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REVIEW ARTICLE

Global Effects Of Early Life Stress On Neurons And Glial Cells

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Abstract: Early life stress is considered a risk factor for the development of many diseases in both adolescence and adulthood. It has been reported that chronic stress (for instance, due to maternal separation during breast feeding), causes damage to the central nervous system at the level of neurons and glial cells, which are reflected in behavioral disturbances and susceptibility to the development of primarily emotional psychopathology. The aim of this review is to identify the overall state of the scientific literature that relates the information about the consequences of early life stress, contextualizing the mechanisms that may be altered, the behavioral consequences that have been studied and the possible dimorphic effects and its causes. At the end a short overview of pharmacological treatments that have been proposed to reduce the behavioral and neuroendocrine consequences caused by early life stress is presented. This review pretends to integrate general but relevant information based primarily on studies in animal models, which allow the experimental approach and the study of the mechanisms involved. A series of questions remains for reflection and surely will be answered in the near future.

Keywords: Early life stress, maternal separation, hypothalamic-pituitary-adrenal axis, Glucocorticoids, Chronic stress.

1. INTRODUCTION

There is abundant evidence related with the deleterious effects of early life stress (ELS) on central nervous system development, and the association with the occurrence of different psychopathologies later in life [1-9]. For example, children who have experienced prolonged maltreatment, abuse or neglect during rearing, usually present an increased risk of developing anxiety disorders, depression, attention deficit, learning problems and so on [9]. One of the critical elements for neurodevelopment on early life is the mother-child interaction [11]. This bond goes beyond the nutritional care because it is an important source of sensitive, motor and affective stimulation for the baby, and helps to shape the maturation of neuronal circuits. It has been reported that maternal deprivation or early maternal separation acts as a chronic stressor that has adverse consequences on brain ontogeny [6,11-12].

Most of the studies that inquire about consequences of ELS use animal models, including several methods of prenatal or postnatal stress, these ones elicited as maternal separation or maternal deprivation procedures. The majority of the results agree with the deleterious effect of ELS, related not only with the probability to affect behaviour but also the neuroendocrine regulation, mainly on the hypothalamus-pituitary-adrenal axis (HPA). The essential players of the stress response regulation by HPA axis are glucocorticoids, and affect several genomic and signalling cascades responsible for modulating cognitive processes and behaviour. Apart from the HPA axis, ELS affects the immune system and alters the immune responses in adult life. There is growing evidence of the involvement of the immune system in the development of psychiatric disorders. It has been hypothesized that the clinical symptoms of several mental illness such as depressive disorders, anxiety, or schizophrenia are contributed by a chronic proinflammatory state of the individual [13]. In this sense, glial cells express receptors for glucocorticoids and probably all the cytokines released by glial cells may induce part of the behavioural effects of chronic stress. Thus, in this review we want to integrate the research about ELS and we will focus mostly on maternal separation, mainly 3 or 6 hours period, as an animal model of ELS, to show how ELS affects both neuronal and glial cell functions.

2. HPA-AXIS PROGRAMMING

During the last decades of research, physiological studies are being focused on the adaptative mechanisms of biological organisms with special attention to the allostasis process that implies stability during the change. In fact, the successes in life begin when living beings are able to anticipate the environment conditions after learning experiences [14-16].

Studying the stress responses, the notion of allostasis allow us to understand that some early events may induce long term patterns of responses like early programming that would take place in critical periods during ontogeny [6]. Taking into account that the Hypothalamus-Pituitary-Adrenal (HPA) axis is one of the main stress response regulator and effector mechanisms, it is the most appropriate system to study adaptative mechanisms as well as possible alterations associated with pathological phenomenon.

Classically, the HPA axis has a hierarchical organization commanded by the hypothalamus. The parvocellular neurons of the paraventricular nucleus (PVN) of the hypothalamus are in charge of corticotrophin releasing hormone (CRH) synthesis. This peptide acts on corticotroph cells of the adenohypophysis inducing the expression of proopiomelanocortin (POMC), molecule that when cleaved result in different peptides: melanocyte stimulating hormone, beta endorphins and corticotrophin. While corticotrophin induce glucocorticoid an mineralocorticoid production in the adrenal gland, beta endorphins act on opiod receptors, that mediate pain responses and participate in emotional regulation. Glucocorticoids modulate the immunologic, metabolic and behavioural actions observed under normal and stressful conditions [7].

