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Anxiety does not contribute to social withdrawal in the sub-chronic PCP rat model of schizophrenia

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Abstract

Social withdrawal should not be considered a direct measure of the negative symptoms of schizophrenia as it may result not only from asociality (primary negative symptom) but also from other altered processes such as anxiety. To understand the contribution of these two factors to social deficit, we investigated whether the social withdrawal observed in the sub-chronic phencyclidine (PCP) rat model of schizophrenia could be attributed to increased anxiety.

Compared to saline controls, PCP-treated rats (5 mg/kg, b.i.d. for 7 days, followed by a washout period) spent significantly less time in social interaction, but did not show anxiety-like behaviors in different relevant behavioral paradigms. In addition, their social deficit was not affected by a behavioral procedure known to reduce anxiety-like behavior (repeated exposure to the same partner) nor by systemic administration of the classical anxiolytic diazepam. In contrast, PCP-induced social withdrawal was reversed by the cannabinoid agonist CP55,940, a drug with known anxiogenic properties. Furthermore, when using the social approach task, PCP-treated animals performed similarly to control animals treated with diazepam, but not to those treated with the anxiogenic compound Pentyleneetetrazole (PTZ).

Taken together, our results indicate that PCP-induced social withdrawal cannot be attributed to increased anxiety. These data are discussed in the context of primary versus secondary negative symptoms and the deficit syndrome of schizophrenia.

Keywords

cannabinoid; deficit syndrome; diazepam; Phencyclidine; schizophrenia; social interaction

Introduction

The negative symptoms of schizophrenia can be parsed into primary negative symptoms, which are etiologically related to the core pathophysiology of schizophrenia, and secondary

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negative symptoms, which results from other factors, such as positive symptoms, medication, depression and anxiety (Kirkpatrick, 2014a). The negative symptoms, which include blunted affect, alogia, asociality, anhedonia, and avolition, contribute to poor functional outcomes, affect patients' quality of life more than positive symptoms and are more difficult to treat (Foussias and Remington, 2010; Hanson et al., 2010). This scenario is unchanged after controlling for the influence of potential sources of secondary negative symptoms (Fervaha et al., 2014). Whereas this distinction might appear trivial, the rationale behind it is essential to understand the pathophysiology of negative symptoms and identify novel therapeutic targets. Indeed, while primary negative symptoms have been linked to fronto-parietal neural dysfunction (Kirkpatrick, 2014a), secondary negative symptoms have more diffuse underlying mechanisms, depending on their origin (from positive symptoms, medication, depression or anxiety). Therefore, it is important to understand the specific causes of social withdrawal in a given model, as each neuropsychological component may have a different underlying neurobiology.

Social withdrawal, one of the core negative symptoms of schizophrenia, and perhaps the most widely examined in animal models (Gururajan et al., 2010; O'Tuathaigh et al., 2010a), could not be considered as a direct measure of negative symptoms as it may result from several altered processes ranging from asociality (primary negative symptoms) to anxiety (File and Seth, 2003). As a matter of fact, there is a strong relationship between negative symptoms and anxiety, which interact with and exacerbate each other (Huppert et al., 2001), and, social withdrawal in schizophrenic patients has been correlated to their anxiety level (Lysaker and Salyers, 2007). On the other hand, patients with deficit syndrome, which is characterized by negative symptoms that are both enduring and primary (Kirkpatrick and Galderisi, 2008), are less anxious than non-deficit schizophrenia patients (Beck et al., 2013; Subotnik et al., 2000; Tek et al., 2001), and their anxiety level does not differ from that of healthy subjects (Subotnik et al., 2000). This contrasts with their poorer social function and greater social isolation (Kirkpatrick, 2014a), and emphasizes the importance of differentiating primary from secondary negative symptoms and understanding the role played anxiety in this context (Kirkpatrick, 2014b).

