Behavioural Brain Research 312 (2016) 93–101

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Towards the development of improved tests for negative symptoms of schizophrenia in a validated animal model

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HIGHLIGHTS

- Sub-chronic PCP (sc PCP) treatment reduces optimism in response to ambiguous stimuli in an optimistic bias paradigm.
- Sc PCP induces indifference to a high-value reward in an affective bias paradigm.
- These behavioural tests have potential to evaluate efficacy of novel compounds for negative symptoms of schizophrenia.

ARTICLE INFO

Article history:
Received 11 May 2016
Received in revised form 9 June 2016
Accepted 11 June 2016
Available online 14 June 2016

Keywords:
Schizophrenia
Negative symptoms
Animal model
NMDA receptor
PCP
Affective and cognitive bias

ABSTRACT

Negative symptoms in schizophrenia remain an unmet clinical need. There is no licensed treatment specifically for this debilitating aspect of the disorder and effect sizes of new therapies are too small to make an impact on quality of life and function. Negative symptoms are multifactorial but often considered in terms of two domains, expressive deficit incorporating blunted affect and poverty of speech and avolition incorporating asociality and lack of drive. There is a clear need for improved understanding of the neurobiology of negative symptoms which can be enabled through the use of carefully validated animal models. While there are several tests for assessing sociability in animals, tests for blunted affect in schizophrenia are currently lacking. Two paradigms have recently been developed for assessing negative affect of relevance to depression in rats. Here we assess their utility for studying negative symptoms in schizophrenia using our well validated model for schizophrenia of sub-chronic (sc) treatment with Phencyclidine (PCP) in adult female rats. Results demonstrate that sc PCP treatment produces a significant negative affect bias in response to a high value reward in the optimistic and affective bias tests. Our results are not easily explained by the known cognitive deficits induced by sc PCP and support the hypothesis of a negative affective bias in this model. We suggest that further refinement of these two tests will provide a means to investigate the neurobiological basis of negative affect in schizophrenia, thus supporting the assessment of efficacy of new targets for this currently untreated symptom domain.

1. Introduction

Despite the wide range of antipsychotic medication available for the treatment of schizophrenia, negative symptoms remain an unmet clinical need [43]. A recent large meta-analysis of 168 studies demonstrated small effect sizes of current therapeutic strategies, ranging from atypical antipsychotics and antidepressants to psychological interventions, that failed to reach the threshold for clinically meaningful improvement [21]. Persistent negative symptoms have a substantial impact on patients' functional outcome, leading to disability and reduced quality of life [20]. Davis et al. provide a recent update on new pharmacological treatments for negative symptoms including examining important methodological consideration for future clinical trials [16]. Recent on-going clinical trials are reviewed in Ref. [5]. However in spite of recent advances in this area, effective therapies are still lacking. Carefully validated animal models combined with appropriate tests for negative symptoms in animals form a critical part of the development of new therapeutic strategies for negative symptoms [6,39]. However, many aspects of negative symptom domains have been poorly investigated in animal studies and when they have,

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http://dx.doi.org/10.1016/j.bbr.2016.06.021
0166-4328/

the most successful work has been conducted on the social withdrawal domain in rats [23,37–39,44]. These studies are certainly valuable and of ethological relevance in such a gregarious species. However such studies lack analysis of emotional regulation or affective state, processes essential for effective human social interaction and communication. There have been some attempts to investigate anticipatory motivation in animals, but the relevance of these tests for schizophrenia symptomatology is debatable [6]. Perhaps surprisingly, the other debilitating aspects of negative symptoms have almost never been investigated in a well-validated animal model for schizophrenia. That is of course partly due to the uniquely human characteristics of certain domains such as poverty of speech and blunted affect. However, there is still a major gap in the animal studies addressing other aspects of negative symptoms and with carefully developed tests even these aspects may be studied. In order to improve our understanding of the neurobiological mechanisms underlying negative symptom domains and therefore develop improved treatment strategies, it is essential to assess these using carefully constructed test procedures in well validated animal models for the disorder.