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In the nervous system, glucocorticoids reach areas where their receptors are located, such as the amygdala and hippocampus, and are involved in processes of learning, memory and emotional regulation against stressful experiences [17-24]. This seems to be one of the keys to understanding the predictive programming of the HPA axis responses. In fact, the negative feedback mechanisms that allow censoring and adjust the HPA axis responses involve these areas of the limbic system, as well as other interconnected areas: the prefrontal cortex and the striatum. They all send excitatory and inhibitory projections that act collaterally to modulate HPA axis responses and they are sensitive to the hormones produced by it in its various segments [24-27].

On the other hand, arginine-vasopressin (AVP) and oxytocin, hormones produced by the neurohypophysis are also important for early ripening of the HPA axis and have reported their modulatory effects of behaviour in regions of the cortex and subcortex . Indeed, some authors note that AVP has a more flexible behaviour that CRH in early stages of development, so that it would become the most important signal for regulating the release of ACTH [28].

3. EFFECTS OF MATERNAL SEPARATION TROUGHOUT LIFE

As suggested, the effects of all these signals are especially important in the early ontogeny. Several experimental models of early adversity have been proposed to address its effects, and it has been shown that they resemble many of the features observed in ELS patients. These models induce a state of chronic stress in the pups by means of altering their environmental conditions. For instance, maternal separation (MS) consists of the separation of the pups from the mother for prolonged periods of time (3, 4 or 6h) and through a daily basis (10, 14 or 21 days) starting one or 2 days after birth [29]. Other models of ELS induce a state of anxiety in the mother by limiting the material to build a nest. This procedure alters the normal pattern of maternal behavior and induces a stressful state in the pups [30]. Other procedures consist of the permanent separation of the pups from their mother at an early age, for instance, at postnatal day (PN) 15 (Early weaning) [31], or combine both the MS procedure with early weaning.

Significantly, the variability of the stress protocols by maternal separation, consisting of differences in the daily period of separation, the number of days that is done, blocking or access to olfactory stimuli and vocalizations of the mother, the isolation from other infants, the type of acute stress in adult life prior to biochemical or behavioural measurements and strains of animals used, among others, has generated important, and even contradictory changes in the research results. It should also be noted the intrinsic variability of individual responses to stress, whose mechanisms are not sufficiently clarified [6]. However, several characteristics are common to most of the animal models.

Studies in rats have characterized a period of "low stress responsivity" between postnatal day 2 and 21, in which it was assumed that the immaturity of the HPA axis prevented the activation of hormonal responses to mild stressors. However, stress models in rat pups such as isolation, early weaning and especially maternal separation (MS), have shown that the shaft is capable of producing the release of glucocorticoids and this reactivity can become a pattern of programmed response in later ontogeny [32]. The long-term effects of these changes have been reported in numerous studies, from the pioneering work of Levine in the early fifties [33] until today.

To illustrate the consequences of MS, the findings of some of these investigations are summarized. A study by Colorado *et al.* [34] included the test in the open, light-dark box and sucrose consumption test. The results showed that rats subjected to MS for 6 hours / day for the first 10 days of life showed a decrease in the behaviour of orientation and an increase in anxiety behaviours and impulsivity in adolescence, which were exacerbated in new contexts for the animal [34]. In another study, Riu et al., found that rats that were separated by 3 hours daily during the first 2 weeks of life and after weaning were kept in isolation, presented at two months of life behaviours of hyperphagia and excessive weight gain, in addition to increased mobility in the forced swimming test and increased frequency of defecation tests in ambulatory activity; extrapolated behavioural symptoms of anxiety and depression. For these authors, these results could be explained by the fact that the isolation after weaning creates an evocation of early stress given by the MS [35]. Furthermore, Zimmerberg *et al*. found that maternal separation was associated with changes in the acoustic characteristics of ultrasonic vocalizations issued by the offspring during the period of separation and an increase in the behaviour of maternal care when pups were returned [36]. Also Macri *et al*. reported a change in the active maternal care in rats subjected to short periods of maternal separation (15 min), which are within the spectrum of time that the mother is away from the pups in the day to find food. These animals were manipulated by the researcher while they were separated from their mothers (Early Handling). The authors conclude that the beneficial effect observed in the EH group in the normo-activation of the HPA axis, could be due to this increase in maternal care at the time of the return and not to the effect of stimulation by the fact of being manipulated. In addition, the comparative design between multiple groups allowed the authors to report that the MS and the level of maternal care act independently and opposite on anxiety and impulsivity behaviours assessed in the open field test [37].

