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Repeated daily restraint stress induces adaptive behavioural changes in both adult and juvenile mice

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HIGHLIGHTS

- Repeated daily restraint stress in mice induced a complex behavioural adaptation of anxiolytic-like and anhedonic responses
- Juvenile mice showed a wider range of behavioural responses following repeated daily restraint stress than adults
- Novel features of the methodology show behavioural changes result from lasting, not acute, effects of restraint stress
- Behavioural responses to repeated restraint stress were qualitatively similar in C57BL/6 and "stress-sensitive" BALB/c mice
- Adaptive behavioural changes seen may reflect resilience and be beneficial in future stress challenges

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ABSTRACT

Chronic stress is known to be a risk factor for the development of depression and anxiety, disorders which often begin during adolescence. Restraint stress is a commonly used stressor in adult rodents, however the effects of repeated restraint stress in juvenile mice have not been well characterised. Here we have shown for the first time the behavioural and hormonal effects of repeated restraint stress in both adult and juvenile BALB/c and C57BL/6 mice. Repeated daily restraint stress (2 h/day for 3, 7 or 14 days) provoked a robust physiological response evident as increased corticosterone levels and decreased body weight after 14 days. However, habituation of the stress-response was evident during repeated exposure to the stressor in both adult and juvenile mice. The behavioural changes seen in response to repeated restraint stress were complex. In juvenile mice, repeated restraint stress evoked an increase in exploratory behaviours in the elevated plus maze, a decrease in time spent immobile in the forced swim test and a decrease in sucrose preference. In adult mice fewer behavioural changes were seen. Interestingly BALB/c and C57BL/6 mice showed qualitatively similar response to 3 days repeated restraint stress. The behavioural changes we observed, as a result of prior stress exposure, may represent an adaptive stress-coping response or resilience. Both the hormonal and behavioural effects of stress were more pronounced in juvenile mice than in adults. This wider range of behavioural responses seen in juvenile mice might reflect a greater ability to engage in adaptive stress-coping strategies that likely have beneficial effects evident in future stress challenges.

1. Introduction

Chronic stress is known to be a major risk factor for the development of many psychiatric disorders, including depression [1–3]. During adolescence, brain development and physical changes associated with puberty are thought to make individuals particularly vulnerable to the effects of stress [4–6]. There is also increasing evidence that the onset of many psychiatric disorders occurs during adolescence, with up to half of adult disorders having begun by the teenage years [7,8].

Changes in the function of the hypothalamic-pituitary-adrenal (HPA) axis also occur during adolescence [9]. In humans, levels of basal cortisol increase with age and pubertal development throughout the adolescent years [9–11]. This pattern has also been shown in rodents, with an increase in corticosterone from birth through to adulthood [12,13]. Changes in the reactivity of the HPA axis in response to stress have also been reported during adolescence. For example, increases in cortisol release in response to a social stress test have been seen in adolescents over the age of 13 years, compared to those under 13 years old [9]. In rats, chronic restraint stress induces greater increases in corticosterone release in juvenile animals compared with adults [14,15]. These differences in stress-responsiveness between adolescents

Abbreviations: EPM, elevated plus maze; FST, forced swim test; HPA, hypothalamic-pituitary-adrenal; SPT, sucrose preference test.

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and adults may result in increased vulnerability to the effects of stress during adolescence [13].

Restraint stress is a widely used model of stress in rodents, as it is straightforward to administer, painless and does not cause physical harm to the animals [16]. There have been several studies examining the effects of repeated restraint stress on changes in depression and anxiety-related behaviours. For example, in adult C57BL/6 and NMRI mice, repeated restraint stress has been shown to increase depression and anxiety-related behaviours, in the forced swim test, tail suspension test and elevated plus maze [17–19]. These changes in behaviour have been shown to persist for up to 3 months after the period of stress [20]. In contrast, there are few reports of the behavioural effects of restraint stress in juvenile animals.

Adolescence is a period of vulnerability for the development of depression. Here, we have examined whether repeated restraint stress in juvenile mice induces a behavioural change consistent with an increase in depression-related behaviour. Juvenile mice aged 4–6 weeks are considered to model human adolescence, as at this age they undergo a growth spurt, sexual maturation and developmental brain changes similar to those seen in adolescent humans [5]. In this study, we have investigated the effects of different durations of repeated restraint stress in both adult and juvenile BALB/c mice on depression- and anxiety-related behaviours, coupled with an assessment of HPA function by measuring corticosterone levels. Furthermore, in some studies we have compared both the behavioural and neuroendocrinological effects of stress in BALB/c mice, with those in C57BL/6 mice. It has previously been suggested that BALB/c mice are more sensitive to the effects of stress than the C57BL/6 strain which are relatively stress resilient [21,22].

