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Age-dependent sensitivity to glucocorticoids in the developing mouse basolateral nucleus of the amygdala

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Summary Experiences of severe trauma during childhood are thought to be risk factors for developing mental disorders, such as anxiety and mood disorders, later in life. Correspondingly, exposure of rodents to early-life stress has been shown to affect neuronal circuitry and emotional behavior in adulthood, indicating a significant impact of stress on brain development. One current hypothesis proposes that the developing central nervous system is more sensitive to environmental influences, such as stress, than the adult. To test this hypothesis, we compared long-lasting effects of systemic corticosterone (CORT) administrations in two distinct early developmental periods. Mice exposed to early-neonatal CORT treatment on postnatal days (PD) 2—4 exhibited strongly enhanced excitability of neurons of the basolateral nucleus of the amygdala (BLA) in early adolescence and displayed impaired extinction of contextually conditioned fear memory, a type of behavior in which the BLA plays an important role. Furthermore, gene-expression of NMDA receptor subunits as well as calcium-activated K^+-channels was reduced in the amygdala. In contrast, exposure to the same CORT concentrations in a late-neonatal period (PD17—19) did not significantly affect BLA electrophysiology or extinction learning in adolescence. These results suggest age-dependent consequences of neonatal CORT exposure in amygdala neurons and provide evidence for a detrimental influence of early-neonatal stress on adolescent fear-memory processing.

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1. Introduction

The neuroendocrine stress response has an important role in the adaptation of many physiological processes in the body to specific environmental conditions. However, excessive or chronic stress may lead to persistent maladaptation of neuronal circuits and may promote the development of psychiatric disorders, such as mood or anxiety disorders (Lupien et al., 2009; Popoli et al., 2012; Rice and Barone, 2000). These disorders often emerge in adolescence (Kessler et al., 2001) and are associated with altered physiology of the amygdala, the brain's center for the regulation of emotions (Altshuler et al., 1998; Etkin and Wager, 2007; Frodl et al., 2002; Lange and Irle, 2004).

In adults, the amygdala interacts reciprocally with the hypothalamic—pituitary—adrenal (HPA) axis, the neuroendocrine system that induces the stress response via the release of stress hormones. On one hand, the basolateral complex, containing the lateral and basolateral nuclei of the amygdala (LA and BLA), regulates the actions of glucocorticoids on a variety of higher cortical functions, including memory consolidation and working memory (Roozendaal et al., 2004, 2009). On the other hand, stress or corticosterone (CORT), the major stress hormone in rodents, have been shown to promote dendritic hypertrophy as well as hyperexcitability of amygdala neurons (Correll et al., 2005; Mitra and Sapolsky, 2008; Rosenkranz et al., 2010; Zhang and Rosenkranz, 2012). However, little is known about the effects of stress hormones on the electrophysiology of developing amygdala neurons.

The exposure of rodents to stress in early life is associated with changes in the sensitivity of the HPA axis, social behavior, and learning and memory (Callaghan and Richardson, 2011; Leshner and Schwartz, 1977; Lin et al., 2006). Although maternal care renders neonatal rodents largely unresponsive to mild stressors, referred to as the stress hypo-responsive period (SHRP), the absence of the dam as well as life-threatening situations can induce stress responses in pups (Levine, 2001; Schmidt et al., 2003). Interestingly, disruption of the SHRP by early-life stress exposure is associated with changes in the HPA axis responsivity in later life (Enthoven et al., 2008; van Oers et al., 1997, 1998a) and these changes are reversible by tactile stimulation simulating maternal care (van Oers and de Kloet, 1999).

Importantly, early brain development is characterized by high rates of neurophysiological changes both in humans (Andersen and Teicher, 2008; Tau and Peterson, 2009; Taylor, 2010) and animals (McCormick and Prince, 1987; Spigelman et al., 1992; Tyzio et al., 2003). It is hypothesized that these periods of rapid development present windows of opportunity for environmental factors, such as stress, to influence neuronal development of various brain regions and, correspondingly, behavior (Andersen, 2003; Andersen and Teicher, 2008; Bock et al., 2005; Rice and Barone, 2000). In this respect, the amygdala is one of the key structures in the connection between early-life trauma and the later development of emotional disorders (Heim and Nemeroff, 2001; Levine, 2001; Lupien et al., 2009; Taylor, 2010).

Certainly, differences in the duration and timing of postnatal brain development call into question whether studies on developmental aspects in animals possess any clinical validity (Rice and Barone, 2000; Tau and Peterson, 2009). However, previous reports described similar age-dependent sensitivities to early life stress on neuronal circuits and the HPA axis across mammalian species, suggesting the use of animal models as a valid approach to investigate basic aspects of early life stress (Agid et al., 1999; Brown et al., 1977; Kendler et al., 1992; Lin et al., 2006; Roy, 1978; van Oers et al., 1998a).

Thus, there is clinical and pre-clinical evidence for a substantial influence of stress on developing brain areas which regulate emotions and the effects of stress on adult amygdala neurons have been thoroughly investigated. Nevertheless, information about electrophysiological consequences of neonatal stress exposure on amygdala neurons is lacking. Therefore, the aim of this study was to investigate the impact of systemic CORT exposure in different neonatal periods on later-life BLA electrophysiology and related behavior.

2. Methods

2.1. Animals

For CORT-studies, pregnant C57BL/6J mice were obtained from Clea (Tokyo, Japan) at gestational stage E15. They were housed in individual cages under controlled ambient temperature ($25 \pm 1\ ^{\circ}C$) and lighting (12-h light/dark cycle) conditions and provided with food and water ad libitum. Only male animals were used in all experiments to avoid variability caused by sex differences in the stress response system (Kudielka and Kirschbaum, 2005). All experimental procedures were conducted in strict accordance with the regulations of the National Institute of Neuroscience (Tokyo, Japan) for animal experiments and were approved by the Institutional Animal Investigation Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Early-neonatal CORT treatment

At PD1, litters were culled to a total of 7—8 pups (male:female ratio was 3:4, 4:3 or 4:4) per dam. At PD2, each litter was randomly divided into three subgroups (vehicle-treated, 1 mg/kg CORT, and 10 mg/kg CORT). Pups remained with the dam until PD28 because of possible influences of weaning stress on behavioral or electrophysiological experiments at PD25—28. See supplementary material for a detailed description.

2.3. Late-neonatal CORT treatment

Male mice were treated at PD17—19 as described for early-neonates with minor modifications. Animals recorded 6—12 days after treatment (PD25—31) remained with the dam and those recorded three weeks after treatment (PD40—46) were weaned at PD21. See supplementary material for a detailed description.

2.4. Non-handled controls

To evaluate possible effects of the injection procedure and neonatal handling, we also recorded non-handled (NH)