In work carried out on MS using the elevated plus maze, we found an increase in exploration risk behaviours and hyperactivity in a group of adult MS females. Animals underwent MS twice daily during the dark cycle from postnatal day 1 to postnatal day 21. Behavior was tested when rats were 65-70 days old. We found that separated females spent more time in the open arms and showed more head dipping behavior compared with controls. The separated males spent more time in the center of the maze and engaged in more stretching behavior than the controls. Furthermore, adult MS males presented higher exploration - avoidance conflicts than controls [38, 39]. Other authors found a significant increase in anxiety behaviours in MS rats also evaluated in the elevated plus maze [40].

In another study by Aisa *et al*., the effects of MS on markers of plasticity were studied with relation to spatial learning in rats. While this type of learning is correlated with an increase in the expression of the total levels of the neural cell adhesion molecule (NCAM) in both the MS group and the control, the increase was significantly lower in the MS group, mainly for NCAM-140 isoform. In addition, this group had lower levels of brain derived neurotrophic factor (BDNF) mRNAs and of synaptophysin protein in the hippocampus, compared to the control group. Cell proliferation was also decreased in the dentate gyrus of the MS group [41]. Other study from our group showed that MS decreases hippocampal neurogenesis right after the stress period, i.e., at postnatal day 15. This effect was associated to a depressive – like behavior in adulthood and to a dysregulated HPA axis [42]. All these results suggest the possible deleterious effect of MS in at least some neuronal mechanisms involved in neurogenesis, cell proliferation and dendritic arborization, postulating it as a factor affecting the long-term neuronal plasticity and HPA axis function.

Furthermore, a study showed that the effects of MS could not only be associated with manifestations of anxiety and depression, but a potential vulnerability for the development of neurodegenerative disorders in adulthood, particularly Parkinson's disease. In that report the authors investigated the effect of dopaminergic neurons damage induced by intrastriatal injection of 6-hydroxydopamine (3μg/ml). Animals with a history of MS showed significant changes in the mobility of their vibrisae and presented manifestations of akinesia, compared with the control group. These results suggest

that MS could be associated with increased vulnerability to damage the dopaminergic circuits in later ages of ontogeny [43].

Meanwhile, other authors have focused on the serotonergic system as one of the main targets of the MS and other forms of stress early as social isolation, studying their effects on the structure and function of some regions of the nervous system, primarily the hippocampus. A review by Weaver *et al*. showed evidence to assume that alterations in the expression of glucocorticoid receptors (GR) in the hippocampus of rats subjected to MS, would be associated with a hyper activation of the ascending serotonergic pathways, with subsequent changes in the expression of transcription factors that could act on the promoter regions of the gene encoding these receptors [44].

Another study showing the broad spectrum of the impact of MS on different circuits of the nervous system was developed by Genest *et al*. Here, they studied the mechanisms involved in the deficit of respiratory control reported in male rats with a history of MS that present an increase in ventilatory responses to hypoxia 25% greater than the controls. This was taken as a criterion of hyperresponsiveness in the chemoreflex mechanisms that appear linked to various disorders of the respiratory control. Their hypothesis was that an alteration of the inhibitory control by gabaergic neurons to the PVN nucleus may be correlated with the elevated response to hypoxia in rats that were separated from their mothers. The results showed that, indeed, the injection of 1 mM GABA or muscimol attenuated the ventilatory response to hypoxia in a much more pronounced fashion in animals with a history of MS than in controls. This attenuation was associated with an increase in the binding site of the GABAA receptor by 22% in the PVN of MS animals relative to controls, in measurements made by receptor autoradiography. However, despite this increase in receptor density, MS animals had a deficient GABAergic modulation, possibly associated with an apparent increase in the effectiveness of excitatory inputs that converge on this nucleus.

In line with the findings of a compromised function of the GABAergic system in the MS, Caldji *et al*. showed that MS animals showed a significant decrease of orthosteric binding sites of high affinity (30 nM) of the GABAA receptor in the medial prefrontal cortex, the nucleus of the solitary tract and the locus coeruleus [45]. In another study, these authors showed that the MS was associated with a decrease in the number of binding sites benzodiazepines, dependent on a decrease in mRNA expression of the subunit γ2 in the same structures mentioned above [45].

