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Chronic mild stress inhibits BDNF protein expression and CREB activation in the dentate gyrus but not in the hippocampus proper

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Abstract

Chronic stress is linked to development of depression and may trigger neurobiological changes underlying the disease. Downregulation of the secretory peptide brain-derived neurotrophic factor (BDNF) and the transcriptional regulator calcium/cyclic-AMP responsive binding protein (CREB) have been implicated in stress and depression-related pathology in animal studies. When animals are exposed to the chronic mild stress (CMS) protocol, multiple depression-like symptoms are observed. Here we investigated the effect of CMS on BDNF protein expression and CREB activation in the dentate gyrus and hippocampus proper. Rats exposed for 5 weeks to repeated, unpredictable, mild stressors showed reduced BDNF expression and inhibited phosphorylation of CREB (Ser-133) in the dentate gyrus ($-25.0\% \pm 3.5\%$ and $-29.7 \pm 7.3\%$, respectively), whereas no significant effects were observed in the hippocampus proper. CMS-treated rats consumed less sucrose compared to control rats, indicating a state of anhedonia. Moreover, phospho-CREB levels in the dentate gyrus were positively correlated with the animals' sucrose intake at the end of the CMS protocol. These results couple chronic mild stress to a downregulation of CREB activity and BDNF protein expression specifically within the dentate gyrus and support the possibility that the BDNF-CREB system plays an important role in the response to environmental challenges.

Keywords: Neurotrophins; Sucrose intake; Anhedonia; CMS; Animal model of depression

1. Introduction

The chronic mild stress (CMS) model has been shown to induce lower consumption of sucrose postulated to reflect anhedonia in animals (diminished capacity to experience pleasure), one of the core symptoms of depression (Willner et al., 1987). The link between taste for sweet solutions and the hedonic state is based on evidence that positive and negative visceral information impact higher level cognitive and behavioural processes (Berntson et al., 2003). 'Taste' is a typical example of brain stem-mediated hedonic evaluation (Badia-Elder et al., 1996; Yamamoto and Sawa, 2000).

The CMS protocol includes mild and uncontrollable daily stressors (e.g. tilted cage, food or water deprivation, paired caging, continuous light, wet bedding). The protocol is regarded as being close to model the human situation, consisting more of daily hassles than traumatic events (see review in (Willner, 2005)). After 4–6 weeks of CMS the animals show a wide variety of symptoms that parallel some features of human

Abbreviations: AMP, adenylmonophosphate; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; BSA, bovine serum/albumin; CA, cornus ammonis; CMS, chronic mild stress; CREB, calcium/cyclic-AMP responsive binding protein; EEG, electroencephalogram; EMG, electromyogram; SPD, Sprague-Dawley; TBST, Tris-buffered saline/0.1% Tween-20; TrkB, tyrosine kinaseB; VTA, ventral tegmental area; 5-HT, serotonin; 5-hydroxytryptophan.

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depression, contributing to the face validity of the model (Willner et al., 1987; Willner, 1997). In our experience, exposure of naive rats to CMS induces lower sucrose intake (Gronli et al., 2004, 2005) consistent with anhedonia (Willner et al., 1987), selective changes in sleep (Gronli et al., 2004) consistent with those classically observed in human depression (Benca, 1996), increased locomotor behavior (Gronli et al., 2005) suggested to reflect psychomotor agitation in humans (Ho et al., 2000), and reduced sexual activity (Gronli et al., 2005) also consistent with human depression (Shabsigh et al., 2001). The reliability and the reproducibility of the model have been questioned especially in relation to the inconsistent occurrence of the anhedonic effect as measured by consumption of a sucrose solution (Matthews et al., 1995; Nielsen et al., 2000). Therefore, to confirm the effectiveness of the CMS protocol and in line with our previous reports, the current study also includes the animals' sucrose consumption.

