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Disruption of social cognition in the sub-chronic PCP rat model of schizophrenia: possible involvement of the endocannabinoid system

Alexandre Seillier and Andrea Giuffrida

Department of Pharmacology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78229-3900, USA

Abstract

Previous studies have shown that social withdrawal in the phencyclidine (PCP) rat model of schizophrenia results from deficient endocannabinoid-induced activation of CB₁ receptors. To understand the underlying cognitive mechanisms of the social deficit in PCP-treated rats, we examined the impact of pharmacological manipulation of the endocannabinoid system on sociability (i.e. social approach) and social novelty preference (which relies on social recognition).

Control rats showed a clear preference for a “social” cage (i.e. unfamiliar stimulus rat placed under a wire mesh cage) versus an “empty” cage, and spent more time exploring a “novel” cage (i.e. new stimulus rat) versus a “familiar” cage. In contrast, rats receiving PCP (5 mg/kg, b.i.d. for 7 days, followed by a 7 day-washout period) showed intact sociability, but lacked social novelty preference. This PCP-induced deficit was due to increased activity at CB₁ receptors as it was reversed by systemic administration of the CB₁ antagonist AM251 (1 mg/kg). In agreement with this hypothesis, the cannabinoid agonist CP55,940 (0.003 – 0.03 mg/kg) dose-dependently suppressed social novelty preference in control animals without affecting sociability.

Taken together, these data suggest that PCP-treated rats have a deficit in social cognition, possibly induced by increased stimulation of CB₁ receptors. This deficit, however, is distinct from the social withdrawal previously observed in these animals, as the latter is due to deficient, rather than increased, CB₁ stimulation.

Keywords

endocannabinoid; odor discrimination; phencyclidine; social cognition

Corresponding author: Alexandre Seillier, Department of Pharmacology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78229-3900, USA, Tel.: +1 210 567 4211 - Fax: +1 210 567 4303, alex.seillier@free.fr.

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Contributors

Alexandre Seillier designed the study, performed the experiments and the statistical analysis and wrote the manuscript. Andrea Giuffrida supervised the project and reviewed the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

1. Introduction

Social withdrawal, a hallmark of several psychiatric disorders, is a behavioral construct that can be assessed in humans and animals (Wilson and Koenig, 2014). Chronic administration of the N-methyl-D-aspartate (NMDA) glutamate receptor antagonist phencyclidine (PCP) in rodents has been widely used to model schizophrenia (Enomoto et al. 2007) and negative symptoms, as it mimics the complex clinical and pathological features of the disease (Jentsch and Roth, 1999). In particular, PCP-treated rats represent the best pharmacological model of social withdrawal (for schizophrenia) in term of construct, face and predictive validity (Gururajan et al. 2010). Nevertheless, the ability of PCP to model schizophrenia varies greatly depending on the treatment regimen, and only withdrawal from repeated PCP administration, rather than acute exposure, produces behavioral, neurochemical and brain circuit alterations matching those observed in schizophrenia (Dawson et al. 2014b; Jentsch and Roth, 1999). Moreover, NMDA antagonists (e.g. PCP, MK801 and ketamine) cannot be used interchangeably when modeling schizophrenia (Dawson et al. 2014a), as only PCP has been shown to produce behavioral deficits reminiscent of positive/negative symptoms, and the characteristic cognitive deficits observed in the disease (Seillier and Giuffrida, 2009).

Social dysfunction plays a central role in schizophrenia (Billeke and Aboitiz, 2013), and negative symptoms seem to intersect with alterations in social cognition and emotional processing in particular (Ochsner, 2008). Indeed, social withdrawal is mainly associated with asociality, a domain of negative symptoms that results from indifference or lack of desire to have social contacts, as well as with impaired social cognition - a domain of cognitive deficits referring to the mental operations underlying social interactions (e.g., ability and capacity to perceive the intentions and dispositions of others) (Hoernagl and Hofer, 2014). Thus, a comprehensive understanding of social withdrawal requires the study of the altered psychological processes underlying this behavioral deficit.

This issue may be addressed by employing multiple testing paradigms capable of capturing several aspects of social behavior (e.g. social approach, social cognition, etc.) (O'Tuathaigh et al. 2010b). Social behavior in rodent models of schizophrenia is typically assessed by measuring social interactions between two freely moving animals (Wilson and Koenig, 2014). Using this paradigm, we recently demonstrated that PCP-induced social withdrawal results from lack of cannabinoid CB₁ receptor stimulation (Seillier et al. 2013). However, to quote Millan and Bales (Millan and Bales, 2013), a “challenge with this basic version of the task is that both animals are freely moving, making it difficult to disentangle factors such as social motivation, emotional status, attention and motor drive, etc. Further, and of particular importance for translational research in schizophrenia, the unmodified procedure does not specifically address the issue of social cognition”. Therefore, in line with the CNTRICS initiative for improving animal models of cognitive impairments in schizophrenia (Millan and Bales, 2013), we developed a behavioral test adapted from “the mice three-chamber social approach task” (Nadler et al. 2004) that allows the evaluation of two fundamental aspects of social behavior: sociability and social novelty preference. Sociability - defined as the motivation to spend more time with a social stimulus - is assessed by comparing the time spent by an animal interacting with a conspecific vs. a non-social object. Social novelty preference - defined as the propensity to spend more time with an unfamiliar animal - relies

on the ability to remember a conspecific and discriminate between familiar vs. novel individuals (social recognition).

