

5-HT_{2C} Agonists Modulate Schizophrenia-Like Behaviors in Mice

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All FDA-approved antipsychotic drugs (APDs) target primarily dopamine D₂ or serotonin (5-HT_{2A}) receptors, or both; however, these medications are not universally effective, they may produce undesirable side effects, and provide only partial amelioration of negative and cognitive symptoms. The heterogeneity of pharmacological responses in schizophrenic patients suggests that additional drug targets may be effective in improving aspects of this syndrome. Recent evidence suggests that 5-HT_{2C} receptors may be a promising target for schizophrenia since their activation reduces mesolimbic nigrostriatal dopamine release (which conveys antipsychotic action), they are expressed almost exclusively in CNS, and have weight-loss-promoting capabilities. A difficulty in developing 5-HT_{2C} agonists is that most ligands also possess 5-HT_{2B} and/or 5-HT_{2A} activities. We have developed selective 5-HT_{2C} ligands and herein describe their preclinical effectiveness for treating schizophrenia-like behaviors. JJ-3-45, JJ-3-42, and JJ-5-34 reduced amphetamine-stimulated hyperlocomotion, restored amphetamine-disrupted prepulse inhibition, improved social behavior, and novel object recognition memory in NMDA receptor hypofunctioning NRI-knockdown mice, and were essentially devoid of catalepsy. However, they decreased motivation in a breakpoint assay and did not promote reversal learning in MK-801-treated mice. Somewhat similar effects were observed with lorcaserin, a 5-HT_{2C} agonist with potent 5-HT_{2B} and 5-HT_{2A} agonist activities, which is approved for treating obesity. Microdialysis studies revealed that both JJ-3-42 and lorcaserin reduced dopamine efflux in the infralimbic cortex, while only JJ-3-42 decreased it in striatum. Collectively, these results provide additional evidence that 5-HT_{2C} receptors are suitable drug targets with fewer side effects, greater therapeutic selectivity, and enhanced efficacy for treating schizophrenia and related disorders than current APDs.

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INTRODUCTION

Atypical antipsychotic drugs (APDs) are the first choice for treating schizophrenia (SZ) and they act primarily through blockade or partial antagonism of dopamine (DA) D₂ receptors and inverse agonism at serotonin 2A (5-HT_{2A}) receptors, together with activities at other DA and 5-HT receptors (Meltzer *et al*, 1989; Roth *et al*, 2004). Psychotic disorders include SZ, schizoaffective disorder, bipolar disorder, major depression, and various neurological conditions (Arciniegas *et al*, 2001; Wigman *et al*, 2012; Landqvist-Waldö *et al*, 2015). The limited efficacy of atypical APDs for

treating negative symptoms and cognitive impairment in SZ, and the role of *N*-methyl-D-aspartate receptor (NMDAR) antagonists to induce cognitive deficiency in man and laboratory animals implicates glutamate (Glu) and γ -aminobutyric acid (GABA) receptors in some aspects of SZ (López-Gil *et al*, 2010). It is now established that atypical APDs are superior to selective D₂ antagonists for improving cognitive function in SZ (Désaméricq *et al*, 2014; Meltzer, 2015), while having modest effects on negative symptoms (Leucht *et al*, 1999; Miyamoto *et al*, 2005).

There is some evidence that 5-HT_{2C} agonists have antipsychotic efficacy (Marquis *et al*, 2007). This receptor interacts with G_{q/11} protein to activate phospholipase C β , leading to intracellular calcium release (Conn *et al*, 1986; McCorvy and Roth, 2015). In rodents, 5-HT_{2C} mRNA is present in dorsal and ventral striatum and is coexpressed primarily in substantia nigra and ventral tegmental area (VTA) neurons containing glutamic acid decarboxylase mRNA (Bubar *et al*, 2011; Eberle-Wang *et al*, 1997), where the 5-HT_{2C} receptor may indirectly inhibit DA neurons

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through GABA interneuronal activation. Neurochemical and electrophysiological studies confirm this point, as a 5-HT_{2C} inverse agonist increases firing in VTA and substantia nigra, enhances VTA bursting activity, and leads to DA release in the nucleus accumbens (NAc) and striatum—with more pronounced effects on DA mesolimbic neurons (Gobert *et al*, 2000). Intrastriatal administration of this inverse agonist increases DA release in the lateral striatum (Alex *et al*, 2005). Conversely, the 5-HT_{2C} agonist Ro-60-0175 decreases DA release in the NAc and striatum (Gobert *et al*, 2000). This agonist blocks cocaine-induced open field hyperactivity and the selective 5-HT_{2C} antagonist SB 242084 reverses this effect (Grottick *et al*, 2000). However, Ro-60-0175 fails to alter cocaine-induced DA release and a 5-HT_{2C} inverse agonist does not influence amphetamine (AMPH)-stimulated DA release in NAc or striatum of halothane-anesthetized rats (Porras *et al*, 2002; Navailles *et al*, 2004). Hence, the effects of 5-HT_{2C} agonists may involve striatal non-dopaminergic postsynaptic mechanisms. Further evidence for a modulatory role of 5-HT_{2C} receptors on DA function derives from *Htr2c* disruption, resulting in elevated DA release in dorsal striatum and enhanced AMPH-induced stereotypies in mice (Abdallah *et al*, 2009). In humans, the *HTR2c* polymorphism Cys23Ser is associated with augmented striatal DA release during pain stress (Mickey *et al*, 2012). Since non-medicated SZ patients may have elevated DA synthesis and release in caudate (Kegeles *et al*, 2010), 5-HT_{2C} ligands may represent a new generation of APDs that modulate dopaminergic neurotransmission in the striatum.

Recently, we have published the synthesis and initial behavioral characterization of novel 5-HT_{2C} agonists with promising APD properties in mouse models of SZ (Cheng *et al*, 2015, 2016). SZ is a neurodevelopmental disorder characterized by positive and negative symptoms, and cognitive impairment. There is an urgent need to control APD-induced side effects, such as neuroleptic malignant syndrome, psychomotor retardation, weight gain, and blood and bone marrow complications. Notably, novel 5-HT_{2C} ligands show promise in treating obesity (Smith *et al*, 2010) and improving glycemic control in type 2 diabetes patients (O'Neil *et al*, 2012). Preclinical studies suggest possible procognitive actions of 5-HT_{2C} agonists in novel object recognition memory (NORM) (Cheng *et al*, 2016).

The present study examined effects of three novel compounds with selective 5-HT_{2C} agonism in pharmacological and genetic models of SZ. For positive symptoms, AMPH-induced hyperactivity and disruption of prepulse inhibition (PPI) were used. For negative symptoms, we focused on motivational impairment and avolition using a breakpoint assay. We also used the sociability test with the NMDAR hypofunctioning NR1-knockdown (NR1-KD) mice (Mohn *et al*, 1999; Milenkovic *et al*, 2014; Park *et al*, 2016). For cognition we included NORM and a visual discrimination reversal test. For possible extrapyramidal side effects, we used the horizontal bar test for catalepsy. Finally, we measured the effects of one of the compounds on extracellular levels of neurotransmitters in the infralimbic cortex and striatum of mice.

MATERIALS AND METHODS

Detailed descriptions of the methods and procedures can be found in the Supplementary Information.

Animals

Adult male and female C57BL/6J and female C3H/HeJ mice were purchased (Jackson Laboratories, Bar Harbor, ME), whereas wild-type (WT) and NR1-KD mice were bred and maintained as described (Park *et al*, 2016). Mice were housed 3–5 per cage on a 14:10 h light:dark cycle (Duke: lights on 0700 h) for behavioral studies and on a 12:12 h light:dark cycle (Northwestern: lights on 0500 h) for microdialysis experiments, with food and water provided *ad libitum*. Six cohorts were used: cohort 1, open field activity and PPI; cohort 2, motivation; cohort 3, sociability and NORM; cohort 4, visual discrimination learning and reversal; cohort 5, catalepsy; and cohort 6, microdialysis. At least 10 days were interposed between tests within any cohort. All experiments were conducted with approved protocols from the Duke and Northwestern Universities Institutional Animal Care and Use Committees.

Drugs

The lorcaserin (Selleckchem LLC, Houston, TX), AMPH (Sigma-Aldrich, St Louis MO), MK-801 (Sigma-Aldrich), and JJ-3-45, JJ-3-42, and JJ-5-34 were reconstituted in sterile 0.9% saline. Clozapine (Sigma-Aldrich) was dissolved in 0.2% glacial acetic acid with saline, whereas haloperidol (Sigma-Aldrich) was dissolved to a final concentration of 3% dimethylsulfoxide. All treatments were administered (intraperitoneally) in 5 ml/kg volume, except as noted.

Open Field Activity

C57BL/6J mice were injected with vehicle or different doses of the 5-HT_{2C}-selective compounds or lorcaserin, placed into the open field (Omnitech, Columbus, OH) for 15 min, removed and injected with vehicle or 3 mg/kg AMPH, and immediately returned to the apparatus for 105 min. Activity was monitored as cumulative distance traveled.

Prepulse Inhibition

C57BL/6J mice were administered vehicle, different doses of the 5-HT_{2C}-selective compounds, or lorcaserin in their home cage. Ten minutes later, animals were given vehicle or 3 mg/kg AMPH and acclimated to a 65 dB white-noise background in the PPI apparatus (San Diego Instruments, San Diego, CA). Five minutes later, mice were presented with combinations of startle (120 dB), prepulse-pulse (4, 8, and 12 dB over the 65 dB background followed by 120 dB), and null trials over 25 min (Park *et al*, 2016). Activity was recorded during all trials. The data were presented as percent PPI = $(1 - (\text{prepulse trials}/\text{startle-only trials})) \times 100$, and as startle and null activities.

