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Research Report

Periodic maternal deprivation may modulate offspring anxiety-like behavior through mechanisms involving neuroplasticity in the amygdala

Ariel Kupfer Berman [a], Rhonda B. Lott [b], S. Tiffany Donaldson [b,*]

[a] Department of Psychology, Brandeis University, Waltham, MA 02454, USA
[b] Developmental and Brain Sciences Program, Department of Psychology, University of Massachusetts Boston, 100 Morrissey Boulevard, Boston, MA 02115, USA

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ABSTRACT

Maternal care has been shown to affect the development of behavioral and endocrine systems. In rats, periodic maternal deprivation (PMD) serves as an early life stressor that directly influences maternal care by promoting more pup-directed behaviors in stressed dams. To further assess the qualities of PMD that may ameliorate long-term anxiety effects in trait anxiety animals, we coded behaviors across lactation (postnatal day (PND) 5, 16, 21) in dams phenotyped as high (HAn) and low-anxiety (LAn). We assessed anxiety-like behavior in male offspring using the elevated plus maze (EPM), focusing on percent open arm (%OA) time and latency to enter OA (OA LAT) as measures of anxiety-like behavior. Finally, we examined the brains of representative male pups to determine if the stress-related protein brain-derived neurotrophic factor (BDNF) might show persistent changes in the amygdala. Dams phenotyped as HAn had lower %OA time and longer OA LAT relative to dams designated as LAn. During PMD, HAn dams had higher incidences of licking-grooming (L/G) and more pup-directed behaviors on PND 5 and 16 compared to LAn dams. Further, as adults, HAn male offspring exhibited less anxiety traits than their maternal line with greater %OA time and %OA entries relative to LAn. HAn offspring showed markedly more BDNF immunoreacted cells in the amygdala than LAn. The combination of these findings suggests that the mild stressor, PMD alters anxiety-like behavior in offspring likely by influencing HAn dams' L/G activity and altering stress related proteins in the amygdala.

1. Introduction

The early environment has received much attention for the critical role it plays in the development of the neural, behavioral and stress reactivity of children. Early adverse experiences can have a negative impact on the development of the hypothalamic pituitary adrenal (HPA) axis, emotional behavior and stress reactivity of affected offspring (Roth and Sweatt, 2011). In clinical populations that have suffered childhood verbal, physical and sexual abuse and neglect, there is a correlation of greater instances of emotional distress in later life (Heim and Nemeroff, 2001) and hyperactivity of the HPA axis (Wismer Fries et al., 2008).

Long periods of maternal deprivation in rodents and primates are used to mimic human-infant early life stress given that it leads to similar impacts on the neural, hormonal and behavioral systems described in clinical populations (Kikusui and Mori, 2009; Macri et al., 2011). In contrast, short-term socio-environmental interventions can help to improve HPA axis functioning in compromised animals through sustained alterations in proteins implicated in plasticity and stress adaptability (Branchi, 2009; Ravenelle et al., 2013). For instance, in periodic maternal separation models in inbred trait anxiety animals (e.g., animals that show high anxiety-like behavior), adult offspring experience recovery of the HPA axis such that in response to anxiogenic stimuli they show a *blunted* adrenocorticotropic response (Neumann et al., 2005). Moreover, in an animal model of activity-based anorexia, if rodents experience long maternal separation (LMS) (i.e., daily separation for 180 min during the pre-weaning period), all offspring experience greater weight gain and females have markedly increased survival time.

Brain derived neurotrophic factor (BDNF) is a neurotrophin implicated in brain plasticity and stress adaptability and is a likely mechanism of change associated with social enrichment paradigms (Branchi, 2009). Indeed, increased levels of brain derived neurotrophin (BDNF) gene and protein levels are often correlated with adaptive neural and behavioral changes found in populations that

* Corresponding author. Tel.: +1 617 287 6249/+1 617 287 6429; fax: +1 617 287 6336.
E-mail address: Tiffany.Donaldson@umb.edu (S.T. Donaldson).

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received early insult and later life socio-environmental intervention (Roth and Sweatt, 2011). In adults with mood disorders and in adult animal models of emotional decline following early life stressors there is an associated decrease in overall BDNF functioning (for review, see Calabrese et al., 2009). Since BDNF appears to be at the center of stress adaptation at critical moments in development and into adulthood, and extreme trait or state anxiety animals appear to show uncharacteristic abilities to adapt, changes in BDNF were investigated in a periodic maternal deprivation (PMD) model using dams and their litters. We also focused on the BDNF levels in the basolateral amygdala since this region is implicated in fear memory storage (Schafe et al., 2001) and new synthesis of BDNF parallels emotional memory formation (Schafe and LeDoux, 2000). In the current study, dams were divided along high (HAn) and low anxiety (LAn) lines using the elevated plus maze, and after birth pups experienced PMD across the lactation period. We measured anxiety-like behavior in male offspring at adulthood as well as post-mortem immunoreactivity for the BDNF protein in the basolateral amygdala.

