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Research Report

Hypothalamic expression of serotonin 1A, 2A and 2C receptor and GAD67 mRNA in female cynomolgus monkeys with different sensitivity to stress

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ABSTRACT

Like women, female cynomolgus monkeys show differential sensitivity to stress-induced reproductive dysfunction. A combined social and metabolic stress (mild diet+moderate exercise+relocation) will rapidly induce anovulation in a third of female cynomolgus monkeys (stress-sensitive; SS); a third will ovulate once and then become anovulatory (medium stress-resilient; MSR) and a third are highly stress-resilient (HSR) and exhibit normal menstrual cycles through two stressed menstrual cycles. In a non-stressed menstrual cycle, SS animals have lower levels of estrogen and progesterone, lower activity of the serotonin system and lower expression of genes related to the serotonin system in the dorsal raphe nucleus. In this study, we examined the expression of 5HT1A, 5HT2A, 5HT2C receptors and GAD67 in the hypothalamus of SS, HSR and MSR monkeys using in situ hybridization. SS monkeys exhibited higher expression of 5HT2A mRNA in the paraventricular nucleus (PVN), higher expression of 5HT2C and GAD67 in the infundibulum, as well as higher expression of GAD67 in the posterior hypothalamus (PH), compared with HSR monkeys. However, the expression of 5HT1A mRNA in the ventromedial nucleus (VMN) was not different between groups. We speculate that the serotonin and GABA systems may be altered in the stress–response and reproductive-related circuits of SS monkeys, and may be participating in altering the sensitivity of the reproductive system to stress in these individuals.

1. Introduction

In several animal species, a stressor can suppress the activity of the hypothalamic-pituitary-gonadal axis and lead to infertility (Akema et al., 1996; Chatterton, 1990; Genazzani, 2005; Warren and Fried, 2001). These stressors can be metabolic, such undernutrition and exercise (Couzinet et al., 1999; Wade and Jones, 2001; Williams et al., 2001), as well as psychosocial (Berga and Girton, 1989; Berga et al., 2003; Giles and Berga, 1993). However, it is recognized that there are individual differences in sensitivity of the reproductive system to stress, with some individuals showing a marked

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suppression of reproductive function, while others are stress-resilient. The primary target of the stress stimulus over the reproductive axis appears to be at the central nervous system, affecting hypothalamic GnRH activity (Aono et al., 1975; Chen et al., 1996; Ferin, 1999; He et al., 1999). However, the neural systems that are involved in the individual differences in sensitivity of the reproductive system to stress are unknown.

In the present study, we used non-stressed female cynomolgus monkeys previously characterized as stress-sensitive, medium stress-resilient or highly stress resilient (Bethea et al., 2004, 2005). When exposed to a combination of stresses (psychosocial stress+mild dieting+moderate exercise) for two menstrual cycles, these monkeys showed differential responses in reproductive function. One-third of the animals was stress-sensitive (SS), had an immediate suppression of ovulation, while one-third was highly stress-resilient (HSR), and continued to ovulate through the stress cycles. The final third were ovulatory in the first stress cycle, but became anovulatory in the second stress cycle and were defined as medium stress-resilient (MSR). Stress-sensitive monkeys have lower levels of follicular phase estradiol secretion, lower luteal phase progesterone secretion and higher heart rates compared to stress-resilient individuals, even when they are not exposed to stress (Bethea et al., 2004). In addition, stress-sensitive monkeys have reduced expression of serotonin-related genes as well as a reduced number of serotonin neurons in the dorsal raphe nucleus (Bethea et al., 2005). Stress-sensitive monkeys also exhibited lower prolactin secretion in response to fenfluramine challenge, suggesting lower endogenous serotonin is available for release (Bethea et al., 2004).

