Psychoneuroendocrinology (2015) **52**, 212—228

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/psyneuen

Rat dams exposed repeatedly to a daily brief separation from the pups exhibit increased maternal behavior, decreased anxiety and altered levels of receptors for estrogens (ERα, ERβ), oxytocin and serotonin (5-HT1A) in their brain

Antonios Stamatakis, Theodora Kalpachidou, Androniki Raftogianni, Efstratia Zografou, Athanasia Tzanou, Stavroula Pondiki, Fotini Stylianopoulou[*]

Biology—Biochemistry Lab, School of Health Sciences, National and Kapodistrian University of Athens, Papadiamantopoulou 123, GR-11527 Athens, Greece

Received 2 July 2014; received in revised form 27 October 2014; accepted 17 November 2014

KEYWORDS
Maternal behavior;
Anxiety;
Estrogen receptors;
Oxytocin receptors;
Serotonergic system;
Prefrontal cortex;
Hippocampus;

Abstract In the present study we investigated the neurobiological mechanisms underlying expression of maternal behavior. Increased maternal behavior was experimentally induced by a brief 15-min separation between the mother and the pups during postnatal days 1 to 22. On postnatal days (PND) 12 and 22, we determined in experimental and control dams levels of anxiety in the elevated plus maze (EPM) as well as the levels of receptors for estrogens (ERα, ERβ), oxytocin (OTR) and serotonin (5-HT1AR) in areas of the limbic system (prefrontal cortex-PFC, hippocampus, lateral septum-SL, medial preoptic area-MPOA, shell of nucleus accumbens-nAc-Sh, central-CeA and basolateral-BLA amygdala), involved in the regulation of maternal behavior. Experimental dams, which showed increased maternal behavior towards their offspring, displayed reduced anxiety in the EPM on both PND12 and PND22. These behavioral differences could

---

Abbreviations: 5-HT1AR, serotonin receptor type 1A; ANOVA, analysis of variance; BLA, basolateral amygdala; CA1-3, cornus Ammonis areas 1—3; Cb, cerebellum; CeA, central amygdaloid nucleus; CPu, caudate-putamen; DAB, 3.3′-diaminobenzidine; DG, dentate gyrus; EPM, elevated plus maze; ERα, estrogen receptor type α; ERβ, estrogen receptor type β; GEE, generalized estimated equations; Hipp, hippocampus; LG, licking & grooming; LG-ABN, licking & grooming and arched-back nursing; MPOA, medial preoptic area; nAc-Sh, shell of nucleus accumbens; ND, below detection limit; NDS, normal donkey serum; NGS, normal goat serum; Occ, occipital cortex; OTR, oxytocin receptor; PBS, phosphate buffered saline; PFC, prefrontal cortex; PND, postnatal day; S.E.M., standard error of the mean; SL, lateral septum; Sm, somatosensory cortex.

[*] Corresponding author. Tel.: +0030 2107461467; fax: +0030 2107461489.
E-mail address: fstilian@nurs.uoa.gr (F. Stylianopoulou).

http://dx.doi.org/10.1016/j.psyneuen.2014.11.016
0306-4530/

Amygdala;
Medial preoptic area;
Nucleus accumbens

be attributed to neurochemical alterations in their brain: On both PND12 and PND22, experimental mothers had higher levels of ERα and OTRs in the PFC, hippocampus, CeA, SL, MPOA and nAc-Sh. The experimental manipulation-induced increase in ERβ levels was less widespread, being localized in PFC, the hippocampal CA2 area, MPOA and nAc-Sh. In addition, 5-HT1ARs were reduced in the PFC, hippocampus, CeA, MPOA and nAc-Sh of the experimental mothers. Our results show that the experience of the daily repeated brief separation from the pups results in increased brain ERs and OTRs, as well as decreased 5-HT1ARs in the dam's brain; these neurochemical changes could underlie the observed increase in maternal behavior and the reduction of anxiety.

1. Introduction

Maternal care, especially during the early stages of postnatal life, is the main source of environmental stimuli for the progeny and a major determinant of behavior in adulthood (Bowlby, 1988). It is widely accepted that the quality and quantity of care received by a child can shape its emotionality and social skills, well into adulthood (McEwen, 2003). Many studies, both in humans and in animal models, have investigated the effects of variations in maternal care on the brain and behavior of the off-spring. In contrast, very few have dealt with the maternal brain itself and the neural mechanisms underlying the expression of maternal behavior, although this information is vital for understanding deviant forms of mothering such as abuse or neglect.