The social interaction task, which was initially used to measure anxiety (File and Seth, 2003), can be easily modified (e.g. by habituating animals to the arena and changing light conditions) to minimize the contribution of anxiety to the behavioral outcomes (Wilson and Koenig, 2014). Nevertheless, even under non-anxiogenic conditions, we cannot rule out that anxiety might still contribute to social withdrawal. As anxiety measures have been infrequently assessed in animal models of schizophrenia, we investigated whether increased anxiety played a role in the social withdrawal observed in the sub-chronic phencyclidine (PCP) rat model of schizophrenia, the best pharmacological model for social withdrawal in terms of construct, face, and predictive validity (Gururajan et al., 2010).

Methods

All experiments were carried out in accordance with the National Institute 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.

Animals

Male Wistar rats (200–225 g; Charles River Laboratories, Wilmington, MA) were housed two per cage at 22 ± 1 °C, under a 12 h light-dark cycle with food and water available *ad libitum*, and habituated to the housing conditions for one week prior to the experiments. Animals were treated sub-chronically (twice a day for 7 days, at approximately 8:00 a.m. and 8:00 p.m.) with either vehicle (saline, 1 ml/kg) or PCP (5 mg/kg) via intraperitoneal route (i.p.) and tested 5–9 days after the last drug injection; no PCP was on board or given during the behavioral assessment.

Behavioral assessment

The experimental procedures were carried out in the morning (during the light portion of the cycle) in a room adjacent to the vivarium. Six independent experiments were conducted, each with a new group of rats. In experiment 1 (Fig. 1), 24 animals were tested for anxiety using the elevated plus maze (EPM) task, as well as for social interaction, 5 and 7 days after the last drug injection (see below for the description of these behavioral tests). In addition, the open field data reported in Fig. 1C were recorded from the same animals (during the first 5 min of the familiarization phase of the social interaction task, 6 days after the last drug injection). For the social interaction / habituation test (SI/h), two experiments were conducted with different parameters: five 10-min sessions (experiment 2; 20 animals) or two 5-min sessions (experiment 3; 24 animals). The same sequence of behavioral assessment described in experiment 1 (i.e. EPM followed by social interaction) was used for experiments 4 (Fig. 2; 64 animals) and 5 (Fig. 3; 56 animals), but no measurement was carried out during the familiarization phase of the social interaction task. For experiment 6, a new group of 32 animals was tested for social approach (Fig. 4).

Social behaviors—An arena made of black acrylic (100 cm × 100 cm × 40 cm) and located in a dimly lit room (5 lux at the arena center) was used for the following procedures.

Social interaction: Six days after the last drug injection, animals were placed individually in the center of the arena and free exploration was allowed for 30 min (familiarization). On the following day, they were tested in pairs (two unfamiliar rats receiving the same treatment, and housed in different home cages) matched up according to their body weights. Animals were placed simultaneously into the arena and their behavior recorded by a video camera for 10 min. The total time spent by each rat engaging in the following behavior was scored: (1) investigative sniffing, (2) following and (3) climbing over or under the conspecific.

Social interaction / habituation (SI/h): For the social interaction / habituation test (SI/h), the initial assessment of social interaction (day1) was carried out as for the social interaction task described above. Then, the same pair of rats was tested daily again for 4 additional days. To determine if the animals were inhibited in their social behavior upon repeated exposure with the same partner, the total time spent by each rat in social behavior was also recorded on the last day (day 5 of social interaction), and compared to the time obtained on the initial assessment (day 1 of social interaction).

Social approach: Seven days after the last drug injection, experimental rats were placed individually in one corner of the arena for a 30-min familiarization period, and then transferred to a holding cage adjacent to the arena for 2 min. During this period, 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. The “stimulus” rat was habituated to placement under the wire mesh cage for several days and weight-matched to the experimental rat. A vinyl coated lead O-shaped blue ring (970 g) was placed on the top of each wire mesh cage to prevent displacement. The animal was then placed back (after a 2 min inter-phase interval) in the arena and allowed to explore it for 10 min. During the entire behavioral test, the experimenter remained motionless next to the corner in which the experimental rats were introduced in the arena, and manually recorded the time spent exploring the mesh cages. The test was also videotaped for further analysis, during which the arena was virtually divided in quadrants and the number of entries and the time spent in each virtual quadrant was recorded (for details, see Seillier and Giuffrida, 2016).

