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Mouse social stress induces increased fear conditioning, helplessness and fatigue to physical challenge together with markers of altered immune and dopamine function

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ABSTRACT

In neuropsychiatric research, animal studies that demonstrate causal effects of environmental manipulations relevant to human aetiological factors on emotional and cognitive behaviours relevant to human psychopathologies are valuable. Such valid animal models can be useful for improved understanding of aetio-pathophysiology and preclinical discovery and development of new treatments. In depression, specific uncontrollable stressful life events are major aetiological factors, and subsequent generalized increases in fearfulness, helplessness and fatigue are core psychopathology symptoms or features. In the present study we exposed adult male C57BL/6 mice to an environmental manipulation of 15-day psychosocial stress with loss of social control but with minimal physical wounding. One cohort of stressed and control mice was assessed in a novel 3-day test paradigm of motor activity, fear conditioning and 2-way avoid-escape behaviour on days 16–18, and a second cohort was assessed in a treadmill fatigue paradigm on days 19 and 29, followed by the 3-day paradigm on days 30–32. All tests used a physical aversive stimulus, namely mild, brief electroshocks. The socially stressed mice displayed decreased motor activity, increased fear acquisition, decreased 2-way avoid-escape responding (increased helplessness) and increased fatigue. The socially stressed mice also displayed increased blood levels of the pro-inflammatory cytokine TNF and spleen hypertrophy, and adrenal hypertrophy without hypercorticoidism. In a third cohort, psychosocial stress effects on brain gene expression were assessed using next generation sequencing. Gene expression was altered in pathways of inflammation and G-protein coupled receptors in prefrontal cortex and amygdala; in the latter, expression levels of a number of genes important in dopamine function were de-regulated including down-regulated *Drd2*, *Adora2a* and *Darpp-32*. Therefore, in a mouse model informed by depression aetiology and psychopathology, it was possible to demonstrate that chronic uncontrollable social stress leads to decreased motor activity and increased fear conditioning, helplessness and fatigue, each under physical aversive challenge, and to identify immune-inflammation and dopamine-function markers that were associated with these behavioural changes. This model can be applied to identify novel targets for treatment of specific psychopathologies such as generalized helplessness or fatigue, and to conduct preclinical screening of the compounds/biologics developed to act at these targets.

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1. Introduction

Depression has core symptoms of low mood, fatigue and anhedonia (ICD-10, 1994). The low mood core symptom involves the patient focussing on and emphasising aversive stimuli and events. The stressful life events that precede depression, e.g. psychosocial, financial, health, are often uncontrollable (Kendler et al., 2003; Kessler, 1997). It has been proposed that learned uncontrollability of one life event (specific helplessness) can become generalized to other life events regardless of their controllability, i.e. generalized helplessness (Abramson et al., 1989, 1978; Beck et al., 1974; Diener et al., 2009; Maier and Seligman, 1976; Pryce et al., 2011). Generalized helplessness is proposed to comprise increased emotional responding to, reduced motivation to actively cope with, and reduced cognitive (response-outcome) expectancy to be able to cope with/control, aversive events; these states are clearly linked to low mood. Experimental evidence for these states include increased fear conditioning to stimuli that predict uncontrollable electroshock (Nissen et al., 2010), and increased helplessness during and after exposure to uncontrollable electroshock (Diener et al., 2009; Strigo et al., 2008), in depressed patients relative to healthy controls. The core symptom of fatigue is also complex and comprises experiencing physical and cognitive tasks as requiring extreme effort (Demyttenaere et al., 2005). The aetiopathophysiology of these important psychopathology symptoms and features is poorly understood. Understanding might be enhanced by the development and study of animal models that comprise (1) exposure to stressors that are relevant to human life events; (2) demonstrable effects of this stress on behaviour in tests that measure fear responding to aversive stimuli, helplessness when confronted with controllable aversive stimuli, and physical fatigue in an aversive environment. Such animal models would have aetiological and face validity for these depression psychopathologies (Cuthbert and Insel, 2013; Hyman, 2012; Markou et al., 2009).

There are currently two animal models of depression psychopathologies that are particularly widely used. In chronic unpredictable mild stress, rats or mice are exposed for 21–28 days to various physical and social stressors on an unpredictable schedule, and the major readouts are tests of reward sensitivity such as the sucrose preference test. Reward sensitivity is reduced, thereby providing a rodent model of stress-induced decreased interest (anhedonia) (Tye et al., 2013; Willner, 1997). In chronic social defeat (CSD), mice are exposed to ten days of daily 10-min attack by aggressive dominant mice, and otherwise there is continuous distal exposure to this psychosocial stressor. Stressed CSD mice display submissive behaviour but this fails to deter/control attacks by the dominant mice (Kudryavtseva et al., 1991). Bite wounds are common in the standard CSD protocol (Golden et al., 2011), somewhat impacting on its aetiological validity as an emotional psychosocial stressor. Readout tests of CSD effects have focussed on passive avoidance of the aggressor mouse strain in a social proximity test (Krishnan et al., 2007; Savignac et al., 2011b); that is, on increased emotional reactivity to the specific learned uncontrollable social stimulus (Russo et al., 2012).