In this study, we aimed to: 1) explore the cognitive processes underlying social withdrawal in the sub-chronic PCP rat model of schizophrenia, and 2) assess whether these putatively altered processes shared the same pharmacology observed in PCP-induced social withdrawal as assessed by the classical dyadic social interaction test (Seillier et al. 2013). In contrast to the experimental protocol used for the three-chamber task, our rats were tested in an open arena virtually divided into quadrants. Nevertheless, as in the case of the original paradigm, the “stimulus” rat was placed under a wire mesh cage (unable to move freely), allowing the experimental rat to initiate and terminate any interaction (free choice).

2. Experimental procedures

2.1. Animals

One hundred and eight male Wistar rats (200–225 g; Charles River Laboratories, Wilmington, MA) were housed in pairs at 22 ± 1 °C, under a 12 h light-dark cycle with food and water available ad libitum. All animals were habituated to the housing conditions for one week prior to the behavioral tests. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

2.2. Social approach and social novelty preference task

This task was directly adapted from the three-chamber social approach task developed by Crawley and co-workers (Nadler et al. 2004), using an open arena (100 cm × 100 cm × 40 cm) made of black acrylic. The arena was located in a dimly lit room (5 lux at the arena center), and was virtually divided into quadrants (Fig. 1A). A holding cage adjacent to the arena was used to host the animals during the inter-trial intervals (2 min). During the procedure, a blinded experimenter stayed motionless next to the corner in which the experimental rat was introduced in the arena, and manually scored the time spent by the test rat exploring the “mesh cages” (see below). The procedure, which was videotaped for further analysis, consisted of three phases:

Habituation—The experimental rat was placed in one corner of the empty arena and free exploration was allowed for 30 min. The first 5 min of open field behavior were examined by an experimenter blind to the study by off-line analysis of the videotape (a 7 × 7 grid image was superimposed on the monitor; fig. 1A left panel). The primary measures were the number of entries (E), time spent (T), number of rears (R) and number of squares crossed (S) in the center (3×3 zone; E_{ce} , T_{ce} , R_{ce} and S_{ce} , respectively) or in the periphery (rest of the arena; E_{pe} , T_{pe} , R_{pe} and S_{pe} , respectively).

Sociability—A wire mesh cage (white epoxy-coated aluminum round basket; 25.5 diameter, 22.9 cm H), designated as “empty” cage, was placed at one corner of the arena (6 cm from the walls), and an unfamiliar “stimulus” rat was placed under a second identical

wire mesh cage, designated as “social” cage, at the opposite corner (Fig. 1A center panel). A vinyl coated lead O-shaped blue ring (970 g) was placed on the top of each cage to prevent any displacement. After a 2 min inter-trial interval, the experimental rat was placed back in the arena and allowed to explore it for 10 min. In addition to the time spent exploring the mesh cages, data were recorded in five-minute time bins scoring the number of entries and the time spent in the virtual quadrants (Fig. 1A center panel). Preliminary experiments were performed to assess any possible influence of the relative positions of the mesh cages (right or left sides of the arena relative to the experimenter). No side preference nor interaction between the position (right or left) and the type of the stimulus (“social” or “empty”) were observed (data not shown).

Social novelty—A new “stimulus” rat was placed under the previously empty wire mesh cage, now designated as “novel” cage, whereas the unfamiliar “stimulus” rat (now familiar) remained under the same cage, now designated as “familiar” cage (Fig. 1A right panel). After the 2 min inter-trial interval, the experimental animal was reintroduced in the arena for 10 min and data were collected and scored as previously described.

The “stimulus” rats were albino Wistar males of the same age that had no previous contact with the experimental rats. They were all acclimated to the apparatus and habituated to the cages for 10-min periods over four days prior to the experiment. After each experimental rat, the wire mesh cages, lead O-shaped rings and arena were wiped clean with 10% alcohol.

2.3. Social odor discrimination task

This task was conducted after the social approach and social novelty preference task (same day / same rats) in the same arena and in the same room (few hours after the former task). A holding cage adjacent to the arena was used to host the animals during the inter-trial intervals (2 min). The procedure, which was videotaped for later analysis, consisted of two phases (Fig. 1B):

Habituation—The experimental rat was placed in the center of the empty arena and free exploration was allowed for 5 min. The open field behavior was quantified by an experimenter blind to the study as described above.

