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Towards a better preclinical model of PTSD: Characterizing animals with weak extinction, maladaptive stress responses and low plasma corticosterone

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ABSTRACT

Most of the available preclinical models of PTSD have focused on isolated behavioural aspects and have not considered individual variations in response to stress. We employed behavioural criteria to identify and characterize a subpopulation of rats that present several features analogous to PTSD-like states after exposure to classical fear conditioning. Outbred Sprague-Dawley rats were segregated into weak- and strong-extinction groups on the basis of behavioural scores during extinction of conditioned fear responses. Animals were subsequently tested for anxiety-like behaviour in the open-field test (OFT), novelty supressed feeding (NSF) and elevated plus maze (EPM). Baseline plasma corticosterone was measured prior to any behavioural manipulation. In a second experiment, rats underwent OFT, NSF and EPM prior to being subjected to fear conditioning to ascertain whether or not pre-stress levels of anxiety-like behaviours could predict extinction scores. We found that 25% of rats exhibit low extinction rates of conditioned fear, a feature that was associated with increased anxiety-like behaviour across multiple tests in comparison to rats showing strong extinction. In addition, weak-extinction animals showed low levels of corticosterone prior to fear conditioning, a variable that seemed to predict extinction recall scores. In a separate experiment, anxiety measures taken prior to fear conditioning were not predictive of a weak-extinction phenotype, suggesting that weak-extinction animals do not show detectable traits of anxiety in the absence of a stressful experience. These findings suggest that extinction impairment may be used to identify stress-vulnerable rats, thus providing a useful model for elucidating mechanisms and investigating potential treatments for PTSD.

Posttraumatic stress disorder (PTSD) is a debilitating psychiatric illness that can manifest after exposure to a highly stressful or life-threatening event (American Psychiatric Association, 2013). Though the last decade has seen important advances in the conceptual understanding and treatment of PTSD, predictive risk factors and mechanisms underlying stress vulnerability continue to be elusive. Preclinical animal models may help in addressing these issues.

For an animal model of PTSD to reach an adequate level of validity it should reliably demonstrate measurable behavioural responses that reflect clinical symptoms of the disorder. As the diagnostic criteria for PTSD specify exposure to a traumatic event as an essential component, this must be at the center of any behavioural model. Animal models proposed to mimic PTSD-like states following a stressful experience include predator exposure (Zoladz et al., 2008), underwater trauma (Moore et al., 2012), restraint stress (Vyas et al., 2002), prolonged exposure to a single stressor (Yamamoto et al., 2009), social instability (Saavedra-Rodriguez and Feig, 2013) and fear conditioning (Daviu et al., 2012). Most of these can reliably induce one or more PTSD-like symptoms, such as increased arousal, long-lasting heightened levels of anxiety and exaggerated startle responses. A common shortcoming of these models, however, is that they ignore evidence of significant behavioural, physiological and genetic variation reported among genetically outbred animals (Gulley et al., 2003; Kabbaj and Akil,

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2001; Kabbaj et al., 2000). Considering that only 20–30% of humans exposed to traumatic events develop PTSD (Breslau et al., 1991; McFarlane, 2000; Ozer et al., 2003), animal models that overlook individual behavioural variation may be neglecting an important aspect of the disorder. Furthermore, it is likely that such behavioural variability is a reflection of physiological differences that may be important during preclinical testing phases of novel treatments.

Previous studies have attempted to distinguish and classify animals into behavioural subtypes by using specific criteria to segregate those with maladaptive behaviours following exposure to stress (Cohen et al., 2003, 2004). While a large proportion (90%) of animals were clearly affected by stress exposure, only 25–30% displayed maladaptive behaviour at long-term (e.g. 30 days) (Cohen et al., 2003, 2004). This type of behavioural classification has been used to demonstrate that animals may be separated into high and low fear-reactivity, as well as fast and slow fear-recovery phenotypes, based on behavioural performance in tests such as fear conditioning and extinction (Bush et al., 2007).

In the present study we applied a simple distribution-based criterion to identify and segregate animals that show impaired extinction of a conditioned fear response (weak-extinction) from those that display fast rates of extinction (strong-extinction). We then re-examined these animals in both short and long-term recall trials, as well as in tests to measure anxiety-like behaviour. In consideration of reported endocrine dysregulation implicated in PTSD pathology (Daskalakis et al., 2013), plasma corticosterone levels were measured at baseline prior to fear conditioning. Our results suggest that weak-extinction rats have lasting deficits in extinction, display increased levels of anxiety, and have low baseline plasma corticosterone levels. As PTSD is associated with extinction impairment, long-term fear responses to stress-related cues and persistent anxiety after a traumatic experience, we suggest that the weak-extinction phenotype described herein may offer a more accurate representation of the disorder.

