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ORIGINAL INVESTIGATION

Clinically effective OCD treatment prevents 5-HT1B receptor-induced repetitive behavior and striatal activation

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Abstract
Rationale Serotonin-1B receptor (5-HT1BR) agonist treatment induces obsessive-compulsive disorder (OCD)-like behaviors including locomotor stereotypy, prepulse inhibition deficits, and delayed alternation disruptions, which are selectively prevented by clinically effective OCD treatment. However, the role of 5-HT1BRs in modulating other repetitive behaviors or OCD-like patterns of brain activation remains unclear.
Objectives We assessed the effects of 5-HT1BR agonism on digging, grooming, and open field behaviors in mice. We also quantified effects on neuronal activation in brain regions overactivated in OCD. Finally, we assessed whether effects of the 5-HT1BR challenge could be blocked by clinically effective, but not ineffective, drug treatments.
Methods Mice were tested in open field, dig, and splash tests after acute treatment with saline, 1, 3, 5, or 10 mg/kg RU24969 (5-HT1B/1A agonist). Behavioral effects of RU24969 were also tested following co-treatment with vehicle, 1 mg/kg WAY100635 (5-HT1A antagonist) and 5 or 10 mg/kg GR127935 (5HT1B/D antagonist). Separate mice were behaviorally assessed following chronic pretreatment with vehicle with 10 mg/kg fluoxetine or 20 mg/kg desipramine and acute treatment with saline or 10 mg/kg RU24969. Brains were analyzed for *Fos* expression in the orbitofrontal cortex, the dorsal striatum, and the cerebellum.
Results RU24969 induced robust locomotor stereotypy and decreased rearing, digging, and grooming. Effects were blocked by GR127935 but not by WAY100635. RU24969 also increased *Fos* expression in the dorsal striatum. Chronic fluoxetine, but not desipramine, alleviated 5-HT1BR-induced effects.
Conclusions We report novel 5-HT1BR-induced behaviors and striatal activation that were alleviated only by clinically effective pharmacological OCD treatment. Studying the mechanisms underlying these effects could provide insight into OCD pathophysiology.

Keywords Mouse model · Serotonin 1B receptor · Serotonin reuptake inhibitor · Repetitive behavior · Exploratory behavior · Striatum · Fos

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Introduction

Obsessive-compulsive disorder (OCD) is a chronic, debilitating psychiatric disorder characterized by the inability to inhibit intrusive thoughts, impulses, and/or repetitive behaviors. OCD is a major cause of global disability (Chaudhury et al. 2006) and is estimated to afflict 2 % of the world's population (American Psychiatric Association 2013; Torres et al. 2006). Several rodent models for aspects of OCD have been proposed as useful tools to elucidate mechanisms and evaluate

potential novel therapies (Shanahan et al. 2011; Welch et al. 2007; Ahmari et al. 2013; Shmelkov et al. 2010), and the utility of these models relies on critical validation (Geyer and Markou 1995).

One recently developed mouse model for studying aspects of OCD is the serotonin (5-HT) 1B receptor agonist-induced model of OCD-like behavior. In this drug-induced model, acute pharmacological challenge of 5-HT1B receptors induces several behaviors relevant to OCD, including locomotor stereotypy, prepulse inhibition (PPI) deficits, and impairments in delayed alternation (Shanahan et al. 2009; Shanahan et al. 2011, Woehrle et al. 2013). The locomotor stereotypy induced in the model is distinct from locomotor patterns produced by psychostimulants (Geyer et al. 1996) and consists of a highly perseverative circling of the open field periphery, to the exclusion of other species-typical behaviors, and lasts for several hours. Similarly, OCD patients exhibit repetitive behaviors, PPI deficits, and impairments in delayed alternation (Hollander 1998; Hoenig et al. 2005; Moritz et al. 2001). OCD patients also exhibit rapid exacerbation of symptoms following acute challenge with antimigraine agents, which are nonselective 5-HT agonists with a high affinity for 1B receptors (Gross-Isseroff et al. 2004; Koran et al. 2001). Furthermore, 4 weeks of treatment with serotonin reuptake inhibitors (SRI) prevents the perseverative hyperlocomotion, PPI deficits, and impairments in delayed alternation induced by 5-HT1B agonists in mice (Shanahan et al. 2009; Shanahan et al. 2011; Woehrle et al. 2013) and chronic SRI treatment provides the only effective pharmacological monotherapy for OCD. Thus, the 5-HT1B-induced model of OCD exhibits a strong predictive validity and warrants further study and development.

Previous studies developing the 5-HT1B-induced model were conducted using BALB/cJ or 129/SvEv inbred strains and a high dose (10 mg/kg) of 5-HT1B/1A receptor-agonist RU24969 (Shanahan et al. 2009; Shanahan et al. 2011). Here, we used C57Bl/6J mice to evaluate the validity of the model in other inbred mouse strains. Furthermore, we investigated whether lower doses of RU24969 might increase other repetitive/compulsive behaviors, such as splash-induced grooming, or reduce other exploratory measures including center activity, vertical rearing, and digging. Because RU24969 shows minor affinity for 5-HT1A receptors (Ki= 2.5 nmol) in addition to major affinity for 5-HT1B receptors (Ki=0.38 nmol) (Peroutka 1986), we tested the ability of selective 5-HT1A and 5-HT1B antagonists to block RU24969-induced behaviors. We additionally quantified RU24969-induced neuronal activation in the orbitofrontal cortex (OFC) and the dorsal striatum, brain regions which were observed to be overactivated in neuroimaging studies of OCD patients (Saxena and Rauch 2000). Finally, we examined the relevance of 5-HT1B-induced behaviors in mice to OCD in humans by testing whether these effects could be blocked by treatment with a clinically effective SRI but not ineffective norepinephrine reuptake inhibitor (NRI).

Methods and materials

Animals

Experimentally naive female C57BL/6J mice aged 9 weeks (Jackson Laboratories, Bar Harbor, Maine) were used for all experiments. Females were used for consistency with prior studies (Shanahan et al. 2009; Shanahan et al. 2011; Woehrle et al. 2013) (see Online Resource 1 for further details).

Chemicals

RU24969 was dissolved in 0.9 % saline and injected intraperitoneally. Drug doses were selected based on results of previous dose-response studies (Cheetham and Heal 1993; De Souza et al. 1986). WAY100635, a selective 5-HT1A antagonist, and GR127935, a selective 5-HT1B/D antagonist, were dissolved in distilled water and injected subcutaneously. Fluoxetine and desipramine, both NRIs, were administered in the drinking water in opaque bottles at 80 and 215 mg/L, to achieve doses of 10 and 20 mg/kg/day, respectively (Dulawa et al. 2004) (see Online Resource 1 for further details).

Fos mRNA quantification

Brains of six behaviorally representative animals per group were used for quantification. Bilateral OFC samples were taken from two consecutive 300-μm sections with 0.5-mm diameter tissue punches; 1.2-mm bilateral punches were taken from three consecutive caudate/putamen sections; and 1.2 mm bilateral punches were taken from five consecutive cerebellum sections (for further details, see Online Resource 1).

Experiments

Experiment 1

Sixty mice received acute injections of saline and 1, 3, 5, or 10 mg/kg RU24969. After 5 min, behavioral testing on mice began with open field (20 min), dig (3 min), and splash tests (5 min). (For details of behavioral tests, see Online Resource 1). Each experiment used separate cohorts of mice.

Experiment 2

Seventy-two mice received injections of vehicle and 1 mg/kg WAY100635 or 5 mg/kg GR127935. Antagonist doses were

selected based on previous studies demonstrating 5-HT1B-specific effects of RU24969 (Shanahan et al. 2009; Lucas et al. 1997; Woehrle et al. 2013). Thirty minutes later, mice received acute injections of saline or 10 mg/kg RU24969. The 10-mg/kg dose was selected because all behavioral measures were affected by RU24969 at this dose. After 5 min, mice underwent the same behavioral testing as in "Experiment 1."

