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Negative Allosteric Modulation of the mGluR5 Receptor Reduces Repetitive Behaviors and Rescues Social Deficits in Mouse Models of Autism

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Abstract

Neurodevelopmental disorders such as autism and fragile X syndrome were long thought to be medically untreatable, on the assumption that brain dysfunctions were immutably hardwired before diagnosis. Recent revelations that many cases of autism are caused by mutations in genes that control the ongoing formation and maturation of synapses have challenged this dogma. Antagonists of metabotropic glutamate receptor subtype 5 (mGluR5), which modulate excitatory neurotransmission, are in clinical trials for fragile X syndrome, a major genetic cause of intellectual disabilities. About 30% of patients with fragile X syndrome meet the diagnostic criteria for autism. Reasoning by analogy, we considered the mGluR5 receptor as a potential target for intervention in autism. We used BTBR T+tf/J (BTBR) mice, an established model with robust behavioral phenotypes relevant to the three diagnostic behavioral symptoms of autism—unusual social interactions, impaired communication, and repetitive behaviors—to probe the efficacy of a selective negative allosteric modulator of the mGluR5 receptor, GRN-529. GRN-529 reduced repetitive behaviors in three cohorts of BTBR mice at doses that did not induce sedation in control assays of open field locomotion. In addition, the same nonsedating doses reduced the spontaneous stereotyped jumping that characterizes a second inbred strain of mice, C58/J. Further, GRN-529 partially reversed the striking lack of sociability in BTBR mice on some parameters of social approach and reciprocal social interactions. These findings raise the possibility that a single

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SUPPLEMENTARY MATERIALS

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Material and Methods

targeted pharmacological intervention may alleviate multiple diagnostic behavioral symptoms of autism.

INTRODUCTION

Autism spectrum disorders affect an estimated 1% of the population (1–4). Intensive behavioral therapy is currently the only effective treatment for the three diagnostic symptoms: qualitative impairment in social interaction, deficits in communication, and stereotyped repetitive behaviors with restricted interests (5–10). To date, only two drugs have been approved by the U.S. Food and Drug Administration for use in patients diagnosed with autism. These two agents, Risperdal and Abilify (11), do not target the core symptoms, but rather treat a cluster of associated symptoms referred to as irritability. Given the high financial and emotional burden to the families and educational and health care systems, affordable treatments for the core diagnostic symptoms of autism represent a severe unmet medical need.

Mouse models of autism spectrum disorders can serve as tools to evaluate the therapeutically relevant efficacy of experimental agents. To incorporate construct validity for the various genetic mutations identified in small numbers of people with autism (12–21), these mutations have been generated in mice (22, 23). Behavioral phenotypes with face validity for some of the diagnostic symptoms of autism have been reported in some of these mutant mouse models (24–47). Experimental interventions, working through diverse genetic and pharmacological mechanisms, rescue subsets of behavioral abnormalities in these mouse models (31, 43, 48–52). Discovery of elevated metabotropic glutamate receptor subtype 5 (mGluR5)–mediated signaling and protein synthesis in fragile X knockout mice (53–56) provided the rationale for testing mGluR5 antagonists in ongoing fragile X clinical trials. A large subset of cases of fragile X meet the diagnostic criteria for autism (57, 58). Because the primary symptoms of autism and fragile X differ in qualitative features, and mouse models of autism and fragile X display sharply divergent phenotypes, testing mGluR5 antagonists in specific assays of mouse behavioral phenotypes that optimize relevance to the diagnostic symptoms of autism would be most informative for translational goals.

Several naturally occurring inbred strains of mice display behavioral features that recapitulate diagnostic symptoms of neurodevelopmental disorders (59–70). These inbred strains are genetically homogeneous and commercially available, maximizing their feasibility as translational tools in medications development (71–73). Inbred strains with robust behavioral phenotypes relevant to the defining symptoms of autism, but without identified genetic mutations, are analogous to individuals with autism for whom the responsible genetic factor(s) remains unknown; at present, this is more than 75% of the cases of autism (21). In addition, inbred strains can incorporate multiple background genes that influence their behavioral deficits, allowing evaluation of two-hit and multiple-hit hypotheses of autism spectrum disorders.

BTBR T+tf/J (BTBR) is a commercially available inbred strain of mouse that displays behavioral phenotypes relevant to all three diagnostic symptoms of autism (60–62, 64, 66, 68–70, 72, 74–78). BTBR engages in low levels of reciprocal social interactions as juveniles

and adults, minimal social approach by both males and females, and low levels of ultrasonic vocalizations in response to social olfactory cues and during reciprocal social interactions compared to other standard inbred strains such as C57BL/6J (B6) and FVB (60–62, 64, 66, 68–70, 74, 76–78). Social interaction and communication deficits in BTBR represent face validity to the first and second diagnostic symptoms of autism, respectively. Long bouts of repetitive self-grooming are the third major characteristic of BTBR, representing face validity to the third diagnostic symptom of autism, spontaneous stereotyped and repetitive patterns of behavior. Absence of the corpus callosum connecting the right and left cortical hemispheres in BTBR (79) is reminiscent of a small subset of individuals with autism who are acallosal (80, 81) and may be a sign of more global abnormalities in white matter connectivity in this mouse strain. Experimentally induced postnatal lesions of the corpus callosum in B6 mice, however, did not recapitulate the social deficits, or the repetitive self-grooming, that characterize BTBR (82). C58/J (C58) is another commercially available inbred strain of mouse that displays high levels of stereotyped vertical jumping behaviors, relevant to the motor stereotypies of the third diagnostic symptom category of autism (65, 67). Unlike mouse models based on identified genetic mutations, the background genes responsible for autism-relevant behavioral traits in these inbred strains of mice remain under investigation (64, 83–85).

