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# Effects of prenatal stress on developmental anatomy of the brain and adult behavioural pathology

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**Abstract**

During critical or sensitive periods of development, the brain is highly plastic and particularly vulnerable to environmental adverse effects, including stress. In response to gestational stress, activation of the sympathetic system, the HPA axis and the central limbic stress loop increase the level of circulating glucocorticoids and catecholamines in both mother and foetus. Exposure to the excess amount of corticosteroids permanently reduces the glucocorticoid and mineralocorticoid receptors; thereby leading to an attenuation of the HPA axis feedback sensitivity and enhanced responsiveness to stress in the adult offspring. In the assessment of consequences at the behavioural, functional, and morphological levels; experimental animal models of maternal stress are extremely useful, in which the timing, intensity and duration of stress exposure can be readily controlled. These animal studies have revealed important links between prenatal stress exposure, life-long changes in the HPA function, and enhanced risk for subsequent psychopathology in a sex-specific manner. The aim of this review is to examine the impacts of prenatal stress on developmental anatomy of the brain, particularly focusing on the relevant emotional and behavioural outcomes within the context of current data obtained from experimental animals.

**Key words:** prenatal stress; HPA axis; development; mental health; neuronal plasticity

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## Introduction

Perinatal period is a time of particular vulnerability to stress which has serious consequences on the developing foetus through both maternal behaviours and physiological changes. Such distress can result from natural or man-made disasters such as earthquakes, floods, storms, war or terrorist acts. Besides, not only in socio-economically disadvantaged regions with less educated background and high unemployment rates, but also in every civilized society; pregnant women are exposed to intimate partner and patriarchal family violence, as well as interpersonal tensions or adverse conditions in the workplace.[1] Moreover, pregnancy related risk factors and dramatic fluctuations in gonadal hormone levels increase the prevalence of mood disorders and anxiety symptoms during this period.[2-4] Regardless of its nature and origin, stressful situations are considered as a 'threat to homeostasis' and a series of morphological, physiological and behavioural responses occur to restore the original condition.[5] Unfortunately, these responses negatively affect the rate of development, mental and physical health of the offspring and increase the incidence of preterm birth, lower birth weights and smaller head circumferences.[6-8] In turn, less optimal birth outcomes can lead to long-term cognitive impairments and motor disabilities. There is also compelling evidence showing that stress exposure during prenatal life enhances susceptibility for emotional problems and behavioural disorders later in

life. Wide range of impairments seen in the offspring might vary from decreased intellectual activity, problem-solving skills, delayed language acquisition, and lower IQ scores to anxiety disorders, hyperactivity, depression, autism and schizophrenia.[9-12] Nonetheless, it is not possible to relate the final outcome in the offspring directly to maternal stress since most studies are not able to control the attendant confounding factors, genetic background and influences of postnatal environmental factors. Especially in retrospective studies, a causal role of prenatal stress has been inferred from interviews with the mothers or their answers to questionnaires. Therefore, a lack of consensus on the definition of maternal stress, appropriate ways to measure it or to account for individual differences in coping with it create challenges for investigation of maternal stress effects on human offspring. Whereas, in experimental animals, not only are environmental and genetic factors more easily controlled, but also timing, duration and intensity of stress exposure are determined by the investigator. Therefore, the most reliable support concerning the effects of prenatal stress on the development and behaviour of the offspring comes from the experiments in rodents and non-human primates.

