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The social defeat/overcrowding murine psychosocial stress model results in a pharmacologically reversible body weight gain but not depression - related behaviours

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ABSTRACT

Depression is a highly prevalent psychiatric disorder, yet its etiology is not well understood. The validation of animal models is therefore a critical step towards advancing knowledge about the neurobiology of depression. Psychosocial stress has been promoted as a prospective animal model of depression, however, different protocols exist with variable responses, and further investigations are therefore required. We aimed to characterise the behavioural and body weight responses to the social defeat/overcrowding (SD/OC) model and to explore the effects of the antidepressant fluoxetine and the peroxynitrite scavenger, Cu^{II}(atsm), therein. Male C57BL/6JArc mice were exposed to a 19 day SD/OC protocol at two levels of aggression, determined by terminating SD bouts after one, or approximately five social defeat postures. This was followed by a battery of behavioural tests including social interaction test (SIT), locomotor activity (LMA), light-dark box test (LDB), saccharin preference test (SPT) and the forced swim test (FST). Mice were dosed daily with vehicle, fluoxetine (20 mg/kg) or Cu^{II}(atsm) (30 mg/kg) throughout the protocol. SD/OC increased body weight compared to controls, which was abolished by fluoxetine and attenuated by Cu^{II}(atsm). Weight gain specifically peaked during OC sessions but was not affected by either drug treatment. Fluoxetine reduced the number of defeat postures during fight bouts on some days. SD/OC otherwise failed to elicit depression- or anxiety-like behaviour in the tests measured. These data raise questions over the SD/OC model as an etiological model of depression-related behaviours but highlight the potential of this model for investigations into mechanisms regulating binge eating and weight gain under conditions of chronic social stress.

1. Introduction

Depression is a debilitating and highly prevalent psychiatric disorder characterised primarily by a depressed mood, loss of interest or pleasure in everyday activities and significant changes in weight or appetite (American Psychiatric Association, 2013). Behind HIV/AIDS, depression is the second largest contributor to the global burden of disease and is predicted to maintain that position through to 2030 (Mathers and Loncar, 2006). Despite this, the etiology of depression remains to be adequately elucidated. Several reasons have contributed to this lack of understanding, including that depression is a complex, heterogeneous disorder with multiple factors contributing to its development (Berton et al., 2012; Krishnan and Nestler, 2008). Furthermore, due to the subjective nature of depression, aspects of its symptomatology have proven challenging to replicate in animal models of the disorder (Cryan and Holmes, 2005; Krishnan and Nestler, 2008; Nestler and Hyman, 2010). The improvement of animal models is therefore an essential step towards advancing the understanding of the neurobiology of depression and the development of effective treatments. For an animal model to be considered valid the symptoms induced must be reasonably analogous to those observed in humans (face validity), the treatments effective in humans must also be effective in the animal model (predictive validity) and the model and human disease should have identical causative factors (construct validity) (McKinney and Bunney, 1969; Slattery and Cryan, 2014). Several rodent models of depression have been developed over the past few decades including chronic mild stress (CMS) (Willner, 1997), olfactory bulbectomy (Harkin et al., 2003), maternal deprivation (Levine et al.,

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1991; Marco et al., 2009; Schmidt et al., 2011) and chronic restraint stress paradigms (Christiansen et al., 2011; Sadler and Bailey, 2016). However, many of these models either fail to reproduce some of the core symptoms of depression, do not satisfy the aforementioned requirements for a valid animal model, or suffer from poor cross-reliability between laboratories.

An emerging class of animal models of depression utilise psychosocial stress paradigms comprising repeated social defeats. Social defeat models involve placing an animal into the home cage of an aggressive resident, enabling physical defeat and subordination from the defeated animal. Such models have demonstrated central aspects of face, predictive and construct validity (Berton et al., 2006; Tsankova et al., 2006) whereby treatment with clinical antidepressants reversed behavioural and physiological effects induced by the model. Depending on the model, mice may be exposed to the resident aggressor for periods of up to between 10 min (for a detailed protocol see Golden et al., 2011) and 2 h (Savignac et al., 2011b). This increases the opportunity for fight wounds to develop (see Golden et al., 2011; and Savignac et al., 2011b) which potentially contribute pain and inflammation to the psychosocial stress (Pryce and Fuchs, 2017). The social defeat/overcrowding (SD/OC) model is an alternative protocol, where mice are separated by barriers following the initial defeat, therefore reducing the opportunity for injuries to occur, and are also intermittently subjected to unpredictable periods of overcrowding with other defeated mice (Finger et al., 2011, 2012; Reber et al., 2006; Tramullas et al., 2012). Studies utilising this model have demonstrated increased social avoidance in the social interaction test (SIT), increased immobility time in the forced swim test (FST), anxiety-like behaviour in the light-dark box test (LDB) as well as alterations in body weight, protein levels, gene expression and other physiological functions (Finger et al., 2011, 2012; Reber et al., 2006; Tramullas et al., 2012). However, results across laboratories have been varied, calling in to question the reproducibility of the model. Additionally, the predictive validity of this model has yet to be ascertained. Further characterisation of this model using a clinically effective antidepressant would be a crucial step towards its more widespread use in preclinical depression research.