The robustness and reproducibility of autism-relevant phenotypes in BTBR and C58 provide an attractive translational platform for the preclinical evaluation of intervention therapies (72, 73). A treatment that attenuates different forms of repetitive behaviors in two different inbred strains is likely to generalize across a range of repetitive behaviors and potentially to generalize across species. Here, we test the hypothesis that a selective negative allosteric modulator of the mGluR5 receptor, GRN-529, will ameliorate autism-relevant behavioral abnormalities in mouse models of autism.

RESULTS

GRN-529 brain penetration and mGluR5 receptor occupancy

We measured the plasma and brain exposure levels of GRN-529 (Fig. 1, B to D) and the relationship between unbound brain levels of GRN-529 and mGluR5 occupancy (Fig. 1F) after systemic administration. The relationship between GRN-529 brain exposure and mGluR5 occupancy was similar in B6, BTBR, and C58 mouse strains (Fig. 1F). A 30- to 60-min timeframe was chosen for occupancy and behavioral experiments because a 2-hour time course of plasma and brain exposure after GRN-529 administration revealed that peak concentrations occurred between 0 and 60 min in B6 mice (Fig. 1E).

Amelioration of repetitive and stereotyped behaviors

GRN-529 and the prototypic mGluR5 antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) reduced the high levels of repetitive self-grooming that characterizes the BTBR strain. Consistent with previous reports (62, 64, 69, 72, 74), BTBR mice treated with vehicle engaged in much longer bouts of self-grooming than did B6 (Fig. 2 and fig. S1). In three replications by two laboratories, acute administration of GRN-529 significantly reduced repetitive self-grooming scores in BTBR (Fig. 2, B and D, and fig. S1F) at doses that

achieved 50 to 90% receptor occupancy (Fig. 1F). 3-((2-Methyl-4-thiazolyl)ethynyl)pyridine (MTEP), another standard mGluR5 antagonist, similarly reduced repetitive self-grooming in BTBR at some doses (fig. S1D) and similarly had no effect in B6 mice (fig. S1C), consistent with current and previous findings with the less selective mGluR5 antagonist MPEP (fig. S1, A and B) (72).

C58 mice displayed high levels of stereotyped vertical jumping (Fig. 2E), consistent with previous findings (67). GRN-529 dose-dependently reduced jumping in C58, almost completely abolishing this repetitive behavior at 3.0 mg/kg, a dose that achieved ~90% occupancy (Fig. 1F). The effects in C58 were not attributable to reduced locomotion or sedation (Fig. 2F and fig. S6).

Complete statistical analyses of all behavioral experiments appear in the Supplementary Materials.

Improvement in social behaviors

Two parameters of social behavior were scored in our automated three-chambered social approach task (86) as described (72, 87). B6 control mice spent more time in the side chamber containing a novel mouse than in the side chamber containing a novel object, meeting the definition of normal sociability in this task, as extensively reported (22, 62, 64, 72–74, 87–89). GRN-529 did not affect the sociability in B6 controls at any dose (Fig. 3, A and C). BTBR mice displayed lack of sociability, defined as not spending more time in the side chamber with the novel mouse than in the side chamber with the novel object, as reported (22, 62, 64, 72–74, 87). GRN-529 reversed sociability deficits in BTBR at a dose of 3.0 mg/kg, as measured by time in the chamber (Fig. 3D). For time spent sniffing the novel mouse versus the novel object, which is a more precise and sensitive measure of true social interaction (87, 90), GRN-529 reversed the deficit in BTBR, again with no detrimental effect on B6 sociability (Fig. 3, A and C). BTBR mice spent significantly more time sniffing the novel mouse than the novel object after doses of 0.3, 1.0, and 3.0 mg/kg (Fig. 3B). The number of entries between chambers, an internal control for general exploration, was not significantly affected by the lower doses of GRN-529 in either strain, but elevated numbers of entries in both strains appeared at a dose of 3.0 mg/kg (Fig. 3, E and F). A second cohort displayed a similar pattern of responses (fig. S2).

Multiple parameters of social behaviors were scored during a freely moving, dyadic reciprocal social interaction test in adult B6 and BTBR mice treated with vehicle or the most effective dose of GRN-529 in the social approach test, 3.0 mg/kg. These detailed interactive social parameters were collected with 129/SvImJ mice as stimulus partners, chosen for their inherently low spontaneous locomotion and aggression, as reported (60, 61, 91). During the reciprocal interaction test, B6 mice consistently exhibited high levels of nose-to-nose sniffing, front approach, and total time spent in social contact (Fig. 4, A and C, and fig. S3A), whereas BTBR mice displayed lack of sociability on these standard parameters (Fig. 4, B and D, and fig. S3B), consistent with previous reports (60, 78, 91). GRN-529 increased sociability in BTBR on some of these parameters, particularly on the most sensitive measures, nose-to-nose sniffing (Fig. 4B) and total time spent in social contact (Fig. 4D), while having no effect in B6 controls (Fig. 4, A and C, and fig. S3, A, C, and E). GRN-529

did not affect general exploratory locomotion in B6 and BTBR mice during the reciprocal interaction session (fig. S3, G to J). Further, during the freely moving, dyadic reciprocal interaction task, BTBR mice treated with vehicle engaged in much longer bouts of self-grooming and repetitive digging than did B6 mice (Fig. 4, E to H). Acute administration of GRN-529 significantly reduced these spontaneous repetitive behaviors within a social context (Fig. 4, F and H).