Experiment 3

Due to only a partial blockade of RU24969-induced locomotor effects following pretreatment with 5 mg/kg GR127935, we ran a dose response of RU24969 against a higher dose of GR127935. Seventy-two mice received co-injections of vehicle or 10 mg/kg GR127935 and 0, 3, or 10 mg/kg RU24969. After 5 min, mice underwent behavioral testing.

Experiment 4

Ninety mice received vehicle, 10 mg/kg/day fluoxetine, or 20 mg/kg/day desipramine for 4 weeks, a minimum for clinical effectiveness in OCD (Ellingrod 1998). On the day of testing, mice received acute injections of saline or 10 mg/kg RU24969. After 5 min, mice underwent behavioral testing. Mice were then immediately sacrificed (85–90 min postinjection) and brains were extracted, snap-frozen on dry ice, and stored at −80 °C for later *Fos* quantification.

Statistical analysis

ANOVAs were applied to assess the effects of RU24969, antagonists, and pretreatment for each dependent measure in the three behavioral tasks and for each brain region analyzed for *Fos* expression. Open field data are presented with time bin collapsed, since patterns of results remained consistent over time bin. To assess a potential relationship between locomotor activity and behaviors observed in the dig and splash tests, we applied an ANCOVA to each digging and grooming measure, with rest time (time spent not locomoting in the open field) as a covariate. Significant interactions were resolved using Tukey's adjustment for multiple comparisons. Significance was set at $p<0.05$.

Results

Experiment 1

Open field test

Effects of RU24969 on locomotor behavior are summarized in Fig. 1. Acute treatment with RU24969 increased total distance [$F(4,55)=30.40$; $p<.0001$]; all doses differed from those of saline (Fig. 2a). RU24969 also decreased the ratio of center to total distance traveled [$F(4,55)=11.54$; $p<.0001$] (Fig. 2b); the 5- and 10-mg/kg doses of RU24969 differed from those of saline and 1 mg/kg, and 10 mg/kg differed from 3 mg/kg. In addition, RU24969 decreased time spent in the center [$F(4,55)=7.29$; $p<.0001$] (Online Resource 3a). The 5- and 10-mg/kg doses of RU24969 differed from those of saline, and 10 mg/kg differed from 1 and 3 mg/kg. RU24969 decreased spatial d [$F(4,55)=18.93$; $p<.0001$] (Fig. 2c); the 5- and 10-mg/kg doses differed from those of saline and 1 mg/kg, and 10 mg/kg differed from 3 mg/kg. All doses of RU24969 eliminated vertical activity [$F(4,55)=6.23$; $p=.0003$] (Fig. 2d) and time spent rearing compared with those of saline [$F(4,55)=5.15$; $p=.0014$] (Online Resource 3b). RU24969 also decreased rest time [$F(4,55)=108.0$; $p<.0001$] (Online Resource 3c); all doses differed from those of saline, and 5 and 10 mg/kg differed from 1 mg/kg.

Dig test

RU24969 treatment reduced digging measures. All doses increased latency to dig [$F(4,50)=19.98$; $p<.0001$] compared with those of saline; 5 mg/kg differed from 1 and 3 mg/kg, and 10 mg/kg differed from 1 and 3 mg/kg (Fig. 2e). RU24969 decreased total time spent digging [$F(4,50)=7.65$; $p<.0001$] (Fig. 2f); the 3-, 5-, and 10-mg/kg doses differed from saline, and 10 mg/kg differed from 1 and 5 mg/kg. Three, 5, and 10 mg/kg RU24969 reduced the number of digging bouts compared with those of saline [$F(4,50)=10.00$; $p<.0001$] (Fig. 2g), with 5 and 10 mg/kg differing from 1 mg/kg and 5 and 10 mg/kg differing from each other. Finally, the 5- and 10-mg/kg RU24969 doses reduced the average duration of digging bouts compared with those of saline [$F(4,50)=9.02$; $p<.0001$] (Fig. 2h); 5 mg/kg differed from 1 and 3 mg/kg, and 10 mg/kg differed from 1 and 3 mg/kg. ANCOVAs confirmed that the effect of RU24969 on average bout duration [$F(4,49)=3.18$; $p=.0212$] remained significant, independent of rest time. However, there were no longer effects of RU24969 compared to saline on dig latency, total time spent digging, and number of bouts, indicating that these measures are directly related to rest time.

Splash test

RU24969 treatment reduced grooming behavior in three of the four measures. Five and 10 mg/kg RU24969 increased the latency to groom compared with those of saline and 1 mg/kg [$F(4,53)=5.43$; $p=.0010$] (Fig. 2i). All doses of RU24969 reduced total time spent grooming compared with those of saline [$F(4,53)=75.64$; $p<.0001$] (Fig. 2j); all doses differed from each other, except 5 and 10 mg/kg. RU24969 significantly increased number of grooming bouts for 1 and 3 mg/kg compared to saline and the 5- and 10-mg/kg groups