## Experimental Animal Models

In order to imitate the effects of maternal stress exposure and investigate the defects originating from such manipulations in the offspring, a number of different experimental paradigms have been developed. In majority of these paradigms; rats or mice are used and pregnant dams are exposed to stress during the last week of gestation, from embryonic day (E) 14 until delivery, although shorter periods of stress exposure are also employed. The most frequently used model has been in the form of the restraint or immobilization (rat limbs are taped to a platform) for 20, 30, 45, 60 or 90 min/once, three times or variable times, daily.[13-15] Other models used in experiments have consisted of exposure to noise (90 or 95 db), cold (4ºC), bright light, foot shocks, sleep or food deprivation, immersion in water (forced swimming) and saline injections.[16-18] In some of the studies, variable (combination of one, two or three different type of stress models) or randomized stress protocols are also applied.[19-21] Although methodological variations can be relevant while comparing the results of different stress protocols; observed effects suggest that mild stressors (noise, saline injection) are as potentially harmful as more severe paradigms. All these stress models, though, share a common property of being repeated and performed on the last week of gestation emphasizing these features are essential for the altered behavioural, functional and molecular outcome.

## Establishment of the Maternal Stress Response

Whatever the nature of stress protocol, response of an organism to environmental challenges activates central and peripheral circuits; namely the hypothalamic-pituitary-adrenal (HPA) axis, the central limbic stress loop, and the sympathetic branch of autonomic nervous system.[22,23] Such response is under the control of stimulating and inhibiting inputs to the hypothalamic paraventricular nuclei (PVN), which control the secretion of corticotropin-releasing hormone (CRH) and vasopressin (VP) into the pituitary portal circulation where they regulate adrenocorticotrophic hormone (ACTH) and β-endorphin release. ACTH, in turn, drives corticosterone (CORT) release from the rat adrenals (or cortisol from the human adrenals). Glucocorticoids easily cross the blood-brain barrier and act predominantly via two intracellular receptors; glucocorticoid (GR) and mineralocorticoid receptors (MR), with low and high affinity, respectively.[24] MR are found in highest concentrations in the hippocampus and appear to be involved in maintaining basal activity of the HPA axis.[25] Whereas, GRs are found everywhere in the brain including the frontal and cingulate cortex, hippocampus, basolateral and medial nuclei of the amygdala, nucleus accumbens and thalamus, but are most abundant in the hypothalamic CRH neurons and the pituitary where they "shut off" the neuroendocrine stress response via negative feedback of CRH release **(Figure 1A**, solid lines). In addition, binding of glucocorticoids to their cognate receptors in hippocampus activates indirect pathways and causes suppression of hypothalamic CRH expression.

Both glucocorticoids and cognitive or emotional stress signals also stimulate the more recently elucidated 'central' limbic stress circuit by converging on the central nucleus of the amygdala (ACe) and activate numerous CRH-producing neurons in this region.[26] ACe send efferent projections to the bed nucleus of stria terminalis (BST), which then projects to CRH expressing cells in PVN and activates the HPA axis. Locally released CRH also acts on cognate receptors on projection neurons of the amygdala and conveys stress-related information directly or indirectly via the entorhinal cortex to the hippocampal formation **(Figure 1A**, dashed lines). Within the hippocampus, CRH binds to its receptors on principle cells where they elicit Fos expression and might mediate either beneficial or adverse effects on synaptic efficacy.[22] This intriguing paradox (excitatory actions of CRH released from typical inhibitory GABAergic interneurons) and contribution of CRH on hippocampal synaptic plasticity are still the focus of ongoing research.

The activation of the sympathetic nervous system in response to stress increases the secretion of catecholamines and norepinephrine. While norepinephrine possesses a stimulatory role on CRH-neurons in the PVN, catecholamines involve in the hippocampal glucocorticoid negative feed back mechanism by modulating corticosteroid receptor levels.[27,28] Therefore, these central and peripheral hormonal cascades are closely interrelated with each other and ultimately cause elevated level of catecholamines and glucocorticoids in both maternal and fetal circulation.