Absence of sedation

Reductions in repetitive self-grooming and stereotyped jumping such as those that we saw could be caused by sedative actions of a pharmacological agent. Conversely, increased numbers of entries in the social approach apparatus could be caused by stimulant actions of a pharmacological agent. To directly detect nonspecific actions of GRN-529 on general activity, we tested the same doses at the 30-min time point in B6, BTBR, and C58 mice on open field locomotion. No evidence of sedation was detected in any strain at any dose during a 30-min test session (Fig. 5 and figs. S4 to S6). Higher total distances traveled were seen in both strains at a dose of 3.0 mg/kg, and higher vertical and center time scores were seen in BTBR at the higher doses in one cohort tested at Pfizer, indicating a moderate increase in general exploratory locomotion. No increases in total distance traveled were observed in C58 mice at any dose of GRN-529 tested (Fig. 2F). No qualitatively unusual behaviors were observed after GRN-529 treatments during any of the grooming, social, or open field test sessions.

DISCUSSION

Autism is a behaviorally diagnosed, lifetime neurodevelopmental disorder. Biological abnormalities have been reported in eye tracking, neuroanatomical pathway connectivity, brain regional volumes, cortical activation during social and communication tasks as measured with functional magnetic resonance imaging and magnetoencephalography, serotonin levels, and other biological assays, but not with sufficient consistency for these to constitute uniform diagnostic biomarkers (92–94). Therefore, therapeutic efficacy is currently evaluated by improvement in the diagnostic behavioral symptoms (11, 50, 95). Compelling neuropharmacological targets for autism remain to be identified. Target strategies draw from therapeutics under investigation for other neurodevelopmental disorders and hypotheses emerging from mutations in synaptic genes identified in small numbers of individuals with autism spectrum disorders (43, 49, 52). One such strategy is modulation of glutamatergic neurotransmission through the mGluR5 receptor (96), which is under investigation for the treatment of fragile X (55).

Using the BTBR mouse model, which recapitulates endophenotypic analogies to the diagnostic social deficits, impaired communication, and repetitive behavioral symptoms of autism, we evaluated GRN-529, a compound with high specificity for the mGluR5 receptor. As demonstrated by Hughes and colleagues (97), in competition binding experiments, GRN-529 competes for [^{3}H]MPEP binding at mGluR5 with high affinity [inhibition constant (K_i) = 5.4 ± 0.43 nM], antagonizes glutamate-induced increases in calcium signaling, but does not directly bind to the orthosteric binding site or affect the affinity of

glutamate for this site, consistent with negative allosteric modulation (97). Using pharmacokinetic and ex vivo receptor occupancy studies, we demonstrated brain exposure and target engagement for GRN-529 across the efficacious dose range (0.3 to 3.0 mg/kg) in B6, BTBR, and C58 mice. Treatment with GRN-529 at these doses reversed the social approach deficits in BTBR on two standardized mouse assays for sociability. In particular, the more ethologically meaningful parameters of time engaged in interactive sniffing of a novel mouse versus time spent sniffing a novel object during social approach, bouts of nose-to-nose sniffing and time in social contact during reciprocal interactions, were restored in BTBR by the acute pharmacological intervention, which had no effect in the normal control B6 strain.

We discovered strong reductions in the repetitive self-grooming phenotype in the BTBR mouse model of autism after GRN-529 treatment, replicated in three cohorts of mice at two research sites. Magnitudes of reduction were consistent with a previous report with the prototypic mGluR5 antagonist MPEP, which required higher doses and is known to also act at N-methyl-D-aspartate (NMDA) receptors (72). Parallel attenuation of self-grooming in BTBR was confirmed at the high dose of MPEP, and for the more brain penetrant and selective mGluR5 analog MTEP, although its dose-response curve was nonlinear. Further, GRN-529 markedly reduced a stereotyped behavior in another inbred strain, vertical jumping in C58.

Lower self-grooming and jumping scores in the treated mice were not the result of overall behavioral sedation, because open field activity was not reduced by GRN-529 at the same doses, time point, and route of administration. Small increases in open field scores, and on number of chamber entries in the three-chambered apparatus, were detected in B6 and BTBR mice after the higher doses of GRN-529, although exploratory locomotion was not significantly affected by GRN-529 in B6 and BTBR mice engaged in social interaction in the PhenoTyper arena. Total distance traversed in the open field was not affected by GRN-529 in C58, did not reach the range considered hyperactive for B6, and did not mimic the qualitative type of fast, unstructured activity that characterizes rodent responses to psychostimulants. Nevertheless, it remains conceptually possible that increased general exploration could contribute to increased sociability. Further, these results may suggest that mGluR5 treatment could prove helpful for the subset of individuals with autism and attention deficit hyperactivity disorder.

