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Distinctive stress effects on learning during puberty

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Abstract

Puberty is a time of significant change in preparation for adulthood. Here, we examined how stressful experience affects cognitive and related hormonal responses in male and female rats prior to, during and after puberty. Groups were exposed to an acute stressor of brief periodic tailshocks and tested 24 h later in an associative memory task of trace eyeblink conditioning. Exposure to the stressor did not alter conditioning in males or females prior to puberty but enhanced conditioning in both males and females during puberty. The enhancement occurred in pubescent females irrespective of the estrous cycle. In adulthood, sex differences in trace conditioning and the response to stress emerged: females outperformed males under unstressed conditions, but after stressor exposure, trace conditioning in females was impaired whereas that in males was enhanced. These differences were not related to changes in gross motor activity or other nonspecific measures of performance. The effects of acute stress on corticosterone, estradiol, progesterone, and testosterone were also measured. Stressor exposure increased the concentration of corticosterone in all age groups, although sex differences were only evident in adults. All reproductive hormones except estradiol increased with age in a predictable and sex dependent fashion and none were affected by stressor exposure. Estradiol decreased in male rats across age, and remained stable for female rats. Together, these data indicate that males and female respond similarly to learning opportunities and stressful experience before and during puberty; it is in adulthood that sex differences and the opposite responses to stress arise.

Keywords: Development; Memory; Eyeblink; Sex difference; Age; Gender; Depression; Corticosterone; Estrogen; Testosterone

Introduction

Clinical studies document that gender differences in mental disorders emerge during or shortly after puberty and many of these disorders are stress related. For example, depression, anxiety, eating, and post-traumatic stress disorders are all more prevalent in women than men, but only after puberty (Kaltiala-Heino et al., 2003; Kessler, 2003; Piccinelli and Wilkinson, 2000; Ruiz et al., 2000). These studies suggest that puberty is a time when women become most vulnerable to stressful experience and some types of psychopathology. Nevertheless, the literature addressing stress and how it affects behavior during puberty is very limited (McCormick et al., 2004). This is particularly surprising given the recent controversies surrounding the prescription of psychotropic drugs to children and adolescents for these disorders.

In humans, the onset of puberty is defined by first menstruation in girls, and first ejaculation/nocturnal emission in boys (Kaltiala-Heino et al., 2003). In rats, puberty is a relatively short period between about 33 and 56 days of age (Gabriel et al., 1992; Ojeda and Urbanski, 1994) and is indicated by canalization of the vagina in females and balanopreputial separation of the foreskin from the glans of the penis in males (Ojeda and Urbanski, 1994). Puberty in most mammalian species is associated with dramatic increases in circulating sex hormones. Anatomically, these changes induce secondary sexual characteristics and behaviorally they alter peer interaction and risk taking behavior (Laviola et al., 2002; Spear, 2000a,b). Within the brain, puberty is associated with changes in neurotransmitter release and concentration (Badr et al.,

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1989; Choi and Kellogg, 1996; Choi et al., 1997; Insel et al., 1990) and gross changes in volume and white matter organization that continue well into the late teens in humans (Gogtay et al., 2004; Paus et al., 1999). It is thus reasonable to assume that these changes in hormone concentration and anatomical structure are accompanied by changes in nonreproductive behaviors such as those associated with learning and memory.

In previous studies, we documented sex differences in learning. Specifically, we observed that female rats outperform males during acquisition of an associative learning task, classical eyeblink conditioning (Wood and Shors, 1998; Wood et al., 2001). These sex differences must emerge before, during or after puberty since males and females condition at similar rates prior to puberty (Ivkovich et al., 2000). Others have found similar effects for spatial learning (Kanit et al., 2000; Krasnoff and Weston, 1976). Together, these data suggest that sex differences in learning arise as the capacity for reproduction is being established. However, little is known about how sex differences affect learning during puberty itself.