Upon binding, activated corticosteroid receptors translocate to the nucleus and modulate the expression of target genes by two independent mechanisms: A direct interaction of receptor dimers with specific DNA sequences known as glucocorticoid response element (GRE) in their promoter region or interaction with other transcription factors (such as c-jun, AP-1, NF-B, STAT5, CREB) activated by binding of different neurotransmitters to their cognate receptors **(Figure 1B).** Thus, glucocorticoid mediated transcriptional effects might be very complex and potentially diverse.[29,30]

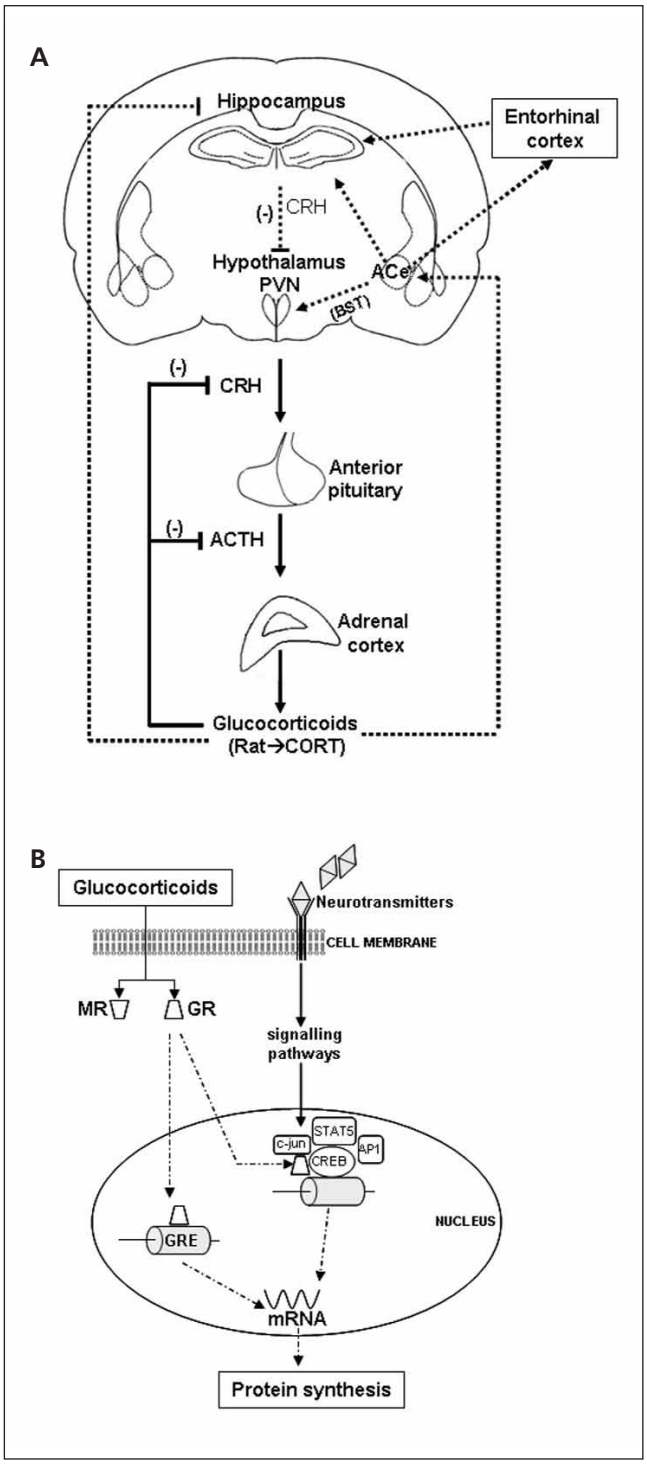


**Figure 1.** Stress-activated pathways comprising the neuroendocrine hypothalamic-pituitary-adrenal axis (**solid lines**) and the central limbic stress loop (**dashed lines**) are shown in **A**; and intracellular interactions of activated corticosteroid receptors are shown in **B**. **BST:** bed nucleus of stria terminalis; **ACe:** central nucleus of the amygdala; **GRE:** glucocorticoid response elements; **AP1:** activator protein 1; **STAT5:** signal transducer and activator of transcription 5; **CREB:** cAMP responsive element binding protein.