Our results show that negative allosteric modulation of the mGluR5 receptor improves social interactions, reduces high levels of repetitive behaviors in BTBR, and reduces stereotyped behaviors in C58, relevant to the first and third diagnostic symptoms of autism. Early clinical indications of beneficial actions of mGluR5 antagonists in fragile X syndrome make this class of therapeutic targets of particular interest (55). The present preclinical findings on reversal of features relevant to autism in two mouse models convey promise for the mGluR5 strategy as a therapeutic intervention for two core diagnostic symptoms of autism.

MATERIALS AND METHODS

Adult male and female C57BL/6J (B6) and BTBR T+tf/J (BTBR) mice tested at the National Institute of Mental Health (NIMH) in Bethesda, Maryland, were bred from adult pairs originally purchased from The Jackson Laboratory (JAX). B6, BTBR, and C58/J (C58) tested at Pfizer in Groton, Connecticut, were purchased as adults from JAX. Behavioral parameters were scored with automated equipment or from digital videotapes by investigators uninformed of treatment. To further ensure absence of unconscious bias by the raters, we recorded identities of subject mice from paw tattoos only after the behavioral test session ended. All procedures were approved by the NIMH and the Pfizer Inc. Animal Care and Use Committees. Complete behavioral and biochemical methods appear in the Supplementary Materials.

Mice were injected with GRN-529 and killed at the time points indicated for pharmacokinetic and ex vivo receptor occupancy assays (Fig. 1, B to F). Plasma and forebrain samples were collected for analysis of drug concentrations by liquid chromatography–mass spectrometry, and of receptor occupancy by binding of 1 nM [^{3}H]MPEPy, similar to the procedures used by Hughes et al. (97). Results from the pharmacokinetic, ex vivo receptor occupancy and/or previous behavioral assays in B6, BTBR, and C58 mice were used to select the doses and posttreatment interval for the present behavioral studies. Complete methods and results appear in the Supplementary Materials.

Repetitive self-grooming was scored from digital videotapes with methods previously published (22, 62, 64, 72, 74, 89) and described in the Supplementary Materials. Briefly, each subject mouse was placed in a bare, empty cage for a 10-min habituation session and then video was recorded for a 10-min test session. Cumulative time spent self-grooming was scored from the videos using a high-accuracy Traceable stopwatch (Thomas Scientific) with the auditory component silenced.

Sociability in the automated three-chambered apparatus developed by our group (86) was conducted as published (22, 32, 62, 64, 72, 74, 87, 89) and described in the Supplementary Materials. Briefly, each subject mouse was placed in the bare, empty three-chambered apparatus for a 10-min habituation session. A novel object, an inverted wire cup, was then placed in one side chamber, and a novel mouse was placed inside a second inverted wire cup in the other side chamber. The subject mouse was given a 10-min test session, offering the choice of spending time in the vicinity of a novel social partner or a novel inanimate object. Time in each chamber, representing proximity to a social partner, was scored by the automated software. Time spent in directly sniffing the novel mouse, representing actual reciprocal social interactions, was subsequently scored from digital videos of the sessions by an observer with a stopwatch.

Reciprocal social interaction was tested in adult B6 and BTBR subject mice during a 10-min session in a Noldus PhenoTyper 3000 arena, as described (91). 129/SvImJ mice were used as interaction stimulus partners to evaluate social behavior in response to social cues from a uniform stimulus mouse during a 10-min session between freely moving dyads (60, 64, 91).

Standard interaction parameters, time spent in repetitive behaviors, and arena exploration were simultaneously scored as published (91) and described in the Supplementary Materials.

Open field locomotor activity was evaluated in a standard AccuScan photocell-equipped open field over a 30-min test session, using methods previously published (32, 72, 89, 98) and described in the Supplementary Materials. Automated parameters including total distance, vertical activity, and center time were generated by the VersaMax software.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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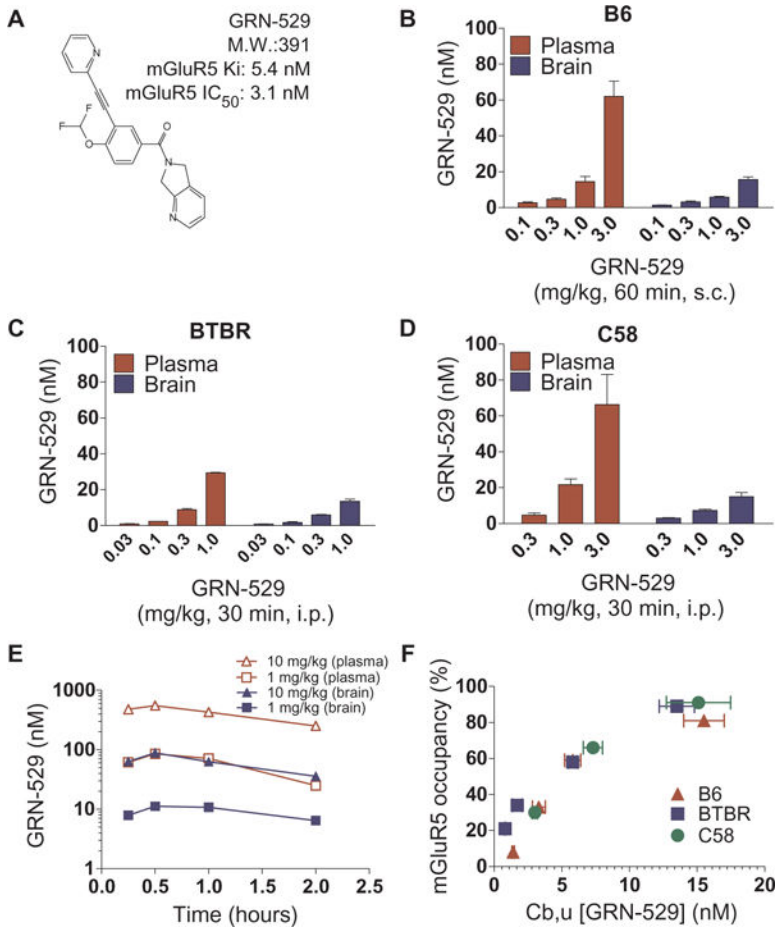

Fig. 1.

