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Establishing a learned-helplessness effect paradigm in C57BL/6 mice: Behavioural evidence for emotional, motivational and cognitive effects of aversive uncontrollability *per se*

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ABSTRACT

Uncontrollability of major life events has been proposed to be central to depression onset and maintenance. The learned helplessness (LH) effect describes a deficit in terminating controllable aversive stimuli in individuals that experienced aversive stimuli as uncontrollable relative to individuals that experienced the same stimuli as controllable. The LH effect translates across species and therefore can provide an objective-valid readout in animal models of depression. Paradigms for a robust LH effect are established and currently applied in rat but there are few reports of prior and current study of the LH effect in mouse. This includes the C57BL/6 mouse, typically the strain of choice for application of molecular-genetic tools in pre-clinical depression research. The aims of this study were to develop a robust paradigm for the LH effect in BL/6 mice, provide evidence for underlying psychological processes, and study the effect of a depression-relevant genotype on the LH effect. The apparatus used for in/escapable electro-shock exposure and escape test was a two-way shuttle arena with continuous automated measurement of locomotion, compartment transfers, e-shock escapes, vertical activity and freezing. Brother-pairs of BL/6 mice were allocated to either escapable e-shocks (ES) or inescapable e-shocks (IS), with escape latencies of the ES brother used as e-shock durations for the IS brother. The standard two-way shuttle paradigm was modified: the central gate was replaced by a raised divider and e-shock escape required transfer to the distal part of the safe compartment. These refinements yielded reduced superstitious, pre-adaptive e-shock transfers in IS mice and thereby increased the LH effect. To obtain a robust LH effect in all brother pairs, pre-screening for minor between-brother ES differences was necessary and did not confound the LH effect. IS mice developed reduced motor responses to e-shock, consistent with a motivational deficit, and absence of a learning curve for escapes at escape test, consistent with a cognitive deficit. When a tone CS was used to predict e-shock, IS mice exhibited increased reactivity to the CS, consistent with hyper-emotionality. There was no ES–IS difference in pain sensitivity. Mice heterozygous knockout for the 5-HTT gene exhibited an increased LH effect relative to wildtype mice. This mouse model will allow for the detailed molecular study of the aetiology, psychology, neurobiology and neuropharmacology of uncontrollability of aversive stimuli, a potential major aetiological factor and state marker in depression.

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1. Introduction

Helplessness, external locus of control and impaired coping ability, are inter-related and major concepts in depression, as predisposing, aetiological and pathological psychological processes (Abramson et al., 1989; Alloy et al., 1999; Harrow et al., 2009). These terms describe the individual's perceived lack of control over environmental events and, although they are not symptoms according to current diagnostic criteria, they are important state markers for therapy, both *per se* and as a potential route to ameliorate core and common depression symptoms such as depressed mood, fatigue, pessimistic views of the future and recurrent thoughts of suicide (DSM-IV, 1994; ICD-10, 1994).

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Learned helplessness (LH) refers to the process in which the experiencing of major aversive life events that are uncontrollable (in terms of their outcome and termination) can lead to the individual responding to subsequent events as if they are also uncontrollable when in fact they are not (Abramson et al., 1978; Miller and Norman, 1979). Learned helplessness has been proposed to comprise the following three dimensions: emotional, in terms of increased arousal, anxiety and fear relative to stimuli/events; motivational, in terms of the reduced level of incentive to terminate the stimulus/event; cognitive, in terms of the reduced expectancy that the stimulus/event can be terminated by own behaviour (Maier and Seligman, 1976). In addition to embracing these three central dimensions of brain-behaviour function, LH is also distinguished by being the first example of a translational concept in psychiatric research. Thus, LH was first demonstrated in animal experimental psychology, including in dog and rat studies (Maier and Seligman, 1976), and the observations and interpretations made with these species were then applied to human behaviour and affective disorders thereof (Abramson et al., 1978; Miller and Norman, 1979; Seligman, 1972). Importantly, the LH effect demonstrated in animals is a deficit in responding to a specific stimulus/event (see below) and not a generalized helplessness, which is the form of helplessness regarded to be important in depression psychopathology (Abramson et al., 1978). Nonetheless, the LH effect model is important in terms of what it could reveal about the underlying neurobiology of specific and generalized helplessness, and therefore depression and its treatment (Amat et al., 2005; Robbins, 2005).