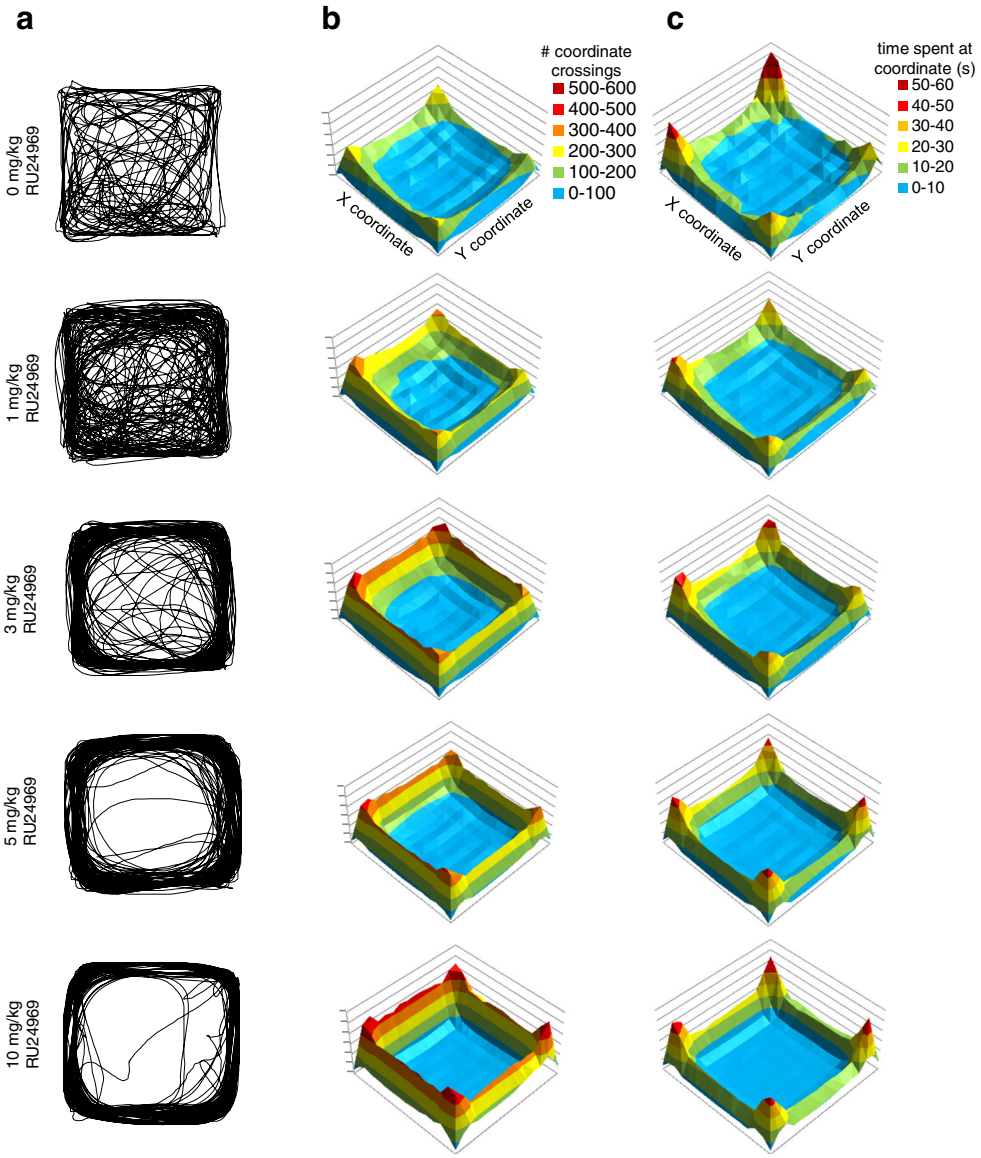

Fig. 1 Effects of RU24969 on locomotor patterns following acute injection. Mice received 0, 1, 3, 5, or 10 mg/kg RU24969 and were tested in the open field. **a** Representative two-dimensional paths taken by mice; **b** three-dimensional representations of average number of crossings at each coordinate (n=12); **c** three-dimensional representations of average time spent at each coordinate (n=12)

[F(4,53)=16.93; p<.0001] (Fig. 2k). Finally, all doses of RU24969 reduced the average grooming bout duration compared with those of saline [F(4,53)=14.19; p<.0001] (Fig. 2l). ANCOVAs confirmed that the effect of RU24969 on total grooming time [F(4,52)=16.39; p<.0001] and number of bouts [F(4,52)=16.39; p<.0001] remained significant, independent of rest time. However, there were no longer effects of RU24969 compared to saline on groom latency and average bout duration, indicating that these measures are directly related to rest time.

Experiment 2

Open field test

ANOVA revealed an RU24969×antagonist interaction on total distance traveled [F(2,63)=7.57; p=.0001] (Fig. 3a). Post hoc tests showed that RU24969 increased distance traveled within each antagonist injection. Within the RU24969-treated group, 5 mg/kg GR127935 reduced distance traveled compared with those of both vehicle and WAY100635. For center/total distance traveled, there was a trend for an RU24969×antagonist interaction [F(2,63)=2.80; p=.0681] (Fig. 3b). While there was no interaction between RU24969×antagonist on center time (Online Resource 3d), there was a main effect of antagonist [F(2,63)=3.61; p=.0327] and a trend for a main effect of RU24969 treatment [F(1,63)=2.96; p=.0903]. ANOVA revealed an RU24969× antagonist interaction on spatial d [F(2,63)=7.57; p=.0011] (Fig. 3c). RU24969 decreased spatial d within the vehicle and WAY100635 groups, but not within the GR127935 group. Both GR127935 and WAY100635 increased spatial d compared to vehicle within RU24969-treated animals. For the measures of vertical activity and time spent rearing,

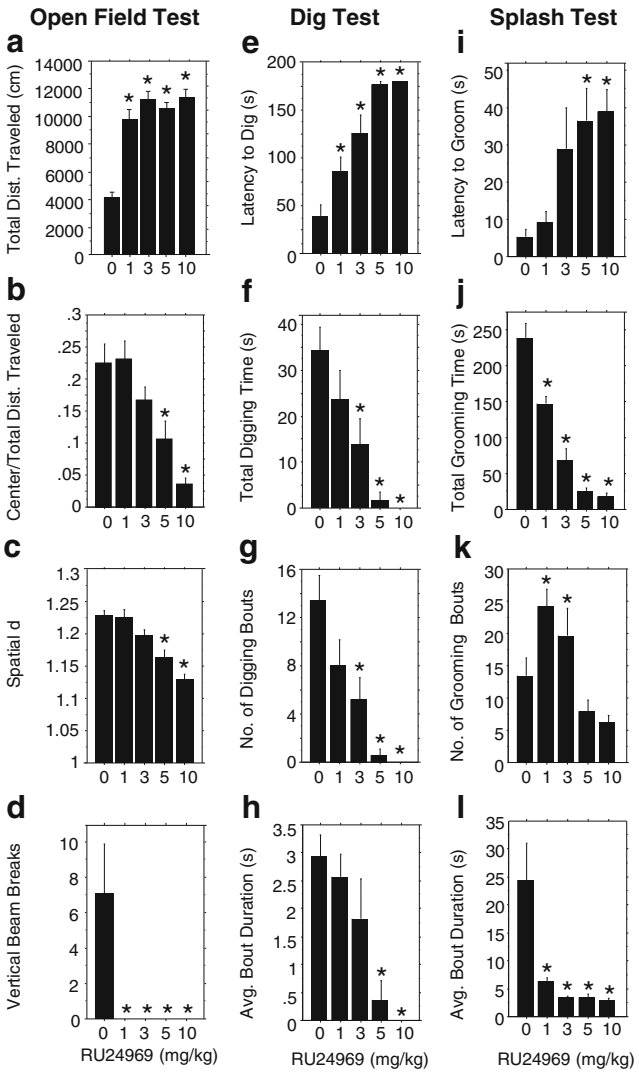

Fig. 2 Effects of RU24969 in the open field, dig test, and splash tests. Mice received acute injections of 0, 1, 3, 5, or 10 mg/kg RU24969 (n=12). Mice were assessed in the open field for **a** total distance traveled, **b** center/total distance traveled, **c** spatial d, and **d** number of vertical rearings. Measures for the dig test were **e** latency to dig, **f** total time spent digging, **g** number of digging bouts, and **h** average duration of digging bouts. Measures for the splash test were **i** latency to groom, **j** total time spent grooming, **k** number of grooming bouts, and **i** average duration of grooming bouts. Results are presented as mean±SEM. *p<0.05 compared to 0 mg/kg RU24969

ANOVAs identified RU24969×antagonist interactions [F(2, 63)=4.614; p=.0135] (Fig. 3d) and [F(2,63)=3.456; p=.0172] (Online Resource 3e), respectively. Post hoc tests showed that RU24969 decreased both rearing measures within the vehicle and WAY100635, but not GR127935, groups. Within the RU24969-treated group, GR127935 increased both rearing measures compared to vehicle and WAY100635. Finally, there was a significant interaction between RU24969 and antagonist injection on rest time [F(2, 63)=15.96; p<.0001] (Online Resource 3f). Post hoc tests showed that RU24969 reduced rest time within each antagonist group. However, GR127935 increased rest time compared to vehicle and WAY100635 within RU24969-treated groups.

Dig test

There was no interaction observed between RU24969 and antagonist on latency to dig (Fig. 3e), but there were main effects of RU24969 treatment [F(1,62)=155.4; p<.0001] and antagonist [F(2,62)=5.311; p=.0074]. ANOVA revealed a trend for an RU24969×antagonist interaction on total time spent digging [F(2,62)=2.63; p=.0800] (Fig. 3f). While there was no RU24969×antagonist interaction on number of digging bouts (Fig. 3g), there was a main effect of RU24969 treatment [F(1,62)=61.59; p<.0001]. ANOVA found an interaction between RU24969 and antagonist on average duration of digging bouts [F(2,62)=3.83; p=.0270] (Fig. 3h). RU24969 reduced bout duration with vehicle and WAY100635, but not GR127935, injections. GR127935 increased bout length within RU24969-treated mice. Within saline-treated mice, WAY100635 increased bout length. When ANCOVAs were applied, no interactions remained between RU24969 and antagonist injection on dig latency, total time spent digging, and number of bouts. There was a trend for an RU24969×antagonist interaction on average bout duration [F(2,61)=2.82; p=.0676], indicating that there may be a component of this measure that is independent of rest time.