GRN-529 chemical structure, plasma and brain concentrations, and receptor occupancy in B6, BTBR, and C58 mice. (**A**) Chemical structure of GRN-529 and binding properties [K_i = 5.4 nM and median inhibitory concentration (IC_{50}) = 3.1 nM] at rat mGluR5. (**B** to **D**) Unbound plasma and brain concentrations of GRN-529 30 or 60 min after systemic administration in B6, BTBR, and C58 mice. s.c., subcutaneously; i.p., intraperitoneally. (**E**) Time course of unbound plasma and brain concentrations (nM) of GRN-529 for 2 hours after systemic intraperitoneal administration in B6 mice. (**F**) Relationship of the concentration of unbound (Cb,u) GRN-529 concentrations (nM) and mGluR5 occupancy in brains from BTBR, B6, and C58 mice. n = 3 to 5 per dose and strain. Data are expressed as the mean for each group.

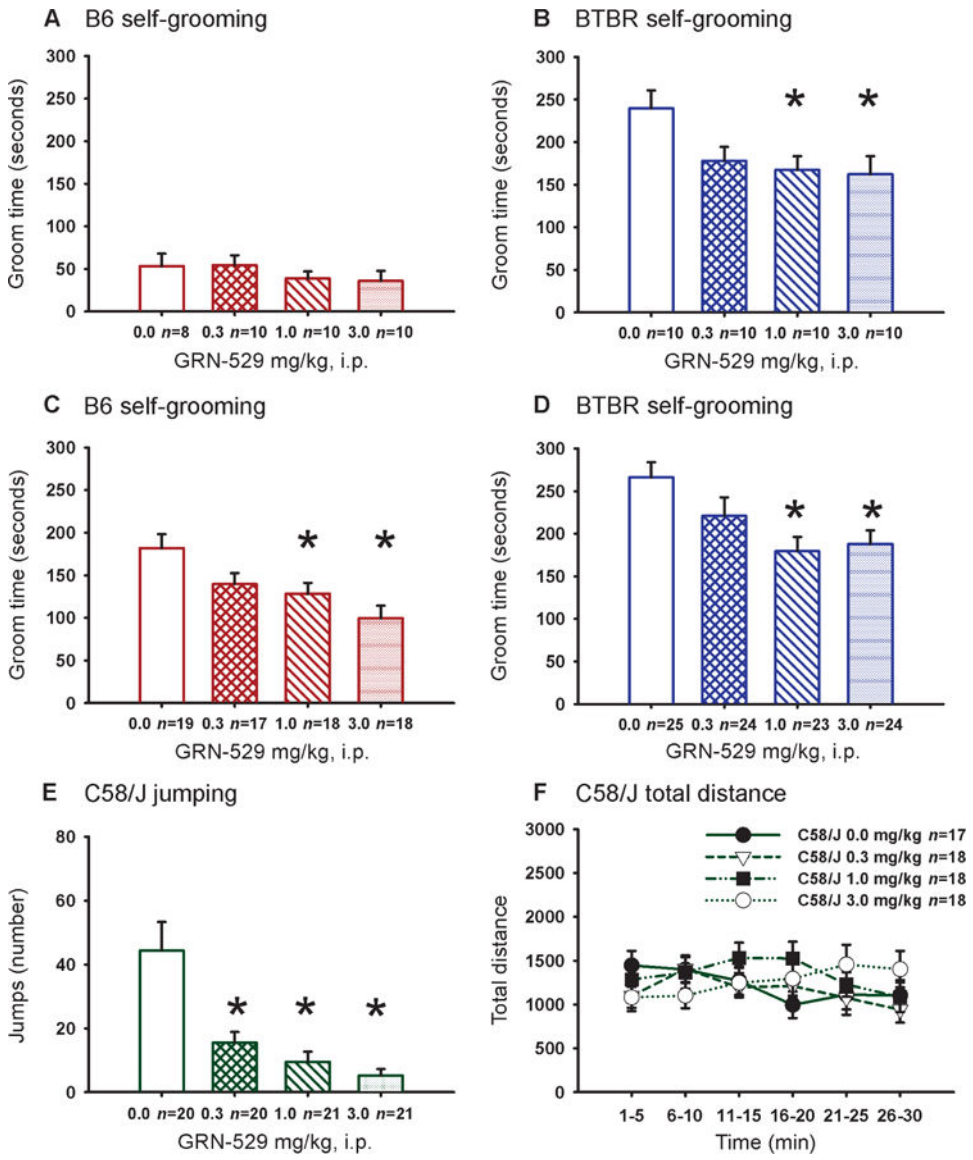

Fig. 2.

