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Maternal separation induces neuroinflammation and long-lasting emotional alterations in mice

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ABSTRACT

Early life experiences play a key role in brain function and behaviour. Adverse events during childhood are therefore a risk factor for psychiatric disease during adulthood, such as mood disorders. Maternal separation is a validated mouse model for maternal neglect, producing negative early life experiences that result in subsequent emotional alteration. Mood disorders have been found to be associated with neurochemical changes and neurotransmitter deficits such as reduced availability of monoamines in discrete brain areas. Emotional alterations like depression result in reduced serotonin availability and enhanced kynurenine metabolism through the action of indoleamine 2, 3-dioxygenase in response to neuroinflammatory factors. This mechanism involves regulation of the neurotransmitter system by neuroinflammatory agents, linking mood regulation to neuroinmunological reactions. In this context, the aim of this study was to investigate the effects of maternal separation with early weaning on emotional behaviour in mice. We investigated neuroinflammatory responses and the state of the tryptophan–kynurenine metabolic pathway in discrete brain areas following maternal separation. We show that adverse events during early life increase risk of long-lasting emotional alterations during adolescence and adulthood. These emotional alterations are particularly severe in females. Behavioural impairments were associated with microglia activation and disturbed tryptophan–kynurenine metabolism in brain areas related to emotional control. This finding supports the preeminent role of neuroinflammation in emotional disorders.

1. Introduction

Emotional disorders, including major depression, are the most prevalent psychiatric disorders worldwide, importantly contributing to the global burden of diseases (Murray and Lopez, 2013). The World Health Organization predicts that depressive disorders will be the greatest contributor to the global burden of disease by 2030 (Mathers et al., 2005; Stuart and Baune, 2014). Major depression is thought to comprise a heterogeneous group of diseases caused by genetic, epigenetic and environmental factors (Nestler, 2014). More than a quarter of depressed patients fail to achieve remission despite trying multiple treatments (Felger and Lotrich, 2013), and a high percentage of patients relapse (Raedler, 2011), highlighting the need for more effective therapies.

Early life experiences are thought to play a key role in brain function and behaviour (Lupien et al., 2009). In humans, detrimental early life events, such as maternal neglect or abuse during childhood, are associated with increased risk of emotional disorders that may persist into adulthood (Gross and Hen, 2004; Heim and Binder, 2012; Heim and Nemeroff, 2001). Experimental and clinical studies have shown that the immaturity and plasticity of the central nervous system during childhood make it particularly sensitive to stress at a young age, which may cause significant changes in brain structure and function (Lupien et al., 2009). In recent years, various rodent behavioural models of early life stress, such as maternal separation, have been used to study the neurobiological basis of emotional and motivational disorders (Fuentes et al., 2014; Martini and Valverde, 2012; Pryce et al., 2001a, 2001b). Maternal separation with early weaning (MSEW) (George et al., 2010) attempts to reduce any potential compensatory maternal care after maternal deprivation.

Recent clinical and experimental data suggest that the pathophysiology of several neuropsychiatric disorders, including depressive

Abbreviations: CA, cornu ammonis; DG, dentate gyrus; HC, hippocampus; IL-1 β, interleukin-1 beta; IL-6, interleukin-6; ISTD, internal standard solution; Iba1, ionized calcium-binding adapter molecule 1; KYN, Kynurenine; LC-MS/MS, Liquid Chromatography Mass Spectrometry; MSEW, maternal separation with early weaning; NMDA, *N*-methyl-D-aspartate; NS, non-significant; PB, phosphate buffer; PBS, phosphate buffer saline; PD, postnatal day; PFC, prefrontal cortex; SRM, Selected Reaction Monitoring; 5-HT, serotonin; SN, standard nesting; TRP, tryptophan; TNF-α, tumor necrosis factor alpha.

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syndromes, involves activation of the immune system in response to inflammatory agents. These studies report that depressed patients present elevated plasma levels of pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin -1β (IL-1β) and the tumour necrosis factor (TNF-α) (Anderson et al., 2014; Dantzer et al., 2011; Miller et al., 2009). The production of these pro-inflammatory mediators has been related to the pathophysiological effects of stress and depressive states (Miller et al., 2009; Myint et al., 2007). Indeed inflammation also appears to be an immunological consequence due to early life neglect (Danese et al., 2007). Thus, the production of cytokines contributes to a deregulation of the hypothalamic–pituitary–adrenal axis and promotes abnormalities in the neural plasticity, including a decrease of the neurotrophic support and an impaired neurogenesis. Further evidence suggests that pro-inflammatory cytokines alter tryptophan (TRP) metabolism, affecting the activity of serotonin (5-HT) neurotransmitter system (Christmas et al., 2011; Miller et al., 2009). Therefore, the metabolic tryptophan route becomes imbalanced during depression, increasing kynurenine (KYN) synthesis and enhancing the alternative TRP metabolic pathway by activating indoleamine 2,3-dioxygenase, (Christmas et al., 2011) (see Figure S1), decreasing the availability of TRP to be metabolized in 5HT. Interestingly, metabolites of the TRP-KYN pathway may regulate the brain homeostasis as well as modulate other different neurotransmitter systems including glutamate and dopamine (Miller et al., 2009; Myint, 2012). In fact, kynurenic acid, an intermediate metabolic product of the TRP-KYN pathway behaves as a NMDA antagonist, displaying neuroprotective actions in the brain and inhibiting the release of the excitatory neurotransmitter glutamate and inhibiting the release of dopamine in discrete brain areas (Borland and Michael, 2004; Klein et al., 2013; Sas et al., 2007). On the other hand, quinolinic acid, one of the pathway's final products, seems to act as an NMDA agonist, promoting glutamate release and contributing to excitotoxicity and oxidative stress in the brain (McNally et al., 2008; Muller and Schwarz, 2007). Similarly, 3-hydroxykynurenine also displays neurotoxic effects by promoting the formation of oxygen species and causing neuronal apoptosis (Okuda et al., 1998; Stone, 2001). Taken together, the imbalance of this metabolic tryptophan route induces a detrimental 5-HT synthesis that has been directly associated to the development of depressive symptoms in humans and in experimental animal models (Gabbay et al., 2010; Laugeray et al., 2011; Steiner et al., 2011). Considering these facts, it seems of relevance the evaluation of the possible imbalance of the metabolic tryptophan route under our experimental conditions in which long-lasting emotional alterations are observed.