The review by Skilbeck *et al*. [46] showed that these effects on GABAergic circuits would be mediated by the increased exposure to glucocorticoids, as a result of the HPA axis hyperactivation stress generated early. Although the diversity of experimental models partially affects the consistency of the findings of different authors, Skilbeck *et al*. suggest that glucocorticoids could alter GABAA receptor phosphorylation via activation of protein kinase C (PKC), which would affect the traffic and expression of surface receptors. In addition, early stress by MS might interfere with the normal turnover subunit α2 by α1 on GABAA receptors, which takes place in the first 21 days of life of the rat and that is associated with the maturation of the GABAergic responses [46].

In work carried out in our research group, we reported alterations in the expression of the α subunit of GABAA receptors in rats with a history of MS. Female rats subjected to MS showed less immunoreactivity positive for the GABAA receptor in the hippocampus, preoptic area and the paraventricular nucleus, while the MS males had a decreased GABAA receptor immunoreactivity in the amygdala, and prefrontal cortex preoptic area, in immunohistochemistry. However, in western blots findings were not fully comparable: a decrease in the amount of GABAA receptor in the paraventricular nucleus and preoptic area for MS females and in the prefrontal cortex and amygdala to MS males [39]. It is important to recognize that more research is needed to clarify the potential influences of MS on the traffic and expression of GABA A receptors and their potential impact, not only to control the HPA axis, but also on the inhibitory connections in cortical and subcortical areas related to emotional processing and reward mechanisms controlling impulsivity.

4. MATERNAL SEPARATION, HPA AXIS RESPONSE AND SEXUAL DIMORFISM

Variations in the stress response associated with gender [47], are important for understanding the epidemiological differences in psychopathological disorders such as depression, schizophrenia and ADHD [48]. Recently, it has been established that there are behavioural differences between men and women accompanied by changes in specific brain structures evoked by stress during critical periods of neurodevelopment. In one study, it was found that the amygdala volume was higher in women whose mothers had high levels of stress during pregnancy, compared with men [49]. On the other hand, the volume of the hippocampus in adulthood was correlated with birth weight in women who had received poor maternal care at an early age, while these changes were evident in the male gender [50].

As suggested, the possible mechanisms of these variations could be related to changes in the hypothalamic- pituitary- response adrenal axis (HPA). A study established that exposure to adverse situations in women at an early age was associated with increased basal levels of corticotrophin, while men with similar backgrounds showed a decrease in basal levels of this hormone. However, reactive secretion of corticotrophin in situations of acute stress was increased in men who had had severe trauma in childhood, without any reported equivalent changes in women [51].

Another study showed that epigenetic changes given by methylation exon 1F in the promoter region of the gene encoding the glucocorticoid receptor (NR3C1), and the presence of genetic polymorphisms in the gene encoding the receptor of α estrogen (ERα) and serotonin transporter (5-HTTLPR) were correlated with changes in salivary cortisol secretion only in women [52]. This study suggests that both genetic and epigenetic influences, in relation to the response to stress may have dimorphic patterns in the human species.

Many studies have explored the molecular mechanisms of sexual dimorphism compared to early stress in animal models such as those mentioned. In a study conducted with mice subjected to a stress model for early weaning (postnatal day 14), the authors showed an increase in anxiety - related behaviour, aggression and changes in the interaction with the mother in male mice, from postnatal day 15. At the age of 8 weeks, with early weaning male mice also had significantly higher baseline levels of corticosterone than controls and females, as well as a decrease in the population of glucocorticoid receptor GR [53]. In other work, the authors reported a decrease in expression of brain derived neurotrophic factor (BDNF) and cell proliferation in the hippocampal of male mice with early weaning [54] as an early myelination in the amygdala, as signal of a possible early maturation process induced by stress [55].

However, the directionality of the changes associated with stress related to sex is not consistent across studies using animal models [56]. Moreover, many of the molecular aspects in this area remain unsolved, mainly due to the difficulty of studying specific signaling pathways, neutralizing interference with other pathways acting concomitantly [7]. In fact, given the complexity of the multiple actions of steroid hormones in the nervous system, most studies have focused on identifying specific molecular mechanisms and have not proposed integrative models that combine different types of ligands. On the other hand, it has been shown that many of the endocrine effects on the nervous system may be different and even in opposing relation to the intensity and duration of hormonal stim-

uli and the location of their target molecules in different areas of the cortex [7].