Social odor discrimination. Four Petri dishes (diameter 9 cm) were taped on the bottom of each corner of the arena (10.5 cm from the walls). They contained either fresh bedding (designated as “neutral”), used bedding from the experimental rat’s home cage (“home”), used bedding from a different cage (“other”) or no bedding (“empty”) (Fig. 1B right panel). After a 2 min inter-trial interval, the experimental rat was placed back in the arena and allowed to explore it for 10 min. In addition to the time spent exploring the Petri dishes, the number of entries and the time spent in the virtual quadrants (Fig. 1B right panel) were scored as reported above.

2.4. Drugs and drug treatments

Animals were treated sub-chronically (twice a day for 7 days) with either saline (1 ml/kg, i.p.) or PCP (5 mg/kg, i.p.; Sigma/RBI, USA) and tested 7 days after the last drug injection.

On the test day, rats received an acute injection of one of the following drugs 10 min before being placed into the unfamiliar arena. The cannabinoid agonist CP55,940 (0.003, 0.01, and 0.03 mg/kg, i.p.; Tocris; $IC_{50} = 0.2$ nM and $K_i = 0.58$ nM for CB_1) was dissolved in Tween-80:polyethylene glycol:physiological saline (0.9%; 5:5:90, respectively) and the CB_1 antagonist AM251 (1 mg/kg, i.p.; Cayman Chemical; $IC_{50} = 8$ nM and $K_i = 7.49$ nM for CB_1) in Tween-80:polyethylene glycol:physiological saline (0.9%; 10:10:80, respectively). Doses and time of injection were chosen based on previous in vivo studies (Genn et al., 2004; Marco et al. 2004; Seillier et al., 2010; 2013).

2.5. Statistical Analysis

For the habituation phase, in addition to the primary open field measures aforementioned, the percent of center squares crossed [$\%S = S_{ce} / (S_{ce} + S_{pe})$], the percent of center rears [$\%R = R_{ce} / (R_{ce} + R_{pe})$] and the average duration of a visit in the center ($DV = T_{ce} / E_{ce}$) were also calculated. For the sociability phase, in addition to the already described measures, a preference index was calculated as the time exploring the “social” cage divided by the total time exploring both “social” and “empty” cages. An index closed to 1 would reflect preference for the “social” cage over the “empty” cage, whereas an index of 0.5 would indicate no preference. Similarly, for the social novelty phase, a preference index was calculated as the time exploring the “novel” cage divided by the total time exploring both “novel” and “familiar” cages.

The number of entries and time spent in the virtual quadrants were analyzed by one-way, or two-way ANOVA, depending on the experimental design (without or with pharmacological treatment, respectively), with Quadrant as a within-subject factor. The time exploring the cages was analyzed by paired, 2-tailed Student's t test or by a two-way ANOVA with Cage as a within-subject factor, depending on the experimental design. The Newman-Keuls test was used for post hoc comparisons when required. The preference index was analyzed by a Student's t test against a reference constant (0.5). Similar analyses were conducted for the social odor discrimination task. The level of significance was set at $p < 0.05$.

3. Results

The first step of our study was to assess whether rats performed similarly to mice in this modified version of the mouse three-chamber sociability task. During the sociability phase (Fig. 2A), animals made significantly more entries (Fig. 2B) and spent more time (Fig. 2C) in the quadrant containing the unfamiliar rat (“social” cage) than in any of the other virtual quadrants. They also spent more time in the quadrant containing the “empty” cage than in the empty quadrants (Fig. 2C), as well as, exploring the “social” cage than the “empty” cage (Fig. 2D), showing a significant preference for a “social” versus an “empty” cage (preference index: 0.79 ± 0.07 ; $t_{(7)} = 11.48$, $p = 0.000009$). During the novelty preference phase (Fig. 2E), animals made significantly more entries (Fig. 2F) and spent more time (Fig. 2G) in the quadrant containing the cages than in the empty quadrants. However, they spent more time in the quadrant containing the “novel” cage compared to the quadrant containing the “familiar” cage (Fig. 2G), and spent more time exploring the “novel” cage than the

“familiar” cage (Fig. 2H), showing a significant preference for a “novel” versus a “familiar” social stimulus (preference index: 0.67 ± 0.13 ; $t_{(7)} = 3.78$, $p = 0.007$).

