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Prenatal Stress Alters Seizure Thresholds and the Development of Kindled Seizures in Infant and Adult Rats

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Stressful events during gestation and in the neonatal period have important effects on the later physical and mental health of the offspring. The present study tested the hypothesis that pre- and/or postnatal stress would affect seizure susceptibility in infant and adult rats, using the hippocampal kindling model. Prenatal stress consisted of daily restraint of the dam under bright light (for 45 min, 3 × / day) during either early gestation or mid/late gestation. Pups were compared to pups born to unstressed dams. Postnatal stress (administered on Days 4 and 5 after birth) consisted of either separation from the dam and placement in the bedding of a strange male for 1 h or injection of dexamethasone. Pups were compared to nonstressed siblings of the same litter. Both early and mid/late-gestation prenatal stress significantly lowered the after-discharge threshold (ADT) in infant, 14-day-old rat offspring, as compared to nonstressed control offspring. This effect on ADT was lost by adulthood. Mid/late-gestation stress increased the rate of kindled seizure development in infant rats and in their adult male, but not female, siblings. Postnatal stress had no significant effect on ADT or kindling rate. These findings indicate that prenatal stress, particularly during the latter half of pregnancy, may play an important role in increasing seizure vulnerability in the unborn offspring. These effects are more pronounced in infancy, but can also extend to adulthood.

Key Words: **adrenal hormones; developing rat; seizure models.**

Pre- and postnatal environments exert profound influences on the development of an organism and can predispose it to adaptive disturbances in later life. Stress during pregnancy results in fetal exposure to chronic high levels of endogenous maternal corticosteroids (Takahashi, Turner, and Kalin, 1998; Stott, 1973). In humans, this has been associated with adverse birth outcomes, including preterm birth (Stott, 1973), fetal growth retardation (Cliver, Goldenburg, Cutter, Hoffman, Copper, Gotlieb, and Davis, 1992), delays in early motor development (Sandman, Wadhwa, Chicz-De-Met, Dunkel-Schetter, and Porto, 1997), behavioral abnormalities (Meier, 1985; Trautman, Meyer-Bahlburg, Postelnek, and New, 1995; Clements, 1992), sleep disturbances in the infant (Weinstock, 1997), and the development of psychiatric disorders, such as schizophrenia and depression in later life (Huttenen and Niskanen, 1978; Meier, 1985). In rats, prenatal stress has been shown to lead to impaired sexual function (Secoli and Teixeira, 1998; Anderson, Rhees, and Fleming, 1985), a vulnerability to anxiety (Takahashi, Haglin, and Kalin, 1992; Fride, Dan, Gavish, and Weinstock, 1985; Makino, Gold, and Schulkin, 1994), an increased propensity to self-administer drugs (Deminiere, Piazza, Guegan, Abrous, Maccari, Le Moal, and Simon, 1992), and impaired feedback regulation of the hypothalamic-pituitary-adrenal (HPA) axis due to decreased numbers of hippocampal corticosteroid receptors (Henry, Kabaj, Simon, Le Moal, and Maccari, 1994; Maccari, Piazza, Kabbaj, Barbanzanges, Simon, and Le Moal, 1995; Barbazanges, Piazza, Le Moal, and Maccari, 1996). With impaired feedback, animals show an increased and prolonged corticosterone secretion in response to stress (Henry *et al.*, 1994; Maccari *et al.*, 1995).

Like prenatal stress, postnatal events can give rise to high, chronic corticosteroid levels, due to activation of

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the organism's own HPA axis (Meier, 1985). In humans, this has been associated with an increased risk of developing major depressions and generalized anxiety disorders (Meier, 1985; Clements, 1992), as well as an increased vulnerability to disease later in life (i.e., atherosclerosis, diabetes, etc.) (Lupien and McEwen, 1997; Sandman *et al.*, 1997). In rats, postnatal glucocorticoid treatment influences emotional behavior (Golub, 1982; Pavlovska-Teglia, Stodulski, Svendsen, Dalton, and Hau, 1995) and maturation of the HPA axis (Felszeghy, Gaspar, and Nyakas, 1996). Thus, stressful events in early life appear to have important effects on HPA function and on the later physical and mental health of the unborn offspring.