## Development of the HPA Axis

Unlike the animals giving birth to mature young (sheep, guinea pigs and primates), in rodents birthing to immature young, a large proportion of the neuroendocrine development takes place during the early postnatal period.[31] However, the HPA axis is highly susceptible to programming and environmental influences during the prenatal period. In rats, PVN develops between E13 and E15, and ability of the fetal HPA axis to respond maternal stress is shown by the observation of increased expression of CRH mRNA in the fetal PVN on E15.[32] Following the stress exposure, fetal HPA activation mirrors maternal HPA activation and increases Fos protein expression in direct relation to the severity of stress treatment. However, unlike maternal PVN neurons, fetal PVN neurons show no adaptation of Fos expression to repeated maternal stress.[33] In the meantime, GR mRNA is present in the hippocampus, hypothalamus and pituitary by E13, but MR expression is not detectable until E16-17.[34] In mouse, on the other hand, MR expression is first detected on E15.5, but GR expression appears on postnatal day 5.[35,36] Therefore, these species differences should be taken into consideration when comparing the effects of maternal stress on the regulation of fetal HPA axis and development.

### Effects of maternal stress on the developing HPA axis

Maternal stress leads to numerous cardiovascular and endocrine changes in the mother including the increases in plasma ACTH, β-endorphin, glucocorticoid and catecholamine concentrations. Elevated maternal catecholamine concentrations may lead to fetal hypoxia by causing constriction of placental blood vessels.[37] The fetal hypoxia then activates the fetal sympathetic nervous system and other neurotransmitter systems in the brain leading to alterations in the physiological responses of the offspring to stress.[38]

Under normal circumstances, the placenta forms a structural and biochemical barrier to many maternal factors. For instance, access of maternal glucocorticoids to the fetus is low due to the placental expression of 11β-hydroxystreoid dehydrogenase (11β-HSD) that converts corticosterone to inactive products.[39] However, repeated prenatal stress exposure leads to a decrease in placental 11β-HSD activity and thereby an increase in maternal corticosterone reaching the fetus.[40] Extended exposure to glucocorticoids attenuates the HPA axis feedback sensitivity by reducing the density of GR and MR in the hippocampus and hypothalamus of the prenatally stressed rats.[41,42] Therefore, these animals exhibit prolonged elevation in plasma glucocorticoid levels following acute exposure to restraint stress. Furthermore, prenatal stress accelerates the age-related HPA axis dysfunctions and circulating basal corticosterone levels in adult offspring.[15,43] Although some of the studies point out that prenatal stress-induced programming of GR and MR, as well as the basal HPA activity is sex specific with the effect being greater in female offspring, there is also remarkable variability in HPA outcome in male offspring.[13,15,31] These differences might be related to the differences in the duration, dose and timing of maternal stress protocols.[44,45]

The development and structure of hypothalamic CRH-containing neurons are also influenced by prenatal stress exposure in a gender- and intensity-dependent manner. During E15-17, daily restraint stress for 3 hours decreases the length of processes on neurons and increases the number of apoptotic cells in the fetal PVN; while 30 minutes stress exposure enhances the cell differentiation as indicated by an increase in both the number of branch points and the total length of the processes from the cell body.[32] Repeated stress exposure for 30 minutes from E17 to E21 induces a significant increase in the number of apoptotic cells in the PVN of female, but not male fetuses.[33]

## Effects of Maternal Stress on the Developing Nervous System

Activation of stress network is very critical during nervous system development, since extended exposure of glucocorticoids can damage growth and maturational processes despite the fact that they are essential for normal brain development.[46,47] The rapid growth rate and high turnover of neuronal connections make the developing brain particularly sensitive to maternal stress hormones. Depending on the critical periods of develop-

ment, interactions of glucocorticoids and CRH with their receptors alter the programming of the fetal HPA axis and in this way increase susceptibility to later psychopathology.

