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Effects of Fingolimod Administration in a Genetic Model of Cognitive Deficits

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Abstract

Notwithstanding recent advances, cognitive impairments are among the most difficult-to-treat symptoms in neuropsychiatric disorders. Deficits in information processing contributing to memory and sociability impairments are found across neuropsychiatric-related disorders. Previously, we have shown that mutations in the DTNBP1 gene (encoding dystrobrevin-binding protein 1 [dysbin-din-1]) lead to abnormalities in synaptic glutamate release in the prefrontal cortex (PFC) and hippocampus and to cognitive deficits; glutamatergic transmission is important for cortical recurrent excitation that allows information processing in the PFC. To investigate possible means of restoring glutamate release and improving cognitive impairments, we assess the effects of increasing endogenous levels of brain-derived neurotrophic factor (BDNF) in a dysbindin-1-deficient mouse model. Increasing endogenous levels of BDNF may aid in remediating cognitive deficits, given the roles of BDNF in synaptic transmission, plasticity, and neuroprotection. To increase BDNF, we use a novel strategy, repeated intraperitoneal injections of fingolimod (Gilenya). Sphingolipids have recently been shown to have therapeutic value in several neurology-related disorders. Both wild-type (WT) and mutant (MUT) genotypes were tested for sociability and recognition memory, followed by measuring endogenous BDNF levels and presynaptic $[Ca^{2+}]_i$ within the PFC. Both genotypes were treated for 1 week with either saline or fingolimod. Relative to WT mice, MUT mice demonstrated impairments in sociability and recognition memory and lower presynaptic calcium. After fingolimod treatment, MUT mice exhibited significant improvements in sociability and recognition memory and increases in presynaptic calcium and endogenous concentrations of BDNF. These results show promise for counteracting the cognitive impairments seen in neuropsychiatric disorders and may shed light on the role of dysbindin-1.

Keywords

fingolimod; glutamate; dysbindin; prefrontal cortex

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ROLE OF AUTHORS

DB-K performed the behavioral experiments and the $[Ca^{2+}]_i$ measures, created the figures, performed statistical analyses, and wrote the article. AHB designed the ELISA methods. QAF and AHB performed the ELISA measures. AL designed and supervised the experiments, reviewed the statistical analyses, and worked on the manuscript and the figures.

CONFLICT OF INTEREST STATEMENT

The authors do not have any conflicts of interest.

Despite their distinction in modern psychiatry, cognitive disabilities in neuropsychiatric and neurodevelopmental disorders share commonalities in phenomenology (Couture et al., 2011) and pathophysiology (Pinkham et al., 2008). Impairments in information processing, particularly social information processing, are some of the most commonly noted deficits shared by neurodevelopmental and neuropsychiatric disorders (Sugranyes et al., 2011). Despite the relevance of cognitive disabilities, there are few therapeutic options to treat them. With the increasing recognition that severity of cognitive disabilities may have the greatest impact on the long-term outcome of neuropsychiatric and neurodevelopmental patients, there is a requirement to develop treatments to improve cognitive function.

A potential therapeutic target, the prefrontal cortex (PFC), is a highly interconnected structure involved with top-down processing of sensorimotor information, and integration of this information affords complex cognitive functions (Miller, 2000; Fuster, 2001). Neuronal microcircuits within the PFC have essential roles in encoding, processing, and maintaining working memory (Goldman-Rakic, 1995; Miller, 2000). PFC neurons show increased activity during memory maintenance, and this memory-related activity has been attributed to glutamate-dependent recurrent excitation within local circuits (Compte et al., 2000). Compromised glutamate release may diminish the ability of cortical circuits to maintain recurrent excitation and may cause failure during information maintenance mechanisms.

Several susceptibility genes associated with deficits in cognition have been targeted as contributing factors in symptom pathogenesis (Modinos et al., 2013). DTNBP1 encodes dystrobrevin-binding protein 1 (dysbindin-1) and is located in the “vulnerability locus” on chromosome 6p24–22 (Straub et al., 1995). Spread across many diverse populations, genome-wide association studies and family-based pedigree studies have made genetic correlations between the DTNBP1 gene and the etiology of schizophrenia (Straub et al., 2002). Although variants of the DTNBP1 gene cannot be linked to every case of schizophrenia, there is evidence that negative and cognitive deficits may be attributed to diminished expression of the dysbindin-1 protein (DeRosse et al., 2006; Donohoe et al., 2007).

