



Review

Early maternal separation: a rodent model of depression and a prevailing human condition

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Abstract:

The early life of most mammals is spent in close contact with the mother, and for the neonate, early maternal separation is a traumatic event that, depending on various conditions, may shape its behavioral and neurochemical phenotype in adulthood. Studies on rodents demonstrated that a very brief separation followed by increased maternal care may positively affect the development of the offspring but that prolonged separation causes significant amounts of stress. The consequences of this stress (particularly the hyperreactivity of the HPA (hypothalamic–pituitary–adrenal) axis) are expressed in adulthood and persist for life. Maternal separation in rodents, particularly rats, was used as a model for various psychotic conditions, especially depression. The most popular separation procedure of a 3-h daily separation from the second to the 12th postpartum day yields a depression model of high construct and predictive validity. The results of studies on maternal separation in rats and monkeys prompt a discussion of the consequences of traditional procedures in the maternity wards of developed countries where attention is focused on the hygiene of the neonates and not on their psychological needs. This alternate focus results in a drastic limitation of mother–infant contact and prolonged periods of separation. It is tempting to speculate that differences in the course and severity of various mental disorders, which are usually less prevalent in underdeveloped countries than in developed countries (as noted by Kraepelin), may be related to different modes of infant care. Only recently has so-called kangaroo mother care (establishing mother–infant skin-to-skin contact immediately after birth) become popular in developed countries. In addition to its instant benefits for the neonates, this procedure may also be beneficial for the mental health of the offspring in adulthood.

Key words:

maternal separation, depression, neonatal care, animal model, kangaroo mother care

All newborn mammals are cared for by their mothers and remain in close contact with them for a period of time. This first period of life is a time of complex interactions related to nursing and plays an important role in the emotional and cognitive development of the offspring. The first days of life shape behavioral, emotional and physiological responses in adulthood [45]. Adverse events in early life, particularly disruptions in the maternal–offspring relationship, cause acute disturbances and produce long-lasting effects. These effects, both acute and long-lasting, develop due to the repeated activation of stress mediators such as glucocor-

ticoids and catecholamines, which have been described by McEwen [74] as allostatic load. The allostatic load affects the behavior and neurochemistry over the lifetime of the mammal, and the consequences of an excessive load on animal behavior may be considered models for some psychiatric diseases.

Because close contact with the mother is very important, early maternal separation results in a strong reaction of protest and despair of the neonate [39] and precipitates a considerable allostatic load, resulting in acute disturbances of physiological and neurohormonal functions with important consequences for social

behavior and neurochemical characteristics in adulthood. The mother's presence and her behavior create an environment in which many factors can specifically influence the neonate's development [82].

In the development of culture, humans formed various patterns of social interactions, including behavior within a family. The transcultural differences in family organization and maternal behavior are considerable [2], and the differences in baby care may influence several aspects of life, including the occurrence and course of psychiatric disorders, as first noted by Kraepelin [52]. The importance of transcultural differences in psychiatry has been recently widely accepted [81], and the differences in the treatment of neonates and infants may in part be responsible for these differences.

Regardless of transcultural differences, the neglect or maltreatment of children in early developmental periods increases the risk of anxiety, depression and psychoses in adulthood [9, 33, 48, 83], which are often resistant to treatment [14, 84]. The mechanisms involved in the influence of distorted maternal care on the neuropsychophysiological development of a child and resulting psychopathologies in adulthood are not yet fully recognized, but the observation that maternal care plays an important role in the development of a wide array of behaviors in rodents and monkeys suggests that molecular changes induced by a disturbance in maternal care in those animals may shed light on the mechanisms underlying the distant consequences of inadequate maternal care in humans [45].