Investigation of postnatal outcomes in the offspring indicates that stress hormones primarily affect the morphology and function of the limbic system structures; causing anxiety, depressive-like behaviour, learning and attention deficits.[48] However, as pointed out previously, behavioural consequences are somewhat ambiguous, as similar maternal stress protocols can elicit different behavioural outcomes, even though it alters regulation of the HPA axis. Therefore, we will concentrate on the primarily affected regions of nervous system and go over the main morphological alterations by reviewing the relevant behavioural consequences:

## Hippocampal and cognitive changes induced by prenatal stress

As both a target of stress hormones and an active participant in the regulation of the stress response being integrated into the limbic system and HPA axis, a number of studies have been focused on the hippocampus to elucidate the molecular and cellular mechanisms by which early life stress induces long-term changes and plasticity in the nervous system.[49] The majority of these studies have been conducted in male rats in order to avoid confronting effects of the eustrous cycle. In most cases, perturbations in cognitive processes and exacerbations in age-related learning and memory impairments have been reported.[21,50] However, depending on the duration and intensity of stress exposure, as well as the gender vulnerability; contradictory results have been indicated on the learning performance. While prolonged stress slows the acquisition of spatial learning in the Morris water maze;[50-52] milder forms of stress do not impair the learning performance[53] or affect that only in males[54,55] or even cause a faster rate of learning performance.[14,56] Offspring of prenatally stressed rats have also demonstrated lesser cognitive performance in other learning tests, such as spontaneous alteration or exploration in the Y-maze, delayed alternation in the T-maze, passive avoidance conditioning and radial arm maze performance.[57,58]

In parallel to learning deficits, reduced postnatal hippocampal weight, reduction of neurogenesis in the dentate gyrus, decreased spine density of pyramidal dendrites in the CA3 region, decreased synaptic density in the hippocampus and suppression of long-term potentiation (LTP) have been demonstrated in the offspring born to prenatally stressed mothers.[59-62] In association with the suppression of LTP, a long-lasting reduction in the NMDA and AMPA receptor function through changes in transcription, translation and localization of its functional subunits.[63,64] On the other hand, opposing effects on the morphological maturation of hippocampal neurons, such as enhanced neonatal neurogenesis, differentiation of hippocampal neuronal processes and LTP have been demonstrated following exposure to short-lasting, mild prenatal stress protocols.[65]

Regarding to cognitive performances and structural plasticity; females seem to be better protected from the effects of prenatal stress than males, but age-dependent deleterious effects of stress exposure on hippocampal cell proliferation have been shown in older females, too.[66] Therefore, these effects might be related to circulating levels of estrogens and testosterone that are known to be shielding or aggravating modulators of cognitive functions, respectively.[67,68] Furthermore, prenatally stressed male rats exhibit higher level of Fos expression under basal conditions and blunted response following stress exposure.[69] Reduction in the activity of metabotropic glutamate receptors in the ventral hippocampus, but increased level of Brain-Derived Neurotrophic Factor (BDNF) and pro-BDNF in the hippocampus of male rats might be relevant changes with decreased synaptic plasticity and compensatory effects of decreased neurogenesis.[70]

## Morphological basis of anxiety and depressive-like behaviour

In majority of the animal studies, maternal stress exposure induces anxiogenic and depressive-like behaviour in the offspring characterized by fewer entries and significantly less time in the open arm of an elevated plus maze, reduced open-field locomotion, exploratory activity, grooming and rearing, anhedonia (ability to feel