Breakpoint as an Index of Motivation

Throughout the experiment mice were food restricted to 85–90% of baseline body weight and housed 4/cage. Mice were trained initially on a continuous reinforcement schedule with condensed milk as a reward. Subsequently, animals were trained on a progressive ratio schedule where the number of bar presses required to earn the reward doubled. Once performance was stable, mice were injected with vehicle, the JJ compounds, or lorcaserin and tested. Breakpoint (highest ratio completed) was recorded.

Sociability

There were three phases to this test: identical non-social (control), a social and non-social (social affiliation), and a novel and familiar social pairing (social preference). Female C3H/HeJ mice were used as partners with WT and NR1-KD tester mice. The tester NR1 mice were injected with vehicle, 0.5 mg/kg clozapine (positive control), or different doses of the 5-HT_{2C}-selective compounds or lorcaserin. Ten minutes later, individual tester NR1 mice were evaluated sequentially in 10 min tests for responses to two identical non-social stimuli (empty wire-mesh cages), to novel social (C3H/HeJ mouse) and non-social stimuli, and to novel social (C3H/HeJ mouse) and now familiar social (previous C3H/HeJ mouse) stimuli. The duration of stimulus contacts was monitored. Preference scores ((time spent with one stimulus – time spent with the other stimulus)/total time spent exploring both stimuli) were calculated where positive scores indicated a preference for the non-social stimulus in the first test, for the social stimulus in the second test, and for the novel social stimulus in the third test.

Novel Object Recognition Memory

Before training, WT and NR1-KD mice were administered the vehicle or different doses of the 5-HT_{2C}-selective compounds or lorcaserin, and 30 min later, they were presented with identical object pairs for 5 min. The next day mice were administered the same treatments and were tested after 30 min. Here, one of the now familiar objects was replaced with a novel object and the mice were tested over 5 min. The duration of object contracts was calculated as a control. Recognition scores ((time spent with novel object – time spent with familiar object)/total time with both objects) were calculated where positive scores signified novel object preference, negative scores indicated familiar object preference, and scores approaching 'zero' denoted no object preference.

Reversal of Visual Discrimination Learning

Since behavioral responses across tests were similar for the three JJ compounds, we only evaluated JJ-3-45 and selected the 2 mg/kg dose because this was the highest dose that did not disrupt the visual discrimination and was close to the optimal dose for NORM. Touchscreens were used to display two separate images for visual discrimination and reversal (Med Associates).

Throughout the experiment mice were food-restricted to 85–90% of baseline body weight and housed 3–5 mice per

cage. At training mice were taught to nose poke at white rectangles to receive a condensed milk (Kroger) reward. Subsequently, mice were trained on a simultaneous discrimination where one of two stimuli was reinforced. Upon reaching a criterion of 80% correct responses, the mouse was given MK-801 or JJ-3-45 to determine which doses would not perturb its visual discrimination performance. Subsequently, mice were assigned to vehicle, MK-801, and MK-801 plus JJ-3-45 groups and these treatments were chronically administered. The mice were tested in the visual discrimination for two additional sessions and then the reward contingencies were reversed. Responses were scored as the number of correct trials, number of errors, and percent correct ((correct/total trials) × 100).

Catalepsy

Baseline responses were recorded and then separate groups of C57BL/6J mice were injected with haloperidol, different doses of the three JJ compounds, or lorcaserin. Mice were tested 60 min later for catalepsy. The latency for a mouse to remove its paws from the bar was recorded as an index of catalepsy with a maximum time of 90 s.

Microdialysis Procedure and Analyses of Neurotransmitters

C57BL/6J males were housed in groups of 3–5 per cage until surgery; afterwards, they were housed individually. Mice were anesthetized with 1.5% isoflurane (Isothesia; Butler-Schein, Dublin, OH) and cannulated with dummy probes in the infralimbic cortex and ventral striatum. Two days later, microdialysis probes were inserted on the morning of the experiment with perfusion at 1 µl/min. Two hours later, microdialysis samples were collected every 30 min. After 90 min, mice were injected with vehicle, 10 mg/kg JJ-3-42, or 1 mg/kg lorcaserin and samples were collected over another 180 min. Parenthetically, the doses for JJ-3-42 and lorcaserin were based on the open field experiment. Samples were submitted to mass spectrometry for acetylcholine (ACh), DA, norepinephrine, 3,4-dihydroxyphenylacetic acid (DOPAC); homovanillic acid (HVA), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), Glu, and GABA. Baseline was designated as 100% and subsequent neurochemical levels were expressed as percent baseline.

Statistical Analyses

The data were presented as means and standard errors of the mean. One-, two-way, or repeated-measures ANOVA (RMANOVA) were used with Dunnett or Bonferroni *post hoc* analyses. Owing to variability in the neurochemistry, some data were analyzed by Kruskal–Wallis, followed by Mann–Whitney tests. A $P < 0.05$ was considered significant.

RESULTS

AMPH-Stimulated Hyperlocomotion in C57BL/6J Mice

AMPH-stimulated hyperlocomotion is used as a model of hyperdopaminergia in rodents and APDs reduce it (Geyer and Moghaddam, 2002). JJ-3-45, JJ-3-42, JJ-5-34, or

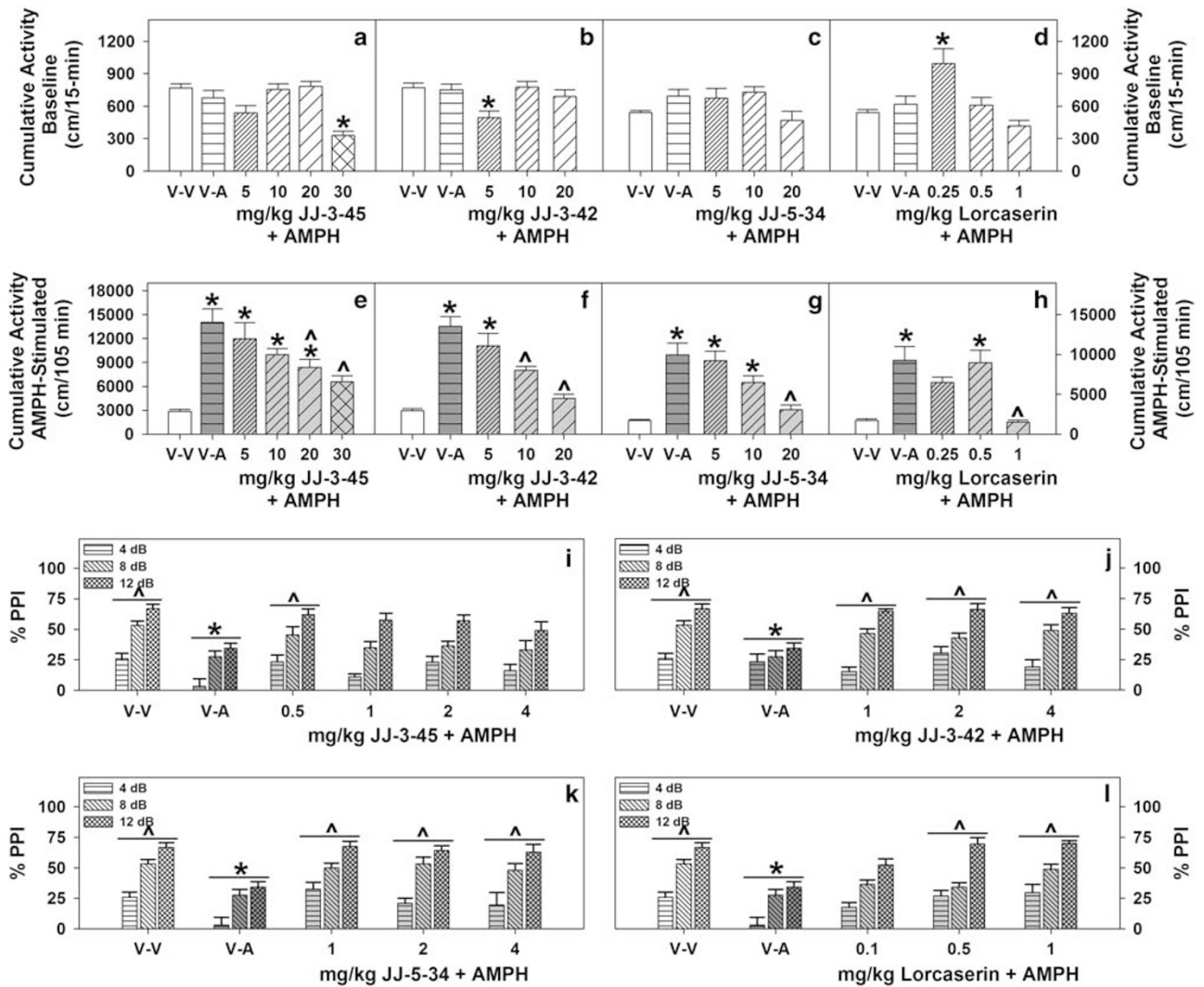


Figure 1 All three JJ compounds and lorcaserin reduced the 3 mg/kg amphetamine-stimulated hyperlocomotion in the open field and restored amphetamine-disrupted prepulse inhibition (PPI) in C57BL/6 mice. (a–d) Open field baseline locomotor activity (0–15 min) in mice administered JJ-3-45, JJ-3-42, JJ-5-34, or lorcaserin. A one-way analysis of variance (ANOVA) observed a main effect of dose: JJ-3-45 ($F(5,81) = 6.740, P < 0.001$), JJ-3-42 ($F(4,75) = 2.604, P < 0.042$), JJ-5-34 ($F(4,62) = 3.511, P < 0.012$), and lorcaserin ($F(4,46) = 6.892, P < 0.001$). (e–h) Amphetamine-stimulated hyperlocomotion (105 min) with dose responses for JJ-3-45, JJ-3-42, JJ-5-34, and lorcaserin. A one-way ANOVA noted a main effect of dose: JJ-3-45 ($F(5,81) = 14.718, P < 0.001$), JJ-3-42 ($F(4,75) = 18.232, P < 0.001$), JJ-5-34 ($F(4,62) = 15.448, P < 0.001$), and lorcaserin ($F(4,46) = 9.987, P < 0.001$). (i–l) Dose responses for JJ-3-45, JJ-3-42, JJ-5-34, and lorcaserin in PPI. A repeated-measures ANOVA (RMANOVA) within-subjects effects for PPI intensity ($F(2,242) = 315.229, P < 0.001$); between subjects effects for treatment ($F(14,121) = 5.302, P < 0.001$). Groups: V–V, vehicle–vehicle control; V–A, vehicle–amphetamine. $N = 9–31$ mice per compound or drug dose in the open field study and $N = 8–15$ mice per compound or drug dose in the PPI experiment; * $P < 0.001$, compared with the V–V control; ^ $P < 0.01$, compared with the V–A group.

