Phencyclidine decreases tickling-induced 50-kHz ultrasound vocalizations in juvenile rats: a putative model of the negative symptoms of schizophrenia?

Denis Boulay, Sophie Ho-Van, Olivier Bergis, Patrick Avenet and Guy Griebel

The objective of the present study was to examine the idea that the decrease in 50-kHz ultrasonic vocalizations elicited by tickling in juvenile rats following the administration of the psychotomimetic drug phencyclidine (PCP) may represent a valid model of the negative symptoms of schizophrenia. Fifty-kilohertz calls in rodents have been suggested to represent an archaic model of human laughter. Our results showed that daily tickling sessions produced a gradual increase in 50-kHz vocalizations, an effect that reached statistical significance from day 3. Administration of PCP (1 mg/kg, intraperitoneally) attenuated the 50-kHz calls induced by 4 consecutive days of tickling. The ability of several clinically effective or potential antipsychotics to reverse the effects of PCP was investigated. The 5-HT_{1A} receptor partial agonist, buspirone (0.3 and 1 mg/kg, intraperitoneally), the dual D_2/5-HT_{1A} receptor ligand, SSR181507 (0.5–0.75 mg/kg, intraperitoneally), but not the atypical antipsychotic, aripiprazole (0.1–1 mg/kg, intraperitoneally), the 5-HT_{2A} receptor antagonist, eplivanserin (0.3–3 mg/kg, intraperitoneally), and the $GlyT_1$ inhibitor, SSR103800 (0.3–3 mg/kg, intraperitoneally) significantly attenuated the effects of PCP on 50-kHz calls. Importantly, in animals not treated with PCP, none of the drugs affected 50-kHz calls elicited by a first handling–tickling session, indicating that the action of buspirone and SSR181507 cannot be explained by an intrinsic effect. To investigate further the specificity of these drug effects, we tested the anxiolytic and antidepressant agents, diazepam (0.1–1 mg/kg, intraperitoneally) and fluoxetine (1–10 mg/kg, intraperitoneally), respectively, in this procedure. Neither drug affected tickling-induced 50-kHz calls in naive or PCP-treated rats. In conclusion, the results of the present study confirm that 50-kHz calls elicited by several tickling sessions in rats can be reduced by acute administration of PCP, and that this effect can be reversed by previous administration of compounds with 5-HT_{1A} receptor agonist properties. As evidence for clinical efficacy of both agents on the negative symptoms of schizophrenia is weak or lacking, the current findings do not allow a definite conclusion to be drawn on the validity of this procedure as a model of this aspect of schizophrenia. ***Behavioural Pharmacology*** **24:543–551 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Sanofi Research & Development, Exploratory Unit, Chilly-Mazarin, France

Correspondence to Denis Boulay, PhD, Sanofi Research & Development, Exploratory Unit, 1 Avenue Pierre Brossolette, 91385 Chilly-Mazarin, France
E-mail: denis.boulay@sanofi.com

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Introduction

Schizophrenia is a severe mental disorder characterized by the presence of positive, cognitive, and negative symptoms. The latter cluster includes social dysfunction, poverty of speech, apathy, anhedonia, avolition, and reduced ability to recognize pleasant or negative emotions (Andreasen, 1990; Henry *et al.*, 2007). The loss of emotional expression, also called the 'blunted affect', is observed at the earliest stage of the illness and generally predicts a poor outcome (Dworkin and Cornblatt, 1995). Current typical and atypical antipsychotics are considered to be only weakly effective in alleviating the negative symptoms and may therefore contribute, through their side-effects, toward a poor societal and functional outcome (Aquila *et al.*, 1999; Bobes *et al.*, 2010). Hence, a compound that would be efficacious against the negative symptoms of schizophrenia would be of greater benefit to patients. Unfortunately, there are few models that have been claimed to relate to aspects of the negative symptoms of this condition (Ellenbroek and Cools, 2000). They generally focus on anhedonia, including measures of reward responsiveness (e.g. transcranial self-stimulation, progressive ratio, sucrose consumption, and social interaction (Markou, 2010).

Phencyclidine (PCP) and PCP-like compounds (such as ketamine or MK-801) are considered as the most relevant pharmacological interventions to produce schizophrenic-like symptoms in human volunteers (Javitt and Zukin, 1991; Krystal *et al.*, 1994). PCP-like compounds are known to interfere with the *N*-methyl-D-aspartate (NMDA) glutamate receptor subtype (Thomson *et al.*, 1985) and this mechanism of action, together with the effects of these compounds in humans (see above), have laid the foundations for the hypoglutamatergic hypothesis of schizophrenia (Javitt, 1987; Krystal *et al.*, 1994). In terms of negative symptoms, PCP-like compounds have been documented to induce a state of

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social withdrawal in humans (Javitt, 1987), primates (Schlemmer and Davis, 1983), and rats (Steinpreis *et al.*, 1994; Sams-Dodd, 1995a, 1995b; Boulay *et al.*, 2004), and produce poverty of speech, apathy, anhedonia, avolition, and blunted affect in healthy volunteers (Adler *et al.*, 1999; Covington *et al.*, 2007).