Splash test

ANOVA revealed RU24969×antagonist interactions on latency to groom [F(2,62)=113.75; p<.0001] (Fig. 3i), total time spent grooming [F(2,62)=7.04; p=.0018] (Fig. 3j), and number of grooming bouts [F(2,62)=16.02; p<.0001] (Fig. 3k). Post hoc tests indicated that RU24969 reduced grooming on these measures within vehicle, but not GR127935 or WAY100635, antagonist groups. Within the RU24969-treated group, both GR127935 and WAY100635 increased grooming on these measures compared to vehicle. For total grooming time, the GR127935 had a greater blockade of RU24969-induced effects compared to WAY100635. There was no interaction found on average bout duration, only a main effect of RU24969 [F(1,62)=39.86; p<.0001] (Fig. 3l). ANCOVAs confirmed that the effect of RU24969 on groom latency [F(2,61)=107.64; p<.0001], total grooming time [F(2,61)=5.61; p=.0058], and number of bouts [F(2, 61)=15.58; p<.0001] remained significant, independent of rest time. There was again no RU24969×antagonist interaction on average bout duration.

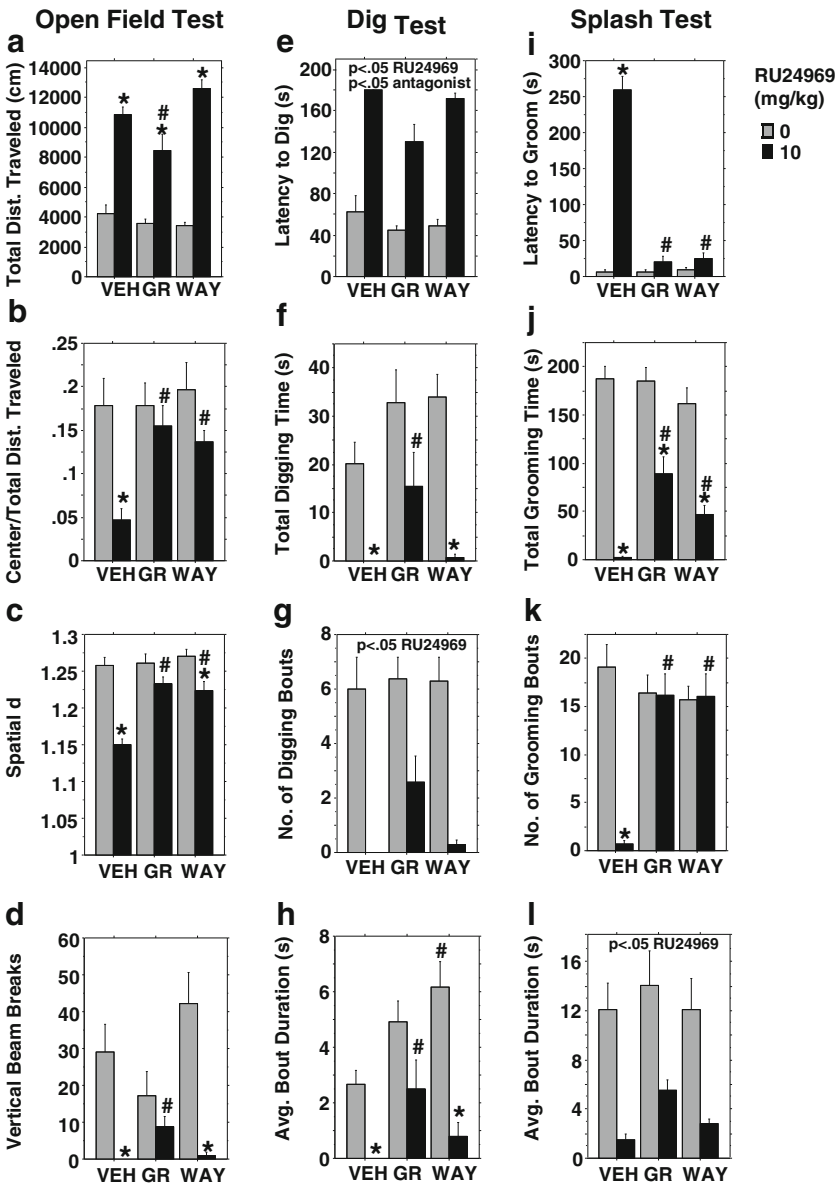

Fig. 3 Effects of RU24969 in the open field, dig test, and splash tests after acute pretreatment with 5-HT1 antagonists. Mice received injections of vehicle, 1 mg/kg WAY100635 or 5 mg/kg GR127935, then 30 min later were injected with 0 or 10 mg/kg RU24969 (n=12). Mice were assessed in the open field for **a** total distance traveled, **b** center/total distance traveled, **c** spatial d, and **d** vertical rearing. Measures for the dig test were **e** latency to dig, **f** total time spent digging, **g** number of digging bouts, and **h** average duration of digging bouts. Measures for the splash test were **i** latency to groom, **j** total time spent grooming, **k** number of grooming bouts, and **l** average duration of grooming bouts. Results are presented as mean± SEM. *p<0.05 compared to 0 mg/kg RU24969; #p<0.05 compared to vehicle injection. *VEH* vehicle, *GR* GR127935, *WAY* WAY100635

Experiment 3

Open field test

ANOVA revealed an RU24969×GR127935 interaction on distance traveled [F(2,64)=8.18; p=.0007] (Fig. 4a). In vehicle-injected mice, both doses of RU24969 increased total distance traveled. Within GR12735-injected mice, 10 mg/kg RU24969 increased distance traveled compared with that of saline and 3 mg/kg RU24969. GR127935 injection decreased total distance traveled within 3- and 10-mg/kg RU24969, but not saline groups. While there was no interaction observed between RU24969×GR127935 on center/total distance traveled (Fig. 4b), there was a trend for a main effect of RU24969 treatment [F(2,64)=2.75; p=.0712]. For center time, there was a trend for an RU24969×antagonist interaction [F(2, 64)=2.99; p=.0576] (Online Resource 3g). ANOVA identified an RU24969×GR127935 interaction on spatial d [F(2, 64)=3.86; p=.0261] (Fig. 4c). Three- and 10-mg/kg RU24969 doses decreased spatial d within vehicle-injected mice. Within GR127935-injected mice, only 10 mg/kg RU24969 reduced spatial d, compared to both saline and 3 mg/kg. GR127935 increased spatial d within the 3- and 10-mg/kg RU24969 groups. For the rearing measures, ANOVA found a trend for an interaction between RU24969 and GR127935 on vertical activity [F(2,64)=2.78; p=.0695] (Fig. 4d) and a significant interaction on time spent rearing [F(2,64)=3.15; p=.0497] (Online Resource 3h). The 3- and 10-mg/kg doses of RU24969 reduced rearing time within vehicle injected animals. GR127935 increased rearing time within the 3-mg/kg RU24969 treatment group. Finally, there was a significant interaction between RU24969 and