Effect of GRN-529 on repetitive self-grooming in BTBR and stereotyped jumping in C58 mice. Cumulative time spent self-grooming by BTBR and B6 mice was scored over a 10-min session in a clean, closed, empty cage after a 10-min acclimation period. Observations of stereotyped jumping behavior in C58 mice were quantified for a period of 10 min. GRN-529 was tested in two independent laboratory environments across three cohorts. (**A**) B6 mice did not display any significant differences in the amount of time spent self-grooming after treatment with vehicle (10% Tween 80/saline) or GRN-529 at doses of 0.3, 1.0, or 3.0 mg/kg intraperitoneally ($n = 8$ to 10 per dose, cohort 1, tested at NIMH, $^*P < 0.05$ versus vehicle). (**B**) BTBR displayed significant reductions in their innately high levels of repetitive self-grooming after treatment with GRN-529 at doses of 1.0 and 3.0 mg/kg ($n = 11$ to 14 per dose, cohort 1, tested at NIMH, $^*P < 0.05$ versus vehicle). (**C**) B6 mice displayed significant reductions in the amount of time spent self-grooming after treatment with GRN-529 at doses of 1.0 and 3.0 mg/kg compared to vehicle (cohort 2, tested at Pfizer). (**D**)

BTBR again displayed significant reductions in high levels of repetitive self-grooming after treatment with GRN-529 at doses of 1.0 and 3.0 mg/kg intraperitoneally ($n = 17$ to 25 per dose for each strain, cohort 2, tested at Pfizer, $^*P < 0.05$ versus vehicle). (**E**) Stereotyped vertical jumping in C58 mice was significantly reduced after GRN-529 administration at doses of 0.3, 1.0, and 3.0 mg/kg intraperitoneally versus vehicle ($^*P < 0.05$, tested at Pfizer). (**F**) No adverse or sedating effects on the general activity of C58 mice were observed during open field locomotion ($P > 0.05$, tested at Pfizer).

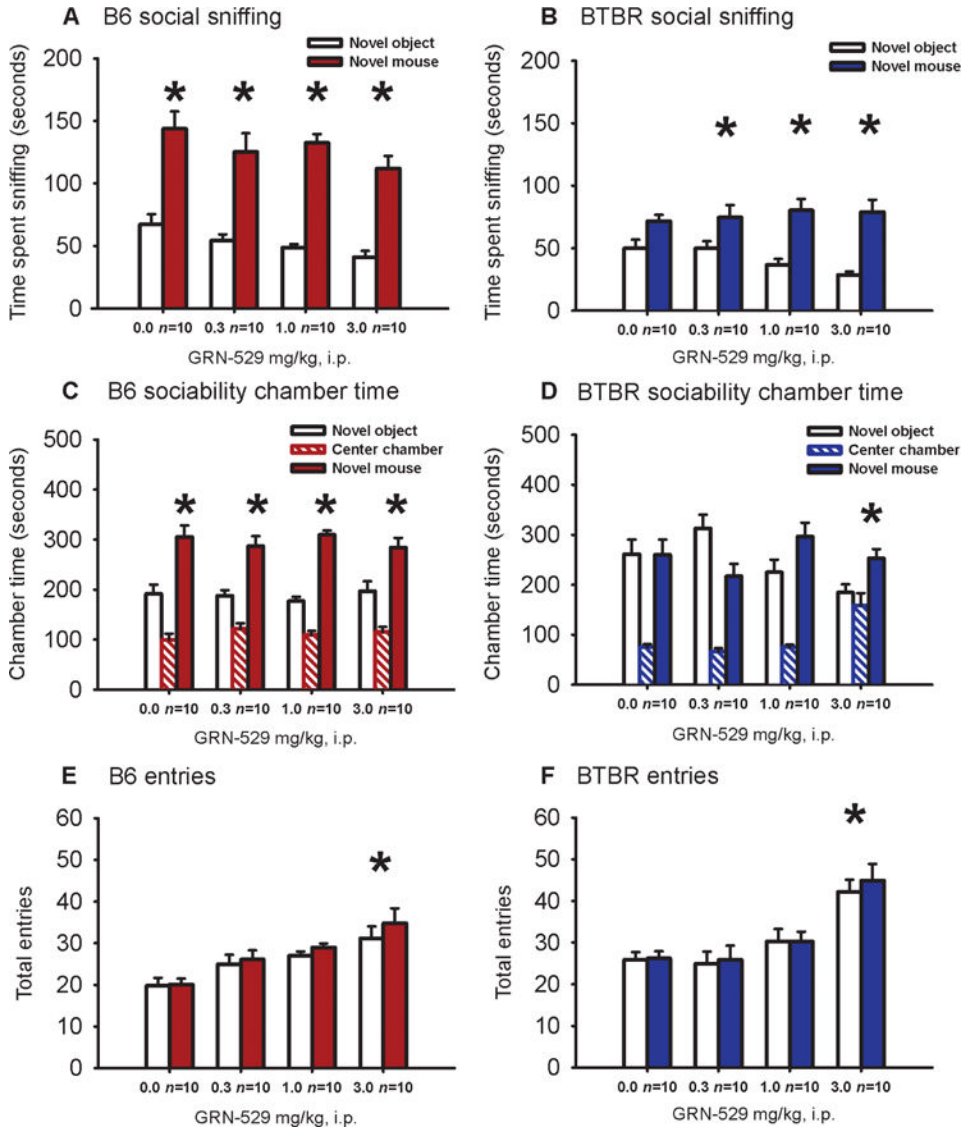

Fig. 3.