Most of the studies on the effects of maternal separation on the development of offspring have been performed on laboratory rats, which appear to have less divergent responses to stress [24, 40] than do mice, which are more resilient to neonatal stress; in mice the distant effects of such stress are often inconsistent [3, 79, 88]. Some effects of maternal separation elicited in rats, such as deficits in prepulse inhibition, could not be reproduced in mice at all [80]. Nevertheless, even experiments limited to rats pose significant methodological problems.

Methodological problems

Although maternal separation is the most robust and common model of the disruption of the mother-offspring

relationship, the reports on its effects in rats are often varied [58, 71]. Separation from dams is always a stressful procedure for rat pups, which respond to it with increased motor activity and decreased cardiac and respiratory rates [36]. The tactile and olfactory stimulation that pups constantly receive from their mother reduces the innate high levels of behavioral arousal of neonates [37]. The distant effects of separation, which may persist to adulthood, vary considerably and depend on gender, the details of the procedure and an adequate choice of controls. Various protocols may dramatically affect the outcome of the experiment and the phenotype of the adult animal exposed to neonatal stress.

Gender differences

In rats, the responses to stress and consequences of early maternal separation are gender-dependent. Male and female rats appear to have different behavioral profiles and coping strategies in many behavioral experiments [6, 44]. Therefore, it is possible that female rats with a history of early adversities express increased emotionality in certain situations differently from males. Female rats experiencing repeated maternal separation as neonates, for instance, show higher emotionality when exposed to a novel environment, while males that have a similar history display suppressed emotionality [99] and decreased anticipatory responses to reward [73]. However, under different conditions, the result may be opposite. Eklund and Arborelius [29] reported a decrease in anxiety in neonatally separated females but no separation-induced changes in males. The difference was that in the first experiment [99], the pups were separated once daily for 270 min, while in the second experiment [29], they were separated twice a day, each time for 180 min.

Early separation versus mother deprivation

The separation may be performed in a number of ways that induce varying degrees of stress for the dams and pups [7]. The mother can be removed from the home cage, leaving the pups without care but in a familiar environment and the presence of their siblings; the litter can also be transferred into a new environment in which it is deprived not only of the mother but also of a familiar environment (thus being exposed to stressful stimuli, particularly new odors) [22]. The latter procedure was denoted "early deprivation", in contrast with the less stressful "maternal

separation". The maternal separation procedure, which is particularly stressful for dams, produced greater interest by the mother in the pups after reunion, while "early deprivation" was more stressful for the neonates and more significantly affected their juvenile behavior [122]. There are possible further variations, e.g., keeping the separated litter together or individually [7]. The results of those studies may be important for the translation of laboratory results to humans (differences between orphans staying at home and those transferred to an orphanage).

The schedule of separation

Frequency of separation

This procedure usually involves a repetitive separation. In most studies, the pups are separated once or twice every day. Alternatively, a single separation for a longer period of time can be used.

Timing

The separation procedure usually starts from the second postpartum day (PD) to day 10 or 14 and extends to PD21 (weaning) in some experiments. The separation period overlaps the stress hyporesponsive period, extending between PD4 and PD14 [105]. This natural defense against stress in neonates may blunt the effect of maternal separation.

The duration of separation

The duration of the mother's absence differs between studies. The brief periods of separation were originally referred to as "early handling" while longer periods of separation, usually lasting 180 or 360 min, were described as "maternal separation". To avoid confusion, most recent papers use the code MSxx, where xx denotes the period of separation in minutes. Thus, the original "early handling" became MS15. In some experiments, the separation lasted 24 h and is referred to as MS1440 in this paper.

The controls

Non-handled pups left with their mother could be considered an appropriate control; however, such pups are reared under conditions of extreme environmental impoverishment [41]. Any handling involved in pup separation, as an environmental enrichment, should positively influence the offspring [11]. Thus, briefly handled pups (MS3–MS15) are sometimes used as controls for MS180 pups [4]. Alternatively, controls are pups kept under animal rearing facility conditions (ARF) with their cages and bedding changed twice or three times per week. In many studies, no differences were observed between MS15 and ARF pups [98]; however, this is not always the case (vide infra).