To distinguish any apparent social novelty preference from contextual (non social) novelty preference (Pearson et al. 2010), we also assessed whether the location of the “novel” and “familiar” cage affected the animal preference during the novelty preference phase. Specifically, for half of the animals, the position of “familiar” and “novel” cage was switched (this condition was designated as “reversed”), whereas the other half of the rats received the typical cage placement (referred to as “same” condition) (Fig. 3A). During the sociability phase, animals from both conditions showed an identical preference for a “social” versus an “empty” cage (data not shown). During the novelty preference phase, animals from both conditions made more entries (Fig. 3B) in the quadrant containing the “novel” cage than in the empty quadrants. Regardless of the conditions, they also spent more time in the quadrant containing the “novel” versus “familiar” cage (Fig. 3C), as well as exploring the “novel” rather than the “familiar” cage (Fig. 3D), showing a significant preference for a “novel” social stimulus [preference index: 0.65 ± 0.03 ($t_{(5)} = 4.74$, $p = 0.005$) and 0.65 ± 0.02 ($t_{(5)} = 6.46$, $p = 0.001$) for “same” and “reversed” conditions, respectively].

As the experiments above indicate that our modified version of the mouse three-chamber task allows the evaluation of sociability and social novelty preference in rats, we then assessed the behavioral performance of PCP-treated rats using the same paradigm. We also evaluated motor functions and anxiety-like behaviors during the first 5 min of the habituation phase. No difference in any of these measures (see Experimental Procedures section) was observed between saline- and PCP-treated rats (data not shown). During the sociability phase, both saline- and PCP-treated animals showed preference for a “social” versus an “empty” cage as assessed by the number of entries in the different quadrants (Fig. 4A), time spent in the quadrants (Fig. 4B), time spent exploring the cages (Fig. 4C), and preference index [0.85 ± 0.02 ($t_{(11)} = 18.25$, $p = 0.000000$) and 0.84 ± 0.02 ($t_{(11)} = 20.37$, $p = 0.000000$) for saline- and PCP-treated rats, respectively]. During the novelty preference phase, animals from both groups made more entries (Fig. 4D) in the quadrant containing the “novel” cage than in the empty quadrants. However, in contrast to saline-treated rats, which spent more time in the quadrant containing the “novel” versus “familiar” cage, PCP-treated animals spent a similar amount of time in both quadrants (Fig. 4E). Moreover, whereas saline-treated rats spent more time exploring the “novel” versus the “familiar” cage, PCP-treated rats did not show any preference (Fig. 4F), as indicated by their respective preference index [saline: 0.67 ± 0.03 ($t_{(11)} = 6.42$, $p = 0.00005$); PCP: 0.48 ± 0.06 ($t_{(11)} = -0.31$, $p = 0.76$)] and the difference between them (unpaired, 2-tailed Student’s *t* test, $t_{(22)} = 2.80$, $p = 0.01$). Importantly, the total time spent exploring either cages was similar between saline- and PCP-treated rats (298.3 ± 81.0 and 297.1 ± 75.4 seconds, respectively; unpaired, 2-tailed Student’s *t* test, $t_{(22)} = 0.04$, $p = 0.97$), indicating that the treatment did not alter social investigation.

As the lack of novelty preference in PCP-treated rats may result from impaired social recognition, and given that social approach in the three-chamber task is primarily driven by social olfactory cues (Ryan et al. 2008), we tested PCP-treated rats for their ability to (a) discriminate between neutral versus social odors, and (b) show preference for novel over

familiar social odors. Both saline- and PCP-treated animals visited all the virtual quadrants containing the different Petri dishes (“neutral”, “home”, “other” and “empty”; Fig. 5A). However, PCP-treated animals spent less time investigating the four Petri dishes compared to saline-treated controls (105.3 ± 8.8 and 81.9 ± 4.5 seconds, respectively; unpaired, 2-tailed Student's *t* test, $t_{(30)}=2.37$, $p=0.02$). Both groups spent more time in the quadrant containing the social odors (either “home” or “other”) than the neutral odors (either “empty” or “neutral”), but PCP-treated animals spent more time in the “other” quadrant and less time in the “neutral” quadrant than saline-treated controls (Fig. 5B). As shown in Fig. 5C, only saline-treated rats explored more the “other” than the “empty” or “neutral” dishes; the time exploring the “home” Petri dish, although greater than that for the “empty” and “neutral” dishes, did not differ statistically. Importantly, both groups showed a preference for social over neutral odors as indicated by their preference index [0.58 ± 0.03 ($t_{(15)}=2.91$, $p=0.01$) and 0.59 ± 0.04 ($t_{(15)}=2.52$, $p=0.02$) for saline- and PCP-treated rats, respectively], suggesting that the lack of preference for social novelty was not related to an alteration of olfactory sensory function. However, neither saline- nor PCP-treated animals showed a preference for novel (i.e. “other”) over familiar (i.e. “home”) social odors as indicated by their preference index [0.55 ± 0.04 ($t_{(15)}=1.32$, $p=0.21$) and 0.51 ± 0.05 ($t_{(15)}=0.21$, $p=0.84$) for saline- and PCP-treated rats, respectively].