Ultrasonic vocalizations (USVs) in the 50 kHz range in rodents have been suggested to provide an archaic model of human laughter (rat laughter) (Panksepp and Burgdorf, 2003). These 50-kHz calls, which are observed in rodents during rough-and-tumble play behavior, mating, and in anticipation of rewarding events, have been suggested to relate to positive emotional expression, whereas 22-kHz calls, which are observed during social defeat, drug withdrawal, and in anticipation of aversive events, might reflect negative emotional expression (Knutson *et al.*, 1998; Panksepp and Burgdorf, 2000; Burgdorf *et al.*, 2005; Burgdorf and Panksepp, 2006). Rats also emit 50-kHz calls when tickled by a skilled experimenter in a playful way, and rates of 50-kHz calls were found to be positively correlated with the rewarding value of tickle stimulation (Panksepp and Burgdorf, 2000). Interestingly, in a protocol assessing 50-kHz USVs emitted by male rats during a 5-min period before the introduction of a female (Bialy *et al.*, 2000), MK-801 has been shown to decrease precontact vocalizations. Similarly, tickling-induced 50-kHz USVs were decreased by MK-801 (Panksepp and Burgdorf, 2000). Together, these findings suggest that 50-kHz calls may be used as a behavioral response modeling certain aspects of the blunted affect observed among the negative symptoms of schizophrenia. Previous studies have shown that an acute injection of PCP can produce a behavioral impairment reminiscent of aspects of the negative symptoms of schizophrenia (Boulay *et al.*, 2004; Bruins Slot *et al.*, 2005). Therefore, the objective of the present study was to determine whether the psychotomimetic drug PCP can reduce tickling-induced 50-kHz calls in juvenile rats, and to test whether clinically effective or potential antipsychotics can reduce this effect. The compounds tested included the atypical antipsychotic, aripiprazole, the 5-HT_{2A} receptor antagonist, eplivanserin, the $GlyT_1$ inhibitor, SSR103800, and the mixed 5-HT_{1A} agonist/D_2 antagonist, SSR181507. To verify the specificity of drug effects for antipsychotics, the anxiolytics, diazepam, and buspirone, and the prototypical antidepressant, fluoxetine, were tested under the same experimental conditions. Finally, the effects of each drug on 50 kHz induced by tickling in the absence of PCP were also investigated.

Methods

Subjects and housing

Experiments were conducted in male Sprague–Dawley rats (50–75 g and 3 weeks old at arrival from Charles River Laboratories, L'Arbresle, France) in accordance with the 'Guide and Care and Use of Laboratory Animals' (National Institute of Health) and were approved by the Sanofi-Aventis Animal Ethics Committee. Animals were housed four per cage ($45 \times 30 \times 20$ cm high) and kept in temperature-controlled (22°C) and humidity-controlled rooms (50%) with lights on from 07:00 to 19:00 h, with water and food freely available. The handling–tickling as described below started 1 day after the animals' arrival.

Tickling-induced 50-kHz ultrasonic vocalization procedure

Juvenile rats were removed from their home cage and placed in a new cage of the same size and with clean sawdust litter. There, they were handled and exposed to a 9% sucrose solution and food pellets for 5 min and then, they were placed back into their home cage. Ten minutes later, they were individually transferred in a new cage and exposed to 10 tickling trials, each lasting no more than 3 s, under a light intensity of 150 lux. Preliminary experiments from our laboratory have shown that juvenile rats vocalize rapidly and only when tickled. The experimenter noted the number of trials (out of 10) during which juvenile rats emitted 50-kHz vocalizations. At the end of the session, animals were placed back in the cage where they had been handled and exposed to sucrose, and returned to the animal holding room.

Each tickling consisted of initial finger movements across the back, focusing on the neck, followed by rapidly turning the rat over on the back with rapid alternating finger movements on the ventral surface as described by Panksepp and Burgdorf (2000). USVs were recorded using Mini-3 detectors (Ultra Sound Advice, London, UK) placed 15 cm above the cage and connected to a headphone. Tuning was set at 50 kHz on the Mini-3 detector, which allowed the recording of vocalizations emitted within the 48.5 to 51.5 kHz range, as the precision of the apparatus is given by the manufacturer as ± 1.5 kHz. It is important to note that pilot experiments using different tuning ranges (e.g. 20–22 kHz) showed that only 50-kHz calls were specifically emitted during the current tickling procedure.

Experiment 1: Effects of repeated daily tickling on 50-kHz ultrasonic vocalizations in handled juvenile rats

Subjects were exposed to one handling–tickling session, as described above, for 4 consecutive days. The experimenter noted the number of trials during which animals emitted 50-kHz vocalizations for each day.

Experiment 2: Effects of phencyclidine on tickling-induced 50 kHz ultrasonic vocalizations in handled juvenile rats

Animals were exposed to one handling–tickling session, as described above, for 4 consecutive days. Animals not achieving a score of 5 (out of 10 trials) following the 4 of training were not used for pharmacological studies.