Although negative symptoms are defined by five consensus-based domains, two have been especially considered as determinant factors in terms of best describing this facet of the illness. The concept is called the *'two factor model'*. One of the factors in this model reflects 'diminished motivation and pleasure' and the other is related to 'diminished verbal/non-verbal expression or communicative output' [27] describing avolition and expressive deficit domains. As we suggested in our 2014 review, there is a clear argument for separate tests for each specific domain of negative symptoms [39] as MATRICS have recommended for cognition [45]. Sub-chronic (sc) treatment with phencyclidine (PCP) and other *N*-methyl-D-aspartate receptor (NMDAR) antagonists have been widely used in an attempt to mimic certain behavioural and neuropathological deficits observed in schizophrenia [34,36,38,39] with animals tested at least 7 days following the PCP treatment regimen. We have demonstrated that these deficits are reversed by several novel targets and low doses of atypical antipsychotics [22,28,38,39]. When it comes to investigating negative symptoms, we and others have shown that sc PCP induces social withdrawal but not anhedonia in rodents [29,39] with the growing consensus that anhedonia *per se*. is not a feature of negative symptoms [6,19] lending further support for the validity of this model. However, there have been no studies to date demonstrating the effect of sc treatment with PCP or other NMDAR antagonists, probably the best validated animal model for schizophrenia, albeit still requiring further validation, on anticipatory pleasure/motivation or on affective state. Therefore, we aimed to investigate the effect of our sc PCP treatment regime (2 mg/kg i.p. twice daily for 7 days followed by 7 days washout) on anticipatory motivation and affective state in female rats. To address this issue, we employed two different paradigms: affective and cognitive bias tests. To our knowledge, this is the first study attempting to model anticipatory reward and affect in an animal model for schizophrenia.

The task of optimistic cognitive bias used in our study was developed by [13] based on earlier studies with starlings [12,11,30]. The rationale for the task developed by [13] and variations used by other laboratories, was based on Harding's [24] original study. In this study, the concept of cognitive/judgement bias, defined as the propensity of a subject to show behaviour indicating anticipation of either relatively positive or negative outcomes in response to ambiguous stimuli [35] was first introduced in animals [24]. In their work, [13] devised an ethologically relevant methodology for investigating the effect of environmental enrichment on optimistic cognitive bias in rats. They showed that rats transferred from un-enriched to enriched conditions gave more optimistic responses to an ambiguous stimulus than control rats maintained in un-enriched conditions. They argued that testing cognitive bias in this way could provide information on an animal's emotional state and that this method could be used to develop novel therapeutic strategies for mood disorders. We have adapted this test to assess optimistic cognitive bias in our animal model for schizophrenia, sc PCP treatment.

Recently Emma Robinson's laboratory (41) developed a novel behavioural test to measure changes in the affective state of rodents. This test, known as the Affective Bias Test (ABT) is another ethologically relevant test in which animals form an association between an odour and food reward. In this study, strength and valence (positive or negative) of this relationship is altered using pharmacological and environmental manipulations. Replicating findings in healthy human volunteers, this study showed that acute treatment with typical (i.e. fluoxetine, citalopram) and atypical antidepressants (i.e. agomelatine, mirtazapine) induces a significant positive affective bias in healthy rats. Furthermore, certain drugs associated with inducing negative affective state in humans (eg. the anxiogenic agent, FG7142) also induced a negative affective bias in rats as indicated by significantly fewer choices for the odour paired with these agents. This paradigm was also found to be sensitive to manipulation of the absolute reward value. These findings support the translational and predictive validity of the ABT [41].

Our overall aim in this study is to investigate the potential of these two tests to assess affect and anticipatory reward deficits of relevance to schizophrenia. In order to achieve this aim, we adapted Brydges's paradigm to investigate the effect of our sc PCP treatment regimen on anticipatory behaviour of rats in a task of optimistic cognitive bias. Secondly, we investigated effects of sc PCP treatment on affective bias using the ABT. Due to the success of our initial study, we believe we have identified two paradigms that could be used with some further adaptations to enhance understanding of these two distinct aspects of negative symptom domains and assist in the development of new therapies. These tests could also be used to assess affective bias and anticipatory motivation in various animal models for other CNS disorders [26]. However these paradigms have certain limitations that should be taken into careful consideration before adopting them for this purpose. The use of one animal model is another limitation and we recommend always testing efficacy of new targets in several animal models representing different risk factors for the disorder eg. pharmacological, neurodevelopmental and genetic.

2. Experimental procedures

2.1. Animals

Two separate batches of 20 and 14 adult female Lister Hooded rats obtained from Harlan UK, were used as subjects for experiments 1 and 2 respectively, 34 rats were used in total. Rats were housed in groups (4–5 rats/cage) and kept under standard laboratory conditions (room temperature $21 \pm 2\,^{\circ}C$ and humidity 40%–50%) on a 12-h light/dark cycle with lights on at 0700 h. All experiments were carried out between 9 am and 5 pm. Water was available ad libitum, but food was restricted to 10 g per rat per day for the duration of both experiments. Female rats were used as they have consistently demonstrated reliable performance in our laboratory in a variety of cognitive tests at all stages of the oestrus cycle [33,42], and robust deficits following our sc PCP regimen [39]. There has been an unfortunate over-reliance on male rodents in animal studies in the past, a situation fortunately being rectified due to a new and overdue NIH directive [15]. All experiments were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986 and were approved by the University of Manchester ethics committee.