Anxiety-like behaviors—Anxiety-like behaviors were assessed using the Elevated Plus Maze (EPM) or the Open Field (OF) tests.

Elevated Plus Maze task: The EPM test was conducted five days after the last drug injection, as previously described (Seillier and Giuffrida, 2011), except that the EPM was located in a lit room. Data were collected by an experimenter blind to the study as total number of entries ($TE = E_{open} + E_{closed} + E_{center}$) and time spent in each zone (the center zone was coded as neither open nor closed arms). An entry was defined as a rat entering the arm with all four paws. Levels of anxiety were assessed as percent time spent in the open arms [$\%T_{open} = T_{open} / (T_{open} + T_{closed})$] and percent of open arm entries [$\%E_{open} = E_{open} / (E_{open} + E_{closed})$].

Open Field task: The OF test was carried out either in a dimly lit arena (during the first 5 min of the familiarization phase of the social interaction task; see above) or in ActiMot Activity boxes (TSE Systems GmbH; Bad Homburg, Germany) located in a lit room (nine days after the last drug injection). The OF behavior was quantified for 5 min, either by off-line analysis of the videotape recordings (a 7×7 grid image was superimposed on the monitor) or using the ActiMot Activity Measuring System version 6.07. 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) or in the periphery. 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. The OF behavior was also quantified during the social interaction task (10 min).

Drugs

PCP, diazepam and pentylenetetrazole (PTZ) were purchased from Sigma/RBI (St. Louis, MO) and the cannabinoid agonist CP55,940 from Tocris (Ellisville, MO). Drugs were dissolved in either saline (PCP and PTZ) or Tween-80:polyethylene glycol:saline (5:5:90;

CP55,940 and diazepam). Drugs were prepared freshly and injected via intraperitoneal route 30 min before the EPM or social interaction test (CP55,940 and diazepam) or 10 min before the social approach task (diazepam and PTZ). The dose of diazepam and CP55,940 were chosen from previous *in vivo* studies carried out in rats (Costall et al., 1991; Genn et al., 2004; Marco et al., 2004; Seillier and Giuffrida, 2011). The dose of PTZ was determined through a dose-response curve in the EPM task (data not shown).

Statistical Analysis

For comparison of two independent groups (saline- vs. PCP-treated rats; Fig. 1), unpaired 2-tailed Student's *t* test was used. Correlation analyses were carried out by Pearson's correlation. For the SI/h test, data were analyzed by Analysis of Variance (ANOVA) with Treatment (saline, PCP) as between-subject factor and Day (Day1, Day 5) as repeated measure. Data from social interaction and EPM (Fig. 2 and 3) were analyzed by two-way ANOVA with Treatment (saline, PCP) and Drug (vehicle, diazepam / CP55,940) as between-subject factors. For the social approach (Fig. 4), data were analyzed by two-way ANOVA with Drug (vehicle, diazepam, PCP, PTZ) as between subject factor and quadrant (4 levels) or cage (2 levels) as within subject factors. When required, pair-wise multiple comparisons were performed using the Newman-Keuls test. Outliers were filtered by Carling's method, namely the median rule, using the recommended ideal fourths and the constant k_2 adjusted for the sample size [$k_2 = (17.63n - 23.64)/(7.74n - 3.71)$] (Carling, 2000).