One week later, they underwent another session of tickling before being randomized with respect to their basal scores and, 1 day later, received an intraperitoneal administration of saline or PCP (0.5 or 1 mg/kg) 30 min before the start of the tickling session. The numbers of trials during which animals emitted 50-kHz vocalizations during this session were noted.

Experiment 3: Effects of different pharmacological treatments on the phencyclidine-induced deficit in 50-kHz ultrasonic vocalizations following tickling in handled juvenile rats

Animals were exposed to one handling–tickling session as described above for 4 consecutive days. Animals not achieving a score of 5 (out of 10 trials) following the 4 days of training were not used for pharmacological studies. One week later, they underwent another tickling session during which 50-kHz vocalizations were scored. One day later, rats were assigned to one dose of treatment (vehicle, dose 1, dose 2, dose 3) per test session, as defined by a Latin square randomization method, to avoid sequence effects on the four test sessions (Fig. 1). The drugs or saline were administered 60 min and PCP at 1 mg/kg (i.e. the effective dose as found in experiment 2) 30 min before testing. Pharmacological test sessions were separated by 2 or 3 drug-free days. The experimenter was unaware of the test conditions.

Experiment 4: Effects of different pharmacological treatments on 50-kHz ultrasonic vocalizations following tickling in nonhandled juvenile rats

Unlike in previous experiments, juvenile rats were not exposed to any training session of handling–tickling. They underwent a single tickling session, 60 min prior to which they were administered the same drugs as in the previous experiment.

Drugs

PCP, SSR181507, SSR103800, aripiprazole, and fluoxetine were synthesized by Sanofi Medicinal Chemistry. Diazepam was obtained from Roche (Basel, Switzerland). Buspirone was purchased from Tocris (Illkirch, France). The drugs were dissolved or suspended in sterile saline with a few drops of Tween 80 and injected in a volume of 5 ml/kg. Doses were chosen on the basis of preliminary experiments from our laboratory and refer to the weight of the free base. Drug solutions were prepared fresh daily.

Data analysis

Data are expressed as the mean score of successful 50 kHz tickled-induced vocalizations out of 10 trials. All statistical analyses were carried out using the SAS software (SAS Institute Inc., Cary, North Carolina, USA). Data from experiments 2 and 4 were subjected to the Kruskal–Wallis multiple-comparisons test for the factor treatment, followed by a one-sided upper comparison with the vehicle group for that experiment. Data from experiments 1 and 3 were analyzed using a nonparametric repeated-measures Friedman test, followed when positive by a one-sided upper comparison versus the respective vehicle group. P values of less than 0.05 were considered to be statistically significant.

Results

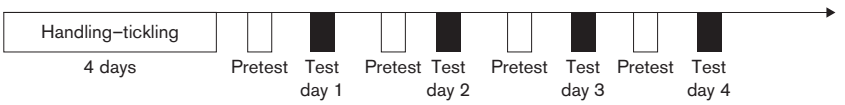
Experiment 1: Effects of repeated daily tickling on 50-kHz ultrasonic vocalizations in handled juvenile rats

Statistical analysis showed a steady increase in 50-kHz USVs over days [$F(3,45) = 32.15$, $P < 0.01$] from three calls on day 1 to 9–10 calls on day 4. Comparison of the scores observed in individual sessions with day 1 showed a significant handling–tickling effect from the third session [$F(1,15) = 3.8$, $P < 0.01$] and a maximal effect on the fourth session [$F(1,15) = 4.8$, $P < 0.01$] (Fig. 2).

Experiment 2: Effects of phencyclidine on tickling-induced 50-kHz ultrasonic vocalizations in handled juvenile rats

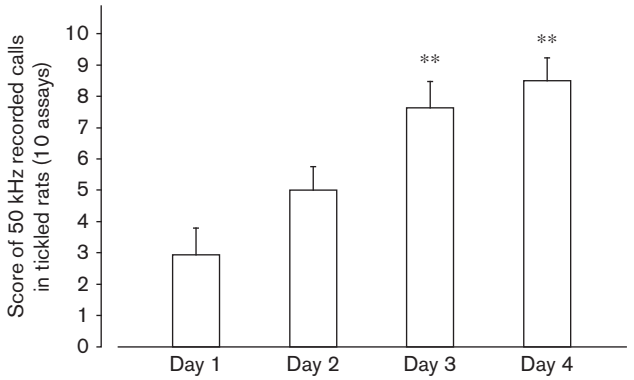
Administration of PCP dose-dependently decreased tickling-induced 50-kHz calls [$\chi^2(2) = 7.4$, $P = 0.02$]. One-sided upper comparisons with the vehicle group showed that the active dose of PCP was 1 mg/kg, intraperitoneally [$F(1,16) = 2.4$, $P < 0.01$]. Saline-treated rats showed comparable performance between the pretest and the test session, respectively, 9.2 ± 0.8 and 10 ± 0.0 (Paired Wilcoxon's test $= -0.5$, $P = 1$), indicating that the injection procedure had no significant influence on tickling-induced 50-kHz calls. The basal performances across groups in the pretest session did not differ significantly: 10 ± 0.0, 10 ± 0.0; 9.9 ± 0.1 for vehicle, PCP 0.5 and 1 mg/kg, respectively [$\chi^2(2) = 1.6$, $P = 0.6$] (Fig. 3).