Effect of GRN-529 on social approach in adult BTBR mice. Social approach was assessed in an automated photocell-equipped three-chambered arena with observer scoring of direct sniffing interactions from videotapes of the social approach. (**A**) B6 mice displayed sociability on the more sensitive parameter, time spent sniffing the novel mouse compared to time spent sniffing the novel object, at each dose of GRN-529 and vehicle. (**B**) BTBR exhibited its characteristic lack of sociability on the sniff parameter after vehicle administration. BTBR treated with a single acute dose of GRN-529, 0.3, 1.0, or 3.0 mg/kg intraperitoneally, exhibited significant sociability on the sniff time parameter. (**C**) The B6 control strain displayed normal sociability, defined as spending more time in the chamber with the novel mouse than in the chamber with the novel object, after a single intraperitoneal dose of vehicle (10% Tween 80/saline) or GRN-529 at doses of 0.3, 1.0, and 3 mg/kg. (**D**) BTBR exhibited its characteristic lack of sociability, that is, did not spend more time in the novel mouse chamber than in the novel object chamber, after treatment with vehicle or the

two lower doses of GRN-529. At the highest dose, 3.0 mg/kg, BTBR displayed significant sociability. (**E** and **F**) B6 (E) and BTBR (F) displayed a greater number of entries into the side chambers after treatment with GRN-529 at the highest dose, 3.0 mg/kg intraperitoneally, indicating a general increase of exploratory activity during the social approach task at that dose. $*P < 0.05$, novel mouse versus novel object in (A) to (D); $*P < 0.05$ versus vehicle in (E) and (F). $n = 10$ per dose for each strain, cohort 1, assayed at NIMH. See fig. S2 for replicated findings in cohort 2.

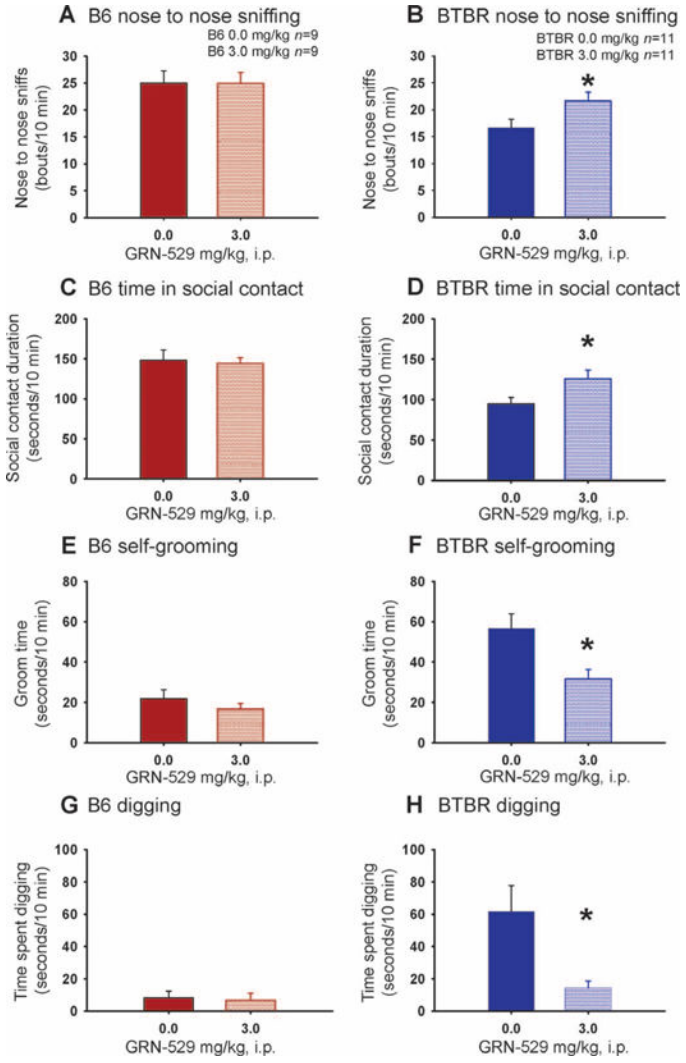

Fig. 4.

Effect of GRN-529 on dyadic reciprocal social interactions in BTBR mice. Social interactions were digitally recorded in dyads of mice in the Noldus PhenoTyper 3000 arena. Coded videos were subsequently scored by an observer uninformed of the treatment condition using Noldus Observer 8.0XT software. (**A**) The B6 control strain displayed normal sociability, as illustrated by high levels of nose-to-nose sniffing with the 129/SvImJ partner stimulus mouse, after a single intraperitoneal dose of vehicle (10% Tween 80/saline) or GRN-529 (3.0 mg/kg). (**B**) BTBR treated with vehicle exhibited its characteristic low sociability, displaying fewer bouts of nose-to-nose sniffing with the 129/SvImJ partner stimulus mouse. GRN-529 (3.0 mg/kg) increased nose-to-nose sniffing bouts in the BTBR. (**C**) B6 displayed high sociability on the parameter, time spent in social contact after GRN-529 or vehicle. (**D**) BTBR exhibited its characteristic low sociability on time spent in social contact after vehicle administration. BTBR treated with a single acute dose of GRN-529 (3.0 mg/kg) exhibited increased time in social contact. (**E**) Cumulative time spent self-grooming was calculated during the 10-min reciprocal social interaction test session. B6 mice treated with either vehicle or GRN-529 (3.0 mg/kg) did not display any significant