Results

PCP-treated rats spent significantly less time interacting with their conspecific (Fig. 1A; $t_{(22)}=2.37$, $p<0.05$), however, their anxiety-like behavior in the EPM (Fig. 1B) and OF (dimly light arena; Fig. 1C) was unaltered when compared to saline controls. Similar results were obtained when the OF measures were carried out in a different environment (i.e. smaller arena and bright light), or during the social interaction task (data not shown). None of the anxiety measures were correlated with the time spent in social interaction (data not shown). By using the SI/h test, we also investigated whether the PCP-induced social withdrawal could be reversed by repeated exposure to a familiar partner (Truitt et al., 2007). This procedure, which was proven effective to reverse social withdrawal induced by diverse anxiogenic stimuli (Lungwitz et al., 2014; Truitt et al., 2007), did not affect PCP-induced social withdrawal (day 1: saline, 111.1 ± 11.3 s and PCP, 85.4 ± 9.9 s; day 5: saline, 76.1 ± 11.6 s and PCP, 57.9 ± 7.6 s). ANOVA revealed a main effect of Treatment ($F_{1,18} = 4.53$, $p<0.05$), but no interaction between Treatment and Day ($F_{1,18} = 0.14$, NS). As the interpretation of the data was confounded by the fact that the time spent in social interaction for both saline- and PCP-treated animals was reduced as a consequence of the repeated exposure to the same partner ($F_{1,18} = 9.45$, $p<0.01$), we tried shorter (5 vs. 10 min) or a smaller number of sessions (2 vs. 5). Nevertheless, these adjustments still lead to a similar decrease in social interaction time (data not shown).

To test the hypothesis that PCP-induced social withdrawal was not due to increased anxiety, we studied the effect of the classical anxiolytic diazepam (1 mg/kg) in our experimental groups. As shown in Fig. 2, diazepam produced the expected anxiolytic-like response in

saline- and PCP-treated animals when tested in the EPM, i.e. increase in %T_{open} (Fig. 2A) and %E_{open} (Fig. 2B). ANOVA revealed a main effect of Drug ($F_{1,60} = 12.57$, $p < 0.001$ and $F_{1,59} = 12.72$, $p < 0.001$; for %T_{open} and %E_{open}, respectively), but no effect of Treatment ($F_{1,60} = 0.81$, NS and $F_{1,59} = 0.15$, NS; for %T_{open} and %E_{open}, respectively) nor any interaction between these two factors ($F_{1,60} = 0.24$, NS and $F_{1,59} = 0.09$; NS, for %T_{open} and %E_{open}, respectively). However, in the social interaction test, diazepam did not affect PCP-induced deficit (Fig. 2C). ANOVA revealed a main effect of PCP ($F_{1,58} = 7.45$, $p < 0.01$), but no effect of Diazepam ($F_{1,58} = 1.25$, NS) nor any interaction between these two factors ($F_{1,58} = 0.10$, NS).

Next, we investigated the effects of increasing anxiety on PCP-induced social withdrawal, using the following rationale. If PCP-induced social withdrawal were caused by increased anxiety, then drug-induced anxiety should either not affect this deficit or make it worse. On the other hand, if increased anxiety could reverse PCP-induced social withdrawal, this outcome would rule out the contribution of anxiety to this social deficit. To address these questions, we capitalized on the bimodal effects of cannabinoids on anxiety (Marco et al., 2004) and on our recent study showing that PCP-induced social withdrawal results from a deficient stimulation of cannabinoid CB₁ receptors (Seillier et al., 2013). As shown in Fig. 3A and B, a high dose of the cannabinoid agonist CP55,940 (0.05 mg/kg) produced the expected anxiogenic-like response in saline- and PCP-treated animals in the EPM. ANOVA revealed a main effect of Drug ($F_{1,49} = 26.79$, $p < 0.0001$ and $F_{1,52} = 7.70$, $p < 0.01$; for %T_{open} and %E_{open}, respectively), but no effect of Treatment ($F_{1,49} = 1.53$, NS and $F_{1,52} = 0.64$, NS; for %T_{open} and %E_{open}, respectively) nor interaction between these two factors ($F_{1,49} = 0.42$, NS and $F_{1,52} = 0.03$; NS, for %T_{open} and %E_{open}, respectively). On the other hand, CP55,940 reversed PCP-induced social withdrawal, and reduced social interaction in control animals (Fig. 3C). ANOVA revealed a significant interaction between the Treatment and Drug ($F_{1,52} = 12.82$, $p < 0.001$), but no main effect of Treatment ($F_{1,52} = 0.10$, NS) nor Drug ($F_{1,52} = 0.16$, NS).