The serotonin neural system is involved in the regulation of diverse functions in response to stress (Azmitia and Gannon, 1986; Jacobs and Azmitia, 1992; Mann et al., 1996; Van de Kar, 1991). Decreased activity of the central serotonin system is found in individuals with increased stress sensitivity and anxiety disorders (Bhagwagar et al., 2002; Lehner et al., 2006; Ressler and Nemeroff, 2000; Stein et al., 2006). In addition, stress affects serotonin function in a variety of ways depending on the intensity and duration of the stress (Botchin et al., 1994; Shively et al., 1995; Filipenko et al., 2002). There are at least 18 serotonin receptor subtypes present in the brain, each with different functions and different brain localizations (Barnes and Sharp, 1999; Uphouse, 1997). The 5HT1A, 2A and 2C receptors are the major serotonin receptors involved in the regulation of mood and anxiety (Blier et al., 1997; Palvimaki et al., 1996; Toth, 2003; Van Oekelen et al., 2003).

The hypothalamus receives abundant innervation from the raphe serotonergic system (Azmitia and Gannon, 1986; Azmitia and Segal, 1978), and expresses serotonin 5HT1A, 2A

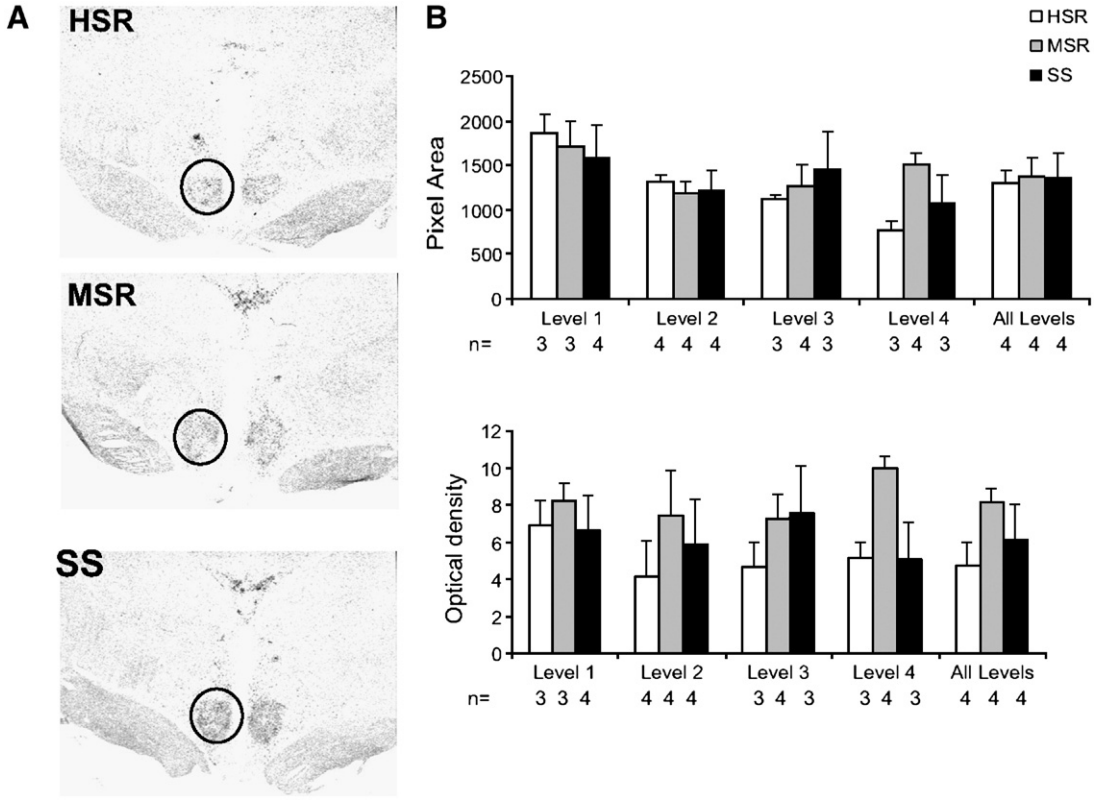

Fig. 1 – 5HT1A receptor mRNA expression in the ventromedial nucleus (VMN) of highly stress-resilient (HSR), medium stress-resilient (MSR) and stress-sensitive (SS) monkeys. (A) Representative autoradiograms of 5HT1A ISH signal in the VMN of one HSR, one MSR and one SS monkey. Outlined is the area considered for the optical analysis. (B) Mean ± SEM optical density and pixel area at different levels of the VMN of HSR, MSR and SS monkeys. There was no significant difference between groups.