Expression of maternal behavior in rodents is linked to gestation. Adult virgin females are neophobic (Jakubowski and Terkel, 1985). On the contrary, pregnant females in the final stages of gestation progressively exhibit aspects of maternal behavior, although the full repertoire appears following parturition (Kristal, 2009) and declines rapidly by the end of the 2nd postnatal week, when pups open their eyes (around postnatal days 13–14) and become more independent (Cramer et al., 1990). Key factors in eliciting maternal behavior are the estrogens, whose high levels prior to parturition prime the maternal brain, so that maternal behavior is expressed following the surge of oxytocin during parturition (Young et al., 1997).

Estrogens act in the brain mainly through two types of estrogen receptors: ERα and ERβ. Both subtypes of ERs have been implicated in the regulation of maternal behavior: ERs are modified in the hypothalamus and extended amygdala during pregnancy and parturition (Wagner and Morrell, 1996; Gréco et al., 2003). Interestingly, both types of ERs participate in the regulation of the oxytocinergic system: ERβs regulate the expression of the oxytocin gene (Patisaul et al., 2003) while ERαs that of the oxytocin receptor (Vasudevan et al., 2001). Natural variations in maternal care are associated with differences in the expression of both ERα and oxytocin receptors (OTR) in the hypothalamus: Mothers exhibiting high levels of licking and grooming as well as nursing in the arched-back position (High LG-ABN) have high levels of ERα and OTR in the preoptic area of the hypothalamus (Francis et al., 2000; Champagne et al., 2001, 2003). Estrogen receptors are also intimately involved in the regulation of serotonergic system function, affecting the expression and function of serotonin receptors and thus serotonergic tone (Bethea et al., 2002).

The oxytocinergic and serotonergic systems play a pivotal role in regulating the expression of maternal behavior. Administration of oxytocin into the cerebral ventricles elicits maternal behavior in virgin rats (Pedersen et al., 1982) and activates brain areas involved in maternal behavior (Ferris, 2008). On the contrary, blocking of oxytocin actions inhibits these same areas (Ferris, 2008) and markedly disrupts maternal behavior (Pedersen and Boccia, 2003; Sabihi et al., 2014), and maternal memory (D'Cunha et al., 2011). OTRs, which mediate the actions of oxytocin, are increased during the post partum period (Insel, 1990). Likewise, the serotonergic system also plays a role in regulating maternal behavior: Neurotoxic lesions of the serotonergic median raphe nuclei, or reduction of the serotonergic input into the hypothalamus disrupt lactation and maternal behavior (Rowland et al., 1978; Barofsky et al., 1983). On the other hand, systemic administration of the SSRI fluoxetine enhances maternal behavior (Johns et al., 2005; Pawluski et al., 2012). Furthermore serotonin turnover is increased during lactation (Lonstein et al., 2003), and dietary modulation of serotonin is accompanied by alterations in nesting behavior (Browne et al., 2012).

Interestingly, the two key players controlling maternal behavior, the oxytocinergic and serotonergic (particularly 5-HT1A receptors—5-HT1ARs) systems are also known to modulate anxiety (Neumann, 2008; Arrant et al., 2013). It is well known that during lactation, anxiety is reduced both in rats (for a review see Neumann, 2003) and in humans (Lonstein, 2007). Based on the above, we hypothesized that an experimental manipulation which alters maternal care, would do so by inducing changes in the levels of ERα and ERβ as well as OTRs and 5-HT1ARs in limbic system areas, i.e. the prefrontal cortex-PFC, hippocampus, medial preoptic area-MPOA, lateral septum-SL, shell of nucleus accumbens-nAc-Sh and amygdala (central-CeA and basolateral-BLA nuclei), which are known to participate in the network controlling the expression of maternal behavior (for reviews see Numan and Sheehan, 1997; Pereira and Morrell, 2011; Ruthschilling et al., 2012). Increased maternal behavior in the experimental group of dams was induced by subjecting them to a repeated brief separation (15 min daily) from their pups on postnatal days 1 to 22, as in the ''neonatal handling'' protocol (Levine, 1957). This experimental manipulation has been used extensively since its first description in 1957 and it is clearly established that it results in increased maternal behavior (Smotherman, 1983; Levine, 1994; Liu et al., 1997; Pryce et al., 2001; Fenoglio et al., 2006; Garoflos et al., 2008). Moreover, we investigated the