Although we performed the social interaction test under non-anxiogenic conditions (Wilson and Koenig, 2014), the lack of pro-social effect of diazepam in control animals (Fig. 2C) might appear surprising (Costall et al., 1991; File and Seth, 2003). Therefore, we used the social approach task, a procedure in which diazepam was reported to be effective (Riedel et al., 2009). We sought to compare the behavioral performance of PCP-treated animals in this task with those of control animals treated with diazepam (anxiolytic drug; 1 mg/kg) or the anxiogenic compound PTZ (15 mg/kg). As shown in Fig. 4, animals from all the groups made significantly more entries (Fig. 4A) and spent more time (Fig. 4B) in the quadrant containing the “social” cage than in any other virtual quadrant (except for PTZ-treated rats which made more entries in the “social” quadrant compared to the “starting” quadrant only). All the experimental groups also spent more time exploring the “social” cage than the “empty” cage (Fig. 4C). In agreement with the anxiogenic profile of PTZ, PTZ-treated animals spent less time exploring the “social” cage than control animals (Fig. 4C). They also spent more time in the “starting” quadrant compared to control animals (Fig. 4B), which was consistent with their low exploratory activity as indicated by the total number of entries in all quadrants. By contrast, diazepam-treated animals spent more time in the quadrant containing the “social” cage than control animals (Fig. 4B), as previously reported (Riedel et

al., 2009). Further supporting our previous observations using the EPM task (Seillier and Giuffrida, 2011), the behavior of PCP-treated animals was similar to those treated with diazepam, with more time spent in the quadrant containing the “social” cage and less time spent in the quadrant containing the “empty” cage compared to control rats (Fig. 4B).

Discussion

Translational research relies on the use of suitable animal models of diseases to investigate the underlying pathophysiological mechanisms and carry out the pre-clinical screening of potential pharmacotherapies. However, the modeling of schizophrenia symptoms, and particularly the negative symptoms, is challenging because of the relatively poor understanding of this illness. Furthermore, in keeping with the idea of primary versus secondary negative symptoms (Kirkpatrick, 2014a), a behavioral phenotype in a given model may reflect changes across several emotional and cognitive domains, which might not be necessarily related to the symptoms under investigation. Given the strong relationship between negative symptoms and anxiety and the contribution of anxiety to social interaction, we investigated whether social withdrawal in the sub-chronic PCP rat model of schizophrenia was secondary to increased anxiety. Using a comprehensive behavioral and pharmacological approach, we showed that: 1) PCP-treated animals did not differ from control rats in the two most commonly used behavioral tests for anxiety, i.e. the EPM and OF; 2) PCP-induced social withdrawal could not be reversed by repeated exposure to the same partner (a procedure known to reduce anxiety-like behavior), or by the anxiolytic drug diazepam, but it could be normalized by the cannabinoid agonist CP55,940 despite its anxiogenic properties.