Dysbindin-1 is part of the biogenesis of lysosome-related organelle complex 1 (BLOC-1; Starcevic and Dell’Angelica, 2004). This complex is involved in multiple cellular functions, including synaptic vesicle dynamics (Larimore et al., 2011; Mullin et al., 2011). At the presynaptic level, its deficiency affects synaptic homeostasis, synaptic vesicle composition, and vesicle fusion (Ji et al., 2009; Newell-Litwa et al., 2009). Previously, using dysbindin-1 deficient mice (henceforth, dysbindin-1 mice), we found decreases in the replenishment of the readily releasable pool of synaptic glutamate vesicles in the PFC, decreases in quantal size and probability of glutamate release, deficits in the rate of endo- and exocytosis, and deficits in memory and working memory tasks (Jentsch et al., 2009; Karlsgodt et al., 2011; Glen et al., 2014; Saggu et al., 2013). Here, we hypothesize that restoring glutamate levels in the PFC may alleviate some of the cognitive deficits exhibited by dysbindin-1 mice. To increase endogenous levels of glutamate, we use a novel drug, fingolimod.

Fingolimod (Gilenya; FTY720) is active as fingolimod phosphate (Groves et al., 2013; Martin and Sospreda, 2014). It has been shown that fingolimod selectively increases endogenous levels of brain-derived neurotrophic factor (BDNF; Deogracias et al., 2012). Evidence supporting a role for BDNF in synaptic transmission and neuronal excitability is strong (Pozzo-Miller et al., 1999; Tyler and Pozzo-Miller 2001, 2003; Chapleu et al., 2009). Activation of tropomyosin receptor kinase B (TrkB) receptors by BDNF induces new protein synthesis (Halegoua et al., 1991; Lewin and Barde, 1996), and Tyler and Pozzo-Miller (2001) have shown that BDNF increases release probability and the number of docked glutamate vesicles in the hippocampus. Furthermore, evidence indicates a link between dysbindin and BDNF. Among three proteins present in regulated secretory vesicles (VAMP7, P14IIα, and BDNF), only BDNF immunoreactivity is reduced in the absence of BLOC-1 (Larimore et al., 2013). Thus, fingolimod's ability to increase BDNF expression may prove useful in the treatment of cognitive symptoms related to neuropsychiatric disorders. This study presents behavioral and biochemical indicators of the effects of fingolimod in social interaction in dysbindin-1 mice and tests the hypothesis that increasing endogenous levels of BDNF improves cognitive deficits in null mice.

MATERIALS AND METHODS

Subjects, Animal Handling, and Housing

All experiments used male wild-type (WT; $dys^{+/+}$) C57Bl/6J mice and a genotype of C57Bl/6J mice carrying a genomic deletion exclusively within the DTNBP1 gene (Li et al., 2003), homozygous dysbindin-1 mutant (MUT; $dys^{-/-}$). Mice were derived from back-crossing with C57Bl/6J (The Jackson Laboratory; Bar Harbor, ME) for at least 10 generations, and genotyping of mice was achieved with previously described protocols (Jentsch et al., 2009). All mice were caged in a temperature- and humidity-controlled 12-hr light-dark cycle housing facility and allowed ad libitum access to food and water. WT mice were littermates of the dysbindin-1 MUT mice. With the exception of three female mice that had to be added because of the small number of MUT males (three females used in our ELISA), all studies used male mice older than 40 days of age. Experimental protocols were approved by the institutional animal care and use committee at the Medical University of South Carolina. No data were excluded from the behavioral or qualitative assays.

General Experimental Setup

For all experiments, mice were divided into two groups; group 1 was injected intraperitoneally (IP) with saline for 7 days, and group 2 was treated with fingolimod (1 mg/kg IP; Sigma-Aldrich, St Louis, MO) dissolved in sterile saline for 7 days. The dose of fingolimod was determined from doses previously reported (Deogracias et al., 2012). To minimize stress, no testing, whether behavioral or molecular, was performed until 24 hr after the last injection.

Sample Preparation for Molecular Analysis

All mice used in behavioral tasks were deeply anesthetized with isoflurane (Abbot Laboratories, Lake Bluff, IL); the brain was rapidly extracted and bathed in a cold isolation buffer (differing per assay used), and the PFC (both infra- and prelimbic) was blocked off

over an iced Petri dish. To make one testable sample, three animals' PFCs per genotype were pooled in a preweighted Eppendorf tube; thus, every sample consisted of three cortices. Each pooled sample was homogenized by following the specific assay-dependent protocol.