Neonate stress and mother behavior during separation

The neonatal rat is dependent upon the mother for thermoregulation, nutrition, the stimulation of urination and protection for the first two weeks of life [104], particularly because its brain at birth is roughly equivalent to a human brain at gestational ages 23–24 weeks [94]. Tactile sensory input from the mother, particularly skin-to-skin contact, augments the pups' hypothalamic oxytocin concentrations [51], which is required for the expression of filial huddling preference [50], an element of mother recognition [1]. Moreover, the licking and grooming of pups during the first ten days of life stimulate the offspring's hippocampal development, spatial learning, and memory [65] and reduce the hypothalamic-pituitary-adrenal (HPA) axis responses to stress [66].

A temporary loss of contact with the dam elicits signs of distress, including alterations in heart rate, circadian rhythms, and hormone levels [36, 37, 53, 78, 110]. The presence of stress is evidenced not only by behavioral indices (immobility and vocalization) and the elevation of stress hormones [109] but also because the distant behavioral effects of handling are prevented by anxiolytic treatment [23]. Complete maternal deprivation (removal of pups on PD4 and rearing them artificially with intragastric feeding) causes deficits in social but not spatial learning [62].

An important confounding factor in the studies on maternal separation is the dam's reaction after returning the pups. When pups are handled in the presence of their mother and then returned to the cage, they became an object of increased grooming and licking [8, 66], and the pups showing the greatest handling effects were those from the litters in which the mothers showed the most intense and prolonged licking and grooming behavior [66]. The increased dam's interest rather than stimulus from the experimenter is responsible for the attenuation of stress responses of rats subject to brief neonate handling [60]. The interest of mothers in their pups is possibly responsible for pups' higher resistance to amphetamine toxicity in adulthood [108] and changes in corticotropin-releasing hormone (CRH) mRNA expression in the amygdala and the locus coeruleus [31, 55]. Not all behavioral effects of mother-pup reunion after separation are necessarily related to the HPA system; some effects may be mediated by the cholecystokinin that is released from the stomach of the pup after feeding [117].

The length of the maternal separation period and its effect on adult phenotype

It could be assumed that stress would increase with the length of the period of maternal deprivation, and the differences in the length of separation period may be a source of variability in results.

Effects of a single 24-h maternal separation period in the neonatal rat

This procedure (single MS1440) is presently not commonly used. It is undoubtedly stressful for pups, and it changes the architecture of sleep and reduces REM sleep [38]. The distant effects of single MS1440 are variable and are usually not persistent. The results largely depend on which postpartum day the isolation takes place, i.e., prior to, in the middle, or at the end of the stress hyporesponsive period [105]. As a consequence, either hypo- or hyperresponsiveness to stress was observed at weaning, but the majority of effects induced by this separation model did not persist to adulthood [104]. The impact of MS1440 could be species-dependent: some behavioral effects persisting to adulthood were described in mice [70].

Long-term consequences of repeated 15-min neonatal maternal separation (MS15) in rats

MS15 decreases behavioral and endocrine responses to stress in adulthood. As adults, MS15 rats show decreased fearfulness in the face of novelty and blunted HPA axis responses to stress. These effects are related to altered CRH gene expression, particularly to changes in the CRH in the central amygdaloid nucleus – locus coeruleus system [31].

One of the first studies demonstrated that brief separation decreased the mortality of stressed rats that experienced the procedure as neonates [118]. The following studies in rats [60] and mice [26] demonstrated that a brief maternal separation (MS15), called “gentling” or, later, “early handling”, repeated daily from PD2 throughout the nursing period “hardened” the pups, which became less reactive to stress as adults, displaying a so-called blunted emotional profile. This profile is related to the blunting of the corticosterone response to stress in MS15 rats [61], which is caused by a selective increase in glucocorticoid receptor in the hippocampus and the frontal cortex, at least in Long Evans rats [77]. The attenuation of emotional arousal in MS15 rats may also be related to a decrease in the branching of dendrites in the locus coeruleus [111].

