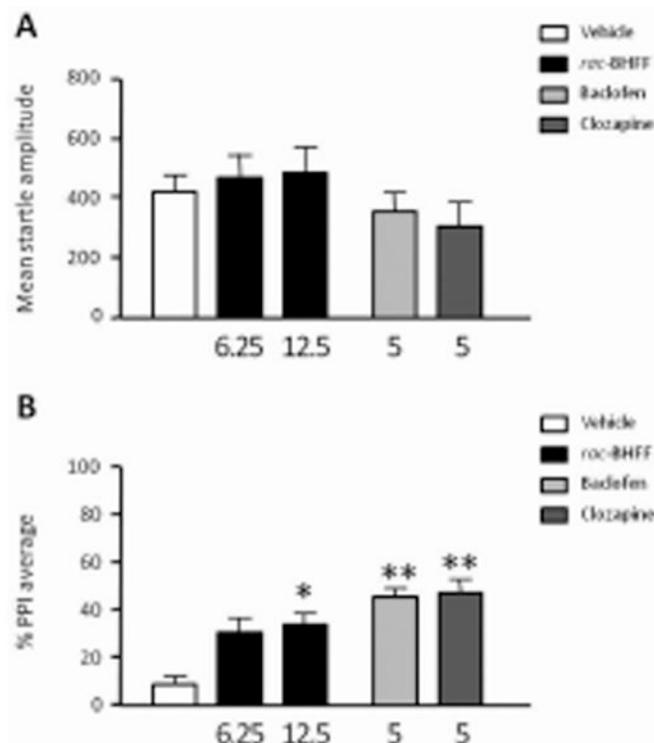


Figure 3. Effects of *rac*-BHFF on the mean startle amplitude (A) and spontaneous prepulse inhibition deficits (B) displayed by DBA/2J mice, compared to baclofen and clozapine. All doses are given in milligrams per kilogram and are indicated below the horizontal axis. Values represent mean \pm SEM for each treatment. Percent prepulse inhibition (PPI) values were collapsed across all three prepulse intensities (4, 8, and 16 dB above 70 dB background noise). For all experimental groups, $n=8-10$. **, $P<0.01$, compared to VEH group. For further details, see text.