Fig. 1



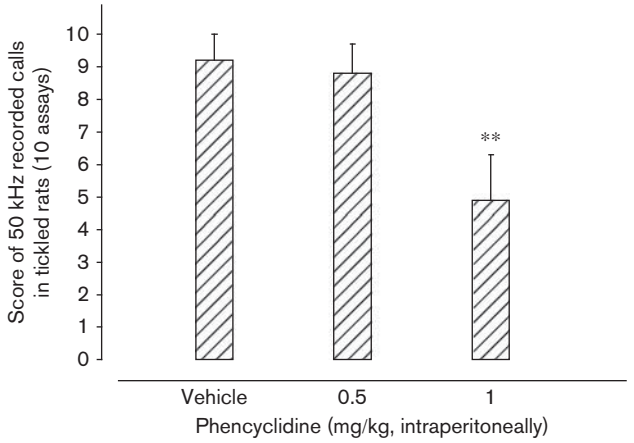
Representation of the experimental design used for experiments investigating the effect of drugs on tickling-induced 50-kHz calls in handled juvenile rats treated with phencyclidine. In the first week, animals were exposed to one handling–tickling session for 4 consecutive days. In the second week, animals were exposed to a pretest tickling session. One day later, rats were assigned to one dose of treatment (vehicle, dose 1, dose 2, dose 3) per test session, as defined by a Latin square randomized design.

Fig. 2



Effect of daily handling and tickling on tickling-induced 50-kHz calls in juvenile rats. Each column represents the mean (±SEM) score of the number of tickled-induced 50-kHz calls over 10 trials observed in each daily test session (one test per day over 4 days of handling–tickling); $n=16$ per group. Statistical analyses used the Friedman test, followed by multiple comparisons test. $^{**}P<0.01$ as compared with day 1.

Fig. 3



Effect of phencyclidine on tickling-induced 50-kHz calls in handled juvenile rats. Each column represents the mean (±SEM) score of the number of tickling-induced 50-kHz calls over 10 trials observed on the test session 30 min following the intraperitoneal administration of phencyclidine (1 mg/kg) or vehicle; $n=6$–9 per group. Statistical analyses used the Kruskal–Wallis test, followed by one-sided upper comparisons with the vehicle group. $^{**}P<0.01$ as compared with the vehicle group.

Experiment 3: Effects of different pharmacological treatments on PCP-induced deficit in 50-kHz ultrasonic vocalizations following tickling in handled juvenile rats

Buspirone and SSR181507 dose-dependently increased 50-kHz vocalizations in PCP-treated rats [buspirone: $F(3,45)=12.85$, $P<0.01$; SSR181507: $F(3,45)=10.77$, $P<0.05$] (Figs 3 and 4). Two-sided comparison versus vehicle groups showed that these effects reached statistical significance for both drugs at the two highest doses [buspirone at 0.3 and 1 mg/kg: $F(1,14)=2.4$, $P<0.05$ and 2.9, $P<0.01$, respectively; SSR181507 at 0.5 and 0.75 mg/kg: $F(1,14)=2.3$, $P<0.05$ for each dose]. Pretest session performance in all groups of rats in the buspirone experiment was identical (i.e. 10 ± 0.0) and those in the SSR181507 experiment were not significantly different [$F(3,45)=0.95$, $P=0.8$].

The administration of aripiprazole (0.1–1 mg/kg), eplivanserin (0.3–3 mg/kg), SSR103800 (0.3–3 mg/kg), diazepam (0.1–1 mg/kg), and fluoxetine (1–10 mg/kg) did not significantly modify the effects of PCP on 50-kHz USVs induced by tickling [aripiprazole: $F(3,69)=0.45$, $P=0.92$; eplivanserin: $F(3,36)=1.01$, $P=0.79$; SSR103800: $F(3,45)=4.06$, $P=0.25$; diazepam: $F(3,21)=1.79$, $P=0.61$; fluoxetine: $F(3,46)=1.31$, $P=0.72$] (Table 1).

It is important to note that pretest session performances in all groups of rats in these experiments were not significantly different: aripiprazole: $F(3,69)=0.98$, $P=0.8$; eplivanserin: $F(3,36)=2.1$, $P=0.55$; SSR103800: $F(3,45)=1.32$, $P=0.72$; diazepam: $F(3,21)=6.06$, $P=0.07$; except for fluoxetine, where the Latin square randomization showed a global preassigned treatment group effect on the basal level of vocalization [$F(3,36)=17.23$, $P<0.01$]. The basal score levels observed in the pretest session for the vehicle and fluoxetine, 1, 3, and 10 mg/kg groups, were, respectively, 9.9 ± 0.1, 9.9 ± 0.1; 9.1 ± 0.3; and 8.6 ± 0.5. Nevertheless, further statistical individual comparisons with the vehicle/PCP assigned treated group did not show any significant difference ($P=1$; 0.1; and 0.14, respectively).

