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Environmental enrichment reduces innate anxiety with no effect on depression-like behaviour in mice lacking the serotonin transporter

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ABSTRACT

Along with being the main target of many antidepressant medications, the serotonin transporter (5-HTT) is known to be involved in the pathophysiology of depression and anxiety disorders. In line with this, mice with varying 5-HTT genotypes are invaluable tools to study depression- and anxiety-like behaviours as well as the mechanisms mediating potential therapeutics. There is clear evidence that both genetic and environmental factors play a role in the aetiology of psychiatric disorders. In that regard, housing paradigms which seek to enhance cognitive stimulation and physical activity have been shown to exert beneficial effects in animal models of neuropsychiatric disorders. In the present study, we examined the effects of environmental enrichment on affective-like behaviours and sensorimotor gating function of 5-HTT knock-out (KO) mice. Using the elevated-plus maze and the light-dark box, we found that environmental enrichment ameliorated the abnormal innate anxiety of 5-HTT KO mice on both tests. In contrast, environmental enrichment did not rescue the depression-like behaviour displayed by 5-HTT KO mice in the forced-swim test. Finally, measuring pre-pulse inhibition, we found no effect of genotype or treatment on sensorimotor gating. In conclusion, our data suggest that environmental enrichment specifically reduces innate anxiety of 5-HTT KO mice with no amelioration of the depression-like behaviour. This has implications for the current use of clinical interventions for patients with symptoms of both anxiety and depression.

1. Introduction

Anxiety disorders and depression are the most common psychiatric conditions in modern societies, with lifetime US prevalence estimates of over 25% and approximately 15% respectively [1]. Aberrant serotonergic signaling has been implicated in the manifestation of both these forms of mental illness and has an established role in regulating hippocampal-dependent emotional processing hypothesized to underpin both disorders [2,3]. Serotonin transporter (5-HTT) gene polymorphisms have been associated with patients presenting with both anxiety and depression-related disorder [4]. In agreement with the clinical evidence, constitutive 5-HTT knock-out (KO) mice have an abnormal innate anxiety phenotype as well as increased depression-like behavior [5]. Environmental enrichment paradigms which seek to enhance cognitive stimulation and physical activity overall, have been shown to correct abnormal innate anxiety and depression-like behaviours in mice [6]. An appropriate period of housing in environmental enrichment can also cause an anxiolytic and antidepressant effect in wild-type (WT) mice [7]. These beneficial effects of environmental enrichment are hypothesized to result from enhancements in home-cage novelty and complexity that raise the levels of sensory, cognitive and motor stimulation experienced by rodents [8]. Sensorimotor gating is another behaviour in rodents that can be modulated by environment enrichment [9,10]. Sensorimotor gating deficits are well established in schizophrenia patients, but there is clinical evidence associating deficits with the diagnosis of both depression and anxiety disorders [11,12]. In this study, we aimed to assess the effects of environmental enrichment on innate anxiety, depression-like behaviour and sensorimotor gating in both WT and 5-HTT KO mice.

The 5-HTT linked polymorphic region (5-HTTLPR) is a functional tandem repeat polymorphism where carriers of the short (s) allele have reduced expression of the 5-HTT and a lowered 5-HT reuptake that elevates extracellular concentrations of 5-HT ([5-HT]ext) [13]. In that seminal study, ‘s carriers’ were associated with an increased risk of developing anxiety disorders. In contrast, the genetic risk of being a 5-HTTLPR ‘s carrier’ does not associate with depression, but an

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association does occur if early life stress is factored into a gene × environment (G × E) design [4]. Stress also elevates [5-HT]ext [14]. The genetic elevation of [5-HT]ext in the 's carriers' combined with the increased [5-HT]ext from early life stress is hypothesized to become sufficient to cause abnormal development of corticolimbic circuitry that creates the vulnerability to depression [15]. In 5-HTT KO mice, [5-HT]ext is raised 7–9 fold and sufficient evidence exists to propose that 5-HTT KO mice are an extreme model of that G × E interaction described above [4,5,16]. Along with providing further evidence of the potential anxiolytic and antidepressant effects of environmental enrichment in a clinically relevant mouse model, our study also investigated whether the loss of 5-HT reuptake through the 5-HTT alters the sensitivity of adult mice to environmental enrichment.

There is often a comorbid clinical presentation of symptoms related to anxiety and depression [17]. Pharmaceutical drugs commonly prescribed for the treatment of both anxiety disorders and depression have a mechanism of action that involves the 5-HTT [18]. These drugs are not always efficacious for patients presenting with symptoms of either condition so novel treatments with mechanisms of action that are not reliant on the 5-HTT are needed [19]. In addition, a meta-analysis determined that 5-HTTLPR s carriers associated with reduced response rate to this type of antidepressant treatment [20] so this population of patients have a specific need for such novel therapies. Evidence has accumulated that clinical environmental interventions, such as cognitive behavioural therapy, do have efficacy for sufferers of both anxiety disorders and depression [21]. We recently published a therapeutic effect of environmental enrichment on innate anxiety in a mouse model of anxiety disorder (5-HT_{1A} receptor KO mice) whose origin is through aberrant developmental serotonergic signaling [22]. As the altered innate anxiety and depression-like behaviour in 5-HTT KO mice is also the result of altered developmental serotonergic signaling, we hypothesized that environmental enrichment would have therapeutic effects to both behaviours in this model. Our study also investigated the potential role of the 5-HTT in mediating these effects.