ELISA: Measuring BDNF

After PFC dissection and sample preparation, concentration of endogenous BDNF in PFC tissue was measured across 30 mice (seven WT + saline, six WT + fingolimod, eight MUT + saline, nine MUT + fingolimod) with the BDNF E_{max} immunoassay system (Promega, Madison, WI). ELISA was performed with the kit protocol provided and referencing procedures, as described elsewhere (Ickes et al., 2000). Pooled tissue samples were homogenized with eight strokes in a Potter homogenizer with 0.5 ml cold isolation buffer containing distilled water (dH_2O), 137 mM NaCl, 20 mM Tris-HCl, 1% Triton X-100, and 10% glycerol. To maximize free protein yield, homogenized samples were acidified with 1 N HCl and neutralized with 1 N NaOH. Before the protocol was started, Tris-buffered saline Tween 20 buffer (dH_2O, 20 mM Tri-HCl, 150 mM NaCl, and Tween 20) was prepared for use as a washing agent before each major incubation step. A 96-well flat-bottom plate, carbonate coated (with added monoclonal anti- BDNF mAb) at room temperature (RT) overnight, was blocked for 1 hr at RT with the provided 1× block and sample buffer. To generate the standard curve, BDNF standard was serially diluted (500–0 pg) in duplicate. The standard and 150 μl of each sample (in duplicate) were incubated at RT for 2 hr on an orbital shaker. Next, the plates were incubated with antihuman BDNF pAb at RT for 2 hr and with anti-IgY HRP conjugate at RT for 1 hr. Finally, TMB One color developing solution was added to each well for 10 min, and the reaction was stopped with 1 N HCl. Plate readings were obtained within 30 min with a VersaMax ELISA microplate reader (absorbance recorded at 450 nm). BDNF levels in the PFC were compared among genotypes and treatment groups. ELISAs were performed by a person blind to the genotype and treatment of the animals.

Fluorescent Calcium Assay

Thirty mice (nine WT + saline, nine WT + fingolimod, six MUT + saline, six MUT + fingolimod) were used for quantification of presynaptic intracellular PFC $[Ca^{2+}]_i$ with a fluorescent calcium assay (both fluo-3 and fluo-4). To measure only intracellular calcium, crude synaptosomes were prepared as previously described (Saggu et al., 2013) with the pooled PFC sample tissue. This was achieved by homogenizing tissue with a Potter homogenizer with 2 ml cold isolation buffer (dH_2O, 320 nM sucrose, 1 mM EDTA, 10 mM Tri-HEPES, and protease cocktail inhibitor [catalog No. P8340; Sigma]) and then centrifuging at 4°C with a specific centrifugation regimen (600*g* for 10 min, then centrifuging supernatant at 9,200*g* for 15 min). A Bradford assay (Bio-Rad; Hercules, CA) was used to determine the specific protein concentration for each sample.

The fluorescent calcium assay was performed with 3–5 mg/ml crude PFC synaptosome preparations with either a fluo-3 AM calcium indicator (Molecular Probes/Life Technologies, Grand Island, NY) or a fluo-4 NW assay kit (Molecular Probes/Life Technologies), according to the company's protocols provided with reference to the methods previously described (Saggu et al., 2013). The synaptosomal preparations were incubated

with 5 μM fluo dye for 30 min at 37°C and centrifuged at 10,000*g* for 10 min. Supernatant was discarded, and the pellet was resuspended in prewarmed Krebs buffer. Newly fluo-loaded synaptosome preparations were placed in a black polystyrene, flat, clear-bottom 96-well plate (CellStar; Greiner Bio-One, Frickenhausen, Germany) along with set-aside WT synaptosome preparations treated with either 0.4% Triton X-100 or 7.5 mM EGTA (pH 8.0) to generate maximum and minimum values, respectively. Readings were obtained with a BioTek (Winooski, VT) FLx800 fluorescence and luminescence microplate reader, with excitation wavelength of 325 nm and emission at 526 nm (Kd = 325 nm) for fluo-3 or excitation wavelength of 494 nm and emission at 516 nm (Kd = 345 nm) for fluo-4. For both dyes, presynaptic intracellular synaptosomal [Ca^{2+}] was calculated with the formula $[Ca^{2+}]_i = Kd \times B\ (R - R_{min})/(R_{max} - R)$, with 0.4% Triton X-100 and 7.5 mM EGTA(pH 8.0) to calculate R_{max} and R_{min} values. Calcium levels were compared among the genotypes and the saline and fingolimod treatment groups.