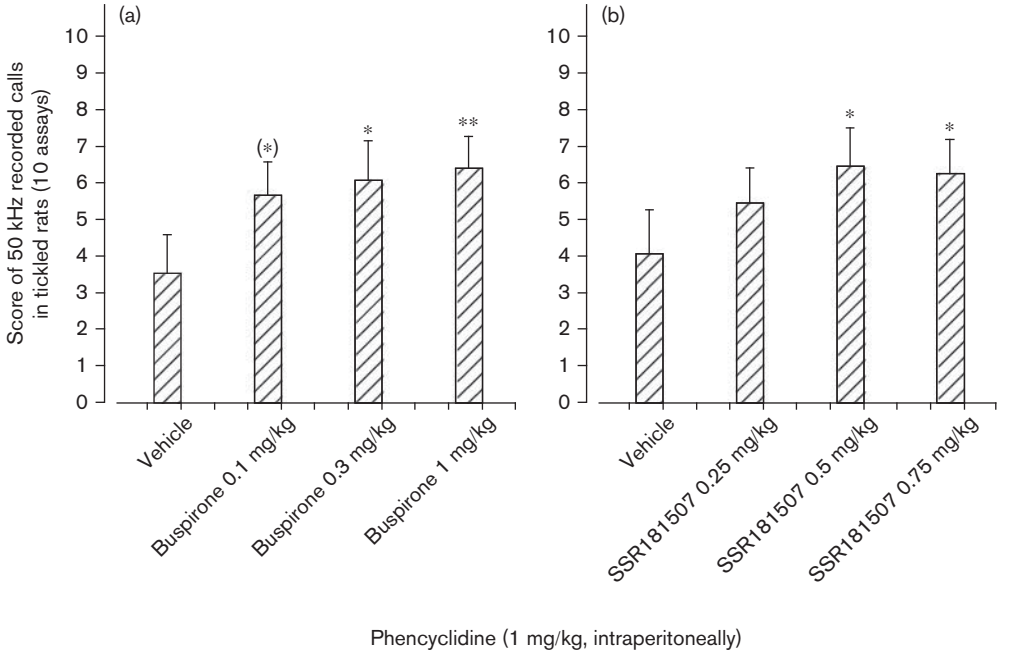
Experiment 4: Effects of different pharmacological treatments on 50-kHz ultrasonic vocalizations following tickling in nonhandled juvenile rats

When tested in the absence of PCP in nonhandled animals, none of the drugs significantly modified 50-kHz USVs induced by tickling [experiment with diazepam, buspirone, and SSR181507 (Table 2): $\chi^2(3)=5.3$, $P=0.15$; experiment with aripiprazole, eplivanserin, and SSR103800 (Table 2): $\chi^2(3)=1.84$, $P=0.6$; experiment with fluoxetine (Table 2): $\chi^2(3)=0.58$, $P=0.9$].

Discussion

The main objective of this study was to investigate the possible deleterious effects of PCP on tickling-induced 50-kHz calls in handled juvenile rats and to validate this procedure as a model of certain aspects of the negative symptoms of schizophrenia. Our results showed that PCP impaired tickling-induced 50-kHz vocalizations and that acute administration of the 5-HT_{1A} receptor agonist, buspirone, and the mixed 5-HT_{1A} agonist/D_2 antagonist, SSR181507, attenuated the effects of this psychotomimetic. In contrast, the atypical antipsychotic, aripiprazole, the 5-HT_{2A} receptor antagonist, eplivanserin, the GlyT1 inhibitor, SSR103800, the anxiolytic, diazepam, and the antidepressant, fluoxetine, did not modify the effects of PCP on 50-kHz calls.

Fig. 4



Effect of buspirone (a) or SSR181507 (b) on tickling-induced 50-kHz calls in handled juvenile rats treated with phencyclidine. Each column represents the mean (±SEM) score of the number of tickle-induced 50 kHz calls over 10 trials observed during the test session 30 min following the intraperitoneal administration of phencyclidine (1 mg/kg) or vehicle and 60 min following oral administration of drug or vehicle (several test sessions randomized as a Latin square design with repeated measures); $n=15$ per group. Statistical analyses used the Friedman test, followed by two-sided comparison vs. the vehicle group. (*)$P=0.06$, *$P<0.05$, **$P<0.01$ as compared with the vehicle group.