1. Methods

1.1. Animals

Outbred male Sprague-Dawley rats (n = 48; 24 used in experiment 1 and 24 in experiment 2) (Charles River, Quebec) weighing 250–300 g, were housed individually on a 12 h light–dark cycle (lights on at 8:00 a.m.), with all training occurring during the light period. Upon arrival, a week of acclimatisation to the facility was allowed with the animals being handled 5 min/day. Water and standard rat chow was available *ad libitum* in the home cages. One week after arrival, all rats began behavioural testing. Protocols were approved by the Animal Care committee of the Centre for Addiction and Mental Health and are in accordance with the Canadian Council on Animal Care (CCAC) guidelines. The timeline for Experiments 1 and 2 is shown in Fig. 1.

1.2. Experiment 1: anxiety measurements after fear learning and extinction

Fear conditioning and extinction were carried out in an operant conditioning chamber (Med Associates, Burlington, VT). The floor consisted of stainless steel bars 1.8 cm apart, connected to a shock scrambler. The chamber was ventilated by a fan and illuminated by a single house light, and housed in a sound-attenuating box (Med Associates, St. Albans, VT). On day 1, rats were presented with six conditioned stimuli (CS; 30 s, 85 dB, 4 hz auditory tones) each co-terminating with a footshock (unconditioned stimulus, US; 0.8 mA, 0.5 s). The intertrial interval was pseudo-randomly varied,

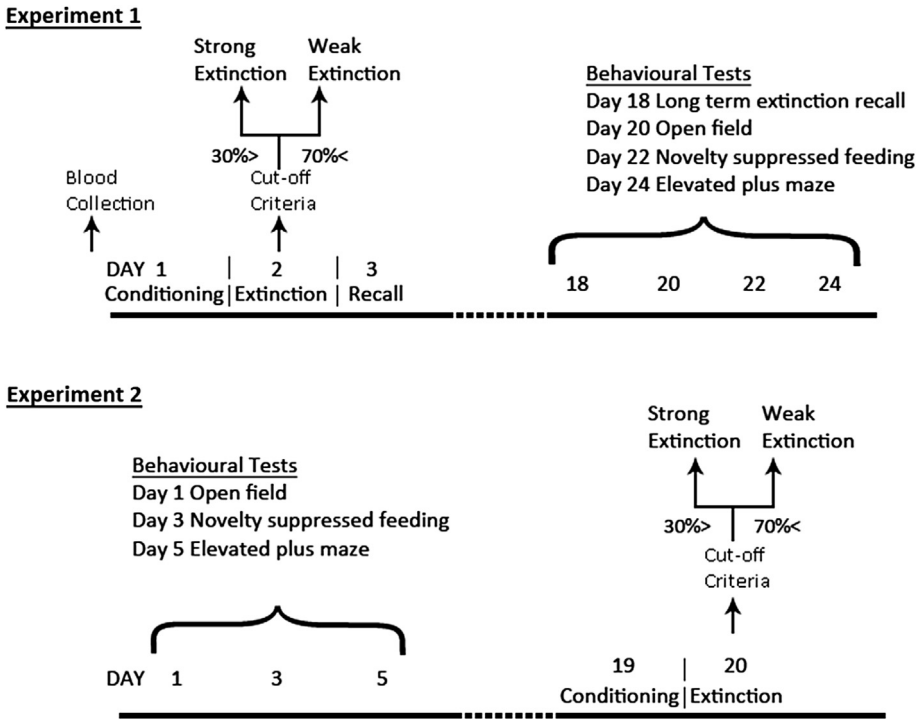

Fig. 1. Experimental timeline. In Experiment 1 the fear learning paradigm took place on days 1–3 and consisted of conditioning, extinction and short-term recall, respectively. Cut-off criteria were applied and animals were separated into strong-extinction (less than 30% freezing average on last 4 extinction trials) and weak-extinction (more than 70% freezing average on last 4 extinction trials) groups, according to their performance during extinction (day 2). Behavioural testing began on day 18. Experiment 2 was conducted in the reverse order, with the behavioural test battery (days 1–5) being administered two weeks prior to fear conditioning and extinction (days 19 & 20). Animals with freezing levels greater than 30% and less than 70% were only included in correlation analyses.