Sociability Test Apparatus

Animals were subjected to the three-chamber sociability test. The three-compartment testing apparatus consisted of a Plexiglas box (41.9 cm long × 41.91 cm wide × 30.5 cm high) without a top. The sides of the box were covered to minimize environmental visual stimuli. Inverted custom wire-mesh cups (diameter 10.16 cm) were placed in each side of the compartments during testing sessions and housed the novel stimulus mouse. The apparatus and wire cups were thoroughly cleaned with distilled water between sessions and after each test mouse.

Sociability Test

Social interaction was measured across 49 mice (12 WT + saline, 12 WT + fingolimod, 12 MUT + saline, 13 MUT + fingolimod) with a social choice/approach task similar to that described elsewhere (Moy et al., 2004; O'Tuathaig and Waddington, 2010; Wilson and Koenig, 2014). In the first session, a test mouse was placed in the middle compartment and allowed to habituate to the apparatus for 5 min. The test mouse was then returned to its home cage for 10 min. A novel stimulus mouse (NM1) was place in an inverted wire cup in the side designated as the social compartment, and an empty inverted wire cup (novel object, NO) was placed in the side designated as the nonsocial compartment. The side opposite of inherent preference, which had been measured during a 5-min habituation period, determined the side designated for the location ofNM1.

The test mouse was then returned to the apparatus and left to explore both chambers for 10 min. Time spent in each chamber was measured. An entry into any of the three chambers was defined as all four paws past the doorway. Social interaction was measured as time spent on the side with NM1. Degree of social interaction was determined by comparing time spent in the presence of NM1 relative to time spent in the middle or the side with the NO. Normal social behavior is assumed to be a range between equal mouse-to-item interactions and a greater novel mouse interaction time. After measurement of social interaction, the mouse subject was returned to the home cage for 10 min, and the test chamber was cleaned with distilled water before the next behavioral assay.

Preference for Social Novelty Task

After 10 min of being in the home cage, test mice used in the social choice/approach task were tested for memory (as measured by recognition ratio) and preference for social novelty with the preference for social novelty task (PSNT) similar to that described elsewhere (Moy et al., 2004; O'Tuathaigh and Waddington, 2010; Wilson and Koening, 2014). Thirty-seven mice were used in this task (nine WT + saline, nine WT + fingolimod, nine MUT + saline, 10 MUT + fingolimod). First, the NM1 that had been used in the social choice/approach task was sequestered on the original side, and a second enclosed novel mouse (NM2) was placed on the side that had originally contained the NO. The mouse subject was place in the middle and given the option of interacting with either NM1 or NM2. Time spent in each chamber was measured for 10 min, and both preference for social novelty and recognition memory were assessed. Preference for social novelty was measured as time spent with NM2 relative to other activity. Recognition memory was measured as time spent with NM2 relative to NM1, i.e., the "recognition ratio." Normally functioning memory (or WT memory) was assumed to be a recognition ratio greater than or equal to one. The basic layout for testing of individual subject mice was start → 5 min acclimation period → 10 min in home cage → 10 min social choice/approach task → 10 min in home cage → 10 min preference for social novelty task → returned to home cage.

Statistical Analysis

For all assays, comparisons are between genotypes and across treatment groups (vehicle and fingolimod). All data presented are mean ± SEM. Repeated measures (RM) ANOVA was used to assess differences following treatment. Prism 6 (GraphPad Software, La Jolla, CA) was used for all statistical comparisons. Statistical significance was defined as $P < 0.05$ (risk of type 1 error, $\alpha = 0.05$).

RESULTS

Sociability Test

Forty-nine mice (24 WT and 25 dysbindin-1) were tested for sociability. Saline WT mice spent 205.9 ± 19.2 sec with NO vs. 248.9 ± 13.5 sec with NM1. There was a trend for significance in the time spent with NM1 vs. NO ($P < 0.08$, *t*-test; Fig. 1). After fingolimod treatment (7 days of daily IP fingolimod injections, 1 mg/kg), WT mice exhibited a significant increase in time spent with NM1 compared with saline mice, 278.0 ± 5.7 sec interacting with NM1 vs. 211.9 ± 9.6 sec interacting with NO ($F_{3,11} = 5.81$, $P < 0.05$, RM ANOVA; Fig. 1A).