We previously reported that PCP-treated rats show reduced anxiety in the EPM, when the behavioral test is carried out in a dimly lit room (Seillier and Giuffrida, 2011). Given that rats' behavior in the EPM is sensitive to light conditions (Bertoglio and Carobrez, 2002), and that the level of illumination can shift the drug effect from anxiogenic to anxiolytic (Handley et al., 1993), we conducted our experiments in a brightly lit room. Under these conditions, we found no difference between controls and PCP-treated rats as previously reported (McLean et al., 2010; Schwabe et al., 2006). While the EPM is one of the most popular tests to measure anxiety in rodents (Ramos, 2008), behavior in the EPM is not always reproducible using other anxiety paradigms. For this reason, we also used a different behavioral test, the OF, under varying lighting conditions. PCP-treated did not differ in any aspect of their behavior in the OF, as previously reported (Enkel et al., 2013; Lee et al., 2005; McLean et al., 2010; Schwabe et al., 2006), although we observed a non significant trend for a decrease in anxiety-like behavior under dim light condition. As the interpretation of these results might be limited without a social context, we measured the OF anxiety-like behaviors during the social interaction task despite the confounding of a conspecific in the arena. Nevertheless, we found no difference between saline- and PCP-treated animals for any of the variables analyzed, nor any correlation between these measures and the time spend in social interaction. Our findings, which point to an absence of increased anxiety-like behavior in PCP-treated animals, are in agreement with other studies using different paradigms, such as the light/dark emergence or light-enhanced startle tasks (Enkel et al., 2013; Lee et al., 2005).

As outlined in the introduction, the social interaction task was developed as a test of anxiety. Similarly to the EPM and OF, this test is based on conflicting innate tendencies to avoid open spaces and explore novel environments (for the former), or to interact with unfamiliar conspecifics (for the latter). The state of anxiety is induced by the test itself (i.e. fear of open spaces), but test conditions (light intensity and familiarity with the arena) can be manipulated to generate different levels of anxiety (File and Seth, 2003). As previously mentioned, PCP-treated animals did not show increased anxiety-like behavior compared to controls in response to open space (EPM or OF) regardless of the test conditions (e.g. light level). Moreover, the anxiety-related components of the social interaction task were minimized (via habituation to the arena and low lighting conditions) to make the test more relevant for assessing the preference of rodents to engage in social behaviors (Wilson and Koenig, 2014). The lack of an anxiolytic effect (i.e., no increase in social interaction) in control animals after administration of diazepam (which had a robust effect in the EPM) confirmed the proposition of an absent/very low anxiety component in our social interaction paradigm. Despite these premises, it is unclear whether a decrease in the time spent in social interaction (dependent variable) indicates an anxiogenic effect or a social dysfunction. Given the robust effect of social anxiety on social function in schizophrenia (Cieslak et al., 2015), the role of social vs. non-social anxiety should not be overlooked. For instance, using a virtual reality social encounter task, Park et al., (2009) reported significantly higher anxiety in response to happy avatars in schizophrenic patients vs. healthy controls. In the social interaction task, the presence of an unknown conspecific and its uncertain behavior, a variable that is absent in the EPM or OF tests, can be an additional source of anxiety. It is important to recall that the presence of a conspecific did not affect the anxiety-related OF measures (during the social interaction task), confirming the absence of increased anxiety-like behavior in PCP-treated animals irrespective of the source of anxiety. Nevertheless, reduced pleasure from social interactions (Schwabe et al., 2006) or altered social cognition (O'Tuathaigh et al., 2010b) could produce distorted perceptions, rendering social cues incomprehensible and possibly anxiety provoking. Thus, to rule out the possibility of a social anxiety factor, we tried to overcome the social deficit by reducing social anxiety using social familiarity through a behavioral procedure (SI/h) that uses the same conspecific partner repeatedly (Truitt et al., 2007). This so-called 'social familiarity-induced anxiolysis' was proven effective to reverse social withdrawal induced by diverse anxiogenic stimuli (Lungwitz et al., 2014; Truitt et al., 2007). We also used a pharmacological approach and tested the effects of the anxiolytic drug diazepam (File and Seth, 2003), which reduced anxiety-like behavior in both saline- and PCP-treated animals when tested in the EPM. However, none of these anxiolytic interventions improved social dysfunction. Furthermore, the cannabinoid agonist CP55,940, which showed anxiogenic efficacy in both saline- and PCP-treated rats in the EPM, reversed PCP-induced social withdrawal, in agreement with the observation that PCP-induced social withdrawal results from a deficient stimulation of cannabinoid CB₁ receptors (Seillier et al., 2013). This is in contrast to the expected outcome if PCP-treated animal would already have an increased anxiety-like behavior (i.e., absence of effect or worsening of the social deficit). Thus, whereas CP55,940-induced social deficit in controls can be attributed to the anxiogenic properties of the drug, PCP-induced social withdrawal is not accounted for by an increase in anxiety. Taken together, these data indicate

