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Temporal profiling of an acute stress-induced behavioral phenotype in mice and role of hippocampal DRR1

Tanja Jene[a,d], Nils C. Gassen[b], Verena Opitz[a], Kristina Endres[c], Marianne B. Müller[a,d,1], Michael A. van der Kooij[a,d,*,1]

[a] Translational Psychiatry, Department of Psychiatry and Psychotherapy & Focus Program Translational Neuroscience (FTN), Johannes Gutenberg University Medical Center, Hanns-Dieter-Hüsch-Weg 19, 55128, Mainz, Germany
[b] Department of Translational Research in Psychiatry, Max Planck Institute of Psychiatry, Kraepelinstr. 2-10, 80804, Munich, Germany
[c] Department of Psychiatry and Psychotherapy, Johannes Gutenberg University Medical Center, Untere Zahlbacher Strasse 8, 55131, Mainz, Germany
[d] German Resilience Center (DRZ), Johannes Gutenberg University Medical Center Mainz, Germany

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ABSTRACT

Understanding the neurobiological mechanisms underlying the response to an acute stressor may provide novel insights into successful stress-coping strategies. Acute behavioral stress-effects may be restricted to a specific time window early after stress-induction. However, existing behavioral test batteries typically span multiple days or even weeks, limiting the feasibility for a broad behavioral analysis following acute stress. Here, we designed a novel comprehensive behavioral test battery in male mice that assesses multiple behavioral dimensions within a sufficiently brief time window to capture acute stress-effects and its temporal profile. Using this battery, we investigated the behavioral impact of acute social defeat stress (ASD) early thereafter (ASD-early, ~4 h), when circulating corticosterone levels were elevated, and late after stress-induction (ASD-late, ~8 h), when corticosterone were returned to timed control levels. ASD-early, but not ASD-late, displayed hippocampal-dependent cognitive impairments in the Y-maze and in the spatial object recognition test. The actin-binding protein (ABP) *Tumor suppressor down-regulated in renal cell carcinoma 1* (DRR1) has been described as resilience-promoting factor but the potential of DRR1 to curb stress-effects has not been investigated. Hippocampal DRR1 mRNA-expression was increased in ASD-early and ASD-late whereas DRR1-protein levels were increased only in ASD-late. We hypothesized that the absence of hippocampal DRR1 protein-upregulation in ASD-early caused the associated cognitive impairments. Hence, virus-mediated hippocampal DRR1-overexpression was induced as putative treatment, but cognitive deficits in ASD-early were not improved. We conclude that hippocampal DRR1-overexpression is insufficient to protect from the detrimental cognitive effects following acute social stress where perhaps a more global response in local actin dynamics, involving multiple stress-responsive ABPs that act synergistically, was warranted.

1. Introduction

Acute stress prepares the body for action by secretion of stress hormones. A short-lasting stressor may cause for temporary impairments but, when stress severity is within limits, homeostatic recurrence in absence of long-term negative sequelae is anticipated. In contrast, chronic and unpredictable stress inflicts many adverse health effects and may lastingly impair cognitive function, social behavior or even precipitate the occurrence of psychiatric disorders (Lupien et al., 2009). Therapeutic strategies, however, may be more effective at earlier, more dynamic stages of mental dysfunction. A recent line of thinking aims to identify potential resilience factors (Krishnan et al., 2007). Understanding how an organism actively copes with stress at an early stage may lead to novel insights into the processes underlying chronic stress-induced long-term detrimental sequelae.

To capture the behavioral effects of experimental manipulations with short-lived consequences is challenging; behavioral test batteries typically span a period of multiple days or even weeks (Kurhe et al., 2015; Lad et al., 2010; Paylor et al., 2006; Wolf et al., 2016; Yonezaki et al., 2015). Thus, in studying the transient effects of acute

* Corresponding author at: Translational Psychiatry, Department of Psychiatry and Psychotherapy, Johannes Gutenberg University Medical Center, Hanns-Dieter-Hüsch-Weg 19, 55128, Mainz, Germany.
E-mail address: m.vanderkooij@uni-mainz.de (M.A. van der Kooij).
[1] Joint last authorship.

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experimental manipulations, lengthy behavioral test batteries are unsuited. Nevertheless, a thorough behavioral characterization shortly after an acute experimental manipulation requires testing in multiple behavioral paradigms. Testing each animal on only one single behavioral test requires many more groups of animals and is both time- and cost-intensive. To circumvent these disadvantages associated with short-lived consequences of acute experimental manipulations, such as acute social stress, we designed a novel comprehensive −but brief- behavioral test battery in which multiple dimensions are assessed, including cognition, anxiety-like behavior and social behavior. The majority of the stressors in humans are social in nature. We employed acute social defeat (ASD) in mice as stress model to ensure a high degree of translational value. Animals were tested early (~4 h, ASD-early) and late (~8 h, ASD-late) after stress-induction in the behavioral test battery to encompass the temporal dynamics of the acute social defeat stress.

The impact of stress on brain and behavior might be various and is affected by many influences such as environment, type of stress or timing and duration of the stressor (Joels and Baram, 2009; Lupien et al., 2009). Previously, it was shown that a single restraint stress and multiple concurrent acute stresses lead to loss of hippocampal dendritic spines and impairments in learning and memory (Chen et al., 2016; Chen et al., 2010; Maras et al., 2014). In stress neuropathology, loss of synaptic plasticity is thought to underlie long-lasting cognitive impairments associated with stress. Social stress was found to affect spine morphology and dendritic reorganization (Iniguez et al., 2016; Kole et al., 2004). The body's stress response involves diverse stress mediators such as neurotransmitters, neuropeptides (e.g. corticotropin-releasing hormone [CRH]) or steroid hormones (e.g. corticosteroids) which have their own functional niche but may also act in concert to appropriately adapt to the prevailing stress condition (Joels and Baram, 2009). For instance, corticosterone and CRH were found to act synergistically in mediating the negative consequences of acute stress on spines and synapses, thereby impairing memory (Chen et al., 2016). On the other hand, distinct, independent effects of both CRH and corticosteroid hormones on complex behavior have been described (Müller et al., 2003). The precise contributions of single stress mediators as well as their potential interactions warrant further research.