pleasure) and learned helplessness.[43,48] Although the clinical symptoms of depression and mood changes cannot be assessed in animals, rodents are able to adopt either active or passive coping strategies in response to anxiogenic stimulus. While the passive strategy is expressed by immobility, freezing and reduced exploratory activity and associated with an increased activity of the HPA axis; the active strategy is expressed as higher loco motor activity and associated with a reduced activity of the HPA axis.[71] In most studies, prenatally stressed rats displayed impaired coping activity, increased duration of immobility in the forced swimming test and decreased preference for saccharine possibly indicating depressive-like behaviour.[72,73] The dopaminergic pathway projecting from the ventral tegmental area to the nucleus accumbens is thought to play a major role in motivation, mediation of the rewarding effects, drug dependence and the initiation of response patterns originating from the frontal corticostriatal loop systems.[74] When pregnant rats subjected to very mild stress, the volume and the total number of cells in nucleus accumbens of adult offspring decrease in a similar extend in both males and females.[75] However, permanent reduction of dopaminergic neurotransmission in the nucleus accumbens is seen only in prenatally stressed females suggesting that females have a greater tendency to depression than males.[76] Recently, an *in vivo* micro dialysis study in freely moving adolescent and young adult rats is shown that prenatal stress differentially modifies basal and stimulated dopamine and noradrenaline release in the nucleus accumbens.[77] These changes play also a key role in the etiology of other psychiatric disorders, such as schizophrenia, autism and drug addiction.[78] Besides, prenatal stress enhances psycho stimulant and neurochemical responsiveness to cocaine and tendency to addiction-related behaviour in adult rats.[79] Therefore, both structural and catecholaminergic alterations induced by gestational stress play a major role in the etiology of psychiatric disorders.

The amygdala is another critical structure involved in mood regulation, mediation of fear and anxiety. Since it contains CRH nerve terminals, cell bodies and receptors, and bi-directionally related to the frontal cortex, hippocampus and hypothalamus, it controls emotional and autonomic responses to stress.[80] Besides, in transgenic mice with a disruption of GR in the cortex and hippocampus, increased anxiety and depressive-like behaviour have been observed in response to greater activity of CRH on CRH1 receptors in specific brain regions.[81]

In prenatally stressed rats, hyperanxiety is associated with an increased response of HPA axis to stress with higher levels of CORT in circulation and CRH in the amygdala.[43,82] Furthermore, anxiogenic behaviour can be induced in normal rats by injection of CRH into the basolateral nucleus of the amygdala.[83] Since neurons in this region are generated during E14-17 in the rat and responsive to maternal CORT and CRH; excessive amount of hormones and receptors may cause permanent changes in the reactivity of the amygdala in response to stress.[82-84] As a matter of fact, prenatally stressed male rats show higher number of Fos expressing neurons in the medial amygdaloid nucleus in response to less anxiogenic environment (exposing to the closed arm of an elevated plus maze).[85] Moreover, challenging them with a more anxiogenic environment (the open arm of an elevated plus maze) results in a less pronounced plasticity in neuro-behavioural responses.

With regard to the developmental and anatomical changes, exposure to prenatal stress temporarily impedes trajectory of lateral, basolateral and central nuclei of amygdala, being smaller in size between P7-25, but resolved after P45.[86] However, expansion in the lateral nucleus, an area in which learned fear is encoded, and increased number of neurons and glia cells has been reported in prenatally stressed adult male rats.[87]

## Effects on the prefrontal cortex and their behavioural consequences

Prefrontal cortex is a very important area for the integration of different information into emotional and cognitive-related behaviours.[88] Similar to the sensory and motor cortical regions, synaptic development of the limbic cortical regions is modulated by emotional experience. Quantitative morphologic analysis has shown that negative emotional experiences significantly alter the neuronal morphology. In the dorsal anterior cingulate and orbitofrontal cortex of rats, gestational stress causes a significant reduction in the spine frequencies of pyram-

idal neurons.[20] In addition, a pronounced decrease in the length and complexity of pyramidal apical dendrites are observed in male, but not female offspring.