We previously established a specific learned helplessness paradigm in mice using 2-way escape behaviour of mild, short electroshocks (Pryce et al., 2012). Mice exposed to electroshocks that can be terminated (controlled) by transfer from one side of an arena to the other readily learn this escape behaviour and maintain a high motivation (motor reactivity) across repeated electroshocks. Mice exposed to identical electroshocks in terms of duration, intensity and interval but now inescapable, i.e. no response-outcome contingency in terms of electroshock termination, exhibit progressively decreasing motivation (reduced motor reactivity) to control the aversive stimulus. When subsequently challenged with escapable shocks they exhibit few escape attempts and fail to learn from reinforced escape responses (Pryce et al., 2012). Using the same apparatus, one aim of the present study was to investigate whether CSD, which constitutes lack of social control, leads to generalized helplessness i.e. altered emotional, motivational and cognitive responding to another form of aversive stimulus, namely electroshock. Specifically, a 3-day test paradigm was used: motor activity test on day 1, contextual fear conditioning on day 2, and 2-way avoid-escape test on day 3. The motor activity test assessed whether CSD decreased activity and/or increased fear-freezing behaviour in a neutral environment; contextual fear conditioning assessed whether CSD increased fear-freezing reactivity to uncontrollable electroshock; the 2-way avoid-escape test assessed whether CSD decreased motivation to control the aversive stimulus (motor reactivity to electroshock) and decreased cognitive expectancy to control the aversive stimulus (avoid-escape learning). Mice were tested sequentially in the different tests across days, so that for the later tests it was the effects of CSD on responses to (coping with) successive physical challenges that was under study. Whilst fatigue is a core symptom of depression, there does not appear to be an animal model for psychosocial stress induced increased fatigability. Using a treadmill combined with electroshock, fatigue induced by enforced running has been demonstrated in mice, and this method has been applied to study factors regulating fatigability, included proinflammatory cytokine administration, for example (Carmichael et al., 2006). Therefore, another aim of the present study was to investigate whether CSD induces increased fatigability under conditions of enforced treadmill running to avoid electroshock. The same mice were subsequently also investigated in the 3-day generalized helplessness paradigm.

A final aim of this study was to investigate CSD effects on specific physiological and molecular genetic parameters that might contribute to the causation of any depression-relevant behavioural effects. One hypothesis of depression aetio-pathophysiology is psychosocial stress activation of peripheral and central immune-inflammation, leading to oxidative stress and neurotoxic disruption of several neurotransmitters including serotonin, dopamine, glutamate and GABA (Dantzer et al., 2008; Felger and Miller, 2012). The proinflammatory cytokines of tumor necrosis factor (TNF) and interleukin-6 (IL-6) are increased in a majority of depression patients relative to matched controls (Dowlati et al., 2010; Maes, 2010). To render CSD aetiologically valid with respect to stress induced inflammation it was essential to introduce refinements to prevent the bite wounding that is frequent in the standard CSD protocol (Golden et al., 2011). The peripheral immune-inflammation markers of plasma TNF and IL-6 levels and spleen mass were measured. Markers of glucocorticoid function were also measured, namely adrenal gland mass and plasma and faecal corticosterone levels. Whilst a number of studies have reported increased basal plasma cortisol levels in a subset of depressed patients relative to matched controls (Brown et al., 2004), there is also evidence for decreased plasma cortisol in depression and other stress-related disorders (Silverman and Sternberg, 2012). Another rationale for studying adrenal/corticosterone status is the evidence that stress can induce glucocorticoid resistance which in turn results in attenuation of its anti-inflammatory function (Rhen and Cidlowski, 2005; Silverman and Sternberg, 2012). Next generation sequencing and canonical pathway analysis were applied to conduct a hypothesis-free, transcriptome-level analysis of effects of CSD on gene expression in specific brain regions. The regions selected for study, ventral hippocampus, medial prefrontal cortex, and central and basolateral nuclei of amygdala, are fundamental to the neurocircuitries underlying the behaviours under study here (Amat et al., 2005; Maren et al., 2013; Moscarello and LeDoux,

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