Contrary to the findings in WT, dysbindin-1 mice spent significantly more time interacting with NO than with NM1 (201.9 ± 14.6 sec with NM1 vs. 247.2 ± 10.7 sec with NO; $F_{1,45} = 9.884$, $P = 0.0109$, ANOVA; Fig. 1A). After fingolimod treatment, dysbindin-1 mice significantly increased the time spent with NM1 vs. NO (NM1 254 ± 10 sec vs. NO 219 ± 11.4 sec; $P < 0.018$, Kruskall-Wallis test, Dunn's multiple-comparisons test; Fig. 1A). These results show that dysbindin-1 mice exhibited deficits in social interaction compared with WT mice. More importantly, fingolimod treatment reversed the deficits in social interaction exhibited by the dysbindin-1 mice.

PSNT

Thirty-seven mice were used in the PSNT (18 WT and 19 dysbindin-1). Preference for social novelty was measured as time spent with NM2 relative to other activity. Assessments of social novelty showed that dysbindin-1 mice exhibited significant deficits in the task (time of interaction with NM2; WT saline, 268.6 ± 9.2 sec; MUT saline, 215.5 ± 11.9 sec; n = 9; $F_{2,49} = 6.09$, $P = 0.0104$, RM ANOVA; Fig. 1B). After fingolimod treatment, dysbindin-1 mice showed significant improvements, reaching levels of social novelty similar to those of WT mice (WT fingolimod, 267.6 ± 12.1 sec; MUT fingolimod, 257.4 ± 10.4 sec; n = 9; Fig. 1B).

Recognition memory was measured as time spent with NM2 relative to NM1, i.e., the recognition ratio. Normally functioning memory (or WT memory) is assumed to be a recognition ratio greater than or equal to one. Analysis of the recognition ratio shows that dysbdindin-1 mice exhibited significant deficits compared with WT mice (WT saline, 1.3 ± 0.9; dysbindin-1 saline, 0.9 ± 0.16; WT vs. dysbindin-1, $F_{2,49} = 8.954$, $P < 0.001$; Fig. 1C). After fingolimod treatment, both genotypes improved significantly relative to saline treatment (WT, 1.3 ± 0.7; MUT, 1.1 ± 0.06; WT vs. MUT, $F_{1,49} = 13.56$, $P < 0.05$, RM ANOVA; Fig. 1C). These results show that dysbindin-1 mice exhibited significant deficits in social novelty and recognition memory and that fingolimod treatment improved performance in social and memory tests.

BDNF ELISA

We used ELISA kits to measure the levels of BDNF in PFC of WT and dysbindin-1 mice. BDNF levels in WT mice were 18.1 ± 1.5 pg/mg tissue (n = 7; Fig. 2), and BDNF levels in dysbindin-1 mice were 15.2 ± 2.2 pg/mg tissue (n = 6; Fig. 2). After fingolimod treatment, the levels of BDNF did not change in WT mice (19.6 ± 1.1 pg/mg tissue, n = 6) and were significantly increased in WT mice (22.2 ± 3.5 pg/mg tissue, $P < 0.02$, paired *t*-test; n = 9; Fig. 2). We did not find any differences in the levels of BDNF measured in female vs. male mice. These results show that basal levels of BDNF in MUT mice were slightly decreased compared with those found in WT mice and that fingolimod treatment increased levels of BDNF.

$[Ca^{2+}]_i$ Levels in Cortical Synaptosomes

Previously, we reported that dysbindin-1 mice exhibited significant decreases in the levels of $[Ca^{2+}]_i$ measured in synaptosomes of the PFC (Saggu et al., 2013). We proposed that the decrease in $[Ca^{2+}]_i$ levels could underlie the decrease in glutamate release reported for the PFC of these mice (Chen et al., 2008; Jentsch et al., 2009). To investigate whether $[Ca^{2+}]_i$ is affected by fingolimod treatment, we used fluo-3 and fluo-4 in combination with a colorimetric assay.

With fluo-4 in cortical synaptosomes of WT mice, the average concentration of $[Ca^{2+}]_i$ was 912.3 ± 163.4 nM (n = 9), and in dysbindin-1 mice it was 544.3 ± 190.6 nM (n = 6). After fingolimod treatment, the levels of $[Ca^{2+}]_i$ were increased in WT and in dysbindin-1 mice, with dysbindin-1 mice showing the greatest increase (WT, 1,649.9 ± 83.2 nM, an increase of 80%, n = 9; dysbindin-1 mice, 1,276.9 ± 452.3 nM, an increase of 134.6%, n = 6; Fig. 3).

Using fluo-3, we repeated the measures in a small group of mice; basal levels of $[Ca^{2+}]_i$ in WT mice were 604.8 ± 108.8 nM (n = 2), and basal levels of $[Ca^{2+}]_i$ in dysbindin-1 mice were 447.4 nM (n = 1). Fingolimod treatment increased the levels by 102.9% in WT mice (1,227.7 ± 519.2 nM, n = 2) and by 86.9% in dysbindin-1 mice (836.6 nM, n = 1).

