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DIFFERENTIAL POSTPARTUM SENSITIVITY TO THE ANXIETY-MODULATING EFFECTS OF OFFSPRING CONTACT IS ASSOCIATED WITH INNATE ANXIETY AND BRAINSTEM LEVELS OF DOPAMINE BETA-HYDROXYLASE IN FEMALE LABORATORY RATS

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Abstract—In female mammals, the postpartum period involves dramatic shifts in many socioemotional behaviors. This includes a suppression of anxiety-related behaviors that requires recent physical contact with offspring. Factors contributing to differences among females in their susceptibility to the anxiety-modulating effect of offspring contact are unknown, but could include their innate anxiety and brain monoaminergic activity. Anxiety was assessed in a large group of nulliparous female rats and the least-anxious and most-anxious tertiles were mated. Anxiety was assessed again postpartum after females were permitted or prevented from contacting their offspring 4 h before testing. Levels of dopamine β-hydroxylase (DBH, norepinephrine synthesizing enzyme) and tryptophan hydroxylase 2 (TPH2, serotonin synthesizing enzyme) were measured in the brainstem and dorsal raphe, respectively. It was found that anxiety-related behavior in the two groups did not differ when dams were permitted contact with offspring before testing. Removal of the offspring before testing, however, differentially affected anxiety based on dams' innate anxiety. Specifically, dams reverted back to their pre-mating levels of anxiety such that offspring removal slightly increased anxiety in the most-anxious females but greatly lowered anxiety in the least-anxious females. This reduction in anxiety in the least-anxious females after litter removal was associated with lower brainstem DBH. There was no relationship between females' anxiety and dorsal raphe TPH2. Thus, a primary effect of recent contact with offspring on anxiety-related behavior in postpartum rats is to shift females away from their innate anxiety to a more moderate level of responding. This is particularly true for females with the lowest anxiety, which may be mediated by central noradrenergic systems, and has implications for their ability to attend to their offspring.

Key words: anxiety, female, peripartum, norepinephrine, serotonin, touch.

INTRODUCTION

The onset and maintenance of motherhood is a time of tremendous neurobehavioral flux for female mammals (Numan et al., 2006; Lonstein et al., 2013; Sisk et al., 2013). This flux involves salient changes in how females process social stimuli, most obviously resulting in heightened positive responses to neonates, as well as changes in the new mother's emotional state that helps or hinders these positive responses. While the early postpartum period has been characterized for some women as a time of particular susceptibility to anxiety and other types of emotional dysregulation, the majority of women and other female animals studied show stable or even improved emotional regulation during the postpartum period (Neumann, 2003; Heron et al., 2004; Ross and McLean, 2006; Lonstein, 2007). Indeed, most studies find that anxiety-related behavior in early postpartum laboratory rodents is lower when compared to females that have not given birth (see Lonstein, 2007 for review). In rats, this reduction depends on recent suckling or non-suckling physical contact with offspring, as dams' anxiety-related behavior rises to levels found in nulliparous females if the litter is removed even for a few hours before testing (Lonstein, 2005; Figueira et al., 2008; Smith and Lonstein, 2008; Miller and Lonstein, 2011). A similar anxiolytic effect of recent suckling or non-suckling contact with infants has been found in human mothers (Heinrichs et al., 2001).

Studies on this topic in laboratory rats have provided valuable information about what can be expected for the anxiety-related behaviors of most postpartum females in response to infant contact. However, postpartum female rats may be heterogeneous in how their anxiety is affected by physical contact with neonates. This is suggested by the fact noted above that women are differentially susceptible to anxiety dysregulation during the postpartum period, with one of the best predictors of women's level of postpartum anxiety being their history of anxiety before giving birth (Engle et al., 1990; Hundley et al., 1998; O'Connor et al., 2002; Heron et al., 2004; Britton, 2008; Grant et al., 2008). Such innate or "trait" anxiety could also contribute to heterogeneity in the anxiety-related behavior of

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Abbreviations: BSTv, ventral bed nucleus of the stria terminalis; DBH, dopamine β-hydroxylase; EPM, elevated plus maze; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; TBS, Tris-buffered saline; TBS-T, Tris-buffered saline with 0.05% Tween-20; TPH2, tryptophan hydroxylase-2.

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postpartum laboratory rats, and there is a burgeoning body of research demonstrating the stability of emotional traits (including anxiety) in individual non-human animals across the lifespan (Burtt, 1967; Lister, 1987; Clarke and Boinski, 1995; Leibsch et al., 1998; Henniger et al., 2000; Gosling, 2001; Landgraf and Wigger, 2002; Cavigelli et al., 2007; Uher et al., 2008; Quinn et al., 2011; Curley et al., 2012). Furthermore, in both rodents and humans, differences among individuals in anxiety or the experimental instillation of anxious states affects somatosensory functioning (Jorum, 1988; van Meeteren et al., 1997; Kain et al., 2000; Rhudy and Meagher, 2000; Geerse et al., 2006; Devall et al., 2009; Aron et al., 2012; Corral-Frias et al., 2013). If the same is true for postpartum rats, mothers with the highest anxiety could be the most sensitive to, and benefit the most from, tactile inputs provided by the litter. One could alternatively conjecture that if maternal tactile sensitivity is too high, interacting with pups could be aversive and not reduce anxiety.