2. Methods

2.1. Animals and housing

Sixteen adult Long Evans females (240–300 g) were purchased timed-pregnant from Charles River Breeding Labs (Wilmington, MA). Prenatal housing for the animals occurred in separate Plexiglas cages with ad libitum access to food and water in a temperature-controlled environment ($21 \pm 1\,^{\circ}C$) with relative humidity ($60 \pm 10\%$). Lights were on from 0700 to 1900 h and dams delivered between gestation days 19 and 21 with an average of 16 pups per litter. Litter sizes averaged 6.1 male pups for the high anxiety dams and 6.6 male pups for the low anxiety dams. On the day of delivery, dams were monitored at a distance and litters were counted but were not adjusted for overall number, only sex (i.e., all females were removed from the litter). Post-delivery dams were housed together with their male offspring until weaning at PND 21. All procedures were approved by the University of Massachusetts Boston Institutional Animal Care and Use Committee and adhered to both state and federal guidelines for humane care of animal research subjects.

2.2. Elevated plus maze

2.2.1. Equipment

The EPM consisted of two opposing open arms (measuring 50×10 cm) and two opposing closed arms (measuring $50 \times 10 \times 40$ cm) constructed of black Plexiglas (Med Associates, St. Alban, VT). The center of the four arms was a 10×10 cm square that was elevated 70 cm above the floor. All sessions were video-recorded for 5 min using a digital recorder with stand elevated above the testing apparatus and testing occurred during the light phase (between 0900 and 1200 h).

2.2.2. Procedure

Dams were tested on the EPM apparatus after a brief handling and acclimation period for 5 days post arrival to the University of Massachusetts animal vivarium. On a testing day, animals were habituated to the testing environment for 15 min then placed in the center platform and video-recorded for 5 min. We used percent open arm (%OA) time and latency to enter OA (OA LAT) as dependent measures, doing a median split separating low %OA time and high %OA time, taking care to choose animals from the lower quartile (low %OA) for the HAn group and the upper quartile (high %OA) for LAn. Male offspring were tested on the EPM at young adulthood (PND 95) using the same procedures as described above.

2.2.3. Behavioral assessment

Raters blind to the experimental condition showing a high level of inter-rater reliability (Pearson $R = 0.99$, $p < 0.E+0$) coded 5-min video recordings of EPM trials for each subject. Raters recorded the times of full body entries into the closed arms (CA) or open arms (OA), beginning with placement into the center platform. From these, we calculated percent time on OA ($OA/(OA+CA) \times 100$) and latency (in seconds) to enter OA (OA LAT).

2.3. Periodic maternal deprivation

2.3.1. Procedure

Pups were removed from their dams on postnatal days 5, 16, and 21 with each period of maternal deprivation (PMD) lasting 30 min. Reunions of dam and offspring were videotaped for 5 min from the moment of re-introduction.

2.3.2. Periodic maternal deprivation

Maternal behavior was scored using an adapted method of assessment described previously (Caldji et al., 1998). Three raters (Cohen's $\kappa = 0.613$) coded 5-min reunion videos for each of the 16 l on postnatal days 5, 16 and 21 according to the predominant behavior observed within 10-s intervals. Thirty 10-s blocks were coded for each video. We hoped to collect data at postnatal day 10 as well but lost data due to mechanical error. While the quality of the reunion behavior changed between postnatal days 5 and 16, a diminution of reunion behaviors was further observed at postnatal day 21.

The following dam behaviors were scored: no observed behavior/self-cleaning, exploring/rearing behaviors, nesting behaviors, pup retrieval, passive/side nursing, blanket nursing, arched-back nursing (ABN)/licking and grooming (L/G), and licking and grooming (L/G). For analysis purposes, we divided these behaviors into three behavioral categories, no contact, non-pup-directed, and pup-directed behaviors. For the pup-directed behaviors, we scored the number of ABN/L/G and L/G.

2.4. Brain-derived neurotrophin factor (BDNF) immunocytochemistry

At the termination of the behavioral monitoring, 4–5 representative male offspring were overdosed with sodium pentobarbital (1 ml, 50 mg/kg) and transcardially perfused with isotonic saline (50 ml) followed by 4% paraformaldehyde (200–250 ml). Brains were extracted and cryoprotected in sucrose-formaldehyde (10% followed by 20% for 48 h each). Serial cross-sections (30 μm) were cut on a cryostat and immunocytochemistry processing was done on free-floating sections. In order to detect BDNF protein in the basolateral amygdala of representative male offspring, we ran free-floating sections using the avidin–biotin system method (Vectastain ABC System, Vector Laboratories, Inc., Burlingame, CA, USA). Sections were rinsed in Tris-buffered saline and then incubated in H_2O_2 and 10% methanol to block endogenous peroxidase activity followed by 48 h incubation at 4 °C with anti-BDNF (1:1000) diluted in 0.01 M PBS (pH 7.4), containing 0.5% Triton X-100 and normal goat serum. For the avidin–biotin system, sections were incubated with the appropriate biotinylated secondary antibodies for 1 h (1/250, Vector Labs) followed by the avidin–biotin–peroxidase complex (1:200; Vector) and developed in a solution of 0.015% 3,3-diaminobenzidine, 0.0024% H_2O_2 in 0.05 M Tris–HCl (pH 7.6). After adhesion on gelatin-coated slides, sections were dehydrated and coverslipped with permount. Digital images (Spot Camera/software) of the basolateral amygdala were captured under light microscopy. Images were later analyzed