Please cite this article in press as: Brzózka MM et al. OSO paradigm – A rapid behavioral screening method for acute psychosocial stress reactivity in mice. Neuroscience (2015), http://dx.doi.org/10.1016/j.neuroscience.2015.11.043

Neuroscience xxx (2015) xxx–xxx

OSO PARADIGM – A RAPID BEHAVIORAL SCREENING METHOD FOR ACUTE PSYCHOSOCIAL STRESS REACTIVITY IN MICE

M. M. BRZÓZKA,[a*] T. UNTERBARNSCHEIDT,[b,c] M. H. SCHWAB[b,c] AND M. J. ROSSNER[a,b*]

[a] *Department of Psychiatry, Ludwig-Maximilian-University, Nussbaumstrasse 7, 80336 Munich, Germany*

[b] *Max Planck Institute of Experimental Medicine, Hermann-Rein-Strasse 3, 37075 Göttingen, Germany*

[c] *Cellular Neurophysiology, Hannover Medical School, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany*

Abstract—Chronic psychosocial stress is an important environmental risk factor for the development of psychiatric diseases. However, studying the impact of chronic psychosocial stress in mice is time consuming and thus not optimally suited to 'screen' increasing numbers of genetically manipulated mouse models for psychiatric endophenotypes. Moreover, many studies focus on restraint stress, a strong physical stressor with limited relevance for psychiatric disorders. Here, we describe a simple and a rapid method based on the resident–intruder paradigm to examine acute effects of mild psychosocial stress in mice. The OSO paradigm (*o*pen field – *s*ocial defeat – *o*pen field) compares behavioral consequences on locomotor activity, anxiety and curiosity before and after exposure to acute social defeat stress. We first evaluated OSO in male C57Bl/6 wildtype mice where a single episode of social defeat reduced locomotor activity, increased anxiety and diminished exploratory behavior. Subsequently, we applied the OSO paradigm to mouse models of two schizophrenia (SZ) risk genes. Transgenic mice with neuronal overexpression of *Neuregulin-1* (*Nrg1*) type III showed increased risk-taking behavior after acute stress exposure suggesting that NRG1 dysfunction is associated with altered affective behavior. In contrast, *Tcf4* transgenic mice displayed a normal stress response which is in line with the postulated predominant contribution of *TCF4* to cognitive deficits of SZ. In conclusion, the OSO paradigm allows for rapid screening of selected psychosocial stress-induced behavioral endophenotypes in mouse models of psychiatric diseases. © **2015 Published by Elsevier Ltd. on behalf of IBRO.**

Key words: OSO paradigm, acute psychosocial stress, social defeat, psychiatric endophenotypes, *Neuregulin-1* (*Nrg1*), *Transcription factor 4 (Tcf4)*.

INTRODUCTION

Converging lines of evidence suggest that most psychiatric diseases are caused by interactions of environmental circumstances with risk genes determining vulnerability for mental illness (European Network of Schizophrenia Networks for the Study of Gene-Environment Interactions, 2008; van Os et al., 2008; Gillespie et al., 2009; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; European Network of National Networks studying Gene-Environment Interactions in Schizophrenia (EU-GEI) et al., 2014).

Psychosocial stress is one of the most crucial environmental factors contributing to the development of psychiatric diseases in vulnerable individuals (Kessler, 1997; McEwen, 2000; Lataster et al., 2006; reviewed in: Lupien et al., 2009;). In preclinical research, different experimental approaches using a variety of stress paradigms exist; however, many of them do not resemble conditions under which social stress-related diseases develop (Tamashiro et al., 2005). The most promising stress models for investigation of psychiatric diseases are those where the stressor is of social nature, a condition more relevant to the situation in humans as compared to common laboratory stressors such as restrain stress, forced swim or a footshock (Björkqvist, 2001; Tamashiro et al., 2005; Thomas et al., 2007; Bartolomucci and Leopardi, 2009; Bartolomucci et al., 2009; Slattery et al., 2012; Vasconcelos et al., 2015). The resident–intruder paradigm is one of the most popular models of chronic social defeat (Koolhaas et al., 1997; Sakai and Tamashiro, 2005) which resembles bullying at school and workplaces, whereby the bully represents the dominant and the victim the submissive part of the conflict (Björkqvist, 2001). This setup is in analogy to the resident and intruder, respectively. Moreover, the resident–intruder paradigm displays some parallels with an experimental assessment of acute stress response in humans, such as the Trier social stress test (Kirschbaum et al., 1993), combining social-evaluative threat and uncontrol-

*Corresponding authors. Tel: +49-89-4400-52737 (M. M. Brzózka). Address: Department of Psychiatry, Ludwig-Maximilian-University, Nussbaumstrasse 7, 80336 Munich, Germany. Tel: +49-89-4400-55891 (M. J. Rossner).
E-mail addresses: Magdalena.Brzozka@med.uni-muenchen.de (M. M. Brzózka), Unterbarnscheidt.Tilmann@mh-hannover.de (T. Unterbarnscheidt), Schwab.Markus@mh-hannover.de (M. H. Schwab), Moritz.Rossner@med.uni-muenchen.de (M. J. Rossner).

Abbreviations: HPA, hypothalamus-pituitary adrenal; OF, open field test; OSO, *o*pen field – *s*ocial defeat – *o*pen field; RMs, repeated measures; SZ, schizophrenia.

http://dx.doi.org/10.1016/j.neuroscience.2015.11.043
0306-4522/© 2015 Published by Elsevier Ltd. on behalf of IBRO.

lability, resulting in social defeat and reliable simulation of biomarkers of psychosocial stress including response in hypothalamus-pituitary adrenal (HPA) function (Kirschbaum et al., 1993; Foley and Kirschbaum, 2010).