Currently, a bimodal model for glucocorticoid action in nerve tissue, which is mediated by a functional balance in the action of two types of cytoplasmic receptors accepted: the mineralocorticoid (MR) and the glucocorticoid (GR) receptors [6]. In general, signals mediated by the MR maintain a basal tone on glucocorticoid stimulation when serum levels are relatively low. Because of circadian fluctuations, these receptors remain saturated during the day and their signals are important to the activation of metabolic pathways and cell survival. This was demonstrated in experiments using adrenalectomized rats that showed high levels of apoptosis in the hippocampal dentate gyrus, an effect that could be restored by low doses of exogenous corticosterone (20 mcg / ml), capable of activating the MR [57]. In addition, while GR response elements (GREs) usually dissociate from the GR-DNA complex after 1 h, the MR tends to stay longer attached to the DNA, ensuring fast-feedback tonic effects [58].

When glucocorticoid (GC) serum level increases in response to stressors, the hormone interacts with GR receptors, whose affinity is activated ten times lower than the MR (Kd : 0.5 nM and 5 nM respectively). Its effects are associated with recovery processes, learning and adaptation to stress [6]. However, if glucocorticoid levels are unusually high and / or sustained high in time it occurs a change in the intracellular signal spectrum, which produces deleterious effects as oxygen- overproduction of reactive species (ROS), mitochondrial dysfunction and activation of apoptosis [6,59-60]. The mechanisms that define this threshold from which the "positive" effects shift to "negative" effects are not fully elucidated [27].

Moreover, several studies have focused on the effect of ovarian steroids on nerve cells. The most studied hormone: the 17β-estradiol (E2), exerts multiple actions in the central nervous system associated with sexual dimorphism in cognitive and behavioural processes, through the strengthening of mechanisms that favor brain plasticity in specific areas [61,62].

In summary, while MS elicits multiple changes in neurotransmitter systems and in neuronal function within different cerebral structures, these changes cannot account for all the observed behavioral effects induced by stress. Further, when analyzing the effects of ELS on male and female subjects, several differences are evident, implying that gender confers a differential response to stress. On the other hand it has been shown that chronic stress exposure, particularly during early ages, can induce glial cell dysfunction, which could play a role in the vulnerability to develop several psychopathologies.

5. MATERNAL SEPARATION AND EFFECTS ON GLIAL CELL FUNCTION

Other studies have analyzed the impact of early maternal separation and have focused on other aspects of the histological organization, covering the changes that occur on the glial cells. The population of glial cells comprises mainly the astrocytes, the microglia and the oligodendroglia. Astrocytes are multifunctional cells of the nervous system; they contribute to the formation of synapses and are involved in neurotransmitter turnover, since they wrap the neuronal synapses, allowing the neurotransmitter to stay longer in the synaptic cleft [63]. Further, astrocytes are involved in the maintenance of neurons, providing nutrients, controlling water and ionic balance, and clearing excess of glutamate [64]. Microglial cells are the main surveillors of the immune system in the brain. They change their morphology in response to a number of insults subserving different roles, and secreting cytokines. In low concentrations these molecules function as growth factors, promoting cell proliferation and differentiation during development, but in high concentrations they convey neurochemical changes that result in inflammatory processes.

5.1. Effects of MS on Astrocytes

Several studies have addressed the role of maternal separation and other models of early stress on various functional and anatomical parameters in the nervous system. Many of these changes take place in areas characterized by a high capacity for tissue remodeling such as the hippocampus [42, 65-69] and prefrontal cortex structures [70,71]. Because of the importance of the role of astrocytes in the process of synaptic connectivity [69-73], the possible effects of stress early in the quantity, morphology and function of these cells become a relevant issue in research. One of the approximations used is the Sholl analysis that allows identifying the degree of astrocytic branching which can be interpreted as an important correlate of neuronal plasticity [65].