that anxiety does not contribute to PCP-induced social withdrawal and that this behavioral measure may represent a direct measure of social dysfunction.

Although PCP-treated animals exhibited social withdrawal in the classical social interaction test, they did not show any deficit in the social approach task, but a slight increase in their preference for the “social” over the “empty” cage compared to controls. Furthermore, this increased preference was similar to that of control animals treated with diazepam (Riedel et al., 2009), but not with the anxiogenic compound Pentylene-tetrazole (PTZ). These observations are consistent with reduced, rather than increased, anxiety-like behavior in PCP-treated animals. However, the lack of a deficit in social approach in PCP-treated rats (Hanks et al., 2013; McKibben et al., 2014), which suggests intact motivation to engage in social interaction, contrasts with the robust disruption of social behavior described in the dyadic social interaction paradigm (Qiao et al., 2001; Seillier and Giuffrida, 2009; Snigdha and Neill, 2008). Such divergence might be inherent to the tasks themselves, as placing the “stimulus” rat under a wire mesh cage (social approach task) might be perceived as “socially safe” by the interacting conspecific (Chabout et al., 2013). Conversely, due to the risk of physical threat, the dyadic social interaction task might provide a “socially unsafe” environment. In line with this hypothesis, Chabout and co-workers (2013) showed that the social behavior of mice lacking the $\beta 2$ subunit of neuronal nicotinic receptors is influenced by the uncertainty of the situation: impaired in the dyadic social interaction task, but intact in the “safe” environment of the social approach task. Other differences between these two assays include the rewarding properties of the social approach vs. social interaction tests, given the limited vs. full physical contact allowed, respectively. Indeed, using the conditioned place preference, Van den Berg et al. (1999) showed that access to socially active partners, but not to a confined “stimulus” rat, is rewarding. As PCP-treated animals show a deficit in conditioned place preference for social contact (Schwabe et al., 2006), we could speculate that PCP-induced social withdrawal results from social anhedonia and that the limited rewarding properties of the social approach task are not sufficient to reveal this impairment. It is noteworthy that the relationship between social anhedonia and social impairment is, at least, partially mediated by the ability to engage attentional control processes (Tully et al., 2014) and that attentional function is impaired in PCP-treated animals (Barnes et al., 2015) among other cognitive processes relevant to schizophrenia (Neill et al., 2010). Thus, the difference observed between the social interaction and social approach tasks may be due to the fact that, in the former, animals have to integrate their motivation for social contact with the potential threat posed by the other animal, whereas in the latter, the animal has a reduced attentional burden (Millan and Bales, 2013). Finally, it can be argued that the time spent with the social stimulus is likely attributable to a conflict between approach- and avoidance-related motivations rather than a simple choice between a “social” vs. an “empty” cage. In agreement with this interpretation, PTZ administration reduced the exploration of the “social” cage, without producing a concomitant increase of the exploration of the “empty” cage. Therefore, in agreement with previous reports (Moy et al., 2009; Pobbe et al., 2011), the social approach task might detect tendencies for social avoidance (as in social anxiety) rather than lack of social approach (as observed in schizophrenia),