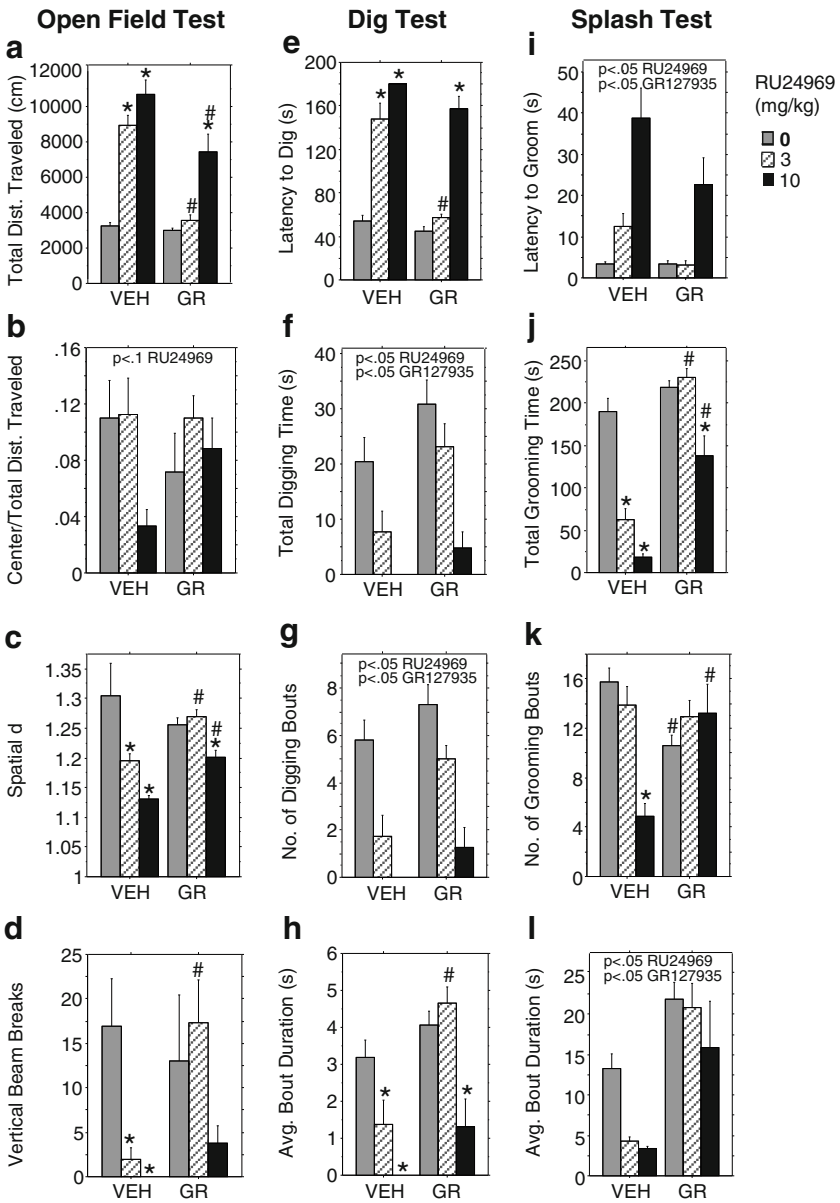

Fig. 4 Effects of RU24969 in the open field, dig test, and splash tests after co-injection with 5-HT1B antagonist. Mice received vehicle or 10 mg/kg GR127935 and 0, 3, or 10 mg/kg RU24969 (n=12). Mice were tested in the open field for **a** total distance traveled, **b** center/total distance traveled, **c** spatial d, and **d** vertical rearing. Measures for the dig test were **e** latency to dig, **f** total time spent digging, **g** number of digging bouts, and **h** average duration of digging bouts. Measures for the splash test were **i** latency to groom, **j** total time spent grooming, **k** number of grooming bouts, and **l** average duration of grooming bouts. Results are presented as mean± SEM. *p<0.05 compared to 0 mg/kg RU24969. #p<0.05 compared to vehicle injection. *VEH* vehicle, *GR* GR127935

antagonist injection on rest time [F(2,64)=13.35; p<.0001] (Online Resource 3i). Both RU24969 doses reduced rest time within vehicle-injected mice, with 3 mg/kg differing from 10 mg/kg. Within the GR127935-injected group, 10 mg/kg RU24969 decreased rest time compared with that of saline and 3 mg/kg RU24969. GR127935 increased rest time within 3- and 10-mg/kg RU24969, but not saline, groups.

Dig test

ANOVA found an interaction of RU24969 and GR127935 injection on latency to dig [F(2,65)=13.23; p<.0001] (Fig. 4e) and a trend toward an RU24969×GR127935 interaction on average bout duration [F(2,65)=3.08; p=.0530] (Fig. 4h). Both doses of RU24969 increased latency to groom in vehicle-injected mice, while only 10 mg/kg RU24969 increased latency to groom within GR127935-injected mice. The 3- and 10-mg/kg RU24969 groups differed from each other within both injection groups. Furthermore, GR127935 prevented 3 mg/kg RU24969-induced increases in latency. Only main effects of RU24969 [F(2,65)=21.62; p<.0001] and GR127935 [F(1,65)=12.22; p=.0009] were found on total time spent digging (Fig. 4g), and on number of bouts[F(2,65)=34.10; p<.0001] and [F(1,65)=11.33; p=.0013], respectively (Fig. 4h). ANCOVAs confirmed an RU24969×GR127935 interaction on dig latency [F(2,64)=5.55; p=.0060] and a trend for an interaction on average bout duration [F(2,64)=2.93; p=.0605], independent of rest time. Again, there were no interactions on total digging time or number of bouts.

Splash test

ANOVA revealed only main effects of RU24969 to increase [$F(2,65)=23.39$; $p<.0001$] and GR127935 [$F(1,65)=5.97$; $p=.0172$] to decrease grooming latency (Fig. 4i). Interactions were found on total time spent grooming [$F(2,65)=23.39$; $p<.0001$] (Fig. 4j) and number of bouts [$F(2,65)=23.39$; $p<.0001$] (Fig. 4k). Both doses of RU24969 reduced total grooming time within vehicle-injected mice. Within GR127935-injected mice, only 10 mg/kg RU24969 reduced time spent grooming. Three and 10 mg/kg differed from each other within both injection groups. GR127935 attenuated RU24969-induced decreases in grooming time for both 3 and 10 mg/kg RU24969. For number of grooming bouts, 10 mg/kg RU24969 reduced bout number compared with that of saline and 3 mg/kg within the vehicle injection group. Within 10 mg/kg RU24969, GR127935 increased grooming bouts. Within saline-treated animals, GR127935 reduced grooming bouts. Only the main effects of RU24969 [$F(2,65)=3.76$; $p=.0285$] and GR127935 [$F(1,65)=27.77$; $p<.0001$] were found on average bout duration (Fig. 4l). ANCOVAs confirmed RU24969×GR127935 interactions on total grooming time [$F(2,64)=7.52$; $p=.0012$] and number of bouts [$F(2,64)=11.90$; $p<.0001$], independent of rest time. No interactions remained on groom latency and average bout duration.

Experiment 4

Open field test

The effects of RU24969 and pretreatment on patterns of locomotor activity are shown in Fig. 5 and Online Resource 2. ANOVA revealed a pretreatment×RU24969 interaction on distance traveled [$F(2,83)=23.13$; $p<.0001$] (Fig. 6a). RU24969 increased distance traveled within each pretreatment group. Within the RU24969-treated group, chronic fluoxetine reduced distance traveled compared to both vehicle and desipramine. Desipramine reduced the distance traveled within the saline treatment group. For center activity measures, ANOVAs identified pretreatment×RU24969 interactions, on center/total distance [$F(2,83)=8.44$; $p=.0005$] (Fig. 6b) and center time [$F(2,83)=4.09$; $p=.0203$] (Online Resource 3j). RU24969 decreased both measures of center activity within the vehicle and desipramine, but not fluoxetine, pretreatment groups. Within the saline treatment group, fluoxetine reduced both center activity measures compared to vehicle and center/total distance compared to desipramine. For spatial d, a pretreatment×RU24969 interaction was found [$F(2,83)=5.85$; $p=.0042$] (Fig. 6c). RU24969 decreased spatial d within each pretreatment group. Within the RU24969-treated group, chronic fluoxetine increased spatial d compared to vehicle and desipramine. ANOVA revealed an interaction of pretreatment and RU24969 on vertical activity [$F(2,83)=6.36$; $p=.0027$] (Fig. 6d) and time spent rearing [$F(2,83)=4.78$; $p=.0109$] (Online Resource 3k). RU24969 decreased both rearing measures within the vehicle and desipramine, but not fluoxetine, pretreatment groups. Within the saline treatment group, fluoxetine reduced rearing compared to desipramine and vehicle. Finally, there was a significant interaction between pretreatment and RU24969 on rest time [$F(2,83)=26.85$; $p<.0001$] (Online Resource 3l). RU24969 reduced rest time within each pretreatment group. Within RU24969-treated group, chronic fluoxetine increased rest time compared to vehicle and desipramine, and desipramine increased rest time within the saline treatment group.