Interestingly, “gentling” for 8 days (PD2–PD10) helps both mice and rats survive food and water deprivation; a longer treatment (PD2–PD20) is beneficial for rats but harmful for mice [25]. The adrenocorticotropic hormone and corticosterone responses to stress were dampened and shortened in MS15 rats [95]. In view of the problem of the mother's behavior discussed above, one may speculate that the hardening effect is due to an increased maternal care induced by brief separation.

The behavioral and neurochemical consequences of MS15 are not harmful, and such separation periods may be considered innate; under natural conditions, Norwegian rat mothers leave the nest for periods of 15–30 min for foraging [17]. Accordingly, several authors have reported that in many parameters, MS15, ARF, and non-handled rats did not differ [41, 98] and were interchangeably used as controls for MS180 rats [5]. However, when investigating the effect of MS15 on the status on cerebral monoamine systems in middle-aged (18 month) female rats, Arborelius and Eklund [4] demonstrated clear differences among ARF, MS15 and MS180 animals. There are few instances in

which the long-lasting effects of MS15 are stronger than those of MS180 or MS360. This is, for example, the case for the elevation of opioid peptides in brain structures [93], but the effects on peptides are dependent on brain area, rat strain, and gender [91, 92].

In most studies, MS15 rats did not display a depression-like phenotype as adults. There were no differences in sugar preference between MS15 and non-handled Sprague-Dawley rats [69] and no differences in a forced-swim test in male Wistar or Fischer rats [87, 103]. MS15 Lewis rats displayed decreased anxiety [28], while MS15 Fisher rats displayed some learning deficits [102].

Long-term consequences of repeated 3-h neonatal maternal separation in rats

The MS180 procedure is the most popular in studies on the consequences of disrupting mother-offspring attachment. In contrast with the hardening effect of MS15, MS180 adversely affects the development of offspring, which in adulthood are more reactive to stress [76]. As discussed below, MS180 is considered a model for depression. The previously discussed acute separation stress experienced by pups in the MS180 procedure is not compensated by increased maternal care. The dams are less attentive to pups upon their return after 3 h, and these pups, in contrast to those separated briefly, appear to be stressed by their reunion with dams [42]. These observations support the notion of Deneberg that the maternal behavior encountered upon reunion may determine individual differences in the stress responsiveness of the progeny [25].

Rats that were subjected to MS180 as pups exhibited a hyperresponsiveness of the HPA axis that persisted for life. These animals permanently maintained high anxiety-like behavior, depression-like syndrome, anhedonia, and a proclivity for increased ethanol intake [12, 43, 46, 54–56, 72, 73, 75, 95, 120].

In parallel with the behavioral effects of MS180, neurochemical changes were observed in the brains of adult rats, particularly the increased activities related to CRH and noradrenaline transmission [30, 55, 64]. These effects included an increase in CRH content in the median eminence related to the up-regulation of CRH gene expression in the paraventricular nucleus, the downregulation of the CRH receptor in the anterior pituitary, the downregulation of the hippocampal glucocorticoid receptor, the binding of mRNA with the concomitant upregulation of the mineralocorticoid

receptor, and decreased glucocorticoid mRNA expression in the medial prefrontal cortex [104]. The brain monoamine systems also appeared to be altered by MS180 [40]. Anhedonia may be related to MS180-induced changes in dopamine [72] and noradrenergic transmission, [111], which are involved with drug-associated rewards [119].

