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Review

Neonatal stress tempers vulnerability of acute stress response in adult socially isolated rats

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Abstract

Adverse experiences occurred in early life and especially during childhood and adolescence can have negative impact on behavior later in life and the quality of maternal care is considered a critical moment that can considerably influence the development and the stress responsiveness in offspring. This review will assess how the association between neonatal and adolescence stressful experiences such as maternal separation and social isolation, at weaning, may influence the stress responsiveness and brain plasticity in adult rats. Three hours of separation from the pups (3-14 postnatal days) significantly increased frequencies of maternal arched-back nursing and licking-grooming by dams across the first 14 days postpartum and induced a long-lasting increase in their blood levels of corticosterone. Maternal separation, which *per se* did not modified brain and plasma allopregnanolone and corticosterone levels in adult rats, significantly reduced social isolation-induced decrease of the levels of these hormones. Moreover, the enhancement of corticosterone and allopregnanolone levels induced by foot shock stress in socially isolated animals that were exposed to maternal separation was markedly reduced respect to that observed in socially isolated animals. Our results suggest that in rats a daily brief separation from the mother during the first weeks of life, which *per se* did not substantially alter adult function and reactivity of hypothalamic-pituitary-adrenal (HPA) axis, elicited a significant protection versus the subsequent long-term stressful experience such that induced by social isolation from weaning.

Keywords

Maternal care, maternal separation, social isolation, allopregnanolone, HPA axis, rat.

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Effect of maternal separation and social isolation on function and reactivity of hypothalamic-pituitary-adrenal axis in adult male rats

It is well known that in mammalians life adverse events during the early period (either in prenatal or postnatal period) of neuronal development could change the normal pattern of brain growth as well as the vulnerability to stress in adulthood [1, 2]. Accordingly, several studies have reported that early acute or chronic stressful periods predispose for the onset of emotional and affective disorders such as depression and anxiety [3]. The time and duration of any stressful experience seem to be extremely relevant for neuronal organization and can increase the predisposition to behavioral disorders [4]. One of the most strong stressor for pups in the early postnatal period is represented by the relationship with mothers. In fact, repeated and long-lasting maternal separation (MS) is associated with increased adult stress reactivity, together with augmented fluctuations in the secretion of corticotropin-releasing factor (CRF); adrenocorticotropin (ACTH) and corticosterone [5]. Therefore, MS is usually considered as animal paradigm designed to mimic repeated exposure to stress during early life, the final result being adults with behavioral and neuroendocrine signs of elevated stress reactivity. On the other hand, brief daily separation (handling) from the dam during the first periods of life may result in a more positive and efficient adaptive interaction with the environment in adulthood [6].

We have demonstrated that, in rats, a daily brief separation from the mother during the two first weeks of postnatal age did not substantially alter adult function and reactivity of hypothalamic-pituitary-adrenal (HPA) axis [7]. Moreover, it is associated with a significant protection versus a subsequent long-term stressful experience such that induced by social isolation from weaning, as demonstrated by the the fact that 3 h of daily MS failed to change the amount of corticosterone in blood of adult animals reared in group and by the same sensitivity to acute stress. In fact, foot shock stress induced a similar amount of increase of corticosterone blood levels in both maternal and non-maternal separated group-housed animals.

Rats deprived from weaning of social contact experience a form of prolonged stress that leads to long-lasting behavioral alterations [8]. Moreover, social isolated animals show a decrease in brain and plasma concentrations of neuroactive steroids such as 3a-hydroxy-5a-pregnan-20-one (allopregnanolone, or 3a-5a-THPROG), a metabolite of progesterone, with a potent positive allosteric action on the $GABA_A$ receptor [9]; conversely, it enhances the neurosterodogenic effect of acute stress [10]. Accordingly, social isolation increases the sensitivity of the pituitary CRF and impaires negative feedback regulation of the HPA axis [11].

As expected [10], 4 weeks of social isolation induced a dramatic decrease of corticosterone plasma levels compared to group housed rats and foot shock stress resulted much more effective in isolated animals compared with group-housed counterpart.

The combination of the two different stressors, MS and post-weaning social isolation was evaluated in the basal and stress stimulated activity of HPA axis.

The decrease of corticosterone levels induced by social isolation was less prominent in socially isolated animals subjected to MS and the enhancement of corticosterone levels, induced by foot shock stress in social isolated animals exposed to MS, was markedly reduced respect to that observed in socially isolated animals.

Given that, in rats, plasma corticosterone is a very sensitive marker of the emotional state changed by both acute and chronic stressful stimuli [12], our results, showing a reduced efficacy of long-term social isolation stress in lowering plasma corticosterone content in animals previously submitted to MS, suggest that such a stressful condition, experienced in early life, allow rats to become more adaptive to chronic stress when adults. This conclusion is strongly supported by data showing that the efficacy of an acute stressful

event such as foot shock in increasing plasma level of corticosterone was markedly reduced in social isolation rats submitted, during early life, to MS. This finding implicates that MS produces a pattern of corticosterone response that is not different from that which characterizes the sensitivity of adult control exposed to stress, but appears to be associated with a decreased vulnerability to negative behavioral response to stress re-exposure in adolescence. Thus, the decreased corticosterone response to foot shock showed by adult rats that had been exposed to MS and to social isolation from weaning might indicate that neonatal stress exposure strengthen the ability of the HPA axis to respond adequately to stressful stimuli in adulthood.