In addition to sex differences in learning, we have observed robust sex differences in response to stressful experience. In particular, we have shown that exposure to an acute stressful experience of periodic tail shocks or inescapable swimming has opposite effects on classical eyeblink conditioning in male versus female rats (Wood and Shors, 1998; Wood et al., 2001). Exposure to the stressful event enhances conditioning in males, whereas exposure to the same event impairs conditioning in females. These effects of stress on later conditioning are not specific to hippocampal-dependent types of learning tasks and occur during training on hippocampal-independent tasks (Wood et al., 2001). They are also not dependent on sex differences in learning itself since they occur even when performance is similar between unstressed males and unstressed females (Wood and Shors, 1998). They also do not appear to reflect performance effects since stress does not alter the magnitude of the unconditioned response (UR), that is, the blink response to the eyelid stimulation (Bangasser and Shors, 2004; Servatius et al., 2001). Nor does it affect pain, sensitivity, or gross motor activity (Shors, 2001). These effects are, however, dependent on the *psychological* aspects of the stressful event since the opportunity to control the stressor eliminates both the enhanced learning in males and the deficit in females (Leuner et al., 2004).

The opposite effects of stress on learning in males versus females are organized by the presence of sex hormones during very early development and in utero. Thus, manipulations of testosterone in utero and shortly after birth eliminate the effects of stress on learning in males, and reverse the effects in females, as expressed in adulthood (Shors and Miesegaes, 2002). In adulthood, the expression of these behaviors is dependent on the activating effects of ovarian hormones in females and corticosterone in males (Beylin and Shors, 2003; Wood and Shors, 1998). Thus, the critical hormonal and psychological determinants of these sex differences have been described. However, we do not know when during development these sex differences emerge. Therefore, in the present studies, we examined sex differences in learning and the response to acute stress in rats before, during, and after puberty. We also examined how stress alters the production of corticosterone and the reproductive hormones, estrogen, testosterone, and progesterone in males and females before, during, and after puberty.

Material and methods

Subjects

Prepubescent (25–29 days), pubescent (35–40 days), and adult (60 days and older) Sprague–Dawley rats were weaned at 21 days and housed individually prior to and after surgery in the Department of Psychology animal facility at Rutgers University. They were given ad libitum access to water and laboratory chow and maintained on a 12:12 light/dark cycle with light onset at 8 am. For the conditioning studies, groups consisted of prepubescent males (stress = 9; no stress = 10), prepubescent females (stress = 9; no stress = 9), pubescent males (stress = 8; no stress = 11), pubescent females that were trained in diestrus/proestrus = (stress = 7; no stress = 10) or trained in estrus (stress = 8; no stress = 11), adult males (stress = 10; no stress = 11), adult females (stress = 9; no stress = 10). We also obtained blood from separate groups of animals in order to detect hormonal responses to the stressor immediately after its cessation. These groups consisted of male prepubescents (n = 10), female prepubescents (n = 10), male pubescents (n = 10), female pubescents sacrificed in estrus (n = 5) or in proestrus/diestrus (n = 7), male adults (n = 10), and adult females sacrificed in diestrus 2 (n = 12).

Vaginal cytology and determination of estrous cycle

Following vaginal canalization (Ojeda and Urbanski, 1994), smears were collected daily in the morning. A Q-tip immersed in physiological saline was inserted into the vaginal tract and rotated to remove loose cells. Cells were rolled onto a slide, dried and stained with 1% Toluidine Blue. Only adult females with a regular 4–5 days cycle including proestrus, estrus, diestrus 1, and 2 were included. Pubescent females alternated between a cytology consistent with estrus and another with characteristics of proestrus and diestrus 1. To evaluate the effects of the two stages in pubescent females, they were either stressed and trained 24 h later in estrus, or stressed and trained 24 h later in proestrus. As in previous studies, adult females were exposed to the stressor during diestrus 2 and trained 24 h