lorcaserin were injected and baseline activity was assessed over the first 15 min. Relative to vehicle, basal locomotion was attenuated by 30 mg/kg JJ-3-45 ($P < 0.001$; Figure 1a) and 5 mg/kg JJ-3-42 ($P < 0.042$; Figure 1b), but it was increased with 0.25 mg/kg lorcaserin compared with all other groups (P -values < 0.014 ; Figure 1d).

After baseline assessment, vehicle or AMPH (3 mg/kg, intraperitoneally) was injected and mice were returned to the open field for 105 min. The 20 and 30 mg/kg JJ-3-45 decreased the AMPH-induced hyperactivity (P -values < 0.045), such that responses to the highest dose were similar to the vehicle control (Figure 1e). Similarly, 10 and 20 mg/kg JJ-3-42 suppressed hyperlocomotion (P -values < 0.050) to levels statistically indistinguishable from vehicle

controls (Figure 1f). By comparison, only 20 mg/kg JJ-5-34 reduced AMPH-induced hyperlocomotion ($P < 0.001$) to the vehicle control (Figure 1g). Thus, all novel 5-HT_{2C} compounds antagonized the AMPH-stimulated response. By contrast, lorcaserin failed to inhibit the AMPH-stimulated effect until the highest dose was used ($P < 0.001$; Figure 1h).

AMPH-Disrupted PPI in C57BL/6J Mice

PPI is reduced in SZ and APDs can normalize AMPH-disrupted PPI in rodents (Krystal *et al.*, 1999; Geyer and Moghaddam, 2002). JJ-3-45, JJ-3-42, JJ-5-34, or lorcaserin were administered, 10 min later the vehicle or AMPH (3 mg/kg, i.p.) were given, and the mice were tested after

Table 1 Startle Amplitudes and Null Activities in C57BL/6J mice

mg/kg	V-V			V-A			JJ-3-45			JJ-3-42			JJ-5-34			Lorcaserin		
	0	3	0.5	1	2	4	1	1	4	2	4	1	1	2	4	0.1	0.5	1
Pulse Activity (AU) ^{ab}	452 ± 59	471 ± 97	332 ± 60	428 ± 81	301 ± 63	229 ± 38	251 ± 48	270 ± 43	323 ± 29	271 ± 61	322 ± 67	313 ± 51	193 ± 33	276 ± 60	244 ± 35			
Null activity (AU) ^{ac}	7.9 ± 0.6	12.1 ± 1.3	13.6 ± 2.6	14.0 ± 3.5	13.1 ± 1.1	14.9 ± 1.5	9.7 ± 0.9	12.0 ± 1.0	10.3 ± 1.0	10.0 ± 0.6	13.2 ± 1.7	11.1 ± 1.6	16.4 ^d ± 1.3	13.3 ± 1.2	14.8 ^d ± 1.5			

Abbreviations: ANOVA, analysis of variance; V–V, vehicle–amphetamine group; V–A, vehicle–vehicle group.

^aResults are presented as means ± SEM; N = 8–15 mice per treatment per group.

^bANOVA (F(1,4,12)) = 2.087, P < 0.017.

^cANOVA (F(1,4,12)) = 2.673, P < 0.002.

^dP < 0.05, compared with the V–V group.

5 min. AMPH depressed PPI compared with the vehicle control ($P < 0.001$; Figure 1i–l). All compounds reversed this disruptive effect as demonstrated with 0.5 mg/kg JJ-3-45; 1, 2, and 4 mg/kg JJ-3-42 and JJ-5-34; and 0.5 and 1 mg/kg lorcaserin (P -values < 0.006). Startle activity varied with treatment and this was due to a dose-induced reduction in responses (Table 1); however, the *post hoc* tests were not significant. Null (baseline) activity was significantly enhanced with 0.1 and 1 mg/kg lorcaserin (P -values < 0.030; Table 1). Thus, all selective 5-HT_{2C} compounds and lorcaserin restored the AMPH-disrupted PPI.

Breakpoint as an Index of Motivation in C57BL/6 Mice

Motivation is regulated by mesolimbic dopaminergic neurons and is reduced in SZ (Salamone and Correa, 2012). Mice were tested on a progressive ratio schedule for food reinforcement as an index of motivation. They were treated with the vehicle and different doses of the JJ compounds or lorcaserin. The 1 and 5 mg/kg doses of JJ-3-45 significantly decreased the breakpoint relative to the vehicle control (P -values < 0.001) (Figure 2a). Similarly, all doses of JJ-3-42 and JJ-5-34 decreased the breakpoints (P -values < 0.009) (Figures 2b and c). Lorcaserin depressed lever responding beginning at 0.5 mg/kg ($P < 0.029$) with higher doses exerting greater effects on breakpoint (P -values < 0.001) (Figure 2d). Hence, all JJ compounds and lorcaserin reduced breakpoints in dose-dependent manner.

Sociability in NR1 Mice

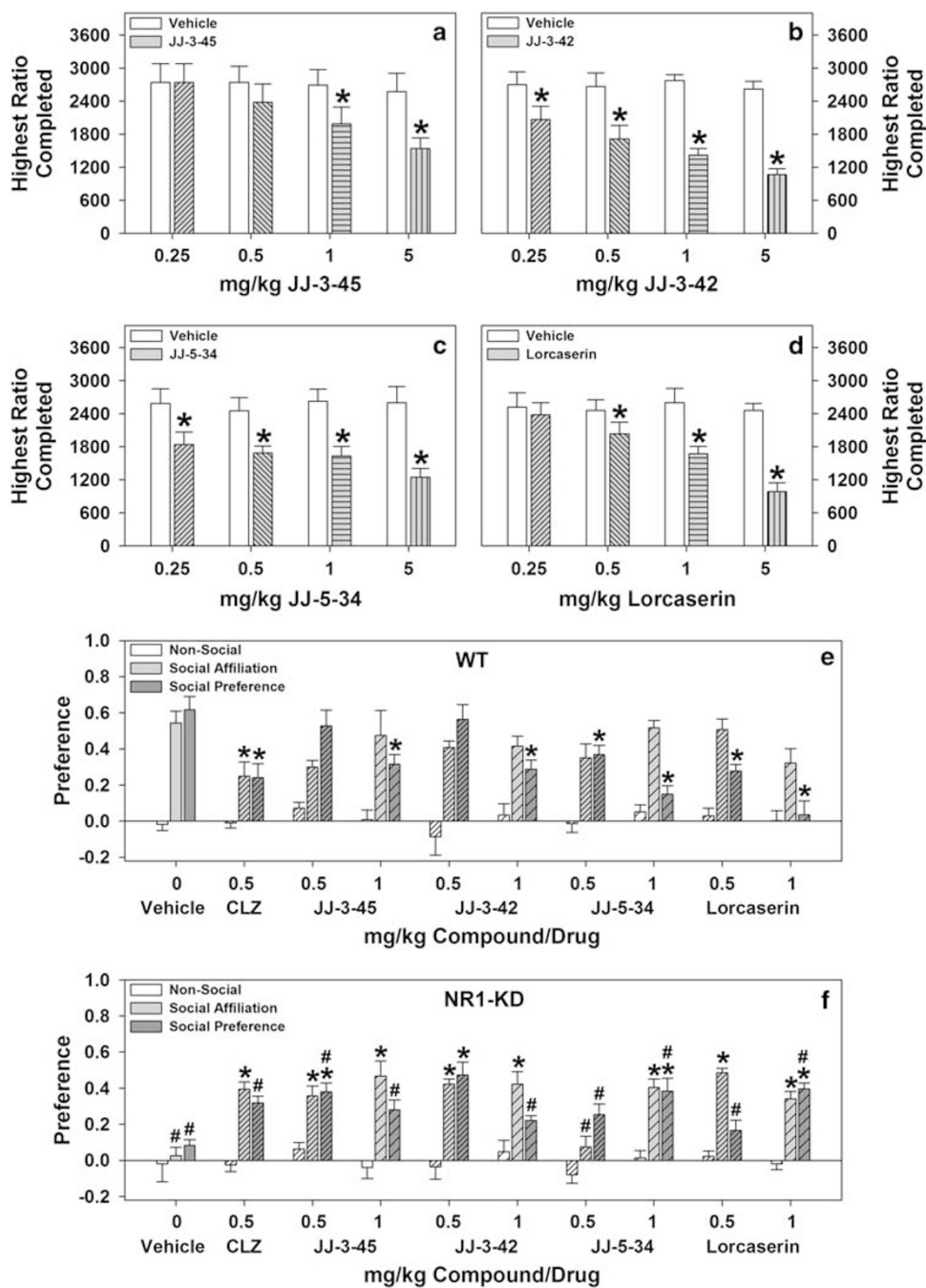
Social withdrawal is a hallmark of SZ and NR1-KD mice display this response (Mohn *et al*, 1999). These mutants are deficient also in sociability and clozapine improves it (Park *et al*, 2016). WT and NR1-KD mice were tested in three phases: a non-social pairing with two identical stimuli, a pairing with social and non-social stimuli (social affiliation), and a pairing with the now familiar and a novel social stimulus (social preference). As a control, the duration of stimulus contacts was examined and no significant differences were observed among groups across test phases (Table 2)—indicating that any deficiencies in social behavior cannot be attributed to a failure to interact with the non-social/social stimuli. During the non-social pairing, no group showed any preference for one over the other identical stimulus (Figures 2e and f). By comparison, vehicle-treated WT mice preferred the novel social stimulus in the social affiliation test relative to NR1-KD mice—which showed no preference for either stimulus ($P < 0.001$). Within genotype analyses revealed clozapine decreased WT performance compared with vehicle ($P = 0.010$) (Figure 2e), whereas it augmented NR1-KD responses ($P = 0.001$) (Figure 2f). Relative to vehicle, there was a tendency for 0.5 mg/kg JJ-3-45 ($P = 0.060$) to reduce WT social affiliation, whereas it was increased in mutants given 0.5 mg/kg JJ-3-45 or 1 mg/kg JJ-5-34 as well as with 0.5 or 1 mg/kg JJ-3-42 or lorcaserin (P -values < 0.006). In NR1-KD mice a dose-dependent increase in social affiliation was observed with JJ-5-34 ($P = 0.008$). Analyses of responses between genotypes demonstrated that social affiliation was higher in WT than NR1-KD mice at both doses of JJ-5-34 (P -values < 0.042). With the exception of 0.5 mg/kg JJ-5-34 responses, all other

