

Desipramine treatment reduces the long-term behavioural and neurochemical sequelae of early-life maternal separation

Glenda M. MacQueen, Karuna Ramakrishnan, Ravena Ratnasingan, Biao Chen
and L. Trevor Young

Mood Disorders Program, McMaster University, Hamilton, Ontario, Canada

Abstract

Primate and rodent models of maternal separation have shown that repeated postnatal separation of young from the mother results in long-term changes to neurohormonal systems relevant to depression. To date, however, it remains unclear whether rodents that experience postnatal maternal separation display specific behavioural or biochemical features of depression in adulthood and whether these changes can be prevented by treatment with antidepressant drugs. We report here that maternally separated mice showed significantly shorter swim times on the forced swim test and significantly lower levels of brain-derived neurotrophic factor in dentate gyrus and CA3 regions of the hippocampus compared to control mice when assessed in adulthood. Neither of these differences was apparent in maternally separated mice that received chronic treatment with the antidepressant desipramine after maternal separation. These results suggest that intervention following early stress may eliminate the long-term vulnerability to behavioural and biochemical dysfunction that occurs following this early chronic stress.

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Introduction

Early trauma (Heim et al., 2000) or loss (Agid et al., 1999) predict occurrence of major depression in adulthood, but the pathophysiological mechanisms through which early stress confers vulnerability to depression in adulthood remain unknown. In primate and rodent models, repeated postnatal separation of young from the mother results in long-term changes to neurohormonal systems relevant to depression (Bremner and Vermetten, 2001; Connor et al., 1999; Levine, 2000). For example, early-life experiences programme hypothalamic–pituitary–adrenal (HPA) axis functioning, including negative feedback derived from stimulation of hippocampal glucocorticoid receptors, to modify subsequent response to stressful experiences (Anisman et al., 1998). It has been proposed that repeated stress causes excitotoxic damage to the

hippocampus (Sapolsky et al., 1990) with remodelling of key cellular elements, involving retraction of dendrites, decreased neurogenesis in the dentate gyrus and loss of glial cells (Czeh et al., 2001; Malberg et al., 2000; Magarinos et al., 1999; Rajkowska, 2000; Sousa and Almeida, 2002; Sousa et al., 2000). Both potentially reversible remodelling and irreversible cell death is likely to be caused by dysregulation of glucocorticoid secretion and elevated activity of excitatory amino-acid neurotransmitters (McEwen, 1999; Sapolsky, 2002). Dysregulation of the HPA axis is a common feature of depression in humans (Young et al., 1994) and the hippocampus is implicated in the pathophysiology of depression by a number of pre-clinical (Sapolsky, 2002), neuroimaging (Sheline, 2000) and neuropsychological studies (MacQueen et al., 2002). Thus it is plausible that the stress of early maternal separation, which leads to longstanding changes in the HPA axis, might also be associated with changes in hippocampal integrity.

A key factor that may be important in hippocampal changes in depression is the neurotrophin, brain-derived neurotrophic factor (BDNF), found in high concentrations in the hippocampus and cerebral cortex

Address for correspondence: G. M. MacQueen, Department of Psychiatry and Behavioral Neurosciences, 4N77A, McMaster University Medical Centre, 1200 Main St. W., Hamilton, ON, Canada, L8N 3Z5.

Tel.: 905 522 1155 (ext. 5496) Fax: 905 304 5376

E-mail: macqueng@mcmaster.ca

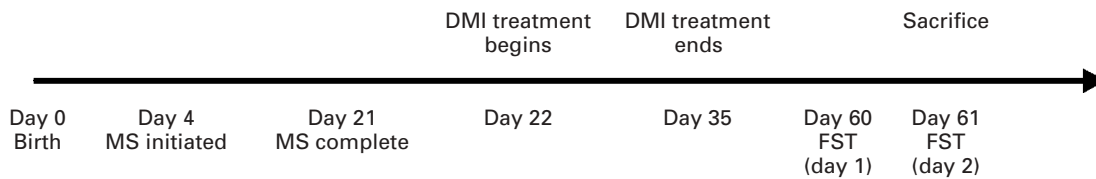


Figure 1. Timeline demonstrating temporal relations between maternal separation, desipramine treatment and testing in the forced swim test (FST).