Social behavior is a complex construct and social withdrawal in schizophrenia may reflect several factors including social anhedonia, social anxiety, and/or deficits in social cognition. While extensively used as a model for negative symptoms of schizophrenia, social withdrawal should not be considered as a direct measure of negative symptoms due to the heterogeneity of the factors that can contribute to this deficit. Overall, social withdrawal in the sub-chronic PCP rat model of schizophrenia did not improve with interventions for anxiety and this behavioral deficit cannot be attributed to increased anxiety. Interestingly, despite their poorer social function and greater social isolation (Kirkpatrick, 2014), schizophrenic patients with deficit syndrome are less anxious than non-deficit schizophrenia patients (Beck et al., 2013; Tek et al., 2001) and do not differ from healthy subjects in this regard (Subotnik et al., 2000). Furthermore, social withdrawal in deficit patients does not result from social anxiety or avoidance of others, but is due to a primary lack of social interest (Subotnik et al., 2000). Given the reminiscence of the behavioral phenotype of PCP-treated animals with the deficit syndrome of schizophrenia (Kirkpatrick et al., 2001; Kirkpatrick and Galderisi, 2008), we propose the subchronic PCP model as an appropriate animal model to study the underlying pathophysiology of (primary) negative symptoms and possible novel treatment approaches.

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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.

Abbreviations

ANOVA	Analysis of Variance
EPM	Elevated Plus Maze
OF	open field
PTZ	pentylentetrazole
PCP	phencyclidine

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Figure 1.

PCP-induced social withdrawal is not accompanied by change in anxiety-like behaviors. **A.** Time spent in social interaction in saline- and PCP-treated rats. **B.** Percent time spent in the open arms (%T_{open}), percent of open arms entries (%E_{open}) and total number of entries (TE) in the EPM. **C.** Number of entries in the center (E_{ce}), time spent in the center (T_{ce}), number of squares crossed in the center (S_{ce}), number of rears in the center (R_{ce}), percent of center squares crossed (%S), percent of center rears (%R) and average duration of a visit in the center (DV) in the open field carried out in a dimly light arena (during the first 5 min of the familiarization phase of the social interaction task). Values are expressed as mean ± SEM (n=10–12 per group). * p<0.05 compared with saline-treated rats (unpaired two-tailed Student t test).

Figure 2.

The anxiolytic drug diazepam does not reverse PCP-induced social withdrawal. Effects of diazepam (1 mg/kg) on the percent time spent in the open arms (%T_{open}; **A**) and the percent of open arms entries (%E_{open}; **B**) in the EPM and on the time spent in social interaction (**C**) in saline- and PCP-treated rats. Values as expressed as mean $\pm$ SEM (n=14–16 per group). ANOVA results are reported in the text.

Figure 3.

The cannabinoid agonist CP55,940 reversed PCP-induced social withdrawal. Effects of CP55,940 (0.05 mg/kg) in saline- and PCP-treated rats on the percent time spent in the open arms (% T_{open} ; **A**) and the percent of open arms entries (% E_{open} ; **B**) in the EPM and on the time spent in social interaction (**C**). Values as expressed as mean $\pm$ SEM ($n=12-14$ per group). ANOVA results are reported in the text. * $p<0.05$ compared to saline-treated animals; # $p<0.05$ compared to vehicle-treated controls (Newman-Keuls post-hoc test).

Figure 4.

PCP-treated animals showed a similar behavioral pattern as diazepam-treated, but not PTZ-treated, animals in the social approach task. **A.** Number of entries in the different quadrants for the four experimental groups. **B.** Time spent in the different quadrants for the four experimental groups. **C.** Time spent exploring the "empty" cage versus the "social" cage for the four experimental groups. Values as expressed as mean $\pm$ SEM (n=7–12 per group). * $p < 0.05$ compared to the other quadrants or cage; # $p < 0.05$ compared to vehicle-treated controls; $\square$ $p < 0.05$ compared to PTZ-treated animals; + $p < 0.05$ compared to diazepam-treated animals (Newman-Keuls post-hoc test).