Dig test

ANOVA found an interaction between pretreatment group and RU24969 on latency to dig [$F(2,81)=8.07$; $p=.0006$] (Fig. 6e). RU24969 increased latency to dig in the vehicle

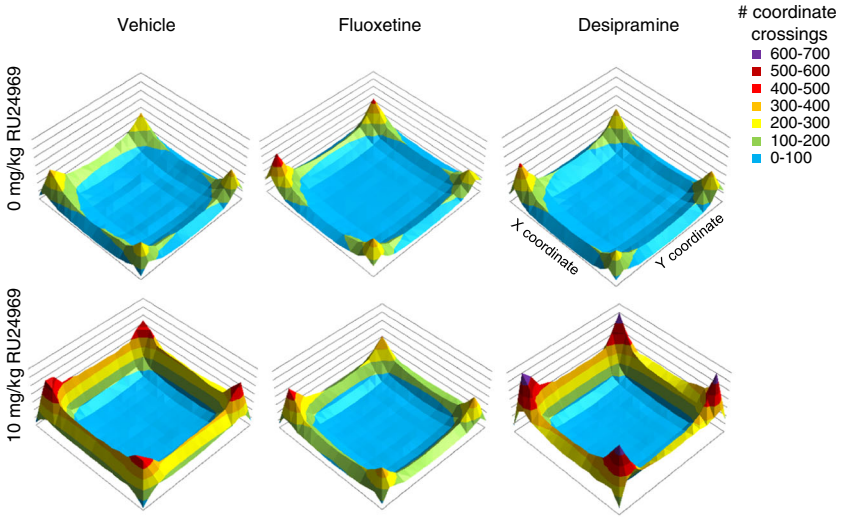

Fig. 5 Effects of RU24969 on locomotor patterns after chronic antidepressant pretreatment. Mice received 4 weeks of vehicle, 10 mg/kg/day fluoxetine, or 20 mg/kg/day desipramine, and acute injection of 0 or 10 mg/kg RU24969 ($n=15$), and were tested in the open field. Three-dimensional representations of average number of crossings at each coordinate are shown

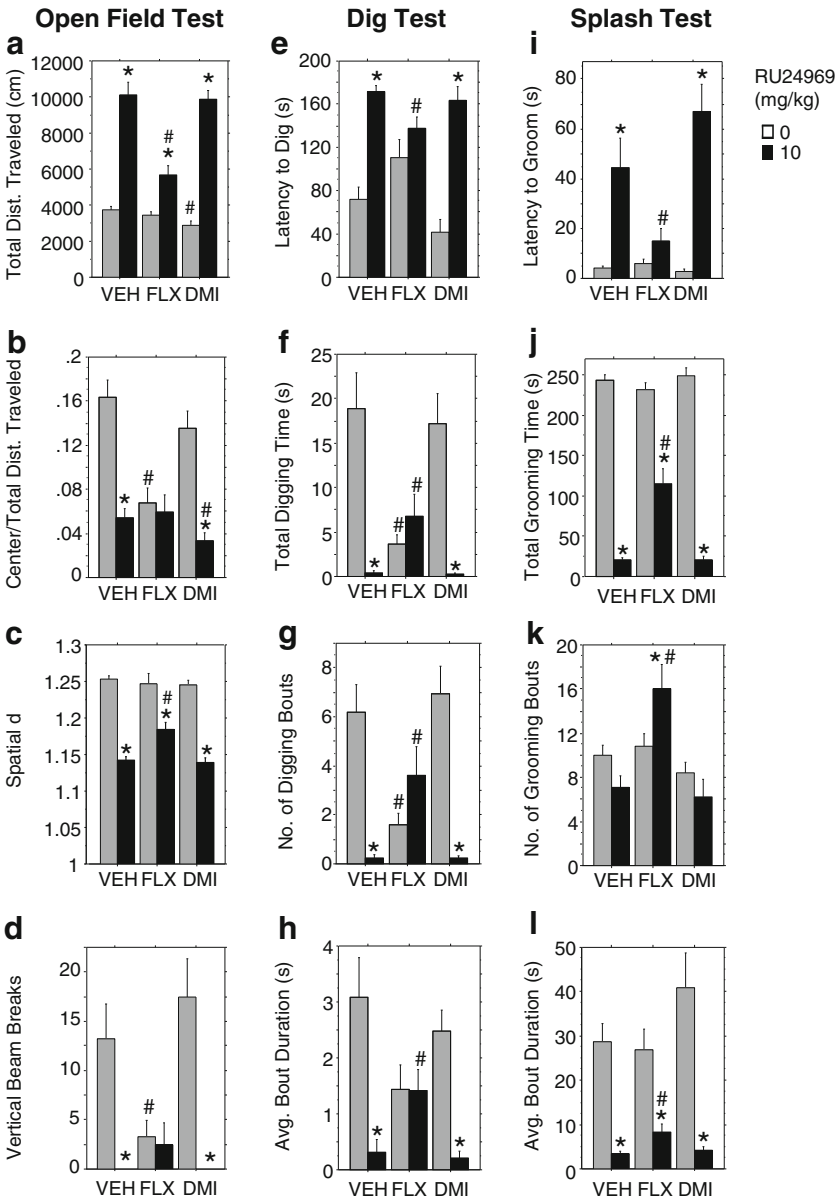

Fig. 6 Effects of RU24969 in the open field, dig test, and splash tests after chronic antidepressant pretreatment. Mice received 4 weeks of vehicle, 10 mg/kg/day of fluoxetine, or 20 mg/kg/day of desipramine, and acute injection of 0 or 10 mg/kg RU24969 (n=15). Mice were assessed in the open field for **a** total distance traveled, **b** center/total distance traveled, **c** spatial d, and **d** vertical rearing. Measures for the dig test were **e** latency to dig, **f** total time spent digging, **g** number of digging bouts, and **h** average duration of digging bouts. Measures for the splash test were **i** latency to groom, **j** total time spent grooming, **k** number of grooming bouts, and **l** average duration of grooming bouts. Results are presented as mean± SEM. *p<0.05 compared to 0 mg/kg RU24969. #denotes p<0.05 compared to vehicle pretreatment. *VEH* vehicle, *FLX* fluoxetine, *DMI* desipramine

and desipramine, but not fluoxetine, pretreatment groups. Fluoxetine reduced latency compared to vehicle within the RU24969 group and increased latency compared to desipramine within the saline group. An interaction was also found between pretreatment and RU24969 for the total time spent digging [F(2,81)=9.34; p=.0002] (Fig. 6f), number of digging bouts [F(2,81)=13.57; p<.0001] (Fig. 6g), and average bout duration [F(2,81)=5.64; p=.0051] (Fig. 6h). RU24969 reduced these measures within the vehicle and desipramine, but not fluoxetine, pretreatment groups. Within the RU24969 group, fluoxetine increased these measures compared to vehicle and desipramine. Within the saline treatment group, fluoxetine reduced total dig time and bout number. ANCOVAs confirmed pretreatment×RU24969 interactions on dig latency [F(2,80)=3.21; p=.0458], total time spent digging [F(2,80)=6.44; p=.0026], number of bouts [F(2,80)=12.93; p<.0001], independent of rest time. There was no longer an interaction on average bout duration.

Splash test

ANOVA revealed an interaction of pretreatment×RU24969 on latency to groom [F(2,82)=8.10; p=.0006] (Fig. 6i), total time spent grooming [F(2,82)=19.36; p<.0001] (Fig. 6j), number of grooming bouts [F(2,82)=5.21; p=.0074] (Fig. 6k), and average bout duration [F(2,82)=6.42; p=.0026] (Fig. 6l). RU24969 increased latency to groom within the vehicle and desipramine, but not fluoxetine, pretreatment groups. Within the RU24969-treated group, chronic fluoxetine reduced latency compared to desipramine.

