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Denial or receipt of expected reward through maternal contact during the neonatal period differentially affect the development of the rat amygdala and program its function in adulthood in a sex-dimorphic way

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Summary Early experiences affect brain development and thus adult brain function and behavior. We employed a novel early experience model involving denial (DER) or receipt of expected reward (RER) through maternal contact in a T-maze. Exposure to the DER experience for the first time, on postnatal day 10 (PND10), was stressful for the pups, as assessed by increased corticosterone levels, and was accompanied by enhanced activation of the amygdala, as assessed by c-Fos immunohistochemistry. Re-exposure to the same experience on days 11—13 led to adaptation. Corticosterone levels of the RER pups did not differ on the first and last days of training (PND10 and 13 respectively), while on PND11 and 12 they were lower than those of the CTR. The RER experience did not lead to activation of the amygdala. Males and females exposed as neonates to the DER or RER experience, and controls were tested as adults in the open field task (OF), the elevated plus maze (EPM), and cued and contextual fear conditioning (FC). No group differences were found in the EPM, while in the OF, both male and female DER animals, showed increased rearings, compared to the controls. In the FC, the RER males had increased memory for both context and cued conditioned fear, than either the DER or CTR. On the other hand, the DER males, but not females showed an increased activation, as assessed by c-Fos expression, of the amygdala following fear conditioning. Our results show that the DER early experience programmed the function of the adult amygdala as to render it more sensitive to fearful stimuli. This

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programming by the DER early experience could be mediated through epigenetic modifications of histones leading to chromatin opening, as indicated by our results showing increased levels of phospho-acetyl-histone-3 in the amygdala of the DER males.

1. Introduction

Early life experiences affect hypothalamus–pituitary–adrenal (HPA) axis reactivity, emotionality and fear-related behavior. Early life stress, in the form of maternal separation increases emotionality and anxiety (Holmes et al., 2005; Penke et al., 2001) and affects fear conditioning (Kosten et al., 2006; Stevenson et al., 2009), whereas a milder early life manipulation, i.e. neonatal handling, reduces emotionality and fear responses (Macri et al., 2004; Madruga et al., 2006). Furthermore, qualitative differences in maternal care, such as high amounts of licking and grooming of the offspring, produces a "neophilic" animal that is more exploratory of novel environments and less emotionally reactive with a lower and more contained glucocorticoid stress response in novel situations; poor maternal care leads to a "neophobic" phenotype with increased emotional and HPA reactivity and less exploration in a novel situation (McEwen, 2007; Meaney et al., 1993).

In rodents, there are sex differences in HPA axis function and emotionality. More specifically, female rats have higher basal corticosterone levels and exhibit a faster and higher stress-induced corticosterone response (Beiko et al., 2004); activity and exploration, as well as unconditioned fear are also higher in female than male rats (Archer, 1975). The use of animal models of early experiences have documented that males are more sensitive to the effects of manipulations of mother-offspring interactions (Slotten et al., 2006; Wells, 1976).

Fear and anxiety-related behaviors depend mostly on amygdalar function (Davis, 1992; LeDoux, 2003), and do not emerge until the functional maturation of the amygdala, which is dependent on corticosterone. In the rat, corticosterone levels during the stress hyporesponsive period (SHRP) are maintained low through contact with the mother (Stanton et al., 1987; Moriceau and Sullivan, 2006) and prevent maturation of amygdala and thus fear learning, which emerges later, toward the end of the SHRP, when stress-induced corticosterone increase permits amygdalar activation (Moriceau et al., 2004). It should be noted that the end of the SHRP coincides with the time at which the mother begins to leave the pups more often in the nest for short periods and to forage in the cage.

As clearly shown by the group of R. Sullivan (Moriceau et al., 2004; Moriceau and Sullivan, 2006) and presented above, contact with the mother is the stimulus regulating stress-induced increase in corticosterone, amygdala maturation, and the ability for fear learning. Thus in the present work we employed a new experimental model (Diamantopoulou et al., 2011) which involves manipulation of the pup-mother contact to study maturation of the amygdala and emotionality in adulthood. More specifically in our model one group of animals is denied the expected reward of maternal contact (DER), while the other is granted it (RER). This model allows the study of the effects of two different early life experiences; one that involves a fair degree of adversity (DER group), while the other one is of minor adversity, as it is terminated by receipt of maternal reward (RER group). Given the stressful characteristics of the DER experience, we hypothesized that it would lead to increased corticosterone release in the neonates, with consequent amygdalar activation, which would influence in a sex dimorphic way emotionality/anxiety and, most importantly, fear memory in adulthood. To evaluate this hypothesis, we measured corticosterone plasma levels in the DER, RER and control rat pups during the neonatal training and amygdalar activation following it, using c-Fos immunohistochemistry. Furthermore, in adulthood emotionality and fear memory of males and females of the three experimental groups were evaluated, using the open field task, the elevated plus maze and fear conditioning. The effects of the latter on corticosterone levels and amygdalar activation were also determined. Epigenetic modifications have been shown to underlie the long-term effects of early experiences (Weaver et al., 2004). We thus also determined in the amygdala under basal conditions the levels of phospho-acetyl-histone-3 phosphorylated on serine 10 and acetylated on lysine 14 (pAcH3), which is present in transcriptionally active chromatin. Furthermore, we performed double immunolabeling for pAcH3 and c-Fos following cue fear memory in order to demonstrate that chromatin opening is linked to c-fos expression.

2. Materials and methods

2.1. Animals

Wistar rats of both sexes born and reared in our colony were used in these experiments. Animals were kept under standard conditions (24 °C, 12:12 h light/dark cycle) and received food (Kounker-Keramari Bros. & Co., Athens, Greece) and water ad libitum. Prior to the day of birth, which was designated as postnatal day 0, each litter was assigned randomly to either of the two experimental groups [pups denied (DER) or receiving (RER) the expected reward], or to the control (CTR – non-handled) group. In order to maintain stable environmental stimulation of the pups, instead of cleaning the cage, wood chip was added every 4–5 days, without disturbing either the pups or the dam. On postnatal day 22, animals were weaned and housed in same-sex, same group (DER, RER, CTR) of three-four animals per cage. Overall, four different cohorts of animals were used for different experimental procedures (Fig. 1). More specifically, one cohort (#1) of animals was used for the experiments during the neonatal period [postnatal days 10–13 (PND10–13)]. In this cohort, each group (DER, RER, CTR) of each postnatal day consisted of $n = 10 \pm 1$ rat pups. Two different cohorts of animals were used for the behavioral experiments in adulthood: One (#2) was used for fear conditioning (FC) and following it, c-Fos immunohistochemistry