Novelty-seeking behavior under free-choice conditions requires the ability to recall and recognize familiar versus novel objects. Therefore, a decrease in novelty preference may result from deficiencies in recognition memory, which has been shown to be altered in PCP-treated rats using the novel object recognition task (Vigano et al. 2009). Since this deficit was accompanied by an increase in the level of the endocannabinoid 2-arachidonoylglycerol (2-AG) in the prefrontal cortex (Vigano et al. 2009), we assessed the potential involvement of CB₁ receptors in PCP-induced lack of preference for social novelty. Systemic administration of the CB₁ antagonist AM251 (1 mg/kg) did not affect motor function or anxiety-like behaviors as assessed during the first 5 min of the habituation phase in either saline- or PCP-treated rats (see Experimental Procedures section; data not shown). During the sociability phase, all animal groups showed preference for a “social” versus an “empty” cage as assessed by the number of entries in the different quadrants (Fig. 6A), time spent in the quadrants (Fig. 6B), and time spent exploring the cages (Fig. 6C), and preference index [0.86 ± 0.02 ($t_{(7)}=15.00$, $p=0.000001$), 0.81 ± 0.04 ($t_{(7)}=8.47$, $p=0.000063$), 0.77 ± 0.05 ($t_{(7)}=5.78$, $p=0.000675$) and 0.82 ± 0.04 ($t_{(7)}=7.85$, $p=0.000103$) for saline-vehicle, PCP-vehicle, saline-AM251 and PCP-AM251 rats, respectively]. AM251 slightly reduced this preference as measured by the time spent in the quadrants (Fig. 6B) by saline-treated animals only. During the novelty preference phase, in contrast to saline-treated rats, PCP-treated animals spent a similar amount of time in the quadrant containing the “novel” than the “familiar” cage (Fig. 6E). Moreover, while saline-treated rats spent more time exploring the “novel” versus the “familiar” cage, PCP-treated rats did not show any preference [Fig. 6F and preference index: [saline: 0.63 ± 0.04 ($t_{(7)}=2.97$, $p=0.02$); PCP: 0.52 ± 0.03 ($t_{(7)}=0.55$, $p=0.60$)]. AM251 restored social novelty preference in PCP-treated rats [Fig. 6E, 6F and preference index: 0.68 ± 0.03 ($t_{(7)}=6.18$, $p=0.000453$ and Newman-Keuls test $p=0.02$ compared to PCP-vehicle rats] but produced a slight deficit in saline-treated animals [Fig. 6E

and 6F, but preference index: 0.62 ± 0.04 ($t_{(7)}=3.28$, $p=0.01$ and Newman-Keuls test $p=0.81$ compared to saline-vehicle rats)].

Since the PCP-induced lack of social novelty preference was reversed by pharmacological blockade of CB₁ receptors (suggesting CB₁ overactivity), we investigated whether pharmacological activation of CB₁ receptors in control animals could mimic this deficit. Systemic administration of the CB₁ agonist CP55,940 (0.003–0.03 mg/kg) did not affect motor function or anxiety-like behaviors as assessed during the first 5 min of the habituation phase (see Experimental Procedures section; data not shown). During the sociability phase, animals showed preference for a “social” versus an “empty” cage as assessed by the number of entries in the different quadrants (Fig. 7A), time spent in the quadrants (Fig. 7B) and time spent exploring the cages (Fig. 7C). Regardless of the dose, CP55,940 did not affect this preference [Fig. 7A–C and preference index: 0.80 ± 0.05 ($t_{(6)}=5.84$, $p=0.001$), 0.89 ± 0.04 ($t_{(7)}=10.24$, $p=0.000018$), 0.84 ± 0.04 ($t_{(7)}=9.68$, $p=0.000027$) and 0.86 ± 0.04 ($t_{(7)}=9.09$, $p=0.000040$), respectively], but slightly increased the time spent in the quadrant containing the “social” cage and decreased the time spent in the quadrant containing the “empty” cage (Fig. 7B). In contrast to vehicle-treated rats, the time spent in the quadrant containing the “empty” cage was not significantly different from the time spent in the empty quadrants in CP55,940-treated animals (Fig. 7B). During the novelty preference phase, in contrast to vehicle-treated rats, CP55,940-treated animals spent a similar amount of time in the quadrants containing the “novel” and the “familiar” cage (Fig. 7E). Moreover, whereas vehicle-treated rats spent more time exploring the “novel” cage rather than the “familiar” cage, this preference was suppressed in a dose-dependent manner by CP55,940 [Fig. 7F and preference index [0.72 ± 0.06 ($t_{(6)}=3.41$, $p=0.01$), 0.65 ± 0.03 ($t_{(7)}=4.45$, $p=0.002978$), 0.57 ± 0.03 ($t_{(7)}=2.85$, $p=0.024546$) and 0.54 ± 0.11 ($t_{(6)}=0.37$, $p=0.72$), respectively].