In rodents, a single social defeat leads to substantial metabolic responses (Keeney et al., 2006), reduction of the survival of new born hippocampal neurons (Thomas et al., 2007) and subtle alterations in behavior (summarized in: Bartolomucci et al., 2004). When applied chronically, social defeat causes long persisting behavioral changes such as deficits in prepulse inhibition, anhedonia, diminished motivation and exploratory drive, and increased anxiety (Bartolomucci et al., 2004, 2005; Rygula et al., 2005; Adamcio et al., 2009; Brzózka et al., 2011; Badowska et al., 2015). Such psychosocial stress-induced behavioral alterations in animals are analogous to symptoms observed in psychiatric diseases (Kessler, 1997; Blanchard et al., 2001; Thomas et al., 2007). In humans, increased emotional reactivity to stress has been observed in psychosis patients and their first-degree relatives (Myin-Germeys et al., 2001), suggesting its genetic determination (Lataster et al., 2009, 2010, 2013). Thus, altered reactivity to acute stress might be an indicator for psychiatric endophenotypes in animal models relevant for psychiatric diseases.

In the present manuscript, we describe a simple and quick method to address the effects of acute social defeat on locomotor activity, anxiety and exploratory behavior. The OSO paradigm (*o*pen field – *s*ocial defeat – *o*pen field) allows examining murine behavior immediately before and after an acute episode of psychosocial stress. The OSO-paradigm does neither require sophisticated nor costly equipment; needed only is an open field arena which is available in a vast majority of behavioral laboratories. Another advantage of this method is that it can be completed within one day. For the proof of the concept study, we exposed C57Bl/6 mice to the OSO paradigm and recorded behavioral alterations after stress exposure characteristic for social defeat. Furthermore, due to the fact that (i) stress reactivity is partially genetically determinated (Lataster et al., 2013), and (ii) a number of human genes have been associated with stress resilience (reviewed in: Wu et al., 2013), we investigated two different mouse models of schizophrenia (SZ): Nrg1-overexpressing mice (*Nrg1*tg) (Velanac et al., 2012; Agarwal et al., 2014) and Tcf4-overexpressing mice (*Tcf4*tg) (Brzózka, 2009, 2010, 2015; Brzózka and Rossner, 2013) in order to examine their behavioral response to acute social defeat.

Therefore, we present the OSO-paradigm as a simple and rapid 'screening' method to approach the complexity of G × E interactions by exposure of genetically manipulated mice to acute psychosocial stress and suggest to include it into the standard battery of behavioral tests as an additional readout.

EXPERIMENTAL PROCEDURES

Mice

All mice were housed with food/water *ad libitum* in 12-h light/dark cycle and room temperature of 21 ± 2 °C. Prior to the OSO paradigm, one-year-old male FVB/N mice (Charles River Laboratories, Sulzfeld, Germany) were tested for aggressive behavior. An unfamiliar intruder mouse was placed in the cage of a FVB/N male and the attack latencies (defined as biting or an attempt to bite) were measured. Only FVB/N males which showed immediate attack (< 5 s) served as residents in the stress paradigm. Residents were housed individually from the age of 2 months onward.

Initially all experimental mice were reared in groups in order not to affect their behavior significantly by early isolation rearing. To allow habituation to the novel home cage, experimental animals were housed individually one week prior to the OSO paradigm.

In the proof of concept study (Experiment 1 and Experiment 2) we used two independent cohorts of adult male C57Bl/6N wildtype (wt) mice (Charles River, Sulzfeld, Germany). In Experiment 1 we employed 19-week-old animals (body weight 28–33 g) assigned into control (n = 22) or stress groups (n = 22). In Experiment 2, we used mice of age between 18 and 22 weeks (body weight 27–35,5 g) subdivided into control (n = 11) and stress (n = 14) group.

Subsequently, we evaluated the OSO paradigm in mouse models for two SZ risk genes.

In Experiment 3, we used in house bred adult (12–16 weeks old, body weight 25–28 g) transgenic male C57Bl/6 mice overexpressing NRG1 type III (*Nrg1*tg) and their wt littermates (Velanac et al., 2012) subdivided into experimental groups as follows: *Nrg1*tg control (n = 8), *Nrg1*tg stress (n = 10); wt control (n = 12), wt stress (n = 11).

In Experiment 4, we employed in-house bred transgenic adult (9–12 weeks old, body weight 22–26 g) male mice overexpressing *Tcf4* (*Tcf4*tg; n = 8) and their wt littermates (n = 14) on a F1-hybrid genetic background (offspring of FVB/N×C57Bl/6) (Brzózka et al., 2010).

OSO paradigm

In the OSO paradigm (*o*pen field – *s*ocial defeat – *o*pen field; Fig. 1a), spontaneous locomotor activity of mice is examined in the open field test (OF) for 10 min (OF1; see: Open field). Subsequently, mice are divided into 'stress' and 'control' groups. Please note that we will refer throughout the manuscript as the 'stressed' group for OF1 and OF2 although animals are non-stressed yet in OF1. Control mice are kept in their home cage for 10 min whereas the stress group is transferred to a separate room and subjected to a social defeat procedure as described previously (Brzózka et al., 2011). Briefly, a test animal ('intruder') is placed in a home cage of a highly aggressive ('resident') mouse. Mice interact freely until the first attack of the intruder by the resident. The intruder is subsequently protected from

Please cite this article in press as: Brzózka MM et al. OSO paradigm – A rapid behavioral screening method for acute psychosocial stress reactivity in mice. Neuroscience (2015), http://dx.doi.org/10.1016/j.neuroscience.2015.11.043