treatment conditions restored NR1-KD social affiliation to that of the WT vehicle controls.

In the social preference test, vehicle-treated WT mice preferred the novel over familiar social stimulus, whereas mutants displayed no preference for either stimulus ($P < 0.001$) (Figures 2e and f). The within genotype comparisons revealed that relative to vehicle, clozapine decreased WT performance ($P = 0.001$) (Figure 2e) with a tendency for it to facilitate social preference in NR1-KD mice ($P = 0.064$) (Figure 2f). Compared with vehicle, social preference in WT controls was depressed with 1 mg/kg JJ-3-45 or JJ-3-42 as well as with 0.5 or 1 mg/kg JJ-5-34 and lorcaserin (P -values < 0.024). In contrast, this behavior was

increased in mutants with 0.5 mg/kg JJ-3-45 or JJ-3-42 or with 1 mg/kg JJ-5-34 and lorcaserin (P -values < 0.050). A dose-dependent decrease in social preference was noted with JJ-3-42 ($P = 0.043$) in WT mice (Figure 2e). Importantly, only 0.5 mg/kg JJ-3-42 restored social preference in NR1-KD mice to that of the WT vehicle controls (Figure 2e and f).

In summary, vehicle-treated NR1-KD mice were significantly impaired in both social affiliation and social preference relative to WT vehicle controls. However, all three JJ compounds and lorcaserin improved both social behaviors in mutants and decreased them in WT mice. Notably, only 0.5 mg/kg JJ-5-34 failed to restore social



affiliation in NR1-KD mice to that of the WT vehicle controls. By comparison, only 0.5 mg/kg JJ-3-42 normalized social preference in mutants.

NORM in NR1 Mice

NORM examines recognition memory and general mnemonic function (Ennaceur and Delacour, 1988), and NR1-KD mice are deficient in NORM (Park *et al.*, 2016). During training, WT and NR1-KD mice demonstrated no object preference between the two identical objects regardless of

drug-dose assignment (Figure 3a and b). A comparison of preference scores between training and testing revealed that at testing WT mice failed to prefer the novel object when administered 5 mg/kg JJ-5-34, whereas NR1-KD animals were deficient when given the vehicle, 1 or 10 mg/kg JJ-3-42, 5 mg/kg JJ-5-34, or 0.5 mg/kg lorcaserin. Analyses of responses within genotype found that in WT mice all treatments—except 10 mg/kg JJ-3-42 and 1 mg/kg JJ-5-34—reduced NORM below that of the vehicle control (P -values < 0.012). In contrast, in mutants 5 mg/kg JJ-3-42, as well as both doses of JJ-3-45, JJ-5-34, and lorcaserin

Table 2 Duration of Contacts in the Sociability Test with WT and NR1-KD Mice

	Veh	Clozapine	JJ-3-45		JJ-3-42		JJ-5-34		Lorcaserin	
mg/kg	0	0.5	0.5	1	0.5	1	0.5	1	0.5	1
<i>Non-social interaction^{ab}</i>										
WT	287.2 ± 40.8	233.7 ± 15.6	137.9 ± 13.2	207.2 ± 31.7	216.5 ± 22.7	224.1 ± 22.3	214.2 ± 27.0	155.8 ± 19.0	157.0 ± 23.9	162.1 ± 24.1
KD ^a	194.2 ± 25.9	205.5 ± 27.8	191.8 ± 31.3	216.7 ± 29.3	197.4 ± 24.3	245.0 ± 34	151.7 ± 27.4	162.7 ± 32.6	153.9 ± 12.3	197.3 ± 23
<i>Social affiliation^{ab}</i>										
WT	285.6 ± 50.6	255.2 ± 17.5	162.5 ± 18.8	213.7 ± 29.7	189.9 ± 27.0	220.2 ± 26.6	231.8 ± 39.0	182.2 ± 31.5	190.5 ± 33.2	172.5 ± 31.6
KD	207.5 ± 24.2	223.3 ± 23.6	196.2 ± 37.6	208.4 ± 34.9	174.7 ± 19.3	229.1 ± 28.0	143.7 ± 17.0	146.7 ± 23.2	205.5 ± 23.5	235.2 ± 31.6
<i>Social preference^{ab}</i>										
WT	260.8 ± 49.4	197.6 ± 23.5	146.9 ± 16.4	205.7 ± 31.8	224.2 ± 20.8	189.0 ± 31.1	231.5 ± 41.8	133.7 ± 36.0	204.4 ± 26.0	198.5 ± 30.6
KD	150.4 ± 20.8	172.6 ± 10.6	196.9 ± 38.2	159.0 ± 20.0	189.1 ± 23.3	225.6 ± 35.1	177.6 ± 10.7	185.4 ± 25.6	203.8 ± 34.5	222.1 ± 33.3

Abbreviations: NR1-KD, NR1-knockdown; RMANOVA, repeated-measures analysis of variance; Veh, vehicle; WT, wild type.

^aResults are presented as means ± SEM; $N = 8-9$ mice per drug or compound per dose.

^bRMANOVA: only the within-subjects time by treatment interaction was significant ($F(18,294) = 1822, P = 0.022$).

Figure 2 The JJ compounds and lorcaserin decreased lever pressing under a progressive ratio schedule for food reinforcement in C57BL/6 mice, but increased social behavior in NR1-knockdown (NR1-KD) mice. (a–d) Dose–responses for JJ-3-45, JJ-3-42, JJ-5-34, and lorcaserin are shown as breakpoints for assessment of motivation. A repeated-measures analysis of variance (RMANOVA) for JJ-3-45 within-subjects effect of session ($F(1,39) = 31.395, P < 0.001$); session by dose interaction ($F(3,39) = 4.898, P < 0.01$). A RMANOVA for JJ-3-42 within-subjects effect of session ($F(1,42) = 121.664, P < 0.001$); session by dose interaction ($F(3,42) = 4.103, P < 0.012$). A RMANOVA for JJ-5-34 within-subjects effect of session ($F(1,42) = 50.513, P < 0.001$). A RMANOVA for lorcaserin within subjects effect for session ($F(1,40) = 57.079, P < 0.001$), session by dose interaction ($F(3,40) = 8.876, P < 0.001$); between-subjects effect of treatment ($F(3,40) = 2.746, P = 0.055$). (e and f) In the sociability test, mice were subjected to three test phases involving pairings of two identical non-social stimuli, a novel social and a non-social stimulus, and familiar and novel social stimuli. (e) Experiments were conducted with wild-type (WT) mice administered the vehicle, clozapine (CLZ), or different doses of JJ-3-45, JJ-3-42, JJ-5-34, and lorcaserin. (f) The same treatment conditions were imposed on NR1-KD mice. A RMANOVA for the vehicle condition: within-subjects effect of test phase ($F(2,30) = 17.896, P < 0.001$), test phase by genotype interaction ($F(2,30) = 10.584, P < 0.001$); between-subjects effect of genotype ($F(1,15) = 54.524, P < 0.001$). A RMANOVA for the vehicle vs CLZ: within-subjects effect of test phase ($F(2,58) = 37.586, P < 0.001$), test phase by genotype ($F(2,58) = 3.595, P = 0.034$), and test phase by genotype by treatment ($F(2,58) = 9.284, P < 0.001$) interactions; between-subjects effect of genotype ($F(1,29) = 20.688, P < 0.001$), genotype by treatment interaction ($F(1,29) = 46.142, P < 0.001$). A RMANOVA for the vehicle and CLZ vs JJ-3-45: within-subjects effect of test phase ($F(2,118) = 71.591, P < 0.001$), test phase by treatment ($F(6,118) = 2.157, P = 0.052$) and test phase by genotype by treatment ($F(6,118) = 3.373, P = 0.004$) interactions; between-subjects effects of genotype ($F(1,59) = 12.307, P < 0.001$) and treatment ($F(3,59) = 2.780, P = 0.049$), genotype by treatment interaction ($F(3,59) = 13.728, P < 0.001$). A RMANOVA for the vehicle and CLZ vs JJ-3-42: within-subjects effect of test phase ($F(2,118) = 90.784, P < 0.001$), and the test phase by genotype ($F(2,118) = 3.512, P = 0.033$), test phase by treatment ($F(6,118) = 3.737, P = 0.002$), and test phase by genotype by treatment ($F(6,118) = 3.209, P = 0.006$) interactions; between-subjects effects of genotype ($F(1,59) = 10.208, P = 0.002$) and treatment ($F(3,59) = 3.336, P = 0.025$), genotype by treatment interaction ($F(3,59) = 15.487, P < 0.001$). A RMANOVA for the vehicle and CLZ vs JJ-5-34: within-subjects effect of test phase ($F(2,118) = 82.860, P < 0.001$), and the test phase by genotype ($F(2,118) = 3.964, P = 0.022$), test phase by treatment ($F(6,118) = 2.632, P = 0.020$), and test phase by genotype by treatment ($F(6,118) = 5.779, P < 0.001$) interactions; between-subjects effects of genotype ($F(1,59) = 22.227, P < 0.001$) and treatment ($F(3,59) = 3.255, P = 0.028$), genotype by treatment interaction ($F(3,59) = 19.790, P < 0.001$). A RMANOVA for the vehicle and CLZ vs lorcaserin: within-subjects effect of test phase ($F(2,114) = 85.422, P < 0.001$), test phase by treatment ($F(6,114) = 3.208, P = 0.006$) and test phase by genotype by treatment ($F(6,114) = 7.020, P < 0.001$) interactions; between-subjects effect of genotype ($F(1,57) = 6.775, P = 0.012$), genotype by treatment interaction ($F(3,57) = 28.218, P < 0.001$). $N = 8-13$ mice per compound or drug dose in the breakpoint experiment and $N = 8-9$ mice per genotype per treatment condition in the sociability investigation; * $P < 0.05$, compared with the vehicle group or the vehicle within genotype; # $P < 0.05$, compared with the WT vehicle control.