4. Discussion

The three-chamber social interaction task, which was originally designed to assess autistic-like behavior in mice, allows to focus on a defined set of behavioral parameters such as reduced social approach and diminished interest in novelty (Moy et al. 2004). Using a modified version of this test, we showed that PCP-treated rats have intact sociability, but lack social novelty preference. This deficit was reversed by the selective CB₁ antagonist AM251 and mimicked by the CB₁ agonist CP55,940 in control rats, suggesting that increased activity at CB₁ receptors contributes to the social novelty preference deficit in PCP-treated animals.

PCP-induced social withdrawal is a phenotype relevant to the negative symptoms of schizophrenia (Gururajan et al. 2010; Seillier et al. 2013; Wilson and Koenig, 2014); however, other processes underlying this deficit (e.g. altered social cognition) have not been fully explored. In most studies, social interaction procedures involve placing two freely moving animals in an arena, an experimental setup that makes the assessment of cognitive mechanisms difficult. The method used in this study - a modified version of the three-chamber social approach task developed by Crawley and co-workers (Moy et al. 2004) - focuses on social approach tendencies that are initiated and terminated by the experimental rat only (most likely, driven by its own motivation) as the stimulus rats is placed in a cage.

In addition, the nature of this social interaction is investigatory only since there is no direct physical contact between the two rats. Thus, this paradigm can be used to measure and isolate different behavioral constructs, such as sociability and social novelty preference. Although PCP-treated rats exhibited social withdrawal in the classical dyadic social interaction test (for review, see Gururajan et al. 2010; Millan and Bales, 2013; Wilson and Koenig, 2014), these animals did not show any deficit in sociability when tested in our social approach task, suggesting that PCP-treated animals have an intact social 'drive' or motivation to engage in social interaction under these conditions. To date, the few studies that have examined sociability in the sub-chronic PCP model of schizophrenia using the three-chamber task have reporting produced conflicting results. Indeed, while some groups have shown lack of effect of sub-chronic PCP on sociability (Hanks et al. 2013; McKibben et al. 2014), others have described a partial attenuation (Brigman et al. 2009) or an abolition of sociability (O'Tuathaigh et al. 2010a). The reasons behind these discrepancies remain unknown. Nevertheless, the inconsistency of the data - or the absence of a PCP effect on sociability (Hanks et al. 2013; McKibben et al. 2014) obtained from the three-chamber task or its modified version (present study) - is in contrast with the robust and consistent disruption of social behavior described with the dyadic social interaction paradigm (Qiao et al. 2001; Seillier and Giuffrida, 2009; Snigdha and Neill, 2008). Intrinsic differences between these two assays may explain these divergent observations. Indeed, Chabout and co-workers (2013) proposed that choosing between multiple rewards within a social context is affected by the uncertainty of the situation. According to the authors, the three-chamber assay, which involves placing the stimulus rat under a wire mesh cage, represents a "socially safe" environment, whereas the dyadic social interaction paradigm, due to the risk of a physical threat, could be defined as "socially uncertain" situation. Indeed, when comparing the behavior of mice lacking the $\beta 2$ subunit of nicotinic receptors in these two situations ("safe" versus "uncertain"), Chabout et al. (2013) showed a behavioral impairment in the dyadic social interaction paradigm, but no deficit in the three-chamber task. Other divergences between the dyadic and three-chamber tasks might also play a role, including different rewarding properties of social interaction under these two situations (e.g., limited vs. full physical contact with the conspecific). Indeed, conditioned place preference, a widely used behavioral paradigm for studying reward, revealed that unrestrained access to a conspecific, but not to a rat confined behind a screen, is rewarding (Van den Berg et al. 1999). Interestingly, sub-chronic PCP treatment suppressed conditioned place preference for social contact (Schwabe et al. 2006), underlying a potential contribution of social anhedonia to the PCP-induced social withdrawal. Finally, even though both assays are based on choices (social vs. non social), the motivation/reward associated with these choices is not equal: animals in the three-chamber task have to choose between a social reward ("social" cage) and the lack of it ("empty" cage), whereas in the dyadic task, the choice is between two rewarding stimuli: a social (conspecific) versus a non-social one [arena exploration; note that some dyadic paradigms also include an object in the arena (e.g., Snigdha and Neill, 2008)]. Therefore, sociability (i.e. time spend with a social stimulus), as measured by the three-chamber task, is likely attributable to a conflict between approach- versus avoidance-related motivations toward the social stimulus (rather than a real choice between the two stimuli). As such, this procedure is useful to study tendencies for social avoidance, which are relevant to animal models of autism (Moy et al. 2004), rather than deficits in social

approach, which occur in schizophrenia (Moy et al. 2009; O'Tuathaigh et al. 2010a). On the other hand, in the dyadic paradigm, rats have to choose between exploring a novel arena (or an arena that was explored the day before) and seeking social contact with a novel conspecific, and it is well established that exploratory and social behaviors compete with each other in the social interaction task (for example, see supplemental Fig. S1 of Avale et al. 2011). Taken together, these observations suggest that the dyadic social interaction task may more rigorously explore normal social behavior by integrating the motivation (or lack of it) for social contact with an uncertain environment (e.g., potential threats from the conspecific).