(Hofer et al., 1990) and important in neuronal survival (Johnson et al., 1986). Stress is associated with decreased BDNF and trkB levels (Duman et al., 1997) and chronic, but not acute, treatment with antidepressants and electroconvulsive shock therapy increase levels of BDNF and trkB in animal models (Zetterstrom et al., 1998) and post-mortem brain studies from depressed individuals (Chen et al., 2001). Administration of BDNF itself has antidepressant-like effects on the forced swim test (FST) and in the learned helplessness behavioural model of depression (Siuciak et al., 1997).

To date, however, it remains unclear whether rodents that experience postnatal maternal separation display specific behavioural features of depression in adulthood and whether biochemical changes, such as changes in BDNF levels, can be observed in the hippocampus following maternal separation. This study was designed to examine these issues and to further assess whether observed behavioural or biochemical changes following maternal separation could be ameliorated by chronic treatment with the prototypical antidepressant desipramine (DMI).

Methods

Maternal separation

The study was conducted following approval by the Animal Research Ethics Board at McMaster University. Figure 1 outlines the experimental timeline. C57Bl/6J pups were born on site and maternal separation was conducted from days 4 to 22, by removing the dam from the pups for 3 h/d. Animals were weaned on postnatal day 22, and DMI (15 mg/kg, intraperitoneal injection, administered once daily between 10:00 and 12:00 hours) treatment began for half of the animals in both the maternally separated and non-separated groups while control animals received equivalent volumes of vehicle administered intraperitoneally. Following completion of the DMI treatment, animals were left group housed and undisturbed except for routine cage cleaning until the FST was begun on day 60. Mice reach early adulthood at approx. day 56, and we were interested in examining whether the

effects of the early separation had an impact on behaviour in early adulthood, the time when depression is most likely to onset in clinical samples (Wittchen et al., 1994). On day 60, four groups of mice were assessed for behavioural evidence of vulnerability to depression: those that had been separated but received chronic DMI treatment (MS+DMI+), those separated that received saline (MS+DMI-), those not separated who received either chronic DMI treatment (MS-DMI+) or saline (MS-DMI-).

FST

On postnatal days 60 and 61, animals were tested in the FST following the method described by Porsolt et al. (1977). The FST is a measure of behavioural despair that possesses high predictive validity and good face validity as a screen for depressive behaviour (Willner, 1984).

The apparatus consisted of a Plexiglas cylinder (diameter, 20 cm) immersed in 30 cm of water maintained at 23 °C. Animals were placed in the water and videotaped for 10 min on day 1 of the test and for 5 min on day 2. Videotapes were scored by two independent observers who were unaware of animals' group assignment. Swimming was defined as time spent engaged in active swimming or struggling movement.

In-situ hybridization for BDNF mRNA

Two hours following the FST, brains were removed for assessment of BDNF mRNA levels. BDNF sense (5'-GATGACCATCCTTTTCCTTACTATGGTTATTT-CAT-3') and antisense oligonucleotides were 3'-labelled with digoxigen (DIG) using a terminal transferase in a template-independent reaction (Boehringer Mannheim, 1996). Tissue sections (15 µm) were fixed with DEPC-treated PBS containing 4% paraformaldehyde and permeabilized and acetylated prior to pre-hybridization for 2 h. Sections ($n=6$ per animal) were incubated with 10–30 ng of DIG-labeled BDNF oligonucleotide overnight at 37 °C. Each slide contained one tissue sample from an animal in each of the four groups. Antibody conjugate was applied for 2 h at

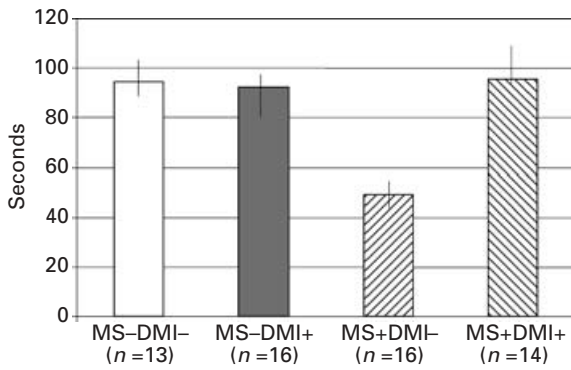


Figure 2. Behavioural assessment: performance on the forced swim test (FST). Time shown refers to seconds spent engaged in active swimming by animals on day 61, the second day of the FST. Decreased time spent in active swimming is interpreted as behavioural despair or hopelessness, an analogue of depressive behaviour.