The neurochemicals underlying postpartum anxiety in general or its modulation by offspring contact are not very well understood. Research on this topic has traditionally focused on ovarian hormones (e.g., estradiol, progesterone) and peptides (e.g., oxytocin, prolactin) (Neumann, 2003; Lonstein, 2007), but classic neurotransmitter systems that modulate anxiety in nulliparous animals, such as norepinephrine and serotonin, are also involved. Noradrenergic neurons located in the locus coeruleus, ventrolateral medulla, and elsewhere in the brainstem have reciprocal connections with many areas of the limbic system and hypothalamus associated with emotion regulation (McKeller and Loewy, 1982; Woulfe et al., 1990). Elevated activity of these noradrenergic pathways is associated with anxiety in both laboratory rats (Tanaka et al., 2000; Neophytou et al., 2001; Dazzi et al., 2002; Fendt et al., 2005; Debiec and LeDoux, 2006) and humans (Sullivan et al., 1999; Tanaka et al., 2000; Ravindran and Stein, 2010; Kalk et al., 2011). Compared to nulliparous rats, postpartum rats have lower noradrenergic activity in some areas of the forebrain involved in the behavioral and physiological responses to anxiogenic stimuli (Toufexis and Walker, 1996; Windle et al., 1997; Toufexis et al., 1998; Douglas, 2005) and this may partly be mediated by brainstem noradrenergic neurons that are sensitive to tactile cues from pups (Li et al., 1999). The serotonin-synthesizing neurons in the brain are mostly located in the midbrain dorsal raphe nucleus and are also interconnected to many neural structures underlying anxiety and other emotional behaviors (Feldman et al., 1987; Chen et al., 1992; Hensler et al., 1994; Dinan, 1996; Ziegler and Herman, 2002; Lechin et al., 2006). The relationship between serotonin and anxiety in rodents is equivocal, though, as experimental manipulations of central serotonin systems have been seen to either increase or decrease anxiety-related behavior (Briley et al., 1990; Critchley et al., 1992; Kalueff et al., 2007; Olivier et al., 2008; Mosienko et al., 2012). Even so, peripartum plasticity of serotonergic cells in the dorsal raphe may render this system particularly influential for how postpartum state and physical interaction with pups affect maternal anxiety (Klink et al., 2002; Robichaud and Debonnel, 2005; Holschbach and Lonstein, 2013).

In the present experiment, we examined if mother laboratory rats differ in how contact with pups influences their anxiety-related behavior, based on whether the mothers were characterized as having a low-anxiety or a high-anxiety profile before giving birth. We then assessed the relationships between their anxiety-related behavior and brainstem expression of dopamine β-hydroxylase (DBH, the rate-limiting enzyme for norepinephrine synthesis), which is very highly correlated with levels of brain norepinephrine (Coyle and Axelrod, 1972; Hartman et al., 1972), and midbrain dorsal raphe expression of tryptophan hydroxylase-2 (TPH2, the rate-limiting enzyme for serotonin synthesis), which is highly correlated with brain serotonin content (Walther et al., 2003; Donner and Handa, 2009). We hypothesized that, unlike randomly selected postpartum laboratory rats that mostly show reduced anxiety in response to recent contact with the litter (Lonstein, 2005; Figueira et al., 2008; Smith and Lonstein, 2008; Miller and Lonstein, 2011), mother rats with the highest anxiety would be the most sensitive to the anxiolytic effect of physical contact whereas mothers with the lowest anxiety would not be affected at all due to a floor effect. Considering the relationship between noradrenergic activity and anxiety in non-postpartum mammals, we predicted an inverse relationship between brainstem levels of DBH and dams' anxiety-related behavior, while the relationship between dams' anxiety and dorsal raphe levels of TPH2 was more exploratory.

EXPERIMENTAL PROCEDURES

Subjects

Subjects were adult female Long–Evans rats, descended from rats purchased from Harlan Laboratories (Indianapolis, IN) that were born and raised in our colony and housed as described previously (Smith and Lonstein, 2008). Beginning at 65 days of age, subjects' estrous cycles were monitored daily by vaginal smear and pre-mating anxiety testing occurred on a day of diestrus (details below). Diestrus was chosen because of it is characterized by low circulating ovarian hormone titers that are similar to lactational diestrus (Tsukamura and Maeda, 2001). Between 90 and 100 days of age, estrous cycles were monitored daily with a vaginal impedance meter (Fine Science Tools, Foster City, CA, USA) and on a day of proestrus females were housed with sexually experienced males from our colony for 2 days. After mating, females were housed with another pregnant female until being singly housed 5–7 days before expected parturition. Litters were culled to contain four males and four females within 24 h after birth. All work was conducted in accordance with the National Institutes of Health Guide for Care and Use of

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