To this end, we aimed to further characterise the SD/OC model in male C57BL/6JArc mice, including analyses of behaviour during SD sessions and a battery of behavioural tests for depression and/or anxiety-like behaviours at the conclusion of the SD/OC protocol. We also aimed to assess the effects of the clinically effective antidepressant fluoxetine, and the potential antidepressant properties of Cu^{II}(atsm), in the SD/OC model. Cu^{II}(atsm) is a member of the bis(thiosemicarbazone) (BTSC) class of compounds. BTSCs are stable, low molecular weight compounds capable of crossing cell membranes including the blood-brain barrier (BBB) (Fodero-Tavoletti et al., 2010). BTSCs show therapeutic potential in neurodegenerative diseases (Hung et al., 2012; Kenche and Barnham, 2011; Roberts et al., 2014) via mechanisms that include the scavenging of the highly reactive nitrogen free radical, peroxynitrite (reviewed in Mckenzie-Nickson et al., 2016). Recent evidence suggests that depression is associated with neuroinflammatory and oxidative/nitrosative stress mechanisms (Hannestad et al., 2011; Heneka et al., 2014; Hurley and Tizabi, 2013; Maes et al., 2009, 2011; Raedler, 2011). Targeting nitrosative stress has also previously been demonstrated to have antidepressant effects in animal models (Doucet et al., 2013; Harkin et al., 1999; Peng et al., 2012). We therefore sought to assess Cu^{II}(atsm) in the SD/OC model due to its anti-neuroinflammatory and anti-nitrosative stress properties.

2. Materials and methods

2.1. Animals

Two separate trials were conducted using male C57BL/6JArc mice (Trial 1: n = 48, 10 weeks of age; Trial 2: n = 40, 9 weeks of age). Mice of various strains (SJL (48% of total bouts fought), Swiss (23%), SJL/BL6 (15%), C57BL/6 (6%), CD1 (5%), Sv129 (3%)) and age (9–52 weeks of age) were used as resident aggressors in Trial 1. In Trial 2 aggressor mice used were almost exclusively of the SJL strain (92%; 17–24 weeks of age). All mice were purchased from Animal Resources Centre (C57BL/6JArc, SJL; Canning Vale, WA, Australia) or bred in-house at The Florey Institute of Neuroscience (Melbourne, Victoria, Australia). All mice involved in the study were single housed for at least one week prior to commencement of their respective trials and remained single housed for the duration of the experiment (except during the overcrowding procedures). Experimental mice were housed in standard open top cages (26.5 × 14 × 12 cm) and aggressor mice in standard transparent cages (29.5 × 16 × 13 cm), both with sawdust bedding and tissue paper nesting material. The holding room was temperature controlled (18.5 ± 1 °C) and under a 12 h light/dark cycle (lights on at 0700 h). Standard rodent food and water was available *ad libitum*. All experimentation was performed in accordance with the Prevention of Cruelty to Animals Act (2004), under the guidelines of the National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia (2013) and approved by The Florey Animal Ethics Committee (AEC number: 15–020). All efforts were made to minimise animal suffering.

2.2. Social defeat/overcrowding (SD/OC) protocol

The SD/OC protocol was carried out as previously described (Finger et al., 2011, 2012; Reber et al., 2006). All aggressor mice were screened for aggressive behaviour on at least three individual days prior to the first day of experiments. The mice were exposed to a test C57BL/6 intruder until the first attack followed by defeat posture (Fig. 1) (Miczek et al., 1982), or for a maximum of 10 min. Mice displaying the shortest attack latencies were chosen as aggressors for the trials. Experimental C57BL/6JArc mice were randomly assigned to one of four groups: 1. No SD/OC and treated with vehicle (Control (Con)), 2. Exposed to SD/OC and treated with vehicle (Veh), 3. SD/OC with fluoxetine treatment (Fluox) or 4. SD/OC with Cu^{II}(atsm) treatment (Cu(atsm)) (Trial 1: n = 12; Trial 2: n = 10 per group). Control mice remained undisturbed except for daily oral gavaging. SD/OC mice were exposed to a 19 day unpredictable stress protocol consisting of social defeat (SD) and overcrowding (OC) sessions (Fig. 2). SD sessions were carried out once (on days 1, 2, 4–7, 9, 12 and 19) or twice (on days 8 and 16) per day. Experimental mice never encountered the same aggressor mouse more than once. Aggressors were ranked according to latency to attack and pseudorandomly assigned to ensure a balanced and consistent presentation of aggression. SD sessions consisted of placing the experimental mouse into the home cage of the aggressor and allowing interaction until defeat posture/s were displayed by the experimental mouse (Fig. 1). The mice were then separated by a wire mesh barrier for 2 h to allow visual, auditory and olfactory, but not physical contact. After 2 h the barrier was removed and another bout and defeat was allowed to take place. The thirteen SD sessions were thus comprised of two SD bouts each, resulting in a total of 26 SD bouts across the protocol. The number of defeat postures displayed was measured for each of these 26 SD bouts. In Trial 1, SD bouts were terminated following one defeat posture regardless of whether an attack occurred or not. In Trial 2, SD bouts were terminated following at least five defeat postures and definitive attack behaviour from the aggressor. If attacks were particularly ferocious and containing extensive biting behaviour by the aggressor, the SD bout was terminated early. All SD bouts were filmed (side-on, four cages at a time) using a standard video camera. Number and latency of defeat posture and latency to attack in Trial 2 were quantified from video recordings. For the overcrowding protocol all mice from one treatment group were housed together in a standard transparent cage for 24 or 48 h with free access to food and water. No injuries were observed in any of the overcrowded mice. Overcrowding occurred on days 3–4, 10–11, 13–15 and 17–18 (Fig. 2).