37 °C, and the colorimetric solution was applied in the dark overnight. Localization and quantification of mRNA was assessed using the BioQuant imaging system (BioQuant TCW 98). Ten representative samples were taken from each hippocampal region (i.e. dentate gyrus, CA1 and CA3) and the average optical density was used to calculate relative optical density for statistical analyses following a method previously described by our laboratory (Chen et al., 2001) to obtain a semi-quantitative measure of BDNF mRNA in the hippocampus. Background was obtained from 10 surrounding regions and the average value subtracted from values obtained from each region.

Results

There were no differences in swimming duration on the first (training) day of the FST. MS+DMI- mice had the shortest swim times on day 2 of the FST (Figure 2), reflecting the long-term behavioural consequence of early chronic stress. The MS+DMI+ group had swim times equivalent to animals that had not experienced the early stress of maternal separation. A 2×2 ANOVA found a significant interaction between maternal separation and DMI treatment [$F(1,56)=15.09$, $p<0.001$], and post-hoc Tukey's test confirmed that MS+DMI- animals had shorter swim durations than other groups ($p<0.005$), while MS+DMI+ animals did not differ from MS-DMI- or MS-DMI+ animals. Animals that had not been maternally separated (MS-DMI+, MS-DMI-) showed no evidence of an effect of the remote DMI treatment, with an equivalent time spent engaged in active swimming.

BDNF mRNA levels paralleled the behavioural results: MS+DMI- animals had lowest levels of BDNF in the CA3 region (repeated-measures ANOVA using Helmet contrasts [$F(1,8)=6.9$, $p=0.03$] and dentate gyrus region [$F(1,8)=5.2$, $p=0.05$]), while MS+DMI+ animals had BDNF levels no different from never-separated animals (Figure 3). Again, animals that had not been maternally separated showed no effect of remote DMI treatment: the MS-DMI+ and MS-DMI- animals had equivalent levels of BDNF mRNA. The CA1 region of the hippocampus was also examined but there were no differences between any groups in this region, although previous studies have shown that levels of nuclear glucocorticoid receptor immunoreactivity in the CA1 hippocampal field may be altered by maternal separation in rats (Biagini et al., 1998). Whether differences in cell number or density partially account for the observed differences in BDNF staining was not formally examined in this sample.

Discussion

Maternal separation was associated with long-term changes in behaviour analogous to depression; whether these changes were the result of home-cage mother-pup interactions following the separation was not specifically assessed. The vulnerability apparent by the shorter swim times in the FST is evidence of lasting behavioural sequelae that appears to be a consequence of early maternal separation. Fourteen days of treatment with antidepressants following the stress of maternal separation, but still well before testing on the FST, prevented the expression of vulnerability that was apparent in animals that did not receive the antidepressant. Thus antidepressant treatment may have conferred lasting changes in neural systems that regulate such behaviour as the effects were seen several weeks after the drug was last administered. There are no published reports that have demonstrated behavioural effects of maternal separation consistent with depression later in life although it is well-known to result in a myriad of other effects. Maternal separation reliably induces long-term changes in HPA axis tone in rodents (Anisman et al., 1998) and HPA axis abnormalities are amongst the most reliably demonstrated biological changes evident in individuals with depression (Young et al., 1994). In this regard, it is not surprising that an early intervention that changes HPA axis tone might confer vulnerability to stress or behavioural manifestations consistent with depressive-like behaviour. Clinical studies have indisputably demonstrated the importance of early childhood loss and trauma on the