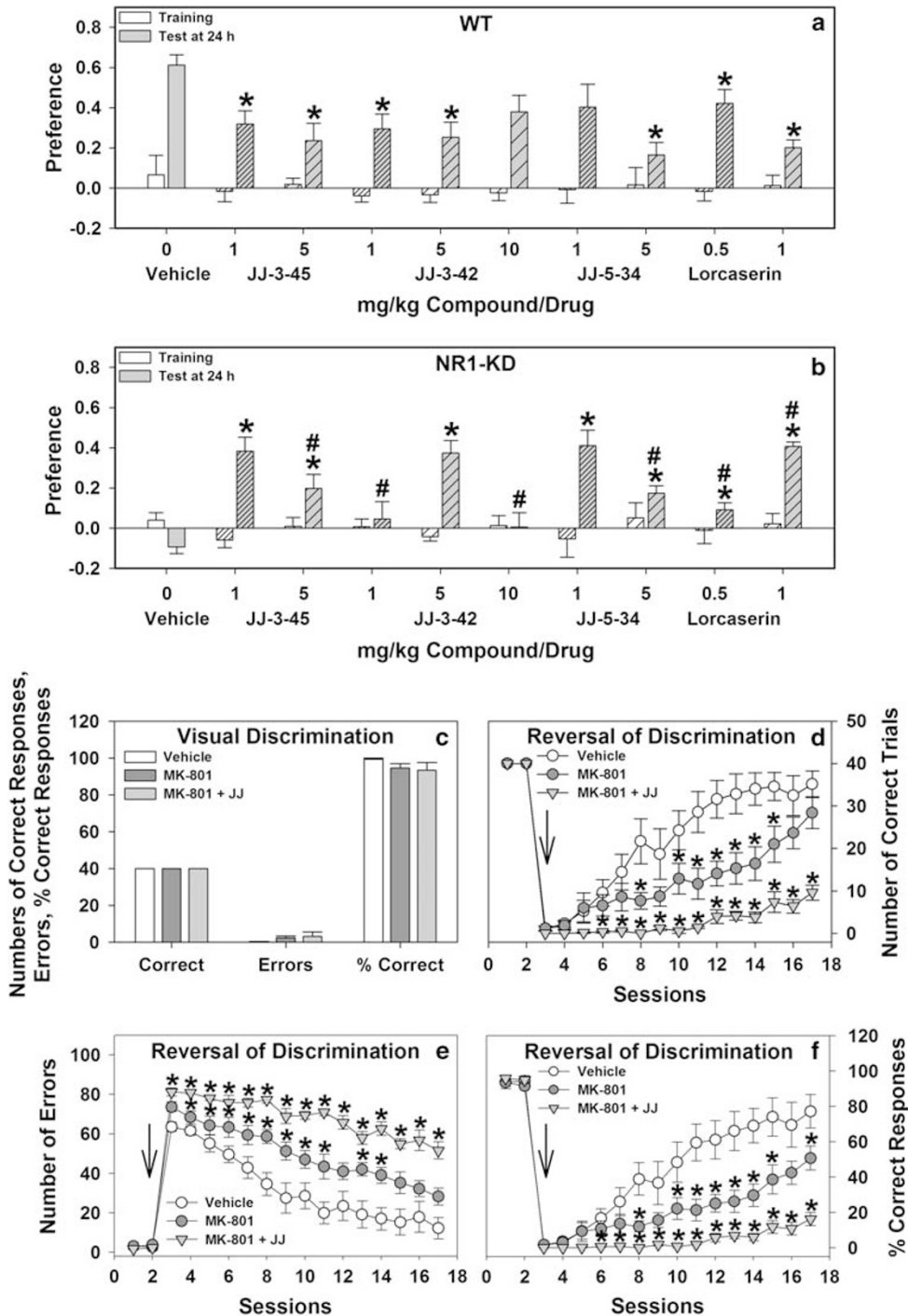
augmented NORM over that of vehicle (P -values < 0.024). In comparing responses to the WT vehicle control, 1 mg/kg JJ-3-45, 5 mg/kg JJ-3-42, and 1 mg/kg JJ-5-34 restored NORM in the NR1-KD mice. It is noteworthy that 1 mg/kg JJ-5-34 maintained NORM in WT mice, whereas this treatment normalized NORM in mutants.

As a control, the duration of object contacts was examined. Although the object interaction times in RMANOVA failed to reach statistical significance, the overall duration of contacts was enhanced in NR1-KD mice during both training and testing (Table 3). As a consequence, any NORM

deficiencies in mutants cannot be attributed to a failure to engage the stimulus objects.

Reversal Learning with MK-801 in C57BL/6 Mice

Reversal learning is a test for plasticity or perseverative responding and it is used in a rodent test battery for SZ-like behaviors (Bussey *et al*, 2012). C57BL/6J mice were trained on a visual discrimination, administered vehicle, MK-801, or MK-801 plus JJ-3-45 during the latter part of the discrimination, and tested with reinforcement contingencies



reversed. The preassigned groups did not differ at baseline in the simple visual discrimination (Figure 3c).

Relative to vehicle controls at reversal, the number of correct trials was reduced with MK-801 on trials 8 and 10–15 (P -values < 0.048), whereas this metric was suppressed even further in the MK-801 plus JJ-3-45 group across trials 6–17 (P -values < 0.033) (Figure 3d). Analyses of reversal performance according to the number of errors revealed increased errors in the MK-801 group on trials 3–11 and 13–15 (P -values < 0.051), whereas errors were further potentiated across all trials in the MK-801 plus JJ-3-45 group (P -values < 0.002) (Figure 3e). After contingency reversal, the percentages of correct responses were decreased in MK-801-treated mice on trials 8, 10–15, and 17 (P s < 0.054) and those in the MK-801 plus JJ-3-45 group were further depressed across trials 6–17 (P -values < 0.029) (Figure 3f).

Collectively, MK-801 alone and MK-801 plus JJ-3-45 reduced reversal performance below the vehicle control. Moreover, performance in the MK-801 plus JJ-3-45 group was further suppressed below that of MK-801 alone at a dose of JJ-3-45 that did not perturb the visual discrimination.

Cataleptic Potential of Compounds in C57BL/6 Mice

An important aspect for new generations of APDs is determining whether they possess motor side effects. In the horizontal bar test mice were evaluated before (baseline) and 60 min after treatment. Catalepsy at baseline was similar across all groups (range 0.0–1.0 s). Compared with vehicle, haloperidol produced significant catalepsy at 1 and 10 mg/kg (P -values < 0.001), which were not significantly different

Table 3 Duration of Contacts in the Novel Object Recognition Memory Test in WT and NRI-KD Mice

	Veh		JJ-3-45			JJ-3-42			JJ-5-34		Lorcaserin	
mg/kg	0	1	5	1	5	10	1	5	0.5	1		
<i>Training^a</i>												
WT	65.9 ± 17.0	57.8 ± 11.0	38.8 ± 7.8	48.0 ± 6.2	34.5 ± 5.8	34.8 ± 5.8	44.6 ± 5.2	44.4 ± 8.4	46.1 ± 5.5	42.4 ± 2.0		
KD	84.3 ± 15.0	109.7 ± 18.0	93.0 ± 13.7	91.9 ± 18.0	91.8 ± 16.0	70.4 ± 11.0	119.9 ± 18.0	87.6 ± 14.0	74.0 ± 9.0	79.5 ± 12.0		
<i>Test^b</i>												
WT	59.5 ± 4.3	65.9 ± 13.0	42.4 ± 8.3	39.1 ± 8.1	19.4 ± 4.5	29.8 ± 5.9	43.9 ± 6.8	49.6 ± 20.5	72.9 ± 11.0	31.9 ± 5.0		
KD	78.5 ± 17.0	107.2 ± 11.1	76.4 ± 11.0	79.8 ± 4.0	70.7 ± 10.0	42.9 ± 5.7	122.8 ± 13.0	84.1 ± 17.0	54.7 ± 8.0	77.1 ± 11.0		

Abbreviations: NRI-KD, NRI-knockdown; RMANOVA, repeated-measures analysis of variance; Veh, vehicle; WT, wild type.