Dorsal and ventral part of the medial prefrontal cortex are shown to detect whether a stressor is under the organism's control and inhibit stress-induced neural activity and behavioural responses.[89] The medial prefrontal cortex consists of the anterior cingulate cortex, prelimbic and infralimbic regions in rats. Prenatal stress exposure has a negative effect on the ratio of mushroom spines in these regions without affecting spine densities.[90] Since these spines have relatively strong synaptic strength, more synaptic vesicles and a larger postsynaptic density; it might be related to reductions in the expression of plasticity related neuronal proteins. In fact, the expression of synaptophysin, one of the markers of functional synapses, is significantly correlated with stress-induced CORT release. Prenatally stressed rats with low levels of synaptophysin expression in both medial prefrontal cortex and nucleus accumbens have a higher CORT response to a subsequent stressor and it is not return to baseline as quickly.[91]

Another marker of neuronal plasticity, BDNF, is also decreased in the prefrontal cortex of adult rats exposed to prenatal stress.[92] Interestingly, a significant reduction in the expression of basic fibroblast growth factor (FGF-2), playing role both as a neuroprotective molecule and neuromodulator involved in psychiatric disorders, has also been demonstrated within this region.[93,94] In addition to neurotrophic factors, dopaminergic and glutamatergic dynamics are influenced by gestational stress in different forebrain regions of male rats.[95,96] In a very recent study, it has been shown that gestational stress attenuates the responsiveness of the glutamatergic system following a challenge at adulthood, without altering basal glutamate receptor expression.[97] Again, these changes show gender and anatomical specificity since they primarily affect function of the prefrontal cortex in male rats.

### Cerebellar alterations

Over the last decade, a growing literature demonstrates that the cerebellum critically involves in higher cognitive functions, behaviour, emotion, many forms of learning and memory.[98] Although the cerebellum comprises only 10% of the total brain volume, the mature cerebellum contains more than 50% of all the central nervous system neurons.[99] Despite the cerebellum is one of first brain structures to differentiate, it is the last one to achieve maturity. Therefore, this protracted developmental schedule makes it particularly susceptible to disruptions during the perinatal period. In our experimental studies, stereological analysis of the synapse-to-neuron ratios in the granular layer of the cerebellar cortex revealed that maternal stress significantly reduces the interneuronal connectivity and synaptophysin expression in the developing rat cerebellum.[100] We also showed that prenatal exposure to stress changes the morphology and numerical density of cerebellar neurons by primarily affecting the actively dividing Purkinje cells during the selected stress period.[101] Intriguingly, in the rat brain, cerebellum is one of the most prominent site of CRFR-1 expression which occurs predominantly in Purkinje cells beginning from early development through to adulthood.[102] The binding of CRH to this receptor has a depolarizing effect on the Purkinje cells and play a beneficial role in the regulation of neuronal survival through activation of a number of putative neuroprotective intracellular signalling pathways.[103]

However, CRF is capable of influencing cerebellar neuronal development and increasing neuronal survival of embryonic GABAergic neurons only after these cells exposed to negative conditions threatening their survival in cultures.[104] Since deficits in GABAergic transmission have been well documented in the prefrontal cortex, limbic system, and cerebellum of individuals with schizophrenia and autism; prenatal stress might possibly contribute to the etiopathogenesis of these disorders by causing Purkinje cell abnormalities and decreasing interneuronal connectivity.

## Concluding Remarks

Maternal stress during pregnancy increases both maternal and fetal plasma CORT and causes down regulation of fetal GR and MR, which then impair the feedback regulation of the HPA axis in the offspring. Besides, excess amounts of CRH reaching through placenta or produced by fetal limbic system structures interact with their cognate receptors and predispose to attention

deficits, anxiogenic and depressive- like behaviour through changes in neurotransmitter activity.

The perinatal period is an extremely sensitive stage for neuroplastic changes which may lead to long-lasting or permanent changes in the structure and function of the nervous system. Such vulnerability is not widespread but, instead, affects specific regions and neuronal pathways that might be relevant to different psychiatric disorders. Reduced neuronal plasticity, especially in the limbic system structures including hippocampus, amygdala and prefrontal cortex might represent a common and relevant component underlying different impairments. Therefore, enhancing neuronal plasticity and cellular resilience might be a novel therapeutic approach for the more effective treatment of psychiatric impairments. And lastly, but certainly not leastly, in considering the higher medical and social cost of emotional and behavioural diseases; women should keep away from stress and receive better protection and sympathy from all parts of the society during pregnancy.

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