In contrast to their intact sociability, PCP-treated animals showed a selective impairment of social novelty preference that was not olfactory-based (no alteration in their ability to discriminate social over neutral odors) nor linked to anxiety [no alteration in motor functions and anxiety-like behaviors during the first 5 min of the habituation phase; confirming previously published data (McLean et al., 2010; Seillier and Giuffrida, 2011)]. Interestingly, this selective impairment of social novelty preference has been reported across a broad range of animal models of schizophrenia (Kaminitz et al. 2014; O'Tuathaigh et al. 2007; Shimamoto et al. 2014; Zoubovsky et al. 2015, but O'Tuathaigh et al. 2010a). Eagle and co-workers (Eagle et al. 2013) proposed that this selective impairment might be related to the differences between sensation-seeking versus novelty-seeking behaviors, the latter requiring to recall and recognize a familiar versus a novel object. Therefore, a major challenge in the interpretation of our findings is to understand whether the lack of preference for social novelty is caused by a social recognition/memory deficit or not. Several studies have shown impaired social recognition in animal models of schizophrenia (Wilson and Koenig, 2014), including PCP-treated rats (Harich et al. 2007; Terranova et al. 2005). However, since social recognition/memory tests generally use a 30-min (or longer) delay between the initial presentation of the stimulus and the actual recognition phase, it can be argued that the 2 min inter-phase interval used in our study might not be sufficient to induce forgetting. However, a 1-min interval has been shown to be sufficient to produce a memory deficit in PCP-treated rats in other behavioral tasks (working memory: Jentsch et al. 1997; Seillier and Giuffrida, 2009, novel object recognition: Snigdha et al., 2010). Furthermore, as social abilities require non-social cognitive processes, alterations in social recognition can be a consequence of impaired non-social cognition. In this context, and in contrast to other models of schizophrenia (Kaminitz et al. 2014; Zoubovsky et al. 2015), PCP-induced impairment in recognition might not be specific to social stimuli, as observed in a non-social recognition task (Vigano et al. 2009). Based on these observations, any attempt to dissociate between social and non-social cognition deficits in PCP-treated animals would be only speculative, and studies aimed at better understanding the underlying mechanisms of these two domains are warranted.

Despite the difficulties to discern whether the lack of novelty discrimination in PCP-treated rats results from impaired social recognition or a lack of preference for social novelty, the CB₁ antagonist AM251 reversed this deficit and produced a deficit on its own in saline controls. This observation, together with the deleterious effects of direct stimulation of CB₁ receptors observed in control animals, suggest that PCP-induced lack of social novelty preference is due to increased activation of CB₁ receptors. The latter cannot be attributed to

enhanced CB₁ expression or function, as neither one are affected in this model (Seillier et al. 2010; Seillier et al. 2013), but might be caused by an excessive production of endocannabinoids. Interestingly, evidence for increased endocannabinoid levels has been reported in the nucleus accumbens and prefrontal cortex of PCP-treated rats (Seillier et al. 2010; Seillier et al. 2013; Vigano et al. 2009); these observations, however, cannot be generalized to the above behavioral paradigm, given the 'on demand' nature of endocannabinoid production (Seillier et al. 2013). Two recent studies have addressed the role of the endocannabinoid system in the three-chamber task by using conditional knockout mice (Brown et al. 2014; Häring et al. 2011). Häring and co-workers (2011) showed that mice lacking CB₁ receptors in GABAergic neurons had intact sociability, but increased social novelty preference. On the other hand, mice lacking CB₁ receptors in glutamatergic neurons showed impaired sociability. Using a different approach based on the genetic inactivation of GABAergic neurotransmission in CB₁-expressing neurons, Brown and co-workers (2014) showed a similar phenotype (intact sociability and enhanced social novelty preference).

Although the increased social novelty preference reported in both studies should be interpreted with caution as none of the control groups showed any preference toward the "novel" over the "familiar" cage, these results are puzzling. Given the nature of these two manipulations, opposite phenotypes should have been expected: why did the removal of CB₁ receptors from GABA neurons (Häring et al. 2011), and the consequent suppression of the endocannabinoid-induced inhibition of GABA release, produce the same effect of the permanent inactivation of GABA release from these same neurons (Brown et al. 2014)? Even though we do not have an answer to this question, these two studies are in line with our findings in control animals treated with either the CB₁ antagonist AM251 or the CB₁ agonist CP55,940; indeed both manipulations, despite their opposing pharmacological actions, produced a deficit in social novelty preference. Interestingly, a similar pharmacology was reported in the case of PCP-induced working memory deficit, which was also reversed by AM251, the latter having a deleterious effect in saline control animals, like other manipulations that elevated endocannabinoid tone (Seillier et al. 2010). Overall, our parsimonious interpretation that the lack of social novelty is due to a general cognitive deficit rather than a selective impairment in social cognition is strengthened by the observation that either CB₁ receptors agonism or antagonism has a disruptive effect on cognition across a variety of behavioral paradigms (Egerton et al. 2006; Seillier et al. 2010).