Table 1 Effect of aripiprazole, eplivanserin, SSR103800, diazepam, or fluoxetine on tickling-induced 50-kHz calls in handled juvenile rats treated with phencyclidine

	Mean	SEM	*N*
Vehicle	2.4	0.7	15
Aripiprazole 0.1 mg/kg	2.5	0.6	15
Aripiprazole 0.3 mg/kg	3.0	0.7	15
Aripiprazole 1 mg/kg	2.7	0.6	15
Vehicle	2.3	0.8	13
Eplivanserin 0.3 mg/kg	2.7	1	13
Eplivanserin 1 mg/kg	2.7	0.7	13
Eplivanserin 3 mg/kg	2.8	0.9	13
Vehicle	3.45	1.02	16
SSR103800 0.3 mg/kg	3.45	1.06	16
SSR103800 1 mg/kg	4.18	1.09	16
SSR103800 3 mg/kg	5.55	1.15	16
Vehicle	3.5	1.3	8
Diazepam 0.1 mg/kg	2.9	1.2	8
Diazepam 0.3 mg/kg	3.4	1.3	8
Diazepam 1 mg/kg	2.6	1.3	8
Vehicle	2.38	0.76	8
Fluoxetine 1 mg/kg	2.23	0.84	8
Fluoxetine 3 mg/kg	1.15	0.52	8
Fluoxetine 10 mg/kg	2.46	0.81	8

Each value represents the mean (±SEM) scores of the number of tickling-induced 50-kHz calls over 10 trials observed during the test session 30 min following the intraperitoneal administration of phencyclidine (1 mg/kg) and 60 min following oral administration of drug or vehicle (several test sessions randomized as a Latin square design with repeated measures).
N, number of animals per group.
Statistical analyses used the Friedman test, followed by two-sided comparison vs. the vehicle group.

Table 2 Effect of buspirone, SSR181507, diazepam, aripiprazole, eplivanserin, SSR103800, or fluoxetine on tickling-induced 50-kHz calls in naive juvenile rats

	Mean	SEM	*N*
Vehicle	4.25	1.28	8
Buspirone 1 mg/kg	3.88	1.33	8
SSR181507 0.3 mg/kg	4.75	1.39	8
Diazepam 1 mg/kg	1.13	0.72	8
Vehicle	6.10	1.29	10
Aripiprazole 1 mg/kg	4.90	1.20	10
Eplivanserin 3 mg/kg	5.30	1.08	10
SSR103800 3 mg/kg	4.20	1.35	10
Vehicle	5.00	1.09	10
Fluoxetine 1 mg/kg	5.60	1.08	10
Fluoxetine 3 mg/kg	4.30	1.26	10
Fluoxetine 10 mg/kg	5.20	1.14	10

Each value represents the mean (±SEM) scores of the number of tickling-induced 50 kHz calls over 10 assays observed on a single test session 60 min following the oral administration of drug or vehicle.
N, number of animals per group.
Statistical analyses used the Kruskal–Wallis test, followed by multiple comparisons test vs. the vehicle group.

50-kHz calls following tickling in handled juvenile rats

A gradual increase in 50-kHz calls was observed when juvenile rats were handled and tickled daily. This result resembles that observed by Mallo *et al.* (2007), and Burgdorf and Panksepp (2001), using a slightly

different procedure. These authors measured 50-kHz calls over a 2-min period following four 15-s sessions of tickling, respectively, in Wistar and Long–Evans hooded rats. They showed that daily tickling sessions produced a progressive increase in 50-kHz calls. It is noteworthy that although isolated animals have been shown to be far more playful than socially housed animals (Panksepp and Beatty, 1980), showing higher rates of 50-kHz calls (Burgdorf *et al.*, 2001), the current experimental condition using socially housed rats was not an obstacle to producing a maximal response to tickling stimulations.

Effect of PCP on tickling-induced 50-kHz calls in handled juvenile rats

The observation that the administration of PCP dose-dependently reduced 50 kHz induced by tickling is consistent with the findings of Panksepp and Burgdorf (2000), who showed that MK-801 could similarly decrease these calls. We tentatively hypothesize that the impaired 50-kHz vocalizations in juvenile rats following NMDA receptor antagonism could relate to aspects of flat affect or impaired emotional sensitivity or expression observed in schizophrenic patients.

Experiments using the administration of amphetamine have shown that 50-kHz calls are linked to the activation of the mesolimbic dopamine system (Wintink and Brudzynski, 2001). Thompson *et al.* (2006) have observed that the enhanced dopamine release induced by the local administration of amphetamine into the shell of the nucleus accumbens (NAcc) was correlated with the production of 50-kHz calls in adult rats, indicating an appetitive state and a positive affect. Although never directly tested, it can be speculated that repeated tickling produces an increase in dopamine levels in the NAcc of juvenile rats. It is noteworthy that PCP has been reported to increase dopamine release in the NAcc of rodents following peripheral administration (Carboni *et al.*, 1989; Steinpreis and Salamone, 1993) or local application on NAcc slices (Hernandez *et al.*, 1988; Ault and Werling, 1999). However, other groups have reported that PCP inhibits NMDA-stimulated release of ACh and DA on NAcc slices (Jones *et al.*, 1987a, 1987b). Negative emotional affect may not only result from a decreased dopaminergic activity in the NAcc as other limbic structures, such as the prefrontal cortex, and other neurotransmitters, such as glutamate and serotonin, may play an additional important role in such an emotional expression.