RU24969 decreased time spent grooming and average bout duration within each pretreatment group, but fluoxetine attenuated this effect compared to vehicle and desipramine. Finally, the number of grooming bouts was increased by only fluoxetine within RU24969-treated animals and by RU24969 within only fluoxetine-pretreated animals. ANCOVAs confirmed RU24969×GR127935 interactions on latency to groom [$F(2,81)=6.13$; $p=.0033$], total grooming time [$F(2,81)=7.33$; $p=.0062$] and number of bouts [$F(2,81)=9.05$; $p=.0003$], independent of rest time. There was no longer an interaction on average bout duration.

qPCR

ANOVA revealed an interaction of pretreatment×RU24969 on messenger RNA (mRNA) expression of *Fos* in the caudate putamen [$F(2,30)=13.01$; $p<.0001$] (Fig. 7b). Post hoc tests showed that RU24969 increased *Fos* mRNA expression in the vehicle and desipramine, but not fluoxetine, pretreatment groups. Within the saline-treated group, fluoxetine also increased *Fos* mRNA expression. No significant effects of either pretreatment or RU24969 were found in the OFC (Fig. 7d). In the cerebellum, RU24969 increased *Fos* mRNA expression [$F(1,30)=28.35$; $p<.0001$] across pretreatment groups (Fig. 7f).

Discussion

Here, we show that a wide range of doses of RU24969 induces locomotor stereotypy and reduces digging and grooming. We also found that chronic fluoxetine, but not desipramine, prevented multiple effects of RU24969, including hyperactivity, as well as reduced spatial d, digging, and grooming. In addition, RU24969 treatment induced neuronal activation in the dorsal striatum, which was also prevented by the chronic fluoxetine, but not desipramine, pretreatment. Thus, our present findings support and extend upon the 5-HT1B-induced mouse model of aspects of O.

Our dose response studies of RU24969 in the open field (Fig. 1a–d) showed that doses of 1–10 mg/kg RU24969 significantly increased hyperactivity and reduced rest time and rearing behavior in the open field. Five and 10 mg/kg RU24969 also significantly reduced center measures and spatial d, the latter indicating that mice receiving these doses exhibited straighter, less circumscribed locomotor paths

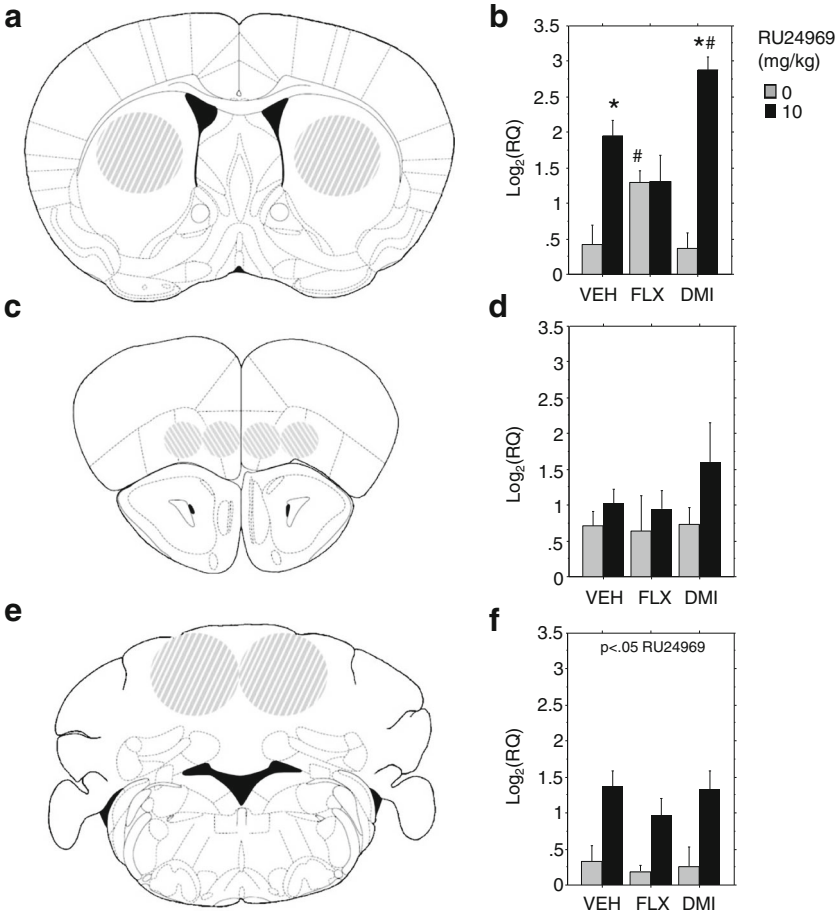

Fig. 7 Effects of RU24969 on *Fos* mRNA expression after chronic antidepressant pretreatment. Tissue punches of the **a** caudate putamen, **c** orbitofrontal cortex, and **e** cerebellum were taken from mice given four weeks of vehicle, 10 mg/kg/day fluoxetine, or 20 mg/kg/day desipramine in the drinking water, and acute injection of 0 or 10 mg/kg RU24969 ($n=6$). *Fos* mRNA expression was quantified using qRT-PCR in the **b** caudate putamen, **d** orbitofrontal cortex, and **f** cerebellum. Results are presented as mean±SEM. *$p<0.05$ compared to 0 mg/kg RU24969. #$p<0.05$ compared to vehicle pretreatment. *VEH* vehicle, *FLX* fluoxetine, *DMI* desipramine

(Paulus and Geyer 1991). Previous investigations of RU24969 in mice have shown robust locomotor effects at 10 mg/kg in Balb/cJ (Shanahan et al. 2009; Shanahan et al. 2011) and C57Bl/6J strains (Cheetham and Heal 1993). However, to our knowledge, no reports have shown significant hyperactivity with a 1-mg/kg dose of RU24969 in mice. Additionally, we show for the first time that RU24969 treatment reduces center activity, spatial d, and rearing in C57Bl/6J mice. Alhough RU24969 reduces center activity, it has no effects on other assays of anxiety-like behavior, such as food or water-motivated conflicts (Gardner 1986). Robust RU24969-induced reductions in rearing were likely detected in the current study due to relatively high baseline levels of vertical activity in C57Bl/6J mice compared to BALB/cJ mice (Lalonde and Strazielle 2008).

We found that RU24969 dose-dependently reduced all measures of digging and grooming behavior (Fig. 1e–l), with the exception of number of grooming bouts. Specifically, the one and three doses of RU24969 increased the number of grooming bouts, while higher doses had no effect. The RU24969-induced increases in grooming bouts at low doses is interesting, because OCD patients insert non-functional acts into motor rituals, resulting in more starts and stops (Zor et al. 2011).

Results of ANCOVAs revealed that effects of RU24969 treatment on total grooming time as well as number of grooming bouts were independent of locomotion throughout all four experiments. In contrast, digging behaviors were confounded by RU24969-induced hyperactivity. Our results suggest that as higher doses of RU24969 induce greater hyperlocomotion, digging behavior is eliminated through response competition. This finding is consistent with recent work demonstrating reduced home cage drinking with increasing doses of RU24969 in rodents (Aronsen et al. 2014). It is unclear how the effects of RU24969 treatment on splash-induced grooming, but not digging, are independent from locomotor activity. One possibility is that splash-induced grooming is elicited by a strong stimulus, while digging is not. Treatment with RU24969 doses lower than 1 mg/kg might further reveal effects on species-typical behavior that are independent from locomotor activity.