In summary, our data indicate a deficit in social novelty preference in PCP-treated rats, possibly induced by increased stimulation of CB₁ receptors. However, it seems unlikely that this deficit contributes in a major way to the social withdrawal observed in the same animals, as the latter is due to deficient, rather than increased, CB₁ stimulation (Seillier et al. 2013).

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Figure 1.
Schematic representation of the test apparatus showing the virtual quadrants and the consecutive phases for the (A) social approach and social novelty preference task and (B) social odor discrimination task. See Experimental Procedures for a detailed description of the tasks.

Figure 2.

Control rats show sociability and preference for social novelty. Schematic representation of the “sociability” (A) and the “social novelty” (E) phases and their respective color code for each virtual quadrant. The figure illustrates: number of entries (B and F) and time spent (C and G) in each of the virtual quadrant for the “sociability” and “social novelty” phases, respectively; time spent exploring the “social” and “empty” cages (D) or the “familiar” and “novel” cages (H), for their respective phases. Values are expressed as mean $\pm$ SEM (n=8). * $p < 0.05$ compared to: (all) other quadrants (B, C, G; Newman-Keuls test), or other cage (D, H; Student’s *t* test); # $p < 0.05$ compared to the empty grey quadrants (F; Newman-Keuls test).

Figure 3.

Social novelty preference is not affected by cage position. Schematic representation of the experimental design (A). Number of entries (B) and time spent (C) in each virtual quadrant, and time exploring the “familiar” and “novel” cages (D) during the “social novelty” phase are indicated. Values are expressed as mean $\pm$ SEM ($n=6$ per group). * $p<0.05$ compared to (all) other quadrants (C), or other cage (D); # $p<0.05$ compared to the empty grey quadrants (B); Newman-Keuls test.

Figure 4.

PCP-treated rats lacked preference for social novelty. The figure shows the number of entries (A and D) and time spent (B and E) in each virtual quadrant for the “sociability” and “social novelty” phases, respectively, as well as the time spent exploring the “social” and “empty” cages (C) or the “familiar” and “novel” cages (F) during their respective phases. The bar color code is reported in Fig.2A and 2E. Values are expressed as mean $\pm$ SEM (n=12 per group). * $p < 0.05$ compared to (all) other quadrants (A, B, E) or other cage (C, F); # $p < 0.05$ compared to the empty grey quadrants (D, E); \$ $p < 0.05$ compared to saline-treated animals (E); Newman-Keuls test.

Figure 5.

Social odor discrimination is not impaired in PCP-treated rats. Number of entries (A), time spent (B) in each virtual quadrant, and time spent exploring each Petri dish (C) are indicated. Values are expressed as mean $\pm$ SEM (n=16 per group). # p<0.05 compared to the “empty” and “neutral” quadrants (A, B, C); \$ p<0.05 compared to saline-treated animals (B); Newman-Keuls test.

Figure 6.

The CB₁ antagonist AM251 restored social novelty preference in PCP-treated rats. The figure shows the number of entries (A and D) and time spent (B and E) in each virtual quadrant for the “sociability” and “social novelty” phases, respectively, as well as the time spent exploring the “social” and “empty” cages (C) or the “familiar” and “novel” cages (F) during their respective phases. The bar color code is reported in Fig.2A and 2E. Values are expressed as mean ± SEM (n=8 per group). * p<0.05 compared to (all) other quadrants (A, B, D, E) or other cage (C, F); # p<0.05 compared to the empty grey quadrants (B, D, E); \$ p<0.05 compared to saline-treated animals (C, E); ° p<0.05 compared to saline-vehicle-treated rats (B, E); + p<0.05 compared to vehicle-treated animals (B, E); Newman-Keuls test.

Figure 7.

The CB₁ agonist CP55,940 suppressed social novelty preference dose-dependently. The figure shows the number of entries (A and D) and time spent (B and E) in each virtual quadrant for the “sociability” and “social novelty” phases, respectively, as well as the time spent exploring the “social” and “empty” cages (C) or the “familiar” and “novel” cages (F) during their respective phases. The bar color code is reported in Fig.2A and 2E. Values are expressed as mean $\pm$ SEM (n=8 per group). * $p < 0.05$ compared to (all) other quadrants (A, B, E) or other cage (C, F); # $p < 0.05$ compared to the empty grey quadrants (D, E); \$ $p < 0.05$ compared to vehicle-treated animals (B, E); Newman-Keuls test.