The changes in CRH and GR gene expression in the amygdala and medial prefrontal cortex, the activation of the noradrenergic system in the locus coeruleus, and changes in the GABA and oxytocin receptors in various brain areas may also be associated with anxiety, hypervigilance, preference for ethanol, anhedonia, and mild cognitive impairment observed in MS180 rats (see [104]) and a concomitant decrease in tone of the inhibitory GABA system. This decrease is reflected by, among other factors, a reduction of GABA_A receptors and benzodiazepine binding in various amygdaloid nuclei and the frontal cortex, which is associated with the decreased expression of mRNA encoding for the benzodiazepine site of the GABA_A receptor [16]. It is plausible that the reduction of the gabaergic inhibition by MS180 is responsible at least in part for enhanced noradrenergic transmission and CRH expression in the amygdala in response to stress [30].

The neurochemical effects of MS180 may, however, be area-specific. Thus, maternal separation, both brief and long, reduced noradrenaline levels in cingulate cortex [4]. Additionally, studies on another species, degu (*Octodon degus*), a guinea pig-like rodent characterized by highly social behavior, have shown that MS followed by social isolation for 24 days after weaning resulted in changes in the innervation of frontal areas (which are considered analogs of the human medial prefrontal cortex). Immunocytochemical studies have suggested a decrease in noradrenergic innervation and an increase in the number of serotonin fibers in various subdivisions of the medial prefrontal cortex, with the changes apparently resulting from an interaction with axonal sprouting and arborization [13].

MS180 also appears to affect brain-derived neurotrophic factor (BDNF), an agent important in stress, depression, and schizophrenia. BDNF content appears to be reduced in the hippocampus and striatum, increased in the ventral tegmental area, and unaffected in the cortex, nucleus accumbens and amygdala of MS180 rats [63]. However, other studies on the effects of MS180 on BDNF in the hippocampus yielded no effect [100] or an increase [34].

Early maternal separation as a model of depression

A valid animal model of psychotic depression may serve as a potent tool for both understanding the mechanisms of pathological changes and preclinically testing a variety of antidepressant treatments. Following 30 years of studies on the effects of early handling and maternal separation in rats, it was proposed that mother-infant separation may be considered an evolutionary model for human depression [112], and it is generally agreed that the MS180 procedure appears to fulfill the criteria for a depression model of high construct value [10]. This model has mechanistic validity: the separation alters the regulation of the HPA axis in both humans [114] and rats, in which the adult depressive-like behavior induced by MS180 is associated with HPA hyperactivity [57]. For a review, see [40].

As summarized by Lippmann et al. [63], rats experiencing MS180 display, as adults, behavioral deficits, abnormal HPA axis function in response to an acute stressor, and long-term alterations in BDNF protein levels. Several characteristics, including decreased activity, increased stereotypic behavior, increased responses to acoustic startle, heightened response to an acute stressor and abnormal levels of mature and immature BDNF in certain brain regions, suggest that these changes result from specific long-term central nervous system alterations and, in many aspects, resemble the changes observed in depression. Moreover, these changes in rats show homology with the effects of mother deprivation in humans, and human studies have demonstrated that the deprivation of maternal care renders children vulnerable to depressive-like states in adulthood [86, 90, 115]. Thus, maternal separation has been proposed as an animal model of early life stress and the subsequent development of depression-related behaviors in adulthood [5, 35, 55]. The MS180 model of human depression has good ontopathogenic validity because it involves manipulations during the early developmental period; disturbances leading to depression similarly take place early in the lives of humans [10].

The fact that several antidepressants or antidepressant procedures are capable of reversing the changes induced by MS180 or similar procedures [20, 21, 27, 43, 49, 68, 113] confirms that maternal separation is a valid model for depression with high predictive validity. Nevertheless, as discussed by Schmidt et al. [107], the outcome

of the model depends on subtle environmental influences and genetic predisposition; in their opinion, none of the established models of early life stress can be considered a robust model of depression. The influence of the adult environment may be of critical value because stress in early life may have adaptive consequences in aversive adult environments but may be maladaptive in an environment that is not aversive [106].