2. Methods

We obtained 5-HTT KO mice and wild-type (WT) littermate control mice from the congenic C57BL/6J mouse colony we established at the Florey Institute of Neuroscience and Mental Health using heterozygous breeding pairs. After weaning, mice were housed in individually ventilated containers by identical genotype until 5 weeks of age and then placed in open-top boxes (standard housing) until the study began at 8 weeks of age. At that point, we randomly assigned mice of both genotypes to either remain in the standard housing condition or to move to the environmental enrichment condition (see Fig. 1 for expanded descriptions). The innate anxiety of the mice was measured after 2 weeks of environmental enrichment and the depression-like behaviour after 3 weeks of environmental enrichment. These time points were chosen because our previous work found that 2 weeks of our environmental enrichment has anxiolytic effects [22] and 3 weeks of our environmental enrichment has antidepressant effects [23]. Furthermore, a recent study investigating the time course of environmental enrichment induced anxiolytic and antidepressant effects in WT mice was in agreement with those time points [7].

We assessed the innate anxiety of mice after 2 weeks of environmental enrichment using two distinct approach-avoidance conflicts, the elevated-plus maze (EPM) and the light-dark box (LDB) test. The elevated-plus maze was performed as previously described [22]. The light-dark box apparatus used was an adaptation of the TruScan Photobeam arenas (Coulbourn Instruments, PA, USA). Briefly, square photo-beam activity arenas (26 × 26 × 38 cm) were divided into light and dark compartments with a black Plexiglas insert. The light compartment was brightly lit between 700 and 750 lux. The insert had a 4 × 4 cm opening in its centre to allow mice free passage between the light and dark compartments. Mice were placed in the dark compartment to begin the task through a lid in the top of the insert. Mice were allowed to explore the arena for 10 min while all aspects of their activity was monitored using Activity Monitor software (Med Associates Inc, Fairfax, Vermont). The innate anxiety of the mice during these approach-avoidance conflicts was indicated by the percentage time spent in the aversive portion of the conflict. For the EPM, this was the percentage time spent in the open arms and for the LDB this was the percentage time in the light compartment. We also derived secondary measures of innate anxiety, the percentage bouts in the open arm (EPM) and the number of transitions between the compartments (LDB). Both these secondary measures control for locomotor activity, but we also report the total distance travelled during both tasks. Depression-like behaviour was assessed after 3 weeks of environmental enrichment. We assessed the level of behavioural despair in the mice once placed into an inescapably stressful situation using the forced-swim test (FST) as previously described [24]. The time mice spent immobile and the latency to the first incident of immobility were used to measure the level of behavioural despair in the animals and thus their depression-like behaviour. Total immobility time was automatically scored using the ForcedSwimScan component of the DepressionScan suite software (Clever Sys Inc., Restin, Virginia). Finally, we also assessed aspects of sensorimotor gating as a measure relevant to both innate anxiety and depression-like behaviour as previously described [25]. Briefly, stimulus-only pulses of 115 dB used to measure the startle amplitude were presented in 4 blocks of eight trials so we presented the data for each block as a measure of startle habituation. The pre-pulse (PP) was presented in a range of non-startling intensities of 2, 4, 8, or 16 dB over the 70 dB baseline (PP2, PP4, PP8 and PP16) so we present the %PPI for each of the four pre-pulse possibilities An inter-stimulus intervals (ISI) of 100 ms was used in all cases. Those experiments were conducted after 4 weeks of environmental enrichment in alignment with our previous work [9]. Two experimental cohorts were used to conduct the behavioural experiments. Both cohorts were used for the anxiety and sensorimotor gating tasks, but data from one cohort only was collected for the FST.

For statistical analysis, we performed a two-way analysis of variance (ANOVA) with genotype and housing condition as between-group factors. If significant interactions between, or main effects of, genotype and housing were identified in our sample population, Bonferroni post hoc comparisons were performed. To adjust for multiplicity, the WT standard housed animals were treated as control animals and we restricted reporting our post hoc comparisons to the three other groups in the study with that control. For the PPI data, repeated measures ANOVAs were performed with pre-pulse intensity and startle block included as within-group factors. For startle amplitude the data is presented as arbitrary units. Genotype and housing were again included as between-group factors. In all cases, the significance threshold was set at $p < 0.05$. For each set of experiments, we observed no significant sex or cohort differences so all data presented were pooled.

3. Results

3.1. Innate anxiety

We measured the innate anxiety of our mice using two approach avoidance paradigms, the elevated-plus maze (EPM) and the light-dark box (LDB) test. The main measure for the level of innate anxiety in mice on those tasks is the percentage time spent in the open arm and the percentage time spent in the light compartment, for the EPM and the LDB respectively (Fig. 2). We hypothesized that environmental enrichment would restore the abnormal innate anxiety 5-HTT KO mice are known to exhibit on both of these tasks [5]. For the percentage time spent in the open arms on the EPM (Fig. 2A), a significant interaction was identified between housing condition and genotypes ($F_{1,47} = 7.30$; $p < 0.01$). Confirming the expected abnormal innate anxiety in 5-HTT KO mice, the post hoc analysis revealed a significant difference between