However, the results in this field have not been entirely consistent. For example, while some authors have found a decrease in the number and activation of astrocytes in different regions of the cortex [74, 75], others have reported significant increases in areas such as the prefrontal cortex [76]. No studies specifically evaluate astrocytic branching patterns relative to the background of maternal separation found. However, some reports indicate that this type of stress can alter the number of astrocytes in the hippocampus. A study by Musholt *et al*. used a double labelling for GFAP protein and phosphorylated CREB (activated downstream of the signaling cascade of cAMP), which quantifies the active astrocytes in different brain slices factor. The results showed a significant decrease in these cells in the hippocampus, both in pups undergoing six hours of maternal separation, such as those undergoing only an hour apart in a model of neonatal stress that spanned the first two weeks of life. Analyses were made 24 hours after the end of the period of separation [74]. In the work of our group with the protocol of maternal separation of 6 hours daily for 21 days of lactation, a significant decrease in the number of astrocytes labeled with GFAP was also found in the prefrontal cortex, the hippocampus and the preoptic area of MS individuals [75]. A recent study using the protocol of daily maternal separation 3h/day (from PN1 to PN14) showed that MS male pups present a reduced number and cell density of astrocytes in the hippocampal hilus at PN15. Interestingly, the number of processes of the astrocytes was also reduced in the MS pups [77].

On the other hand, the research developed by Kwak *et al*. reported that maternal separation was associated with an increase in GFAP labelled astrocytes number in adult male rats. They also reported in this group of animals an increase in hyperactivity behaviour and risk screening, when subjects were tested in the open field and in the elevated plus maze [76,78], using the deprivation protocol of 3h/day (PN1 – PN14), reported no change in the number of neurons and astrocytes in the prelimbic area of the prefrontal cortex [78], Another study showed a reduction in the total number of glial cells in the substantia nigra and the ventral tegmental area of juvenile male rats and in the substantia nigra of female rats subjected to MS as pups [79]. The difference between these results and across the age may reflect the dynamic nature of astrocytic changes at different times of the ontogeny. In fact, the increase in the number of astrocytes in a region of the cortex in adult animals can also be a signal depletion of synaptic connections by a process of reactive astrogliosis, in which these cells partially replace the space left by neuronal loss [76].

5.2. Effects of MS on Microglial Cells

Regarding changes in microglial cell activation related to early life stress, there is evidence to postulate that many alterations documented in different brain regions could be mediated by neuroinflammatory phenomena which are emerging as an important field of study in the coming years [80]. Numerous studies have addressed the effects of chronic stress on the activation of microglial cells in adult animals [81], but in contrast, very few studies have addressed the effects of ELS on this cell type. Actually, some reports postulate that the mechanisms of neuro- inflammation elicited by the increase

in pro- inflammatory cytokines and activation of microglia could be related to early stressful experiences and their long-term changes [82, 83]. It has even been reported that these mechanisms could be associated with sexual dimorphism in response to stress, as in rodent studies in the juvenile stage, only the males showed a significant trend for neuro - inflammatory changes [80].

Initial studies from Kazl *et al*. showed that early life environment plays a significant role in the response of the brain and glial cells to neuronal injury. They grew a group of rats under a MS schedule (3h/day) or under maternal care and enriched environment (EE), and injected kainic acid at PN35. They showed that MS rats displayed an increase in DNA fragmentation and in microglial activation in the hippocampus, compared to the EE rats [84]. Another study combining MS (4h/day, PN2-PN5 and 6h/day PN6 – PN16) and early weaning (EW, PN17) showed behavioral alterations such as greater anxiety in MS-EW adolescent and adult male and female mice. This study also demonstrated a higher activation of microglial cells in several regions of the hippocampus (CA3, CA1) of the MS – EW female mice; this was not observed in MS – EW male mice [85]. These results were associated to a dysregulation of the tryptophan – kynurenine metabolism in the prefrontal cortex of MS – EW male and female mice [85]. A recent study from our group using the MS schedule of (3h/day, PN1 – PN14) showed no changes in the total number and density of microglial cells in the hippocampal hilus, but instead an increased percentage (from 35% to 58%) of activated microglial cells in this area was observed in MS male pups at PN15 [77]. Further, changes in the activated state of microglia together with a reduction of astrocyte density was associated to an enhanced expression of IL-1β and of TNF-α in hippocampal extracts of MS pups. Interestingly, IL-1β expression was greatly enhanced (20 fold) after subjecting a subgroup of MS pups to an acute stressor, suggesting that this response could contribute to the programming of ELS of the HPA axis altering the cytokine responses at hippocampal level [77]. Since cytokines are involved in cell proliferation and differentiation, it is hypothesized that alterations in cytokine expression at hippocampal level during this early stage could contribute to alter the process of neurogenesis (known to be harmed by MS) and the normal maturation of the hippocampus, inducing permanent alterations in its inhibitory control of HPA axis function and in mood regulation [86, 87].