differences in the amount of time spent self-grooming during the session. (**F**) BTBR treated with GRN-529 (3.0 mg/kg) displayed significant reductions in their high levels of repetitive self-grooming versus BTBR treated with vehicle. (**G**) Cumulative time spent digging in the arena floor bedding during the social task was calculated during the 10-min test session. B6 mice treated with either vehicle or GRN-529 (3.0 mg/kg) displayed similar time spent digging during the session. (**H**) BTBR treated with GRN-529 (3.0 mg/kg) displayed significant reductions in their high levels of repetitive digging behavior versus BTBR treated with vehicle. $n = 9$ to 11 per treatment group, GRN-529 (3.0 mg/kg) and vehicle, for each strain, $*P < 0.05$ versus vehicle.

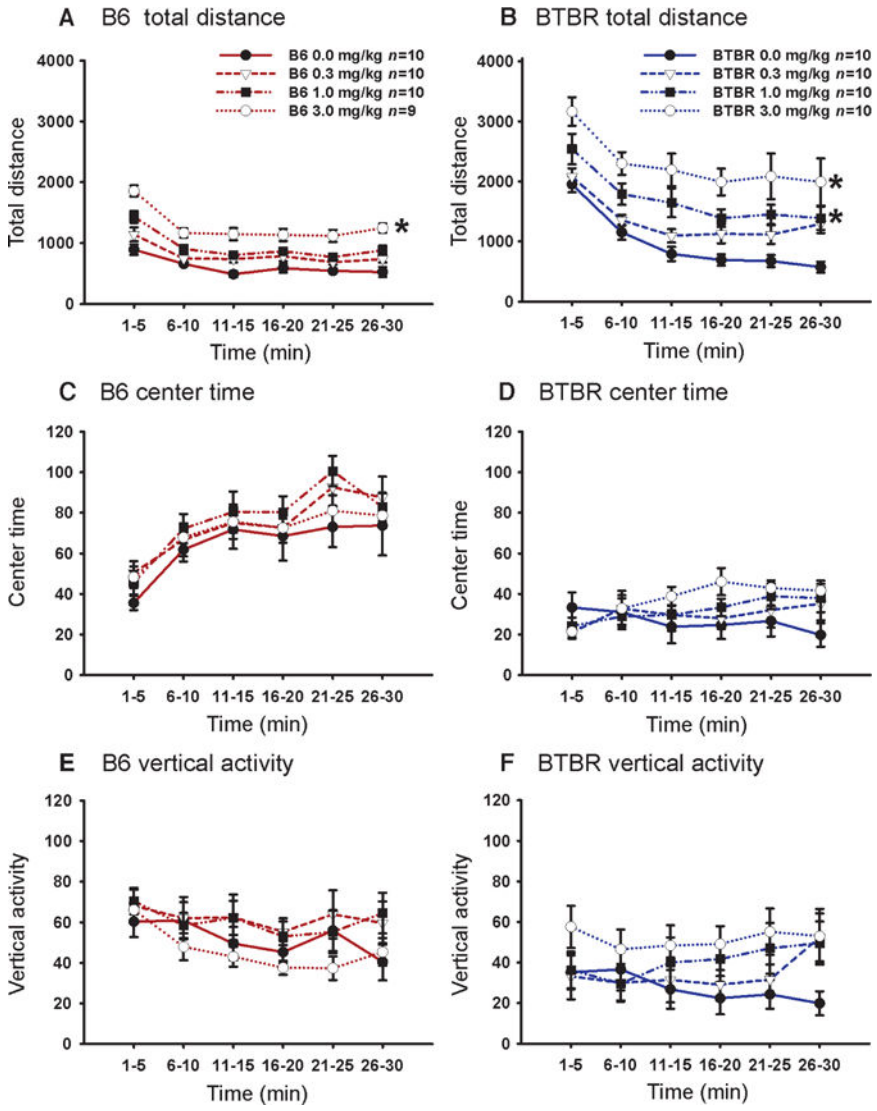

Fig. 5.

Effect of GRN-529 on open field locomotion at doses that reversed repetitive and social deficits. Exploratory locomotion was assayed in a standard automated open field arena, in 5-min time bins across a 30-min session after the identical GRN-529 treatments. (**A**) B6 displayed a significant increase in total distance traversed after GRN-529 at the highest dose, 3.0 mg/kg, intraperitoneally, compared to vehicle. (**B** and **C**) GRN-529 administration had no significant effect on (B) time spent in the center of the arena or (C) vertical activity in B6 tested at NIMH. $n = 9$ to 10 per dose. (**D**) BTBR displayed significant increases in total distance traversed after GRN-529 at doses of 1.0 and 3.0 mg/kg intraperitoneally compared to vehicle, indicating increased exploratory activity. (**E** and **F**) GRN-529 administration had no significant effect on (E) time spent in the center of the arena or (F) vertical activity in BTBR. $*P < 0.05$, $n = 10$ per dose, cohort 1 tested at NIMH. See fig. S4 for open field results replicated at NIMH and fig. S5 for open field results replicated at Pfizer. See fig. S6 for additional open field parameters in C58 mice.