In our examination of 5-HT1B specificity for RU24969-induced behaviors, 5 mg/kg GR127935 was unable to fully block the locomotor effects of 10 mg/kg RU24969 in C57Bl/6J mice, whereas a complete blockade was previously observed in BALB/cJ mice (Shanahan et al. 2009). Because C57Bl/6J mice have higher baseline activity levels than BALB/cJ mice have (Lalonde and Strazielle 2008), we also investigated behaviors following co-treatment with a moderate dose of RU24969 and a higher dose of GR127935. Co-treatment with 10 mg/kg GR127935 and 3 mg/kg RU24969 produced behaviors comparable to saline on all measures, indicating that RU24969 effects are mediated by 5-HT1B at moderate doses in C57BL/6J mice. At higher doses of RU24969, other receptors might be involved. WAY100635 had no effects on RU24969-induced hyperactivity, rearing, or digging, indicating a lack of 5-HT1A involvement for those behaviors. Since both GR127935 and WAY100635 blocked RU24969-induced reductions in center activity and most grooming measures, activation of both 5-HT1A and 1B receptors are likely required for these effects. Consistent with observations in BALB/cJ mice (Shanahan et al. 2009), RU24969-induced reductions of spatial d in C57Bl/6J mice were fully blocked by GR127935 and partially reduced by WAY100635.

Chronic fluoxetine, but not desipramine, pretreatment attenuated several effects of RU24969 treatment (Fig. 6). Chronic fluoxetine, but not desipramine, pretreatment attenuated the hyperactivity and reductions in spatial d induced by RU24969 in C57Bl/6J mice, as we have previously reported in BALB/cJ mice. Although chronic fluoxetine also reduced effects of RU24969 on center activity and rearing, chronic fluoxetine produced robust effects alone, which compromises interpretation of these findings. Chronic fluoxetine, but not desipramine, pretreatment also prevented RU24969-induced increases in dig latency, total time spent digging, and number of digging bouts, independent from locomotor effects. However, chronic fluoxetine also reduced digging behaviors, similarly complicating interpretation of results.

In the novel environments of both the dig and open field tests, chronic fluoxetine pretreatment alone robustly reduced measures of exploration. These measures comprised center/total distance, time in the center, number of rearings, rearing duration, total time spent digging, number of digging bouts, and average digging bout duration. These findings likely reflect reduced exploration rather than increased anxiety (Dulawa et al. 1999), as we and others have previously shown that chronic selective SRI treatment reduces anxiety in several tests, including the novelty-induced hypophagia test (Dulawa and Hen 2005). Furthermore, these fluoxetine-induced reductions in exploration were unlikely due to sedation, since chronic fluoxetine pretreatment did not alter total locomotor activity or grooming behavior. Similar to our findings, chronic fluoxetine has been shown to reduce center activity measures in different rat strains (Durand et al. 1999). However, chronic fluoxetine has not been found to affect center activity measures on various mouse strains, including C57Bl/6J (Dulawa et al. 2004). Additionally, chronic fluoxetine has been shown to increase exploratory behavior in zebrafish in the novel tank test (Wong et al. 2010). Further studies will be required to determine the effects of chronic SRI treatment on exploration in humans and animals.

Chronic fluoxetine, but not desipramine, pretreatment also prevented RU24969-induced increases in grooming latency, reductions in overall grooming time, and reductions in average grooming bout duration. With the exception of average

bout duration, these effects on grooming behavior were independent of locomotor activity. Chronic fluoxetine desensitizes the 5HT-1A autoreceptor (Li et al. 1996) and the 5-HT1B receptors in various brain regions (Shanahan et al. 2011). Since we observed both 5-HT1A and 5-HT1B components to changes in grooming behavior following RU24969 treatment, the observed attenuation of grooming by fluoxetine could result from 5-HT1A or 1B desensitization. Furthermore, we observed no baseline effects of chronic fluoxetine on any of the grooming measures. Because RU24969 effects on grooming behavior were reversed by chronic SRI, but not NRI, treatment, these measures exhibit predictive validity for aspects of OCD. While the present results validate RU24969-induced *reductions* in grooming behavior as OCD-relevant, *increased* grooming has been proposed to phenotypically model OCD symptoms. However, face validity, which refers to the phenomenological similarity of a measure across species, is neither necessary nor sufficient for determining whether a model is useful (Geyer and Markou 1995). Rather, measures with predictive validity, such as RU24969-induced reductions in splash-induced grooming, are more informative for studying the human condition.

We also report for the first time that chronic fluoxetine, but not desipramine, pretreatment prevents RU24969-induced increases in neuronal activation in the dorsal striatum. Previous work with RU24969 and co-treatment with 5-HT1A or 5-HT1B antagonists has shown that activation of 5-HT1B receptors mediate RU24969-induced c-*fos* expression in the striatum (Lucas et al. 1997). Our findings are consistent with human functional imaging studies showing that OCD patients exhibit overactivation of the dorsal striatum that is normalized by chronic SRI treatment (Benkelfat and Nordahl 1990; Baxter and Schwartz 1992; Saxena et al. 1999). However, we did not observe activation of the OFC in mice treated with RU24969. Although overactivation of the OFC and the dorsal striatum are hallmarks of OCD, an animal model is unlikely to mimic all aspects of a psychiatric disorder, which are complex and heterogeneous in nature. Other putative mouse models of OCD have been characterized by either dorsal striatal overactivation, including the SAPAP3 knockout mouse model (Welch et al. 2007; Burguière et al. 2013), or OFC overactivation, such as the SliTrk5 knockout mouse model (Shmelkov et al. 2010). One possible reason we did not observe on OFC activation is that the medial and lateral OFC have dissociable functions that have been suggested to be hypoactive versus hyperactive in OCD, respectively (Milad and Rauch 2012). Thus, our assessment of neuronal activation using whole OFC preparations could have obscured opposing effects of RU24969 treatment on neuronal activation in the medial versus lateral OFC. Additional studies using immunohistochemistry could be used to resolve potential differential neuronal activation of the medial versus lateral OFC.

Interestingly, chronic fluoxetine pretreatment alone increased *Fos* mRNA expression in the dorsal striatum. Although treatment with either RU24969 or fluoxetine increased striatal activation, RU24969 treatment induced locomotor stereotypy while fluoxetine did not. The inconsistent relationship between striatal activation and locomotor stereotypy could be explained by the induction of different populations of striatal neurons by the two drug treatments. For example, RU24969 might activate direct pathway medium spiny neurons (MSNs), while chronic fluoxetine pretreatment might activate indirect pathway MSNs. Activation of direct pathway signaling is thought to facilitate movement, while activation of the indirect pathway has been suggested to suppress movement (Bromberg-Martin et al. 2010). Activation of the indirect pathway by chronic fluoxetine might prevent the effects of RU24969 challenge. In support of our hypothesis, a recent study found that chronic fluoxetine treatment induces delta-FosB in indirect pathway neurons in the dorsal striatum (Lobo et al. 2013).

Finally, although RU24969 increased neuronal activation in the cerebellum, this effect was not altered by any pretreatment.

Our findings show that the 5-HT1B-induced model of aspects of OCD exhibits strong predictive validity in C57Bl/6J in addition to BALB/cJ mice. Furthermore, we show that 5-HT1B activation alters specific measures of splash-induced grooming and digging behaviors that are independent of locomotion and can be attenuated by chronic fluoxetine, but not desipramine, treatment. We also show that RU24969 treatment induces striatal activation, which is prevented by chronic fluoxetine, but not desipramine treatment. In summary, the effects of RU24969 on striatal activation, locomotor activity, spatial d, latency to groom, and total time spent grooming model aspects of OCD. Vertical rearing, latency to dig, total time spent digging, and number of digging bouts are also good candidates because chronic SRI, but not NRI, prevents RU24969-induced effects. However, since our chronic SRI also had baseline effects on these explorative measures, their relevance to OCD requires further validation. Studying the mechanisms underlying 5-HT1B-induced effects and their reversal by chronic SRI treatment could provide insight into OCD pathophysiology.

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