



Neonatal maternal separation in male rats increases intestinal permeability and affects behavior after chronic social stress

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ABSTRACT

Prolonged maternal separation in rats has several effects on health and behavior. Here we investigated how maternal separation might interact with social stress in adulthood on behavior and gastrointestinal permeability. The effects of either daily 180 min long term pup-dam separation (LMS) during the stress hyporesponsive period or daily 10 min brief maternal separation (BMS) on behavior, corticosterone and intestinal permeability were investigated, compared to a non-handling (NH) condition in male offspring. The animals from each separation condition were then randomly assigned to adult stress and control conditions, where the stress condition was exposure to 14 days of social instability (CSI). Sucrose preference, elevated plus maze behavior and corticosterone were measured. Colitis was experimentally induced by dextran sulfate sodium for 7 days, followed by measurement of intestinal permeability using the ⁵¹CrEDTA method. Granulocyte marker protein was measured in feces and colons were examined histologically for inflammation. Prior to the social stress, the LMS offspring showed elevated corticosterone levels, lower elevated plus maze activity and less fluid consumption. After social stress, corticosterone levels were suppressed in LMS animals and again they showed less fluid consumption. LMS animals had significantly higher intestinal permeability, but only when also exposed to the social stress in adulthood. The current results support a two-hit model, whereby early life events interact with adult life events in altering animals' vulnerability.

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1. Introduction

For a number of disorders, a multi-hit model has been proposed, whereby exposure to one event sets or modulates vulnerability following exposure to a later precipitating event. This conceptual model has been used with respect to schizophrenia and stress sensitivity among others [1–3]. The re-upsurge in studies of effects of early postnatal maternal separation on behaviors and endocrinology reflects the implicit acceptance of the model, and has led to spectacular advances, including the demonstration of epigenetic effects. However, the lifetime of the animal is made up of several phases

(such as the early postnatal period, adolescence and adulthood). Events intervening between an early maternal separation and an outcome measure would also be expected to modulate the separation effects themselves. In this study, we have investigated how early life events (here maternal separation in rats – MS) effects later anxiety- and depression-like behaviors together with gastrointestinal vulnerability, and how these effects might be modulated (enhanced or moderated) by an intervening chronic social stress procedure (chronic social instability – CSI).

The concepts of stress and anxiety are closely related, with an overlap in reaction patterns in the neuroendocrinological system and activation of similar areas of the brain [4]. MS is a well established animal model for early life stress. In long-term maternal separation (LMS), pups are typically removed from their mother during the so-called stress hyporesponsive period after birth, usually between postnatal day (PND) 2 and 14, for 180 min each day [e.g. [5]]. This seems to increase anxiety-like behaviors in adulthood as measured in for example the elevated plus maze (EPM) test [6]. Brief maternal separation (BMS – also called brief handling in some literature), however, often leads to reduced anxiety-like behavior [7]. Negative stress is also one of the main etiological factors for depression and not surprisingly, anxiety and depression exhibit high co-morbidity, and a number of clinical studies show that having multiple disorders increases risk of medical disability [8]. One of the distinguishing characteristics

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of depression is the inability to gain pleasure from enjoyable events (anhedonia). The sucrose (or saccharine) preference test for anhedonia seems to be a reliable measure of a depression-like state [see [9], for review]. In rodents, long-term maternal separation appears to induce anhedonic behavior [10], and several studies also report that rats previously exposed to chronic mild stress show a lowered intake of a sweetened solution compared to controls [e.g. [11–13]].

Although considerable progress has been made in gastrointestinal (GI) research, we do not yet know what causes inflammatory bowel disease (IBD) or irritable bowel syndrome (IBS) other than indications that it involves a complex interaction of factors like genetic predisposition, immune defense systems and environmental triggers. Alimentary microbiota may be a risk factor for IBD. Changes of diet and of bacterial exposure associated with migration are associated with a higher incidence of IBD, especially among younger children [14,15]. Although maintenance of mucosal adaptive immunity demands exposure to a wide range of micro-organisms, the absence of or a sudden change in signals from commensal bacteria represent a risk of inflammation. Functional bowel disorders like irritable bowel syndrome (IBS) [16,17] are also associated with abnormal reflex motor responses in the gut and intestinal motility dysfunction. Hazard pathogens pose not only an immunological threat, but are also suggested to underlie defects of the enteric nervous system which controls motility [18]. Due to the close connection between the brain and the body, emotional stress can in principle influence any disease. This is not to claim that stress per se is the cause. Conditions involving GI symptoms, whether functional or inflammatory, have been associated with altered HPA (hypothalamic–pituitary–adrenal) activity, one of the most commonly used markers of stress [19]. Thus, compared to healthy controls, IBS patients show decreased basal levels of ACTH but increased levels of 24 h basal cortisol and higher cortisol levels after stress [20]. Furthermore, IBS-like symptoms in humans are associated with low cortisol suppression following dexamethasone treatment [21].