By combining percentage measures of fluo-3 and fluo-4, the average increase in $[Ca^{2+}]_i$ after fingolimod treatment was 91.9% in WT mice and 86.9% in dysbindin-1 mice. These results replicate our previous findings showing that dysbindin-1 deletions decrease $[Ca^{2+}]_i$. Furthermore, these results show that fingolimod treatment increased the basal level of $[Ca^{2+}]_i$.

DISCUSSION

We previously reported that dysbindin-1 mice exhibited decreases in glutamate release, decreases in basal levels of $[Ca^{2+}]_i$, and decreases in N-type Ca^{2+} channels (Saggu et al., 2013). Those data, together with evidence that dysbindin-1 mice exhibit deficits in working memory (Karlsgodt et al., 2011), allowed us to generate the hypothesis that the decrease in the basal levels of $[Ca^{2+}]_i$ was underlying the decrease in glutamate release and the cognitive deficits exhibited by the dysbindin-1 mice.

As reviewed by Amaral and colleagues (2007), at presynaptic level, BDNF enhances excitatory synaptic transmission by direct presynaptic mechanisms (Lohof et al., 1993), an effect mediated by increased presynaptic Ca^{2+} levels and dependent on extracellular Ca^{2+} levels and presynaptic depolarization (Stoop and Poo, 1996). Enhancement of GABA-mediated inhibitory synaptic transmission by BDNF in cultured hippocampal neurons also seems to be mediated by presynaptic Ca^{2+} signaling because chronic BDNF treatment increases the expression and channel density of presynaptic P/Q- and N-type channels without affecting postsynaptic L-type channels (Baldelli et al., 2000, 2002).

To alleviate the decrease in $[Ca^{2+}]_i$ levels present in dysbindin-1 mice and perhaps alleviate some of the cognitive deficits exhibited by these mice, we decided to increase the endogenous concentration of BDNF. However, BDNF cannot be administered systemically because it does not cross the blood-brain barrier. Deogracias and colleagues (2012) demonstrated that the sphingosine 1-phosphate (S1P) analog fingolimod (FTY720) crosses the blood-brain barrier, selectively increases endogenous BDNF levels, and activates the ERK-MAPK pathway (Deogracias et al., 2012; Doi et al., 2013) in the brain. We tested the effects of fingolimod treatment in WT and dysbindin-1 mice to rescue presynaptic Ca^{2+} levels and restore glutamatergic transmission in the PFC.

ELISA measures show that fingolimod treatment slightly increases levels of BDNF in dysbindin-1 mice but not in WT mice. Moreover, our data show that dysbindin-1 mice exhibit lower basal levels of BDNF compared with WT mice. We did not construct a dose/response curve. However, Deogracias and colleagues (2012) demonstrated a dose/response effect of fingolimod in the levels of BDNF in neuronal cultures; fingolimod at concentrations of 10 or 100 nM but not at doses of 1 μM increased BDNF levels. Perhaps

increasing the IP dose of fingolimod could result in greater increases in endogenous levels of BDNF in dysbdindin-1 mice.

Our experiments did not measure the levels of other neurotrophic factors; however, Doi and colleagues (2013) have shown that fingolimod selectively increases BDNF mRNA (but not nerve growth factor or neurotrophin-3 mRNA) and increases neuronal BDNF production. When basal levels of $[Ca^{2+}]_i$ were measured, we were able to replicate the results reported previously (Saggu et al., 2013), and we now show that fingolimod treatment improves the basal concentrations of $[Ca^{2+}]_i$ measured in cortical synaptosomes of dysbindin-1 mice. These results suggest that fingolimod treatment, by increasing BDNF levels in dysbindin-1 mice, improves $[Ca^{2+}]_i$.

BDNF plays multiple roles in synaptic transmission and neuronal plasticity. As reported by Amaral and colleagues (2007), one of the prominent signaling cascades activated by TrkB receptors, the hydrolysis of phosphatidylinositol-4,5-biphosphate by activated phospholipase (PLC)-γ, is expected to cause Ca^{2+} elevations in neurons (Segal and Greenberg, 1996; Huang and Reichardt, 2003). The amplification of Ca^{2+} signals by mobilization from intracellular stores may allow the enhancement of processes as transmitter release and gene expression. It is possible that the increases in $[Ca^{2+}]_i$ resulting from the fingolimod treatment are mediated directly by the mobilization of Ca^{2+} from the endoplasmic reticulum.