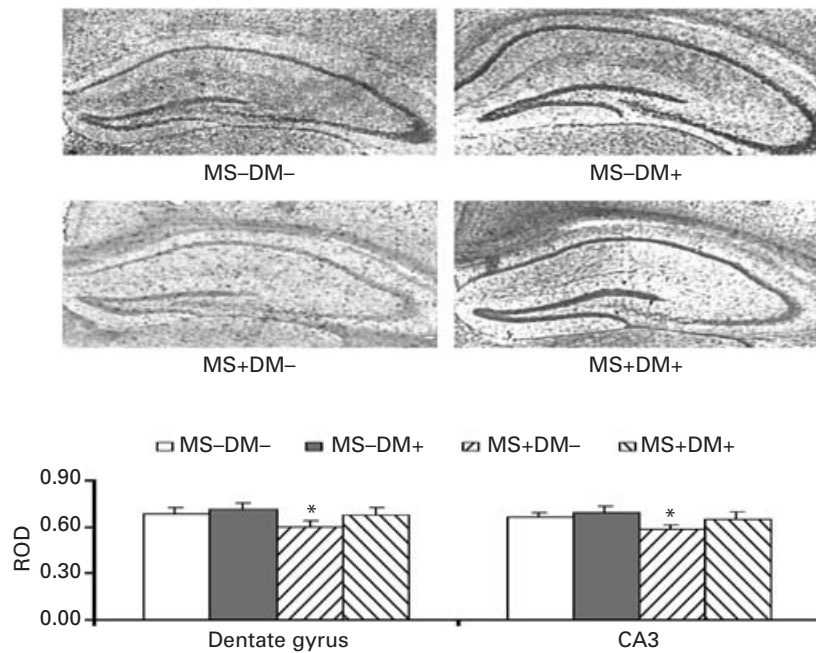


Figure 3. Digoxigenin (DIG)-labelled in-situ hybridization for BDNF mRNA in the dentate gyrus and CA3 regions of the hippocampus.

occurrence of depression in adult life (Agid et al., 1999; Heim et al., 2000). These data demonstrating early-life vulnerability to adult onset of depression in an animal model may provide a simple, valid way to examine the pathophysiological changes that occur in early life and increase vulnerability to the development of psychiatric disorders in adulthood.

In the present study, maternal separation was associated with changes in BDNF levels upon exposure to a stressful situation (the FST) in adulthood. Decreased levels of BDNF are consistent with those found after acute and chronic immobilization stress (Smith, 1996; Smith and Cizza, 1996; Smith et al., 1995) but were clearly more pronounced in animals that had remotely received maternal separation. The reduction in BDNF levels is probably not secondary to the swim associated with FST. All animals were in the tank for 10 min on day 60, and swim times for each group were equivalent on this day. Animals also spent an equal amount of time in the tank on day 61, and while some engaged in more active swimming, overall the magnitude of difference in swim time approached 2 min for the DMI-treated vs. DMI-untreated animals. We are not able to rule out the possibility that BDNF levels are exquisitely sensitive to activity in mice, but argue that it is unlikely that on a background of extensive swimming in all groups 24 h previously, this relatively small difference in swim vs. float time accounts for the differences in observed BDNF mRNA levels. Thus

DMI following the early separation appears to ameliorate the apparent vulnerability conferred by the early stress in such a way that BDNF levels in maternally separated but DMI-treated animals were equivalent to control animals, while animals that received maternal separation but no DMI retained a vulnerability to stress into adulthood. As demonstrated behaviourally, these findings suggest that early-life events result in long-term neurobiological changes in regions such as the hippocampus which are similar to those found in other animal models of depression.

Many neurohormonal changes have been shown in the brain of maternally separated rodents, including altered HPA axis tone and increased CRH levels (Coplan et al., 1996; Francis et al., 1999), altered neurotransmitter systems including noradrenaline (Francis et al., 1999; Harvey et al., 1994), dopamine (Matthews et al., 2001) and serotonin (Braun et al., 2000; Matthews et al., 2001) among others (Grove et al., 2001). Decreased hippocampal BDNF levels apparent weeks after maternal separation extends our understanding of the lasting vulnerability which occurs as the result of traumatic early-life events. DMI treatment was associated with hippocampal BDNF levels which were not different from controls. Fluoxetine treatment following 14 d of maternal separation increased neurogenesis and decreased apoptosis in these same hippocampal regions measured immediately following antidepressant treatment (Lee et al., 2001). These

results are consistent with our findings on BDNF mRNA levels; however, our results also suggest that the neuroprotective effects of antidepressant treatment may persist after the drug is withdrawn. It is not yet known whether these antidepressant effects only occur when administered early in life or whether such potentially beneficial effects may continue after treatment and withdrawal in adults.

In summary, treatment with an antidepressant following chronic stress in early life reduced the long-term behavioural and biochemical consequences of the stress in a murine model of depression. These results suggest that intervention following early stress may eliminate the long-term vulnerability to behavioural and biochemical dysfunction that occurs following this early chronic stress. Future studies on the pathophysiology of vulnerability to depression based on early-life stress and trauma may benefit from similar rodent models.

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