In animal studies, stress in adulthood also appears to affect the inflammation associated with colitis. Mice exposed to chronic social stress show enhanced inflammation following exposure to DSS (dextran sulfate sodium – a commonly used agent for induction of colitis-like symptoms), and they even show an increase in inflammation consequent to the stress manipulation alone, without the DSS [22]. Emotional or psychological stress such as water avoidance also reacts colitis-like symptoms induced by DSS [23]. A defective barrier function is correlated with inflammation and several studies propose an association between increased mucosal permeability and both functional gastrointestinal disorders (such as IBS) and inflammatory bowel diseases [24,25]. Acute stressors like brief foot-shocks have also been shown to increase colonic permeability in DSS-treated rats [26].

There are reports of enhanced colonic permeability in adult rats that had been exposed to maternal separation as pups, and then given TNBS (2,4,6-trinitrobenzenesulphonic acid) in adulthood to induce colonic inflammation [27]. Another study has reported changes in the barrier function of the distal colonic mucosa and increased permeability [28], as well as an increase in bacterial adherence and penetration.

As stress in adulthood can cause disruption of the gastrointestinal tract and also worsen an already existing inflammation, it is of interest to study the effect that maternal separation has on the vulnerability to these adverse effects of later life stress. There are some studies that have looked at this phenomenon [see [29], for review]. These have shown an increased colonic permeability in rats that have been exposed both to MS in infancy and acute “water avoidance stress” as adults [30]. There is however a lack of studies of intestinal permeability using a more chronic stress paradigm following MS.

The present study aimed to investigate (1) the effect of early daily maternal separation (MS) on behavior and corticosterone and (2) the

potential interactions between early maternal separation and chronic social instability (CSI) in adulthood on behavior, corticosterone, intestinal permeability and inflammation.

Our *a priori* hypothesis was that long-term maternal separation during the first two weeks of life would affect animals' body weight, and later stress responsivity assessed by an increase in corticosterone levels as well as intestinal permeability following challenge. Further, we expected that this separation condition, when combined with chronic social instability, would further accentuate anxiety and depression-like behaviors, corticosterone, intestinal permeability and inflammation.

The experimental protocols were approved by the Norwegian Council of Animal Research and registered by the authority. All procedures have been conducted in accordance with Norwegian laws and regulations controlling handling live animals in research.

2. Methods and materials

2.1. Animals

Eighteen female HanTac:Wistar rats, (Taconic Europa, Denmark) time-mated 10 days before arrival, were singled housed in IVC (individually ventilated cages) cages (Model 1500U) with chopped wood as bedding (Aspen bedding, NOVA-SCB Norway), a polycarbonate play tunnel for rats (20-K3325, NOVA-SCB Norway) and soft paper. They were given breeding diet (RM3, Special Diet Services, U.K.) and purified (RO, reversed osmosis) water *ad libitum*. The light and dark cycles were 12:12 with lights on at 0700, and a gradual turn on/turn off at 0600 and 1800 respectively (full light at 0700 and full darkness at 1900). Temperature was held constant at $22^{\circ}\text{C} \pm 1$ and humidity at $52 \pm 2\%$. Ten of the 18 rats were successfully pregnant and a total of 102 offspring were born. After birth, dams and their offspring were randomly designated to one of three groups in the maternal separation conditions: (1) LMS (Long-term maternal separation, $n = 3$), (2) BMS (Brief-term maternal separation, $n = 3$), and (3) NH (Non-handling, $n = 4$). After birth on postnatal day (PND) 0, 26 offspring were cross-fostered to avoid an imbalance in litter size. The litter size ranged from 6 to 13. The staff handling the cross-fostering and maternal separation procedures was the same throughout the whole experiment. Latex-free disposable gloves and full-suit protection were used at all times. The offspring's gender was determined at weaning on PND 22. The female pups were relocated to a separate room and were not used further in this experiment. A total of 47 male pups were used with the following distribution and mean body weights (grams) at weaning: BMS ($n = 18$ (50.3 g)), LMS ($n = 11$ (48.2 g)), and NH ($n = 18$ (53.7 g)). They were housed in groups of four or five in Model 1500U cages. At PND 30, the offspring were rehoused in groups of two or three in Model 1291H cages and again, at PND 34, housed individually in same sized cages with enrichment (polycarbonate play tunnel) and regular food and water *ad libitum*.

2.2. Procedures

The timeline shows the progression of the experiment (Fig. 1)

2.2.1. Maternal separation

2.2.1.1. Long-term maternal separation (LMS). The dams ($n = 3$) and their offspring ($n = 30$) were daily separated for 180 min from PND 2 to PND 14 from 0900 h to 1200 h. The dam was first removed from the home cage and placed in a smaller cage (Model 1291H) with chopped wood bedding, food and purified water *ad libitum*. The new cage was placed in an adjacent room to the offspring. The litter was then moved to a new but similar sized cage with chopped wood bedding and soft paper, but divided by thick cardboard to allow for placement of the three litters at the same time. The litters

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