It is not possible to rule out completely a direct effect of fingolimod on $[Ca^{2+}]_i$. Fingolimod activates S1P1 receptors that are involved in many pathophysiological processes. S1P receptors couple to several G proteins, such as Ras, PLC, and phosphoinositide 3-kinase. Moreover, S1P activation increases glutamate release from presynaptic terminals in the hippocampus (Kanno et al., 2010). Hagihara et al. (2013) have shown that fingolimod stimulates Ca^{2+}/calcineurin signaling in yeast.

More relevant was the finding that fingolimod treatment improves social cognition. Our results showed that dysbindin-1 mice exhibited significantly lowered scores in social tests compared with WT mice. Seven days of daily IP injections of fingolimod at 1.0 mg/kg significantly improved sociability, preference for social novelty, and recognition memory.

CONCLUSIONS

Fingolimod treatment improves social and memory deficits in dysbindin-1 mice via increases in BDNF that, in turn, improve $[Ca^{2+}]_i$ and may normalize synaptic glutamate levels in the PFC.

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SIGNIFICANCE

Neurodevelopmental disorders are devastating health problems that produce enormous costs in terms of medical expenses, social and family stress, and loss of productivity; therefore, there is an urgent requirement to understand the mechanisms underlying these disorders and to explore new therapeutic interventions. To explore the cellular underpinnings of cognitive deficits associated with neurodevelopmental disorders, we use a mouse model, dystrobrevin-binding protein 1 (dysbindin-1) mutant mice, and evaluate the effects of fingolimod (FTY720), a sphingolipid that selectively increases endogenous levels of brain-derived neurotrophic factor. Systemic treatment with fingolimod improved social interaction and working memory in dysbindin-1 mice.

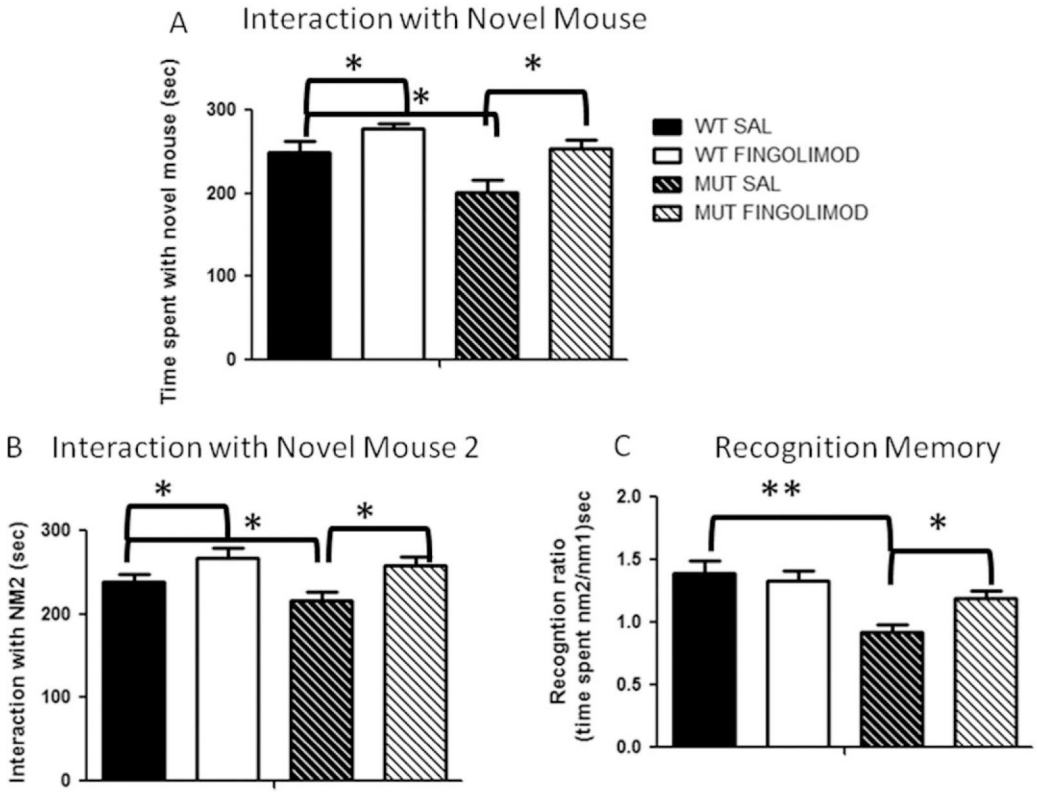

Fig. 1.