^aResults are presented as means ± SEM; $N = 8$ – 10 mice per drug or compound per dose.

^bRMANOVA: within-subjects effects of time ($F(1,146) = 4.527, P = 0.035$) and time by genotype interaction ($F(1,146) = 4.055, P = 0.046$); between-subjects effects of genotype ($F(1,146) = 66.186, P < 0.001$) and treatment ($F(9,146) = 2.648, P = 0.007$); there was a trend for the genotype by treatment interaction ($F(9,146) = 1.785, P = 0.076$) to be significant.

Figure 3 The three JJ compounds and lorcaserin improved novel object recognition memory (NORM) in NRI-knockdown (NRI-KD) mice; however, JJ-3-45 was unable to restore reversal discrimination performance in MK-801-treated C57BL/6 mice. (a and b) Responses at training and at testing 24 h later in NORM with wild-type (WT) and NRI-KD mice. (a) The investigations were conducted with WT mice using different doses of JJ-3-45, JJ-3-42, JJ-5-34, and lorcaserin. (b) The same experiments were run with NRI-KD mice. A repeated-measures analysis of variance (RMANOVA) for JJ-3-45 within-subjects effect of time ($F(1,46) = 59.964, P < 0.001$), time by genotype ($F(1,46) = 8.521, P = 0.005$), time by treatment ($F(2,46) = 3.110, P = 0.054$), and time by genotype by treatment ($F(2,46) = 13.318, P < 0.001$) interactions; between-subjects effects of genotype ($F(1,46) = 12.403, P < 0.001$), genotype by treatment interaction ($F(2,46) = 12.136, P < 0.001$). The RMANOVA for JJ-3-42 within-subjects time effect ($F(1,60) = 73.254, P < 0.001$), time by genotype ($F(1,60) = 32.444, P < 0.001$) and time by genotype by treatment ($F(3,60) = 9.676, P < 0.001$) interactions; between-subjects genotype effect ($F(1,60) = 19.730, P < 0.001$), genotype by treatment interaction ($F(3,60) = 7.672, P < 0.001$). A RMANOVA for JJ-5-34 within-subjects effects of time ($F(1,48) = 40.152, P < 0.001$), time by genotype ($F(1,48) = 6.975, P = 0.011$), time by treatment ($F(2,48) = 4.892, P = 0.012$), and time by genotype by treatment ($F(2,48) = 8.493, P < 0.001$) interactions; between-subjects effects of genotype ($F(1,48) = 7.745, P = 0.008$), genotype by treatment interaction ($F(2,48) = 8.455, P = 0.001$). The RMANOVA for lorcaserin within-subjects main effects for time ($F(1,26) = 59.239, P < 0.001$), time by genotype ($F(1,46) = 16.858, P < 0.001$) and time by genotype by treatment ($F(2,46) = 15.199, P < 0.001$) interactions; between-subject tests for the genotype ($F(1,46) = 20.639, P < 0.001$), genotype by treatment interaction ($F(2,46) = 20.269, P < 0.001$). (c–f) Visual discrimination performance and reversal in C57BL/6 mice treated with vehicle, MK-801, or MK-801 plus JJ-3-45. (c) Baseline performance on the visual discrimination is presented as the numbers of correct responses, numbers of errors, and percent correct responses for groups given vehicle, MK-801, or JJ-3-45 plus MK-801. (d) Number of correct responses during the last two days of visual discrimination followed by reversal testing. A RMANOVA for the numbers of correct trials: within-subject effect of session ($F(15,270) = 50.264, P < 0.001$), session by treatment interaction ($F(30,270) = 4.828, P < 0.001$); between subject effect of treatment ($F(2,18) = 16.831, P < 0.001$). (e) Number of errors over the same course of testing. A RMANOVA for the number of errors: within-subjects effects for session ($F(15,270) = 60.559, P < 0.001$), session by treatment interaction ($F(30,270) = 2.664, P < 0.001$); between-subjects effect of treatment ($F(2,18) = 35.288, P < 0.001$). (f) Percent correct responses over the same course of testing. A RMANOVA for the percent of correct responses: within-subjects effects of session ($F(15,270) = 59.741, P < 0.001$), session by treatment interaction ($F(30,270) = 5.251, P < 0.001$); between-subjects effect of treatment ($F(2,18) = 17.346, P < 0.001$). $N = 8$ – 10 mice per drug or compound per dose in NORM and $N = 5$ – 10 mice per treatment in the reversal visual discrimination test; * $P < 0.05$, compared with the vehicle group or the vehicle within genotype; # $P < 0.05$, compared with the WT vehicle control.

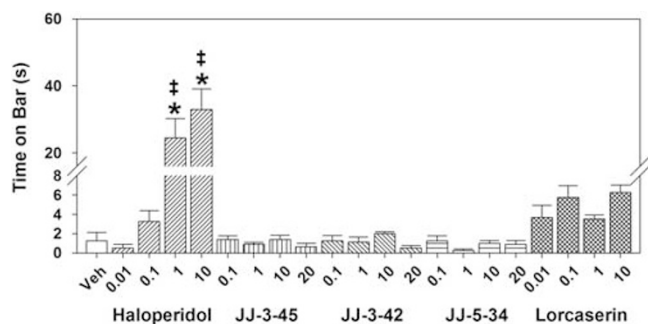


Figure 4 The three JJ compounds and lorcaserin have low cataleptic potential in C57BL/6j mice compared with haloperidol in the horizontal bar test. Dose responses with haloperidol, JJ-3-45, JJ-3-42, JJ-5-34, and lorcaserin for catalepsy. An analysis of variance (ANOVA) between-subjects effects of treatment ($F(20,175) = 18.511$, $P < 0.001$). $N = 8-12$ mice per compound or drug dose: * $P < 0.05$, compared with the vehicle control; † $P < 0.05$, 1 or 10 mg/kg haloperidol compared with all other doses and compounds.

from each other (Figure 4). Additionally, responses to 1 and 10 mg/kg haloperidol were significantly higher than those to lower doses of haloperidol and all other compounds (P -values < 0.001). Thus, the novel 5-HT_{2C} compounds and lorcaserin do not produce catalepsy at doses that elicit it with haloperidol.

Effects of JJ-3-42 and Lorcaserin on *In Vivo* Brain Neurotransmitter Release

5-HT_{2C} receptors are G-protein-coupled receptors expressed on GABAergic, glutamatergic, and dopaminergic neurons (Bubar *et al*, 2011; McCorvy and Roth, 2015; Nocjar *et al*, 2015). These receptors are present in cortical areas, hippocampus, ventral midbrain, striatum, NAc, hypothalamus, and amygdala (Jensen *et al*, 2010). Microdialysis studies were conducted to determine whether a 5-HT_{2C} agonist or lorcaserin could influence neurotransmitter efflux in mouse infralimbic cortex or striatum. In infralimbic cortex, 10 mg/kg JJ-3-42 significantly reduced DA ($P = 0.040$), 5-HT ($P = 0.014$), DOPAC ($P = 0.003$), and HVA ($P = 0.006$), whereas 1 mg/kg lorcaserin decreased DA ($P = 0.021$) and HVA ($P = 0.050$) compared with vehicle (Figure 5a). In the striatum, only JJ-3-42 significantly decreased DA efflux ($P = 0.021$) (Figure 5b). All other neurotransmitter levels were not significantly different among treatments.

Responses to JJ-3-42 and lorcaserin on cortical and striatal DA efflux over time are displayed in Figures 5c and d, respectively. In these brain regions, no significant pre-treatment effects were observed at baseline (−90, −60, −30, and 0 min). At 90 min postinjection, infralimbic cortex extracellular DA levels were decreased with lorcaserin ($P = 0.029$) and they were even more suppressed with JJ-3-42 relative to the vehicle control ($P = 0.004$) (Figure 5c). DA levels were still depressed at 120 min with JJ-3-42 (P -values < 0.031), whereas levels recovered to those of the vehicle with lorcaserin. In contrast, striatal DA levels were reduced at 60, 90, and 150 min postinjection with JJ-3-42 (P -values < 0.029), but not with lorcaserin (Figure 5d). Collectively, JJ-3-42 exerts more marked effects than lorcaserin on DA efflux in infralimbic cortex and only JJ-3-42 reduced DA in the striatum.

DISCUSSION

New medications to treat positive, negative, and cognitive symptoms of SZ that have superior efficacy and fewer side effects than typical and atypical APDs are crucial to achieve better outcomes in various psychotic disorders. In this regard, compounds that can modulate DA release such as 5-HT_{2C}-selective agonists (Cheng *et al*, 2015, 2016) show some promise. In the present study, we provide behavioral profiles of three novel selective 5-HT_{2C} agonists in models of SZ-like behaviors and have analyzed one of the compounds and lorcaserin on neurotransmitter efflux. It should be emphasized that our results are relevant to SZ because clinical trials were completed in 2014 for a 5-HT_{2C}-selective drug (<https://clinicaltrials.gov/ct2/show/results/NCT00563706?sect=X543016#limit>).