The identification of resilience-promoting proteins that play key roles in neuroplasticity and synaptic function, such as brain-derived neurotrophic factor (BDNF) (Krishnan et al., 2007), may thus greatly add in our understanding of the neurobiological mechanisms underlying stress. Synaptic neuroplasticity requires reorganization of the cytoskeleton. In the adult brain, actin is the most prominent cytoskeletal protein at synapses, where its presence is confirmed at both presynaptic boutons as well as postsynaptic dendritic spines (Cingolani and Goda, 2008). The actin cytoskeleton is remarkably dynamic and involved in key neuronal processes such as presynaptic vesicle movement, neurotransmitter release, postsynaptic glutamate receptor trafficking and dendritic spine morphogenesis, thus influencing synaptic plasticity (Cingolani and Goda, 2008; Hotulainen and Hoogenraad, 2010; Lamprecht, 2014). Actin dynamics are governed by actin binding proteins (ABPs), which are critically important for synaptic plasticity and complex behavior, including learning and memory (Cingolani and Goda, 2008; Hotulainen and Hoogenraad, 2010; Lamprecht, 2014). More specifically, it was shown that actin rearrangements through ABP functions are indispensable for hippocampal-dependent memory formation, including spatial memory, fear memory formation and memory extinction (Fischer et al., 2004; Lamprecht, 2014; Nelson et al., 2012).

Glucocorticoid receptors (GRs) to which the stress hormone corticosterone (in rodents) or cortisol (in humans) binds, are present in hippocampal dendritic spines and local synaptic actin dynamics may be regulated by genomic, but also by non-genomic actions through the GR, the latter facilitating rapid rearrangements in the cytoskeleton (Jafari et al., 2012; Stournaras et al., 2014). Importantly, several ABPs are specifically induced by stress (van der Kooij et al., 2016), highlighting the relevance of actin dynamics in stress-related neuropsychiatric disorders (Zhao et al., 2015). *Tumor suppressor down-regulated in renal cell carcinoma 1* (DRR1, also known as Fam107A and TU3A) is an ABP upregulated by various stressors (e.g. food deprivation, social stress) in a GR-dependent process with strong expression in distinct brain areas, notably the hippocampus (Schmidt et al., 2011). The finding that hippocampal DRR1-overexpression, thereby mimicking part of the stress response, improved cognitive integrity led to the hypothesis that hippocampal DRR1 expression may play an important role in conferring stress resilience and to actively counterbalance deleterious stress-induced consequences (Schmidt et al., 2011).

However, a full integration and causal relationship of hippocampal DRR1 with regard to an acute stress response is still lacking. Here we studied the regulation and the functional role of hippocampal DRR1 in response to ASD using our newly established behavioral test battery.

2. Materials and methods

2.1. Animals

Adult male C57Bl/6J mice (8–9 weeks) and CD-1 retired breeders were obtained from Janvier Labs (France). Mice were single housed with food and water *ad libitum* in an air-conditioned ($T = 22 \pm 2\,^{\circ}C$, $\phi = 50 \pm 5\%$) housing room with 12 h/12 h light-dark cycle (lights on at 07:00 a.m.). Mice were allowed one week of habituation prior to the start of the experiments. Male CD-1 mice acted as aggressors in the acute social defeat paradigm, which were trained and selected for their aggressive behavior preceding the social defeat as previously described (latency to attack < 60s) (Golden et al., 2011). All experiments were conducted in accordance with the European directive 2010/63/EU for animal experiments.

2.2. Acute social defeat paradigm

We established a novel model of acute social defeat: adult male C57Bl/6J mice were exposed to three 10 s aggressive encounters with a larger adult male CD-1 mouse. We introduced this model to limit the amount of aggressive injurious behavior while simultaneously making it better quantifiable, thereby limiting variation in the behavioral and physiological outcomes. The intruder mouse was sequentially introduced into the home cage of three different single-housed unknown CD-1 residents to increase the psychological stress aspect of the ASD. After each aggressive encounter, animals were separated for 15 min by a perforated metal partition to allow sensory input, but avoiding the physical contact. We investigated the effects of ASD on the behavioral phenotype in a time window in which circulating plasma corticosterone were increased (~4 h post-ASD, 'ASD-early') and when corticosterone levels returned to timed control levels (~8 h post-ASD, 'ASD-late') in separate batches of animals. We found that ASD consisting of only a single social defeat was, in contrast to the triple social defeat model employed here, insufficient to increase corticosterone levels when measured 4 h after stress-induction (Supplementary Fig. S1).

2.3. Behavioral tests

We designed a comprehensive – but brief- behavioral test battery which in combination with our novel model of Acute Social Defeat enabled the investigation of acute stress effects on multiple behavioral dimensions in a short time frame (lasting ~3 h). Animals were handled for 2 min/day for the three days preceding the behavioral test battery. Testing took place in a sound-attenuated cabinet with closed curtains. During inter-test intervals (i.t.i.), lasting 15 min, set-ups were cleaned with 5% EtOH. The behavioral tests were conducted with presumably the most aversive test (CD-1 encounter test) at the end of the battery and under dimmed light conditions of 40 lx (except for the Light-Dark Box), to minimize anxiety. The behavioral test battery includes tests (in