USVs in rats have been shown to involve respiratory, phonatory/laryngeal, and possibly articulatory communication subsystems. Therefore, it cannot be excluded that the effects of PCP on 50-kHz calls may result from muscular side-effects. However, the observation that 50-kHz calls elicited by tickling are insensitive to drugs such as morphine or naloxone (Panksepp and Burgdorf, 2000) tends to suggest that the effects of PCP on 50-kHz calls were more emotion oriented and have not been contaminated by motor depressant and/or analgesic effects. Moreover, the findings that the anxiolytic and antidepressant drugs, diazepam and fluoxetine, did not modify 50-kHz calls induced by tickling, both in the presence and in the absence of PCP, suggests that this behavior is unrelated to aspects of anxiety or depression.

The atypical antipsychotic aripiprazole is inactive on PCP-induced attenuation of 50-kHz calls

A second important objective of this study was to investigate the predictive validity of the current procedure as a model of the negative symptoms of schizophrenia. We first tested the effect of aripiprazole, a compound known to be clinically active against the negative symptoms of schizophrenia (Kane *et al.*, 2002; Potkin *et al.*, 2003). Aripiprazole is an atypical antipsychotic with dopamine D_2, D_3, serotonin 5-HT_{1A}, and 5-HT_{2A} receptor partial agonist properties (Shapiro *et al.*, 2003). Our results showed that aripiprazole did not block the effects of PCP on 50-kHz calls, questioning the idea that this model may relate to aspects of the negative symptoms of schizophrenia.

The 5-HT_{1A} receptor agonists SSR181507 and buspirone blocked the effects of phencyclidine on 50-kHz calls

The administration of the 5-HT_{1A} receptor partial agonist, buspirone, and the mixed 5-HT_{1A} receptor agonist and full antagonist at the human dopamine D_2L receptor, SSR181507, which has been reported to show antipsychotic-like activity in rodents (Claustre *et al.*, 2003; Depoortere *et al.*, 2003; Terranova *et al.*, 2005) resulted in an attenuation of the effects of PCP on tickling-induced 50-kHz calls in handled juvenile rats. Although, as indicated above, it is not clear whether the current model is relevant for the negative symptoms of schizophrenia, the results obtained with SSR181507 in this study are compatible with earlier data showing that the drug was active against the deleterious effect of PCP in a rat model of poor social functioning, which is part of the cluster of the negative symptoms of schizophrenia (Boulay *et al.*, 2004). It is important to note that neither buspirone nor SSR181507 modified tickling-induced 50-kHz vocalizations in juvenile rats in the absence of PCP, albeit the dose of SSR181507 (0.3 mg/kg) may have been too low, considering that the minimal active dose of the drug in the PCP experiment was 0.5 mg/kg. This finding is in agreement with that of Hamed *et al.* (2009), who reported that buspirone (up to 3 mg/kg) did not affect isolation-induced appetitive 50-kHz vocalizations. Clinical data with 5-HT_{1A} receptor agonists on the negative symptoms of schizophrenia are sparse. One study (Ghaleiha *et al.*, 2010) showed recently in a double-blind, randomized, and placebo-controlled trial that buspirone added to risperidone in patients with chronic schizophrenia was active in negative symptoms.

The role of the 5-HT_{1A} receptor in some effects of PCP has been described in earlier studies (Boulay *et al.*, 2004; Bruins Slot *et al.*, 2005; Snigdha and Neill, 2008; McLean *et al.*, 2009; Nagai *et al.*, 2009). For example, 8-OH-DPAT, a selective 5-HT_{1A} receptor full agonist (Boulay *et al.*, 2004), and buspirone (Bruins Slot *et al.*, 2005) have been shown to reverse the deficit in social interaction induced by PCP in rats. Together, these studies suggest a close interaction between 5-HT_{1A} receptors and the primary target of PCP, namely, the glutamate-NMDA receptor. Postsynaptic 5-HT_{1A} receptors are found in the prefrontal cortex, and more specifically in the dendritic spines in which glutamate receptors are concentrated (Kia *et al.*, 1996). The activation of 5-HT_{1A} receptors has been shown to reduce currents through the NMDA-type glutamate receptor channels, indicating that the NMDA receptor is one of the key targets of 5-HT_{1A} receptor activation in glutamate neurons (Yuen *et al.*, 2005). It has been hypothesized that the 5-HT_{1A}/NMDA receptor-channel interaction in limbic areas could play a significant role in the modulation of cognitive and emotional processes (Yuen *et al.*, 2005). The current results on the effects of 5-HT_{1A} receptor agonists against PCP reinforce the hypothesis of an interaction between the 5-HT_{1A} and NMDA receptors.

