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Anxiety-like behaviour and associated neurochemical and endocrinological alterations in male pups exposed to prenatal stress

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Summary Epidemiological studies suggest that emotional liability in infancy could be a predictor of anxiety-related disorders in the adulthood. Rats exposed to prenatal restraint stress (''PRS rats'') represent a valuable model for the study of the interplay between environmental triggers and neurodevelopment in the pathogenesis of anxious/depressive like behaviours. Repeated episodes of restraint stress were delivered to female Sprague-Dawley rats during pregnancy and male offspring were studied. Ultrasonic vocalization (USV) was assessed in pups under different behavioural paradigms. After weaning, anxiety was measured by conventional tests. Expression of $GABA_A$ receptor subunits and metabotropic glutamate (mGlu) receptors was assessed by immunoblotting. Plasma leptin levels were measured using a LINCOplex bead assay

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kit. The offspring of stressed dams emitted more USVs in response to isolation from their mothers and showed a later suppression of USV production when exposed to an unfamiliar male odour, indicating a pronounced anxiety-like profile. Anxiety like behaviour in PRS pups persisted one day after weaning. PRS pups did not show the plasma peak in leptin levels that is otherwise seen at PND14. In addition, PRS pups showed a reduced expression of the γ2 subunit of $GABA_A$ receptors in the amygdala at PND14 and PND22, an increased expression of mGlu5 receptors in the amygdala at PND22, a reduced expression of mGlu5 receptors in the hippocampus at PND14 and PND22, and a reduced expression of mGlu2/3 receptors in the hippocampus at PND22. These data offer a clear-cut demonstration that the early programming triggered by PRS could be already translated into anxiety-like behaviour during early postnatal life.

1. Introduction

In the pediatric population, anxiety disorders are quite common with prevalence estimates ranging from 5 to 18% (reviewed by Costello et al., 2005; Stein and Stein, 2008; Ramsawh et al., 2010). Epidemiologic studies indicate that children exposed to early adverse experiences are at increased risk for the development of depression and anxiety disorders in the adulthood (Heim and Nemeroff, 2001; Cartwright-Hatton, 2006). Whether adverse early life events cause anxiety also in babies and children is less clear.

We decided to examine the impact of early life stress on anxiety in the early postnatal life using as a model the offspring of pregnant rats exposed to prenatal restraint stress (here indicated as ''PRS rats''). Adult PRS rats show behavioural and endocrinological changes, which are indicative of an anxious/depressive-like phenotype (Darnaudery and Maccari, 2008; Weinstock, 2008). These include (i) anxiety-like behavior in the elevated plus maze (EPM) and the open field tests (Vallee et al., 1997; Mairesse et al., 2007a; Zuena et al., 2008); (ii) a disorganization of circadian rhythms (Koehl et al., 1999) and sleep-wake cycle (Dugovic et al., 1999), which is also seen in depressed patients (Hickie and Rogers, 2011); and (iii) an increased reactivity of the hypothalamic–pituitary–adrenal (HPA) to stress, which is already manifest in the early postnatal life (Henry et al., 1994; Maccari et al., 1995, 2003; Mairesse et al., 2007a). Adult PRS rats also show long-lasting neurochemical changes, which fit nicely with their anxiety-like profile. Adult PRS rats show a reduced density of benzodiazepine recognition sites at $GABA_A$ receptors in the hippocampus and central nucleus of the amygdala (Barros et al., 2006), and a reduced expression of mGlu2/3 and mGlu5 metabotropic glutamate receptors in the ventral hippocampus (Zuena et al., 2008; Morley-Fletcher et al., 2011). The γ2 subunit of $GABA_A$ receptors is essential for binding and activity of benzodiazepines, and mice heterozygous for the γ2 subunit represent a model of chronic or trait anxiety (Crestani et al., 1999). mGlu2/3 and mGlu5 receptors are potential targets for the treatment of anxiety disorders (reviewed by Swanson et al., 2005; Nicoletti et al., 2011). All these features make PRS rats an experimental animal model of depression and anxiety endowed with face, construct, and predictive pharmacological validity (Morley-Fletcher et al., 2003, 2004, 2011; Maccari et al., 2003; Maccari and Morley-Fletcher, 2007).

Here, we studied the influence of PRS on anxiety-like behavior in pups using a battery of behavioural tests based on measurements of ultrasonic vocalizations (USVs). Existing data on prenatal stress and USVs in pups are not homogeneous (Takahashi et al., 1990; Williams et al., 1998; Zimmerberg and Blaskey, 1998; Morgan et al., 1999; Harmon et al., 2009; Jones et al., 2010). For example, prenatal stress induced by uncontrollable electric shock or by exposure to high noise during pregnancy reduced USV emission in 14-day old pups (Takahashi et al., 1990; Morgan et al., 1999). In contrast, increases in USV emission were found in 14-day old after mothers' exposure to unpredictable variable stressors during the third week of pregnancy (Williams et al., 1998; Harmon et al., 2009). Here, we monitored USV emissions in PRS rats at different days after birth during maternal separation (Hofer, 1996; Wohr and Schwarting, 2008), and under conditions that increase the power of USVs as indicators of stress-induced anxiety (Smotherman et al., 1974; Takahashi, 1992a; Hofer et al., 1998). An increased number of USVs emitted by pups following separation from their mothers is considered as a reliable indicator of neonatal anxiety (Gardner, 1985; Hofer and Shair, 1978; Winslow and Insel, 1991; Hofer, 1996; Takahashi, 1992b; Takahashi and Kim, 1994). In the paradigm of ''maternal potentiation'', a brief contact between an isolated pup and a dam results into a marked increase in USV in the subsequent isolation period. This potentiation effect is not seen after identical periods of brief contact with a group of littermates (which has a comparable quieting effect on USV). At the very opposite, USVs are suppressed when pups are exposed to an unfamiliar male odor (Takahashi, 1992a). This particular response is suppressed by adrenalectomy and restored by corticosterone replacement (Takahashi and Kim, 1994).

We also examined the expression of $GABA_A$, mGlu2/3 and mGlu5 receptors in the hippocampus and amygdala, and blood levels of leptin as potential neurochemical and endocrinological surrogates of anxiety in pups. Remarkably, USVs are sensitive to treatments with benzodiazepines (which positively modulate $GABA_A$ receptors) and mGlu5 receptor agonists (Hodgson et al., 2008; Takahashi et al., 2009). Leptin is a feeding hormone that regulates neurodevelopment during early postnatal life (Bouret and Simerly, 2007), and has a pivotal role in linking feeding to emotional responses (reviewed by Bouret, 2010). Plasma leptin levels show a developmental peak during the second week of postnatal life (Smith and Waddell, 2003; Bouret et al., 2004a). Leptin is known to negatively regulate the HPA axis (Heiman et al., 1997). Plasma levels of leptin and corticosterone are inversely related in naive pups, and high neonatal leptin exposure enhances brain expression of glucocorticoid receptors (GRs), thereby increasing the efficacy of the feedback regulation of