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Periodic maternal separation decreases hippocampal neurogenesis without affecting basal corticosterone during the stress hyporesponsive period, but alters HPA axis and coping behavior in adulthood

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Bromodeoxyuridine

Summary Although not directly evaluated, the early rise of glucocorticoid (GC) levels, as occur after exposure to adverse early life experience, are assumed to affect hippocampal ontogeny by altering the hippocampus negative feedback on adult HPA axis. To test whether hippocampal ontogeny is affected by early exposure to stress we estimated the survival of recently formed hippocampal granule cells in rat pups subjected to periodic maternal separation (180 min/day; MS180) from postnatal days (PND) 1 to 14. Accordingly, MS180 pups injected with bromodeoxyuridine (BrdU, 50 mg/kg, ip) at PND 5 showed decreased density of doublecortin (DCX) positive BrdU-labeled cells at PND 15. MS180 and AFR pups showed similar corticosterone (CORT) basal levels between PND 3 and 12, whereas adult MS180 rats presented with higher CORT levels than AFR adults. Nonetheless, both AFR and MS180 pups and adults showed similar transient increments of CORT levels in response to stress. In addition, MS180 had no effect on the adult anxiety-like behavior evaluated in the elevated plus maze, but evoked a passive coping strategy in the forced swimming test. The data show that the decrease in hippocampal neurogenesis is an early onset phenomenon, and suggests that adverse experiences alter hippocampal ontogeny without chronic elevation of GC levels.

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1. Introduction

Adverse early life experience is known to exert detrimental effects on the development of the central nervous system (CNS). Such effects increase the susceptibility to anxiety and depressive disorders, as well as stress vulnerability in adulthood (Heim et al., 1997; Bellis et al., 2002; Chapman et al., 2004; Cohen et al., 2006). This susceptibility has been hypothesized to occur via chronic elevation of glucocorticoid (GC) levels during sensitive time windows (Gillespie et al., 2009), that program the adult hypothalamic pituitary adrenal axis (HPA) responsiveness.

During the first two weeks of life, rat pups show a markedly reduced adrenocortical response to mild stress (Schapiro, 1962; Sapolsky and Meaney, 1986), and this window has been termed the 'stress hyporesponsive period' (SHRP). This blunted neuroendocrine response (absence of ACTH and corticosterone [CORT] release) is thought to originate by an increase in GC receptor (GCR)-mediated negative feedback condition at the level of the brain (i.e., hippocampus, hypothalamus, and pituitary). This ensures the low stable corticoid levels that appear to be optimal for neuronal development (Sapolsky and Meaney, 1986). Periodic maternal separation (MS180) is a potent stressor that activates the HPA axis response even during the SHRP (Sapolsky and Meaney, 1986; Pihoker et al., 1993). Chronic elevations of GC levels during SHRP induced by MS180 could impact GC-sensitive brain regions, such as the hippocampus, that contains high concentrations of mineralocorticoid receptors (MR) and GCR (Sapolsky, 1985; Eldridge et al., 1989; Mitchell et al., 1990). This could lead to hippocampal neuronal loss, alterations in GCR density, and result in alterations of adult HPA axis negative feedback (Sapolsky et al., 1985).

Hippocampal neurogenesis extends from gestation into adulthood (Gould et al., 1999; Alvarez-Buylla and Lim, 2004; Paizanis et al., 2007). MS180 decreases granular cell number in juvenile rats (Oreland et al., 2010) and dentate gyrus (DG) neurogenesis in adults (Mirescu et al., 2004), suggesting that the functional consequences of early adverse experience may involve a diminished pool of nascent neurons. This decrease has been proposed as one of the possible mechanisms underlying the psychophysiological effects of adverse early life experiences and could relate to alterations in the HPA axis negative feedback (Mirescu et al., 2004). In rodents, the DG develops during the first postnatal week of life (Schlessinger et al., 1975; Rickmann et al., 1987) making it vulnerable to stress. Evaluating the effect of MS180 on the hippocampus during the SHRP and the mechanisms of HPA axis developmental programming is necessary.

Because DG granule cell neurogenesis occurs during the same time window as pups are subjected to periodic separation, our aim was to evaluate whether MS180 chronically increases GC levels and affects hippocampal ontogeny.

2. Methods

2.1. Animals

Male and female Sprague-Dawley rats were housed in standard temperature controlled rooms with a 12-h light/dark cycle (light on at 07:00 h) and free access to food and water. When the females were clearly pregnant the males were removed from the cage and the females were checked twice daily (09:00 and 17:00 h) for delivery. The day of delivery was designated postnatal day zero (PND 0). At PND 1, litters were adjusted to eight pups with as little disturbance to mothers and pups as possible. Manipulation was kept to a minimum throughout all the procedures to avoid handling effects. Half of the litters were designated to 180 min of periodic maternal separation (MS180) and the rest were left undisturbed except for routine cage cleaning once a week (animal facility-reared, AFR controls). Some of the animals were then sacrificed at PND15 for histological procedures. The rest of the animals were weaned at PND 21 and group-housed according to treatment (3—5 per cage). Rats remained undisturbed until adulthood. To avoid sex dependent effects of MS180, only males were evaluated for the neurogenesis and behavioral tests.

All experiments were carried out in accordance with the Official Regulations for use and care of laboratory animals of Mexico (NOM-062-ZOO-1999), the General Law of Health of Mexico, and the guidelines of the American National Institutes of Health. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Periodic maternal separation

At PND 1, dams were gently pushed aside from the nest with the aid of a cardboard in the home-cage and the whole litter was removed. Subsequently, pups were placed together in a separate small plexiglas cage filled with a mixture of clean sawdust and lining from the nest, and were then re-located to an adjacent room independent to the main colony. Separated pups were placed on a heating pad set at 30—33 °C for 180 min (MS180). At the end of this period the pups were returned to the original home-cage in the main colony room using the same procedure to keep the mother away from the nest. The whole procedure was repeated daily from PND 1 to PND 14 using the same cages throughout for MS180 pups.

2.3. BrdU administration

To determine whether early life experience affects cell survival in the developing hippocampus during the stress hyporesponsive period, AFR and MS180 litters were injected twice with bromodeoxyuridine (BrdU, Sigma—Aldrich 25 mg/kg) at PND 5 and left to survive for ten days (Fig. 1). At PND15, pups were euthanized with pentobarbital anesthesia and

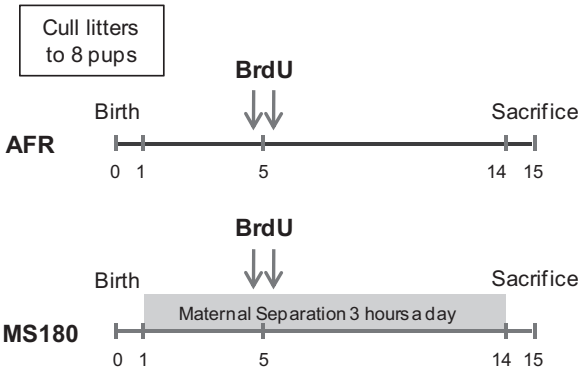

Figure 1 Experimental procedure for BrdU administration.