JJ-3-42 and JJ-3-45 are structurally unique compounds that show good potency and excellent selectivity for the 5-HT_{2C} receptor (Cheng *et al*, 2015, 2016). Both compounds are 5-HT_{2C} agonists (EC_{50} s of 4.2 and 3.4 nM for JJ-3-42 and JJ-3-45, respectively), with ~100-fold selectivity against 5-HT_{2A} as partial agonists ($E_{max} = 56$ and 76% for JJ-3-42 and JJ-3-45, respectively). Most importantly, these compounds show no significant activation of 5-HT_{2B} receptors at concentrations up to 10 μM, whereas lorcaserin is a full agonist with moderate potency in our assay ($EC_{50} = 478$ nM, $E_{max} = 92\%$). JJ-5-34 is comprised of a benzofuran scaffold and it activates the 5-HT_{2C} receptor with an EC_{50} of 23 nM ($E_{max} = 102\%$). It is a very weak 5-HT_{2B} agonist ($EC_{50} = 433$ nM, $E_{max} = 19\%$), with no activation of 5-HT_{2A} receptors up to 10 μM (results to be published). Besides their excellent pharmacological profiles, in CD-1 mice all three compounds have excellent brain penetration properties, with brain/plasma concentration ratios > 5 at all time-points tested (data not shown). These high brain concentrations indicate that the JJ compounds may be actively transported into the brain, which is ideal for CNS-targeted therapeutics. Despite their excellent profile, the compounds may have some activities that were not assayed. Regardless, all three JJ compounds represent excellent pharmacological tools for exploring the therapeutic effects of 5-HT_{2C}-selective agonists in SZ models and they represent potential 5-HT_{2C} receptor drug candidates for further development.

Acute AMPH-induced hyperactivity is used as an animal model for acute psychosis as AMPH administration to SZ patients exacerbates the florid symptomatology (Angrist *et al*, 1980). All three JJ compounds dose-dependently reduced AMPH-stimulated hyperlocomotion. With the exception of 30 mg/kg JJ-3-45, these effects did not affect baseline activity. JJ-3-42 and JJ-5-34 depressed AMPH-induced hyperactivity to levels that were statistically indistinguishable from vehicle controls. JJ-3-45 was not as efficacious since activity with 20 mg/kg was still significantly higher than vehicle, whereas 30 mg/kg significantly depressed baseline locomotion. By comparison, lorcaserin had a stimulatory effect on baseline activity at the lowest dose and the reduction in AMPH-induced hyperlocomotion was not dose-dependent—suggesting a complex mechanism of action (see Devroye *et al*, 2015).

Atypical APDs exert their actions through antagonism of D₂, inverse agonism at 5-HT_{2A}, and various actions at 5-H_{1A}, 5-HT₆, and 5-HT₇ receptors—depending on the drug

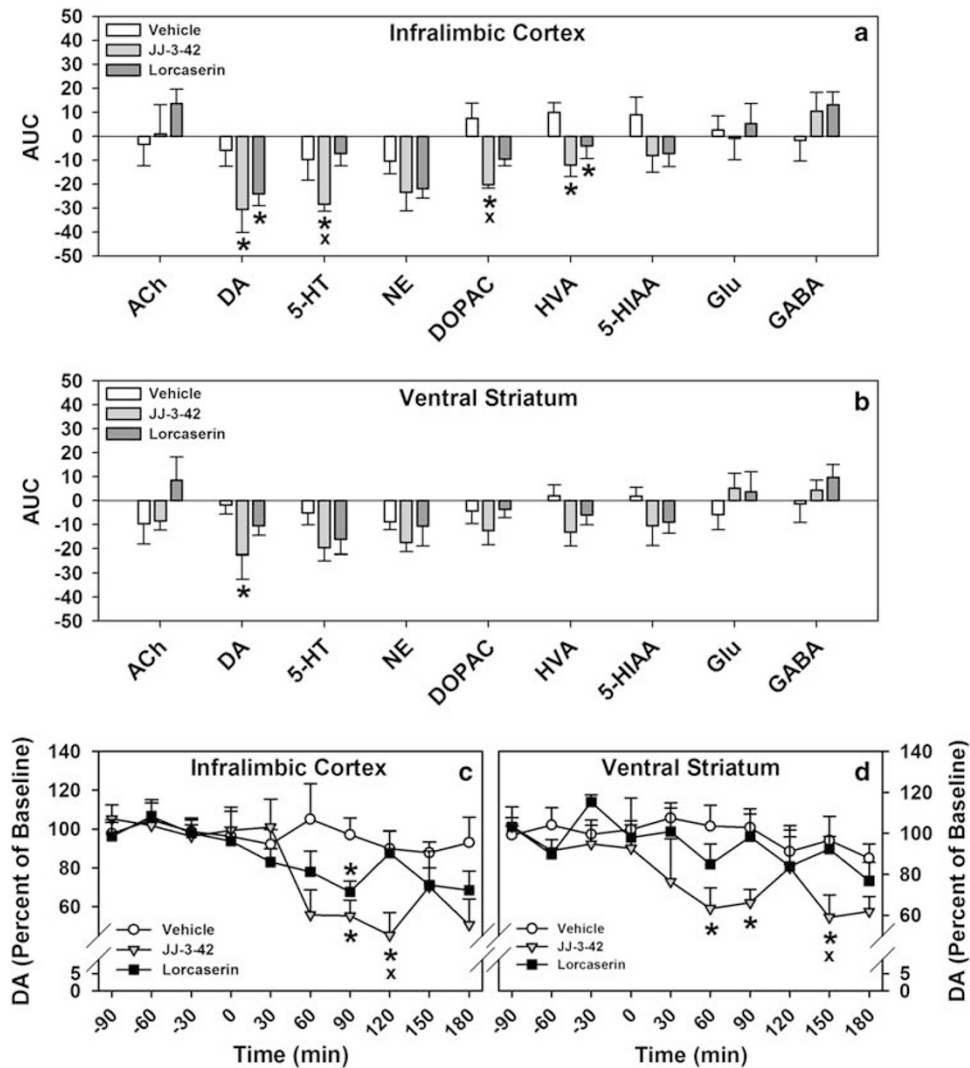


Figure 5 JJ-3-42 and lorcaserin decrease dopamine efflux in infralimbic cortex, whereas only the JJ compound reduced it in the striatum of C57BL/6J mice. Baseline values were collected and then mice were injected (time 0) with vehicle, 10 mg/kg JJ-3-42, or 1 mg/kg lorcaserin and samples were collected over 180 min. Absolute baseline levels can be found in Supplementary Table S1. (a) Extracellular neurotransmitter and metabolite levels in the infralimbic cortex expressed as area under the curve (AUC) representing the mean values from 0 to 180 min. In infralimbic cortex, the following neurotransmitter efflux levels were significant by Kruskal–Wallis *H* tests: dopamine (DA) ($P = 0.035$), serotonin (5-HT) ($P = 0.009$), 3,4-dihydroxyphenylacetic acid (DOPAC) ($P = 0.002$), and homovanillic acid (HVA) ($P = 0.016$). (b) Efflux of neurotransmitter and metabolites in the striatum displayed as area under the curve (AUC). The Kruskal–Wallis *H* test discerned a significant effect for DA ($P = 0.042$) and a trend for HVA ($P = 0.075$). (c) Extracellular DA levels in the infralimbic cortex at baseline (–90, –60, –30, and 0 min) and subsequently (30, 60, 90, 120, 150, and 180 min) postinjection to the vehicle, JJ-3-42, and lorcaserin. A repeated-measures analysis of variance (RMANOVA) for responses at baseline (–90 to 0 min) to 180 min: within-subjects effects of time ($F(6,114) = 3.927, P < 0.001$), time by treatment interaction ($F(12,114) = 1.943, P = 0.036$); between-subjects effects of treatment ($F(2,19) = 5.458, P = 0.013$). (d) Extracellular DA levels in the striatum during baseline and following exposure to the vehicle, JJ-3-42, and lorcaserin. Kruskal–Wallis *H* tests for DA at 60, 90, and 150 min (P -values < 0.054). $N = 7$ – 9 mice per treatment; * $P < 0.05$, compared with the vehicle group; * $P < 0.05$, lorcaserin vs JJ-3-42.

administered (Meltzer and Huang, 2008). Typical APDs act primarily through D_2 receptor blockade. The novel JJ compounds do not block DA receptors (Cheng *et al*, 2016). However, they reduce DA receptor stimulation by decreasing DA release and by acting on 5-HT_{2C} receptors on GABAergic neurons in the substantia nigra and VTA (Gobert *et al*, 2000). This latter mode of action may underlie the lack of significant cataleptic potential of JJ compounds. Although lorcaserin produced catalepsy over baseline; this response was not significantly higher compared with that to vehicle. With regard to JJ compounds, it should be emphasized that at 20 mg/kg, each compound significantly antagonized open-field AMPH-stimulated hyperactivity

while producing no catalepsy in the horizontal bar test. By comparison, typical APDs with relatively selective D_2 antagonism as well as atypical APDs produce similar reductions in AMPH-induced hyperlocomotion at doses that yield significant depressions in baseline activity or produce catalepsy (Schaefer and Michael, 1984).

PPI is disrupted in acute psychosis (Meincke *et al*, 2004) and it is perturbed in rodents by direct and indirect DA agonists (Geyer and Moghaddam, 2002). Both typical and atypical APDs reverse these effects. JJ-3-42 and JJ-5-34 at 1 to 4 mg/kg normalized AMPH-disrupted PPI to the vehicle control; JJ-3-45 and lorcaserin at 0.5 mg/kg were more efficacious. These doses were much lower than those needed

to affect baseline open field locomotion or antagonize the AMPH-induced hyperactivity. By comparison, the doses of conventional APDs used to normalize AMPH-disrupted PPI (Brody *et al*, 2004; Feifel *et al*, 1999; Swerdlow *et al*, 2005) are in the range of doses that can affect baseline locomotion or block AMPH-induced hyperlocomotion, while the atypical APD clozapine restores PPI at 20 mg/kg, it does not normalize locomotion in rats (Cáceda *et al*, 2005). Thus, JJ compounds display superior efficacy in normalizing PPI in the AMPH model and they may possess a broader therapeutic index than typical APDs for treating acute psychosis.