In this study, we investigated behavioural alterations induced by early life adversity in male and female CD1 mice, and explored the interplay between depressive manifestations in behavioural models, neuroinflammation, and alterations in the TRP-KYN pathway. Using MSEW in CD1 mice, we aimed to elucidate behavioural, neuroimmunological and neurochemical changes induced by maternal separation. We used CD1 mice to evaluate the effects of two experimental rearing paradigms on emotional behaviour during adolescence and into adulthood, MSEW and a Standard Nest (SN). We performed a range of tests for anxiety- and emotional-related behaviours, and evaluated neuroinflammatory responses in specific brain areas of mice reared under each paradigm. We evaluated microglia activation in the prefrontal cortex (PFC) and hippocampus, and analysed metabolites of the TRP-KYN pathway to explore the link between depressive disorders and inflammatory reactions.

2. Materials and methods

2.1. Animals

We used 12 male and 12 female outbred CD1 mice as breeders for this study (provided by Charles River, Barcelona, Spain), and shipped to our animal facility, UBIOMEX, PRBB. Animals were 10 weeks old at the start of breeding and were housed individually in standard cages in a temperature- (21° ± 1 °C), humidity- (55% ± 10%), and light-cycle-controlled room. The room was lit between 8:00 h and 20:00 h, and experiments were conducted during the light phase (8:30 h to 15:00 h), except for the evaluation of maternal behaviour, as indicated. Food and water were available ad libitum except during behavioural testing of the offspring. Mice were allowed to acclimatize to the new environmental conditions for at least one week before starting the experiments. Every effort was made to minimize animal suffering and reduce the number of animals used. All procedures were conducted in accordance with national (BOE-2013-1337) and EU (Directive 2010-63EU) guidelines regulating animal research, and were approved by the local ethics committee (CEEA-PRBB).

2.2. Rearing conditions

Mice were randomly assigned to one of two different experimental groups, SN and MSEW. For each group, breeding pairs (one male, and one female) were housed in Plexiglas cages (369 × 156 × 132 mm), and the males were removed when the females were about 10 days pregnant. Pregnant females were observed daily at 9 and 17 h for parturition. For each litter, the date of birth was designated postnatal day (PD) 0. In the MSEW group, offspring were separated from their mothers for 4 h per day on PD2-5 (9:30–13:30) and 8 h per day on PD6-16 (9:30–17:30 h). For separation, mothers were moved to another cage, while the offspring remained in their home cages with a heating blanket (32–34 °C) for thermoregulation. After removing the mothers, offspring were taken to another room to avoid their mothers to become stressed from hearing their vocalizations (George et al., 2010). Pups were weaned at PD17, and to facilitate their access to food and avoid a possible dehydration, wet regular chow and hydrodrogel (Bio-Services, Uden, The Netherlands) were provided in their home cages until PD21. In the SN group, offspring remained with their mothers for 21 days and were then weaned (PD21). Cages remained untouched until PD10, when they were cleaned. After weaning, offspring were housed in groups of 4 to 5 animals of the same sex. For the experiments, 5 and 4 females were assigned to the MSEW and SN groups, respectively. We observed no significant difference between groups in the total number of offspring, or the number of males or females. Average litter size was 13 (53% male). A different group of mice was used to evaluate adult behavioural parameters. In this case, 5 females were assigned to each group, and we observed no differences between groups in the number or sex of the offspring (average litter size, 12; 56% males).

2.3. Maternal care

We recorded the biological mother's spontaneous maternal behaviour 3 times per day (8:15, 17:30 and 20:15) from PD1 to PD16 according to an adapted version of a previously described protocol (Dimitsantos et al., 2007; Fodor et al., 2012). The long break between the morning and afternoon maternal care evaluation session was consistent with the 8 h maternal separation period performed during PD6-16 (9:30-17:30 h). Behaviour was recorded on-line by an observer who remained silent in the room. Observations of the maternal care behaviour were performed at three periods of the day, at 8.15 h, 17.30 h and 20.15 h. Within each observation period, the behaviour of each mother was scored 25 times spaced 3 min each one (25 observations x 3 periods per day × 16 days = 1200 observations/mother). The following behaviours were scored as present or absent and quantified in a check list: 1) mother licking and grooming any offspring (body + anogenital region); 2) mother nursing offspring in an arched-back posture with rigid limbs (“high kyphosis”); 3) mother nursing in a “blanket” posture, i.e. lying on the offspring or her limbs are rigid but she has a low dorsal arch posture (“low/partial kyphosis”); 4) mother nursing in a “passive” posture (“supine nursing”), i.e. lying on her