Altered HPA function might influence seizure susceptibility. In adult epileptic humans, subjective stress and / or unpleasant life events are positively associated with seizure frequency (Neugebauer, Myunghee, Hauser, Nadel, Leppik, and Susser, 1994). In adult animals, studies have similarly shown a direct proconvulsant effect of the corticosteroids. Corticosterone replacement after adrenalectomy, for example, lowers amygdala seizure thresholds and facilitates the development of kindled seizures in adult rats (Edwards, Burnham, Mendonca, Bowlby, and MacLusky, 1999). As well, administration of high, pharmacologic doses of 11-deoxycortisol to adult rats, cats, and monkeys produces tonic-clonic convulsions, leading to status epilepticus and death (Heuser and Eidelberg, 1961). In infants, the effect of corticosteroids on seizure susceptibility is less clear.

Since pre- and postnatal stressors can alter regulation of the HPA axis, and since hormones of the HPA axis can affect seizure vulnerability—at least in adults, we hypothesized that prenatal stress and / or postnatal manipulations might alter seizure susceptibility in infant and adult rats. Previous investigators have demonstrated an effect of prenatal stress (i.e., dams exposed to 20 min of restraint stress on Gestational Day 18) on activity and duration of kainic-acid-induced seizures (Frye and Bayon, 1999). No one to date, however, has tested this hypothesis in infant rats, or has ever used the kindling model to measure seizure vulnerability.

The present study tested the effects of a prenatal stressor (i.e., repeated maternal restraint under bright lights) and a postnatal manipulation (i.e., either a dexamethasone treatment or a form of maternal separation) on hippocampal after-discharge thresholds (ADT) and rates of kindled seizure development in infant rats. The effects of our *prenatal* stressor were also tested on ADT and kindling rate in adult rats. The hippocampus was chosen as the kindling site because of its sensitivity to corticosteroid hormones and its role in HPA feedback regulation (Reul and De Kloet, 1985; McEwen, De Kloet, and Rostene, 1986)

METHODS

Subjects

Male and female Sprague-Dawley rats (250–275 g) were obtained from Charles River Breeding Farms (St. Constant, Quebec, Canada). They were 3–4 months old on delivery. Rats were housed singly in 24 × 24 × 45 cm transparent, plastic cages, with food (Purina Rat chow) and water provided *ad libitum*. The colony was maintained at 18°C on a 12-h light: 12-h dark schedule (lights on at 07:00 h). All procedures involving animals were approved by the University of Toronto Animal Care Committee.

Experiment 1: Effects of Prenatal Stress

Female rats were randomly paired with males 1 day after delivery to the animal care facility. Vaginal smears were performed on the females every morning (7:00–8:00 AM) to look for the presence of sperm. A blunted, plastic 20-μl Eppendorf tip was placed on the vaginal opening and the orifice was flushed twice with 15 μl of 0.9% saline. The saline flush was placed on a microscope slide and observed at 100× magnification. The presence of sperm marked Day 1 of pregnancy.

Pregnant dams were randomly sorted into one of four experimental groups: (1) early pregnancy stress, (2) mid / late-gestation stress, (3) nonstressed controls, and (4) transport controls. The first group consisted of five pregnant females who were stressed daily on Gestation Days 5–12 (i.e., early pregnancy stress). The second group consisted of six pregnant females who were stressed daily on Gestation Days 12–20 (i.e., mid / late-gestation stress). In those two groups, stress involved transport of the home cage to the experimental room and placement of the pregnant female in a restraint chamber (transparent, plastic, cylindrical chamber, 6.5 cm diameter, 18 cm length) under bright light from two lamps (100 W bulbs). Animals were restrained under bright light for 45 min, three times a day (between 9:00 and 10:00, 12:00 and 13:00, and 15:00 and 16:00 h). This procedure has previously been shown to cause alterations in the regulation of the