Brief maternal separation and Kangaroo Mother Care in humans

A vast body of evidence documents the adverse effects of child separation, maltreatment and abuse. The neglect or maltreatment of children in early developmental stages increases the risk of psychological disturbances such as anxiety, depression and psychoses in adulthood [9, 33, 48, 83], which are often resistant to treatment [14, 84]. The earlier the infant encounters the adverse experience, the more prone it is to express anxiety and depression in adulthood [47]. However, as mentioned for brief maternal separation in rats, a mild stress may result in positive effects in rodents. Studies on monkeys demonstrated that maternal separation in primates had only negative consequences including the enhancement of acute response of the HPA axis [89, 96], disturbed sleep architecture [96], symptoms of anhedonia [97], and depression-related low motivation for reward seeking [59]. These findings indicate that maternal separation in primates has no ecological advantage and does not increase fitness [89].

Although child neglect and abuse attracts the attention of medical professionals, social workers, and the general public, the critical perinatal period and first days of life of an infant do not. The obvious stress signal – the neonatal cry – is generally considered natural, and the need for mother-infant body contact is in modern society usually neglected. In most maternity wards, the newborn is immediately washed, weighed, wrapped, presented to mother and then placed in a separate cot or transferred to the “newborn room”, from which it is delivered to the mother only for feeding. Such a procedure appears to be more radical than MS180, particularly because the infant separation takes place even prior to bodily contact with the mother.

This routine has begun to change as obstetricians and neonatal nurses realize that the maternal body is the natural “playground” for the naked newborn baby and that mother-infant skin contact is very important both for the baby (learning odors, listening to heart-beat, preventing hypothermia) and mother (formation of attachment) (see [121]). The Western postnatal care standard of the 20th century was challenged by the introduction of “Kangaroo mother care” (KMC) by Dr. Edgar Rey Sanabria, a Colombian pediatrician who successfully introduced it in 1978 to reduce the mortality of preterm infants in an overcrowded and under-equipped Bogota hospital [18]. His observations have been widely confirmed [18, 19].

KMC involves the placement of the freshly born, unbathed, naked baby between the mother’s breasts. This method has gained wide acceptance, and it has been proposed that KMC should be a standard for all newborns [101]. The NICE guidelines for postnatal care [85] state that women should be encouraged to have skin-to-skin contact with their babies as soon as possible after birth and that the initiation of breastfeeding should take place within 1 h after the birth. Moreover, the separation of a woman from her baby within the first hour of birth for routine postnatal procedures (e.g., weighing, measuring and bathing) should be avoided.

Numerous studies have demonstrated that KMC has many immediate beneficial effects on the infant, including the provision of warmth and nutrition, improving neonatal cardiac and respiratory rate, oxygen saturation, sleep, pain relief, elevating neurobehavioral general development, mental/motor scores, and maternal attachment [19, 67, 101, 121]. Numerous studies have shown the beneficial effects of KMC both for the infant and mother, and the positive effects of skin-to-skin contact during the first 2 h after birth on the mother-infant relationship were observed one year later [15]. Immediate post-birth contact is so important that if the mother is unable to provide it (e.g., after Caesarean section), the father should substitute for her [116].

It is tempting to speculate that traditional neonatal care in Western hospitals may be considered very early maternal separation, which affects a vast majority of the population, and its distant psychological effects may seriously affect the mental health of the society. These effects may be responsible for, as described by Kraepelin [52], more serious courses of mental disease in our civilization compared with “un-

civilized” societies that follow traditional, evolutionarily established “primitive” customs, routines and rituals. It may be assumed that the predominant majority of people born in the 20th century and raised within Western civilization were the victims of early maternal separation. The degree of damage caused by this separation may vary from society to society, as the consequences of separation may be compensated for by additional maternal care, the intensity and quality of which varies depending on local cultural traditions. Nevertheless, maternal care and an enriched environment do not appear to compensate in full for the adverse effects of early maternal separation [32].

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