2. Methods

2.1. Animals

Male BALB/cAnNCrl mice (Charles River UK) and male C57BL/6 mice (University of Bath) were 9–10 weeks old (adults) or 4–5 weeks old (juveniles) at the start of experiments. Mice were either individually housed (BALB/c mice) or housed in groups of 3–4 (C57BL/6 mice) in 35 × 20 × 15 cm polysulfone cages (Plexx, Elst, The Netherlands) with woodchip bedding (Datesand, Manchester, UK) and paper nesting material (Lillico/LBS Biotechnology, Horley, UK). The mice were maintained in a temperature (21 ± 1 °C) and humidity (50–60%) controlled environment, under a 12 h light/dark cycle (lights on 07:00 h), with food and water available ad libitum. All mice were acclimatised to the animal facility for at least 4 days prior to the start of experiments, during which time they were handled on at least two occasions by gentle cupping [23]. Experiments were conducted during the light phase, between 08:00–13:00 h, with the exception of the sucrose preference test which occurred during the dark phase. All procedures were carried out under a Home Office project licence held in accordance with the Animals (Scientific Procedures) Act 1986.

2.2. Restraint stress

Stressed mice were placed head first into a modified 50 ml syringe with ventilation holes, which was plugged with the syringe plunger and adjusted so that mice were unable to move forwards or backwards. Mice were restrained for 2 h each day, for either 3, 7 or 14 consecutive days. Stressed mice were weighed daily, and monitored for signs of distress using a scoring system adapted from Lloyd and Wolfensohn [24] (Supplementary Fig. 1). Non-stressed control mice were weighed daily, but otherwise remained in their home cage.

2.3. Behavioural testing

Behavioural testing in the elevated plus maze and forced swim test occurred in a dimly lit room adjacent to the animal holding room. Mice were left to acclimatise in the behavioural room for at least 1 h prior to testing. Testing in the elevated plus maze, forced swim test and sucrose preference test occurred one day following 3 days restraint, or two days following 7 or 14 days restraint. Separate groups of mice were used for each behavioural test. A diagram outlining the restraint stress protocol is shown in Fig. 1.

2.4. Forced swim test (FST)

Mice were placed in a glass beaker (diameter 22 cm, height 34 cm), filled to a depth of 23 cm with water (25 °C). Each 6 min test session was filmed and behaviour in the last 4 min of the test was scored by an experimenter blind to treatment. Swimming was defined as horizontal movement, and immobility defined as the minimal activity needed to stay afloat. Following the test session, mice were dried with paper towels and placed in a warm holding cage, before being returned to the home cage. The water was replaced, and the beaker cleaned with 70% ethanol, between each mouse.

2.5. Sucrose preference test (SPT)

On the last day of restraint, mice were habituated to drinking from 2 bottles of water, for 12 h (19:00–07:00 h). The following day, mice were given the choice to drink either water or 5% w/v sucrose (BALB/c mice), or water and 2.5% w/v sucrose (C57BL/6 mice) during a 12 h test (19:00–07:00 h). Inbred mouse strains are known to have differing sensitivities to sucrose [25], so the concentration of sucrose required to produce preference was determined in preliminary experiments (Supplementary Fig. 4). Bottles were weighed before and after the test, and the preference for sucrose was determined as a percentage of the total volume consumed. Total volume consumed was also determined.

2.6. Elevated plus maze (EPM)

The elevated plus maze (Campden Instruments) consisted of four arms (38 × 5 cm), arranged at right angles around a central intersection (5 × 5 cm). Two of the arms were open, with a 0.5 cm rim, while the other two were enclosed with 15 cm high walls. The entire maze was elevated 65 cm off the floor. Lighting on the open arms measured 20 lx for experiments with BALB/c mice, and 50 lx for experiments using C57BL/6 mice. Mice were placed in the central intersection facing an open arm and allowed to freely explore the maze for 5 min. Time spent in, and number of entries into the open arms, and total locomotion over the whole maze, were recorded by MotorMonitor™ software using infrared photobeams. The maze was cleaned with 70% ethanol after each mouse.

2.7. Assessment of neuroendocrinological function

Blood samples were taken from all mice at baseline and immediately following the last restraint stress. 40 μl samples were taken from the lateral tail vein [26] and collected in heparinised capillary tubes (Hawksley, Sussex, UK). Blood was transferred to micro-centrifuge tubes containing EDTA (final concentration in sample 3 μg/μl) and kept on ice until being centrifuged at 2000 rcf for 20 min at 4 °C. Plasma was removed and stored at −20 °C until analysis. The concentration of corticosterone in plasma was determined using an ELISA (IBL International, Hamburg, Germany). All blood samples were taken between 11:00–13:00 h. To assess the effect of repeated restraint stress on adrenal gland weight, mice were killed by cervical dislocation two days after the last session of restraint, and adrenal glands were removed and weighed. Function of negative feedback inhibition of the HPA axis was determined using the dexamethasone suppression test (DST), which has been used extensively to indicate abnormalities in negative feedback of the HPA axis in depressed patients [27]. A baseline blood sample was taken between 3 and 4 pm, on the day after the last session of