The 5-HT_{2A} receptor antagonist eplivanserin was inactive against phencyclidine-induced 50-kHz call deficits

Eplivanserin, administered over a wide dose range, did not attenuate the effects of PCP, nor did it modify tickling-induced 50-kHz vocalization in naive rats. Alteration of the 5-HT system has been proposed as an additional hypothesis for schizophrenia (Meltzer, 1989). This is based notably on the observation that second-generation antipsychotics have shown therapeutic activity on the positive, cognitive, and negative symptoms of schizophrenia. Their better effects (Lieberman, 2007) over first-generation antipsychotics have been attributed in part to their antagonist activity at the 5-HT_{2A} receptor. However, selective blockade of the 5-HT_{2A} receptor is not sufficient to produce antipsychotic activity, as shown by several clinical trials in schizophrenic patients using eplivanserin and volinanserin, another 5-HT_{2A} receptor antagonist, which showed, respectively, weak and no activity of these compounds (Kehne *et al.*, 1996; Meltzer *et al.*, 2004). Although eplivanserin was inactive in the current study against PCP, it is noteworthy that previous studies showed that the drug is able to block some of the behavioral effects of NMDA receptor blockade (Boulay *et al.*, 2011). For instance, eplivanserin blocked the hyperactivity induced by the non-competitive NMDA receptor antagonist, MK-801, and partially reversed spontaneous hyperactivity of NMDA $Nr1^{neo-/-}$ mice (Boulay *et al.*, 2011).

The $GlyT_1$ inhibitor SSR103800 was inactive against phencyclidine-induced 50-kHz call deficits

Compounds such as D-cycloserine, glycine, alanine, and D-serine, which putatively increase NMDA receptor sensitivity to glutamate, have been shown to provide an additional benefit when associated with antipsychotics on negative symptomatology and cognitive dysfunction (Evins *et al.*, 2002; Tsai *et al.*, 2006). Recently, it was reported that the $GlyT_1$ inhibitor, RG1678, could improve negative symptoms in schizophrenic patients when administered in combination with risperidone (Umbricht *et al.*, 2010). In the current study, the selective GlyT1 inhibitor SSR103800 alone did not attenuate the effects of PCP, nor did it modify tickling-induced 50-kHz vocalization in naive rats. The drug has been reported to show antipsychotic-like effects in rodents, in models related to positive, cognitive ,and negative symptoms of schizophrenia (Boulay *et al.*, 2008, 2010; Black *et al.*, 2009). In particular, Black *et al.* (2009) reported that SSR103800 increased latent inhibition (LI) in untreated controls, while reversing amphetamine-disrupted LI as well as abnormally persistent LI induced by MK-801, and suggested that this compound may have the potential to alleviate the negative symptoms of schizophrenia. The current findings with SSR103800 do not support such an assumption, or alternatively, further strengthen the above suggestion that the PCP-induced deficit in the 50-kHz call model does not relate to aspects of the negative symptoms of schizophrenia.

Methodological considerations

There are several methodological considerations with this study. Although the currently used heterodyne bat detector system has been used extensively in previous studies to record 50-kHz calls in rodents (Knutson *et al.*, 1998; Panksepp and Burgdorf, 2000; Burgdorf *et al.*, 2005; Burgdorf and Panksepp, 2006), it is important to emphasize that it is not free from pitfalls. Fifty-kilohertz USVs occur within a frequency range of 30–100 kHz. At least three different types of 50-kHz USVs have been described, namely trill, flat, and step, of which only flat 50-kHz USVs have a frequency of about 50 kHz (Burgdorf *et al.*, 2008). The most prominent type during tickling is the trill, with a frequency of about 70 kHz (Burgdorf *et al.*, 2008), whereas the results obtained for the flat type are inconsistent (Burgdorf *et al.*, 2011). The bat detector used in the present study detects only vocalizations emitted within the 48.5 and 51.5 kHz range, indicating that not all types of 50-kHz calls have been recorded. Moreover, heterodyne recording does not completely distinguish artifacts or other USVs call types from 50-kHz ultrasonic calls. For example, 20-kHz USVs are much louder than 50-kHz USVs and the harmonics from these calls can be detected from a heterodyne channel tuned to 50 kHz. Another concern may have been the exposure of juvenile rats to sucrose and food pellets before the trials to obtain consistent levels of USV, as shown by pilot

experiments. It cannot be excluded that this may have been a confounding factor. To overcome these limitations, it will be necessary in future studies to use high-frequency recordings and sonographic scoring. Finally, our findings from the rescue experiments could have been strengthened by the addition of a vehicle + vehicle control group to determine more precisely to what extent the active drugs could normalize the 50 kHz deficit.

Conclusion

The findings of the present study suggest that PCP-induced deficits in 50-kHz calls may relate to aspects of blunted affect, but at this stage, it is not clear to what extent, if any, this model has predictive validity for all the negative symptoms of schizophrenia, as two compounds that are clinically active against these symptoms (i.e. aripiprazole and buspirone) showed a different profile. Future experiments with this model should notably test the effects of a combination of classical, atypical, and/or potential (e.g. GlyT1 inhibitors) antipsychotics.

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Conflicts of interest

All authors are employees of Sanofi Research & Development.

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