Some negative symptoms of SZ amenable to modeling in animals are motivational impairment and diminished social drive (Kirkpatrick *et al*, 2000). Second-generation APDs partially ameliorate negative symptoms (Leucht *et al*, 1999) and D₂/D₃ antagonists may cause secondary negative symptoms (Heinz *et al*, 1998). To assess motivation, we used a progressive ratio schedule of food reinforcement. Breakpoint in rodents is correlated with the magnitude of sucrose reward (Young and Markou, 2015) and with exertion of effort (Salamone and Correa, 2012). Effort-based tasks have been proposed for modeling motivational abnormalities in SZ based on preserved hedonic responses to primary rewards (Young and Markou, 2015). We found all three JJ compounds and lorcaserin reduced breakpoints in the same dose ranges that were effective in antagonizing AMPH-disrupted PPI. In this respect, the selective 5-HT_{2C} ligands behaved similar to typical APDs (Salamone *et al*, 1991). Hence, these compounds may not have the potential to reverse motivation-related deficits in SZ, which continue to be a challenging area in the management of SZ.

Currently, the nature of impaired social interactions in SZ is obscure where they may have complex relationships with positive and negative symptoms, reflect social anxiety (Millan and Bales, 2013), or perturbed executive function (Meltzer *et al*, 1996). Hence, social behavior in animal models of SZ should be interpreted with caution. Nevertheless, a commonly used procedure with rodents is the sociability test (Moy *et al*, 2004). This test examines social affiliation, which may represent motivation to spend time with a conspecific *vs* an inanimate object and social preference, which denotes an ability to differentiate between familiar and novel social stimuli. We applied this test to NR1-KD mice, which are considered to mimic the hypothesized NMDAR hypofunction in SZ and are hyperactive in the open field, impaired in PPI, and deficient in sociability (Mohn *et al*, 1999; Park *et al*, 2016).

All three selective 5-HT_{2C} agonists and lorcaserin significantly improved social affiliation in NR1-KD mice in a dose range similar to clozapine. Although clozapine increased social affiliation in mutants to that of WT vehicle controls, their overall levels of social behavior were lower. The results in NR1-KD mice appear more marked than in SZ patients where clozapine exerts only modest effects on psychosocial functioning as measured with the Quality of Life Scale and other questionnaires that assess social functioning and activities of daily living (Swartz *et al*, 2007; Guo *et al*, 2012). In a previous study, diazepam improved the abilities of NR1-KD mice to prefer social over non-social stimuli (Milenkovic *et al*, 2014). It remains to be seen whether the selective 5-HT_{2C} agonists possess anxiolytic

properties like diazepam. In this respect, an *HTR2c* polymorphism has been associated with enhanced accumbal DA release during pain stress (Mickey *et al*, 2012). Hence, by acting on the mesolimbic DA system, selective 5-HT_{2C} agonists may possess anxiolytic actions and ameliorate stress responses.

The NR1-KD mice were impaired also in social preference. Since there was a 1–2 min delay between the social affiliation and social preference tests, this deficit is unlikely to represent impaired memory function. Clozapine failed to improve social preference in NR1-KD subjects; however, other doses may be more effective. By comparison, lower doses of JJ-3-45 and JJ-3-42 and higher doses of JJ-5-34 and lorcaserin improved social preference in these mutants. While the underlying mechanism requires additional investigation, one process relevant to SZ symptomatology may be deficient encoding of information. For instance, a discrimination of social novelty requires selective attention and this can be disrupted by acute or neonatal phencyclidine treatment that is sensitive to clozapine (Terranova *et al*, 2005). While attention has not been assessed in NR1-KD mice, blockade of NMDARs in frontal cortex reduces accuracy in the 5-choice serial reaction-time task in rodents due to ‘distractability’ (Carli and Invernizzi, 2014). Thus, it is possible that 5-HT_{2C} agonists may affect selective attention independent of memory processes in NR1-KD mice.

In addition to various psychosocial symptoms, non-verbal memory deficits in SZ are well documented, especially for incidental visual memory (Seidman *et al*, 2003). A deficit in a subtype of long-term memory termed episodic memory is considered a core deficit in SZ (Clare *et al*, 1993) and the NORM task requires intact visual memory for a previously encountered item (see Ennaceur and Delacour, 1988). JJ-3-45, JJ-5-34, and lorcaserin reversed the NR1-KD impairment in NORM at 1 mg/kg. Parenthetically, this same dose restored social affiliation in these mutants. By comparison, JJ-3-42 normalized NORM in NR1-KD mice at 5 mg/kg. Higher doses of all compounds were not efficacious—suggesting an optimum for stimulation of 5-HT_{2C} receptors in this task.

Another cognitive symptom of SZ pertains to an inability to suppress a previous response when it becomes irrelevant and this deficiency has been attributed to reduced sensitivity to negative feedback (Leeson *et al*, 2009). This condition, perseveration, can be modeled by reversing the contingencies of a simple visual discrimination (Bussey *et al*, 2012). With this paradigm, we examined effects of chronically administering JJ-3-45 to mice given the NMDAR antagonist MK-801, as another model for cognitive impairment in SZ (van der Meulen *et al*, 2003). We empirically selected a dose of MK-801 that did not perturb visual discrimination performance but significantly retarded both the learning of a new contingency and the extinction of the prepotent response to the irrelevant stimulus. Here, mice treated with MK-801 made more errors after stimulus reversal and only by the end of 14 days of testing did they approach the accuracy of vehicle controls. Reversal learning depends upon the 5-HT system and Alsö *et al* (2015) found that systemic 5-HT_{2C} antagonism alleviated early (ie, occurring before achieving 50% correct responses) perseverative errors. Our results with the agonist JJ-3-45 agree with this finding where this compound potentiated the effects of MK-801-induced

perseveration as demonstrated by a higher proportion of errors from the beginning of reversal through the final sessions, compared with MK-801 treatment alone. Thus, selective 5-HT_{2C} agonists may have the potential to exacerbate this executive-function deficit in SZ.

5-HT_{2C} receptors are expressed on GABAergic, glutamatergic, and dopaminergic neurons (Bubar *et al*, 2011; McCorvy and Roth, 2015; Nocjar *et al*, 2015). 5-HT_{2C} agonists (eg, WAY 163909, CP-809101, and vabicaserin) have been proposed to offer potential antipsychotic efficacy without possessing the traditional side-effects associated with current APDs (Dunlop *et al*, 2006; Marquis *et al*, 2007; Siuciak *et al*, 2007; Aloyo *et al*, 2009; Jensen *et al*, 2010; Rosenzweig-Lipson *et al*, 2012; Liu *et al*, 2014; Cheng *et al*, 2015). These agonists can reduce basal firing rates and bursting activity of DA neurons and exert an excitatory effect on many GABAergic neurons in the VTA (Invernizzi *et al*, 2007; Di Giovanni *et al*, 2000), suggesting a possible mechanism of action for these agonists. In a microdialysis study with rats, 5-HT_{2C} agonists (ie, WAY 163909, Ro-61-0175, and MK 212) decreased DA in the medial prefrontal cortex and NAc, while increasing ACh efflux in the prefrontal cortex and hippocampus (Millan *et al*, 1998; Nair and Gudelsky, 2004; Marquis *et al*, 2007). We found that 10 mg/kg JJ-3-42 and 1 mg/kg lorcaserin reduced infralimbic DA efflux, while only JJ-3-42 successfully decreased it in striatum—suggesting that their mechanisms of actions may be different. Since the DA hypothesis of SZ postulates that extracellular DA levels are low in cortex and high in striatum (see Weinstein *et al*, 2016), it is possible that 5-HT_{2C} agonists may exacerbate some symptoms of SZ by further reducing cortical DA. Nevertheless, these findings support the hypothesis that 5-HT_{2C} receptors in the NAc and VTA negatively regulate DA efflux from dopaminergic neurons (O’Neil, 2010). Our findings suggest that JJ-3-42 may stimulate 5-HT_{2C} receptors in the NAc or VTA, and thereby inhibit dopaminergic neurons that project to the frontal cortex and striatum, respectively. Other APD-like mechanisms of 5-HT_{2C} agonists may include modification of signaling pathways and trafficking, as well as RNA-editing changes that have been associated with SZ (Rosenzweig-Lipson *et al*, 2012).

SZ is a complex neuropsychiatric disorder and responses to APDs are heterogeneous. Despite multiple actions at D₂ and other receptors, most APDs are efficacious in treating positive symptoms but are less robust for treating negative and cognitive symptoms (Leucht *et al*, 2009; Miyamoto *et al*, 2012). In the present studies, we demonstrate that the 5-HT_{2C} agonists—JJ-3-45, JJ-3-42, and JJ-5-34—are mostly efficacious in ameliorating a broad range of SZ-like behaviors in mice. Importantly, these compounds show efficacy in mice with persistent NMDAR hypofunctioning and in the hyperdopaminergic AMPH model of SZ. While we do not know whether 5-HT_{2C} agonists will be efficacious in treating patients, the ability of these ligands to modulate dopaminergic activities in the frontal cortex and striatum may provide a unique opportunity to develop drugs with fewer side effects, greater therapeutic selectivity, and enhanced efficacy for treating SZ and related disorders than current APDs.

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The authors declare no conflict of interest.

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