Apparently, the experience of early life stress is strong enough to permanently program the neuroimmune system of an individual, as suggested by the studies of Diz-Chaves *et al*. [82, 83]. They showed that the induction of prenatal stress (PS) to the fetal mice by immobilizing the mother 3 times/day (45min, from gestational day 12 to delivery) produced a permanent activation of microglial cells in the dentate gyrus of male and female PS adult mice, and an exacerbated response of microglial and astrocytic cells to a further challenge in adulthood [82, 83, 88].

Thus, ELS programs the neuroendocrine system and induces a silent proinflammatory state in different areas of the brain, inducing local changes in cytokine expression and sensitizing the glial cells to respond in an exacerbated way to future challenges, and predisposing the individual to develop a psychopathology.

6. THERAPEUTIC APPROACHES TO COUNTERACT DELETERIOUS EFFECTS OF MS

As stated above, the long term effects of ELS have been thoroughly studied in terms of different mechanisms and neurobiological changes that take place in the brain and are partially responsible for the development of psychopathology. An actual line of research concerns the possible therapeutic treatment, which could counteract or prevent the deleterious effects of ELS.

In this regard, some studies have been made using antidepressants, inhibitors of neurotransmitter receptors, or anti-inflammatory agents. Here we will shortly highlight some examples of these approaches to show the state of the art in the field.

6.1. MS and Antidepressants

Affective disorders are usually treated with several types of antidepressants. Since ELS is an influential factor in the development of this psychiatric illness, attempts have been made to counteract the effects by the use of antidepressants to normalize most of the parameters affected by MS stress.

An early report from Huot *et al*. [89] described the use of paroxetine to inhibit the preference for ethanol observed in MS adult rats. Antidepressant treatment was administered to adult MS rats during 21 days before testing and resulted in improvement of anxiety and HPA responses and a lower consumption of ethanol by the MS rats. Martisova *et al*. [90] reported on the use of the antidepressant venlafaxine and its effect on the glutamatergic/gabaergic circuit of the hippocampus of MS rats. They observed that venlafaxine reversed the upregulation of the glutamate receptor subunits NR1 and NR2A, and GluR4. Studies in vitro demonstrated that this antidepressant also counteracted the increase of glutamate and the decrease of GABA induced by corticosterone. Thus, the authors concluded that an important target is the modulation of the glutamate /GABA circuit by antidepressants to improve behavioral effects of ELS. Cotella *et al*. [91] tested the use of the antidepressant amitriptyline to correct the HPA axis function in MS adult rats subjected to chronic stress also in adulthood. Antidepressant treatment was administered simultaneously with daily stress exposure from PN 50 – PN 74. They reported that MS induces a reduction in ACTH plasmatic levels that the combination of MS and adult chronic stress raises more the concentration of corticosterone, and that antidepressant treatment prevented this increase in corticosterone. Yoo *et al*. [92] utilized fluoxetine, a 5-hydroxytryptamine (5HT) reuptake inhibitor, to reverse some of the effects some of the behavioral effects of MS in female rats. Fluoxetine treatment was started at PN35 and until the end of the experimental period (PN45). MS female adults showed an increase of 5-HT metabolism in both, the hippocampus and the hypothalamus of MS females, while behavioral parameters were not improved in adolescent females. Exposure of an antidepressant during development has been also investigated by the group of Pawluski. Rat pups subjected to prenatal stress were indirectly administered with fluoxetine, by giving this substance to the mother. Maternal treatment with fluoxetine reversed the immobility in the forced swimming test and the decrease in hippocampal neurogenesis of prenatal stress offspring [93]. Together, all these experiments show that antidepressant treatment helps to reverse or to ameliorate several behavioral, neuronal consequences of early life adversity. Clearly, more studies are needed and with different early stress models testing different time schedules and onset of administration of antidepressants to reverse the deleterious effects of ELS.