A: Sociability of dysbindin-1 MUT mice as measured by the social choice/approach task. Relative to WT mice (n = 12), dysbindin-1 MUT mice (n = 12) exhibited lower interaction times with NM1 (★$P < 0.0109$). After fingolimod treatment, dysbindin-1 MUT mice (n = 13) showed normalized levels of interaction with NM1; the interaction is significantly greater compared with saline-treated counterparts (n = 12; ★$P < 0.018$). **B:** Assessing preference for social novelty in dysbindin-1 MUT mice. Given the choice between a past and a novel social interaction, dysbindin-1 MUT mice (n = 9) exhibited significantly lower interaction times with NM2 (★$P = 0.0104$) compared with WT mice (n = 9). After fingolimod treatment, dysbindin-1 MUT mice (n = 10) showed normalized levels of interaction with NM2, significantly greater compared with saline treatment (n = 9; ★$P = 0.0173$). **C:** Recognition ratio as a measure of memory function in dysbindin-1 MUT mice. WT mice showed no deficits in recognition memory when given the choice between a past and a novel social interaction, as measured by the recognition ratio. However, relative to WT mice, dysbindin-1 MUT mice exhibited significant reductions (★★$P < 0.001$) in recognition. After treatment with fingolimod, dysbindin-1 MUT mice showed significant improvement in recognition ratio (★$P < 0.05$) compared with the saline-treatment group. SAL, saline.

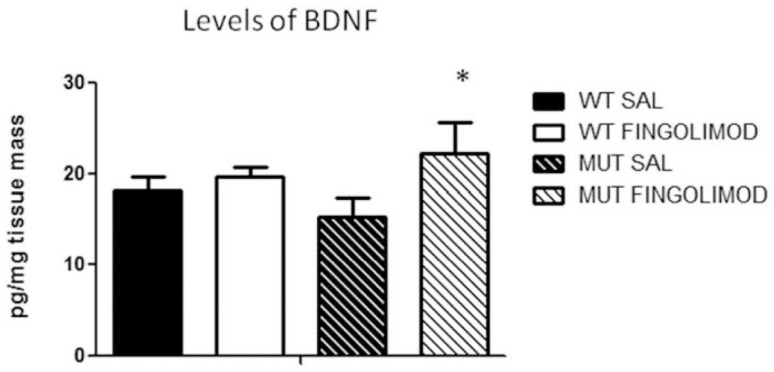

Fig. 2.

ELISA measuring levels of endogenous BDNF in the PFC across both genotype and treatment groups. Dysbindin-1 MUT mice (n = 8) showed slightly lower levels of BDNF in the PFC compared with their WT counterparts (n = 7) and a significant increase (n = 9; paired *t*-test, ★$P < 0.022$) after fingolimod treatment. SAL, saline.

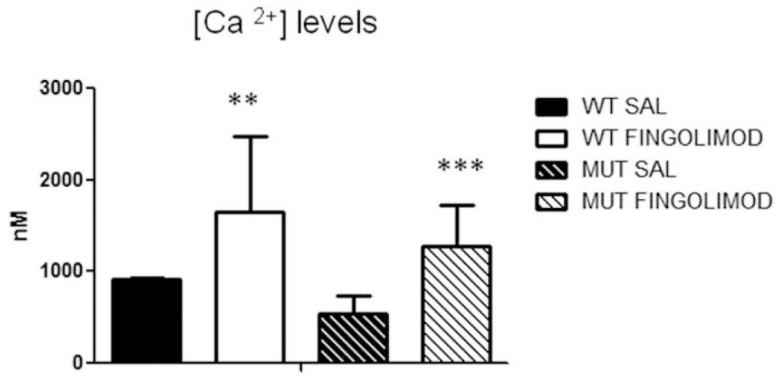

Fig. 3.

Effect of fingolimod treatment on PFC presynaptic calcium concentration as measured by fluorescent calcium indicators. Baseline levels of intracellular calcium did not significantly differ between genotype, as measured by fluo-4 and fluo-3 (WT SAL, n = 9; MUT SAL, n = 6). After treatment, fluo-4 measures showed significant increases in intracellular calcium in both WT (n = 9; ★★P = 0.001) and dysbindin-1 MUT (n = 6; ★★★P < 0.0001) mice. Both dyes indicate a near doubling of intracellular calcium for both genotypes after treatment with fingolimod. SAL, saline.