Most LH experiments have been conducted in the rat. Subjects are allocated at random either to exposure to controllable (escapable) or uncontrollable aversive stimuli and this is followed by a test in which all subjects are assessed in terms of their behaviour relative to controllable aversive stimuli. In the exposure phase, the operant latencies to terminate the escapable stimuli (ES) in the ES group are used as the durations of the inescapable stimuli (IS) in the IS group; as a result of this yoking principle the only difference between ES and IS is the controllability of the aversive stimuli experienced (Maier and Seligman, 1976). The current most commonly used rat LH model comprises exposure to tail electro-shock that can be terminated in the ES group by operant turning of a wheel, and testing in terms of escaping electro-shock by operant running in a two-way shuttle box: IS rats exhibit an escape deficit at test in terms of reduced number of escapes/increased latency to escape, where the escape response involves crossing from one side of the shuttle box to the other and back again (Amat et al., 2005). A distinguishing feature of the LH effect as a model in pre-clinical depression research is that the process underlying the behavioural change and being measured is clear: it is the prior experience of uncontrollability. This is not the case for other models: for example, the forced swim test is often claimed to measure "behavioural despair" (e.g., Castagné et al., 2011), "helplessness" (e.g. El Yacoubi et al., in press), "passive coping" (e.g. Keck et al., 2003) or even "active coping" (e.g. Lu et al., 2008) but, because there is no equivalent of the ES comparison group in the LH effect paradigm, it is difficult to interpret what underlies the reduction in swimming exhibited by rats and mice and, therefore, what the test actually measures.

Despite the high use and potential of mice in pre-clinical research into affective disorders, and despite the high face and construct validity of the LH effect paradigm, there have been relatively few published studies of a mouse LH effect. A recent literature search (Embase, Medline and Psycinfo, from 1806 to 2011) of rat and mouse LH effect studies that used the ES–IS paradigm yielded 38 rat studies and 6 mouse studies (Pryce et al., in press) and the most recent mouse study was published in 2003 (Palermo-Neto et al., 2003). There are a large number of mouse publications which include comparison of an IS group with a group that received no aversive stimuli prior to test, the so-called unconditioned-stimulus (US) pre-exposure effect paradigm (e.g. Chang et al., 2007; Maeng et al., 2008). These studies do not allow for assessment of the effect of aversive uncontrollability *per se* and thereby lack the very essence of the LH effect paradigm. One laboratory that has made a significant contribution to establishing and studying the LH effect in mice is that of Anisman, with the majority of studies conducted with Swiss-Webster mice and using electro-shocks for ES–IS pre-exposure and e-shock two-way escape behaviour as readout (e.g. Anisman et al., 1978, 1979, 1980; Anisman and Merali, 2001). As noted (Anisman and Merali, 2001), one possible reason for the relative scarcity of mouse LH effect studies is the challenge of designing an aversive manipulation such that those mice pre-exposed to the escapable form (ES) continue to exhibit high levels of escape behaviour up to and including the escape test, whilst those mice pre-exposed to the inescapable form of the manipulation (IS) – which is identical in intensity and duration to ES – develop a robust escape deficit that is expressed during the escape test.

Given its common application in molecular-genetic models in pre-clinical depression research, the first aim of the current study was to establish an LH effect paradigm in the C57BL/6 mouse strain. Both ES–IS pre-exposure and escape test were carried out using a two-way e-shock escape apparatus that allowed for concurrent data collection on several relevant behavioural measures. The apparatus was modified across experiments to increase the robustness of the LH effect. The experimental design was based on brother-pairs, such that each ES–IS dyad was also a brother-pair. This was commensurate with the yoked design of the ES–IS paradigm and, given that inter-individual differences are pronounced even within inbred mouse strains such as C57BL/6 (e.g. Siegmund et al., 2009), using brother pairs could also reduce existing individual (trait) differences between mice allocated to the same ES–IS dyad and thereby further increase the robustness of the LH effect obtained. The second aim of the study was to use features and behavioural measures provided by the apparatus to identify the extent to which emotional, motivational and cognitive processes contributed to the LH effect. The third aim was to study the effects of heterozygote knockout (HET) of the serotonin transporter gene (Slc6a4, commonly abbreviated to 5-HTT) on the LH effect. Reduced 5-HTT activity in human due to the *short* variant of the 5-HTT gene-linked polymorphic region is associated with a relatively high risk of depression in individuals exposed to several aversive life events (Caspi et al., 2003; Karg et al., 2011). In mouse, 5-HTT HET mice exhibit reduced two-way escape responding following IS relative to wildtype in the US pre-exposure effect paradigm (Muller et al., 2011). An increased LH effect in 5-HTT HET mice would demonstrate that this phenotype is due to increased uncontrollability and demonstrate the translational validity of the mouse LH effect for neuro-biological and –pharmacological study of depression and its treatment.

2. Materials and methods

2.1. Animals and maintenance

Male C57BL/6J mice (RCC, Füllinsdorf, Basel, Switzerland) were delivered to the laboratory at age 8 weeks and allowed 2 weeks to adjust to a reversed light–dark cycle (lights off at 07:00–19:00 h). Male and female 5-HTT knockout mice on a C57BL/6J background (>20 backcross generations) were transferred from the University of Würzburg (Bengel et al., 1998) and breeding was established in-house with wildtype (WT) dams and 5-HTT heterozygote knockout (HET) sires. Study mice were weaned at 4 weeks and genotyped.

Mice were held in individually-ventilated cages (type 2 long) containing sawdust, a sleeping house and bedding, with continuous access to food and water.