6.2. MS and Anti-inflammatory Agents

Psychiatric illnesses are associated to inflammatory processes in the brain. Therefore, strategies targeting some key players in the inflammatory cascade like prostaglandins and the enzyme cyclooxygenase-2 (COX-2), might turn to be good therapeutic agents to prevent or reduce some of the effects of ELS. Studies of Hennessy *et al*. [94] showed that a proinflammatory state contributes to the behavioral effects observed during isolation of MS pups. They showed that the administration of the prostaglandin synthesis inhibitor indomethacin reduced the passive repertoire of responses elicited by pups during a 3h isolation period. Later, Brenhouse and Andersen [95] blocked the action of COX-2 in the brain of MS rats, since this is an important mediator of the inflammatory cascade, and MS increases COX-2 expression in the prefrontal cortex. They administered a COX-2 inhibitor to the MS rats during preadolescence and showed that this strategy prevented the loss of parvalbumin and improved their performance on a working memory task. Recently, Hennessy *et al*. [94] administered another anti-inflammatory agent naproxen, to guinea pig pups during several

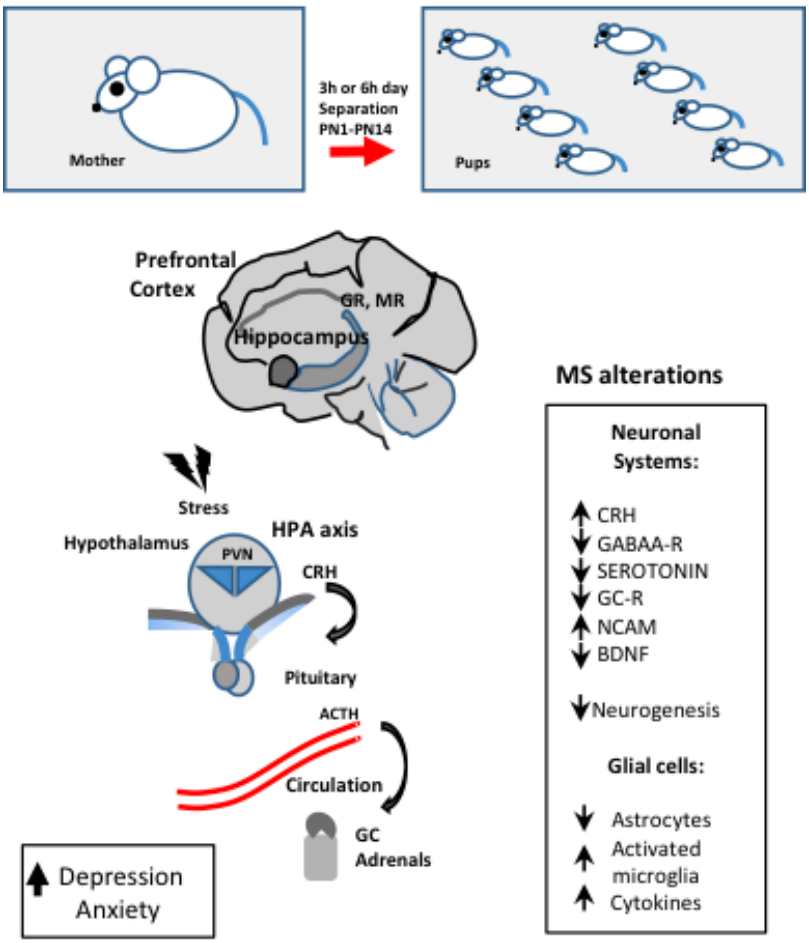

Fig. (1). Schematic representation of the effects of Maternal Separation as Early Stress factor, on different systems, neurons and glial cells.

maternal separation procedures and showed that its administration reduced the depressive – like behavior and fever elicited by the pups after the MS procedures.

The above studies confirm the participation of proinflammatory mechanisms in the behavioral and neuroendocrine effects evoked by ELS. Clearly more studies are needed with different anti-inflammatory agents to target and prevent the deleterious consequences of chronic stress exposure at early ages.

In summary early life stress affects different aspects of the physiology and the mechanisms that are involve still on research. Certainly, the disruption of mother-child interaction during breast-feeding that is frequently in low-income-countries is a chronic stress and alters the children development and their cognitive performance. In figure **1** we summarize the effects of MS, and, in this sense, animal models are one of the best ways to explain the pathways involved and many questions have to be answered.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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