Effects of maternal separation and social isolation on allopregnanolone levels in plasma and cortex of adult male rats

An important mechanism regulating HPA axis activity is the neuronal inhibition exerted by GABAergic neurons within the hypothalamus and the neuroactive steroid allopregnanolone is the most potent endogenous positive allosteric modulator of $GABA_A$ receptor function [13]; this hormone, when administered to animals either systematically or intracerebroventricularly, induced anxiolytic, sedative-hypnotic, and anticonvulsant effects [14, 15]. Allopregnanolone also exerted a potent inhibitory action on HPA axis activity and attenuated the elevation of plasma ACTH and corticosterone elicited by stress [16], an effect similar to that induced by benzodiazepines [17].

To further clarify the functional interaction between the combination of the two different stressors, MS and post-weaning social isolation, we evaluated the plasmatic and cerebrocortical levels of allopregnanolone in adult animals. In fact, this steroid derivate is very sensitive to acute and chronic stressful stimuli [10, 18]. As expected [10], 4 weeks of social isolation after weaning induced a marked decrease of allopregnanolone both in plasma and cerebral cortical levels compared to group-housed rats. On the contrary, MS failed to modify the content of allopregnanolone both in plasma and brain. The finding that, in contrast to social isolation, early MS failed to reduce allopregnanolone level in adult rats might suggest that the normal baseline concentration of allopregnanolone in these animals is sufficient to attenuate stress-induced HPA axis activation leading to more resilient animals.

The marked decrease of plasmatic and cerebrocortical allopregnanolone content induced by social isolation resulted significantly lower in animals that previously underwent to MS. This result is consistent with the evidence that the impairment of the alteration of the HPA axis, apparent in isolated rats [11], was prevented or normalized by administration of exogenous allopregnanolone either during or following social isolation [19].

Effect of pups separation on maternal care and dams corticosterone levels

The neuroendocrine response to acute stress observed in adult rats that had been exposed to neonatal MS and to social isolation from weaning seems to be similar to that found in adult rats that were exposed to a brief (15 min) daily period of separation from their mother during the first few weeks of life [20]. In fact, these animals showed reduced fearfulness, attenuated ACTH and corticosterone responses to stress [6, 20, 21]. All of these changes were suggested to be due to increased dam-pup interactions [22]. Indeed, the quality of maternal care provided to offspring early in life is a direct determinant of developmental programming and calibration of the HPA axis [23], leading to a reduction of adult HPA reactivity for daily 15-min dam-pups separation [7]. Thus, we examined total frequencies of maternal arched back nursing (ABN) and licking and grooming (LG) by all dams across the first two weeks postpartum. ABN and LG are considered important parameters to discriminate the high quality of maternal behavior [24, 25]. Pup-separated dams displayed more ABN and LG than pup-no separated mothers across all day of the observation period.

Our results indicate that 3 h daily separation for the first two weeks of life significantly increased levels of active position ABN of the mother to nurse its pups. ABN, characterized as the only active nursing position with the dam being fully engaged in a quiescent kyphotic posture, is an important parameter to discriminate the high quality of maternal behavior [24]. Moreover, mothers to whom pups were separated showed an increased frequency of LG.

It is well established that increased LG and ABN by mothers alter the offspring epigenome at the hippocampal glucocorticoid receptor gene promoter increasing glucocorticoid feedback sensitivity [23]. Another important determinant in regulating HPA axis response in adult rats is the postnatal exposure to glucocorticoids.

We found that corticosterone level were higher (+ 180%) in dams that underwent pups separation than in control mothers.

It has been extensively demonstrated that administration to the pups of high dose of glucocorticoids induced a permanent and disruptive effect on the HPA system [26], while adult rats nursed by mothers assuming moderate levels of corticosterone showed enhanced ability to cope with different situations [27]. This conclusion is consistent with the concept of general stress inoculation [28]. Moreover, moderate level of corticosterone in the dams modulates maternal behavior and female rats receiving exogenous corticosterone spent more time licking, more time over pups in some form of hovering or crouching behavior [29]. The active maternal care of pup-separated dams may act as a potential buffer against an adverse environment. The protective effect induced by the increased level of maternal care, which is not evident in normal condition, may be unmasked when the subject undergoes a second event of adverse condition, such as social isolation as adolescent.

Conclusion

In conclusion, our findings suggest that early life stress might have a crucial role to program later life stress-responsiveness of key genes involved in emotional regulation and sustain the mismatch hypothesis stating that aversive experiences early in life trigger adaptive processes, thereby rendering an individual to be better adapted to similar aversive challenges later in life [30-32].

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Declaration of interest

The Authors declare that there is no conflict of interest.

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