The monoamine hypothesis of depression suggests a deficiency of serotonin (5-hydroxytryptophan, 5-HT) or noradrenaline in the brain (Schildkraut, 1965; Wong and Licinio, 2004). An emerging hypothesis proposes that problems in information processing within specific neural networks in addition to changes in chemical balance may play a critical role in the pathophysiology of depression. Regulation of intracellular messenger cascades mediate the ability of neuronal systems to adapt in response to pharmacological and environmental stimuli and the effect of antidepressants has been suggested to contribute to regaining the plasticity within intracellular signal transduction pathways (Duman et al., 1994). The neurotrophin brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and the most widespread growth factor in the brain. BDNF has diverse functions in the adult brain as a regulator of neuronal survival, fast synaptic transmission, and activity-dependent synaptic plasticity (Lewin and Barde, 1996; Blum and Konnerth, 2005; Bramham and Messaoudi, 2005). A dysregulation of BDNF has been suggested in the pathophysiology of depression (Duman et al., 1997; Altar, 1999; Hashimoto et al., 2004). Levels of serum BDNF are decreased and negatively correlated with the Montgomery-Asberg-Depression Rating Scale in unmedicated major depressive patients (Karege et al., 2002) and are associated with vulnerability to develop mood disorders in healthy subjects (Lang et al., 2004). In rats, downregulation of BDNF mRNA (Smith et al., 1995; Russo-Neustadt et al., 2001; Rasmusson et al., 2002) and BDNF protein (Franklin and Perrot-Sinal, 2006) is found in several brain regions following stress paradigms. Increases in BDNF synthesis and signaling have been implicated in the effect of chronic antidepressant drug treatment (Nibuya et al., 1995; Altar, 1999; Russo-Neustadt et al., 1999; Duman, 2002; Saarelainen et al., 2003; Castren, 2005).

The transcription of the BDNF gene is regulated by the transcriptional regulator calcium/cyclic-AMP responsive-element binding protein (CREB) (Tao et al., 1998; Conti et al., 2002) and BDNF signalling through its receptor tyrosine kinase, TrkB, is capable of inducing CREB phosphorylation (Finkbeiner et al., 1997; Ying et al., 2002). Phosphorylation of CREB at its transcriptional regulatory residue Serine-133 is necessary to activate transcription of genes containing a cAMP response element (Montminy et al., 1990). Like BDNF, activation of CREB is downregulated following stress and upregulated in response to antidepressant treatment (Nibuya et al., 1996; Alfonso et al., 2006).

The hippocampus plays an important role in regulation of stress responses and it expresses high levels of BDNF protein and mRNA in the normal adult rat (Conner et al., 1997). Stress in rats is associated with reduction of hippocampal BDNF levels (Russo-Neustadt et al., 2001; Shirayama et al., 2002). However, there may be a difference between the dentate gyrus and the cornus ammonis (CA) regions of the hippocampus in the stress-induced effect on the BDNF. Immobilization stress in rats is associated with greater impairments in BDNF mRNA expression in the dentate gyrus compared to the CA region (Smith et al., 1995). Furthermore, induced overexpression of CREB in the dentate gyrus, but not in the CA1 or CA3 regions, is associated with antidepressant-like behavioral effects (Chen et al., 2001a,b).

The present study was designed to evaluate a) if CMS affects the hippocampal BDNF-CREB system; b) if a specific difference exists between the dentate gyrus and the CA region; c); if possible changes are correlated to anhedonia-like effects as measured by sucrose intake.

2. Materials and methods

2.1. Ethical evaluation

The experiment has been approved by the Norwegian Animal Research Authority and registered by the Authority. The experiment has thus been conducted in accordance with the laws and regulations controlling experiments in live animals in Norway, i.e. The Animal Protection Act of December 20th 1974, No 73, Chapter VI sections 20–22 and the Animal Protection Ordinance concerning Biological Experiments in Animals of January 15th 1996. Norway has signed and ratified The European Convention for the protection of Vertebrate Animals used for Experimental and other Scientific purposes of March 18, 1986.

2.2. Animal handling

Male Sprague-Dawley (Mol:SPD) rats (Møllegaard, Copenhagen, Denmark) were used in this experiment. To minimize stress, the animals were allowed to remain in the transport cage for five days before they were separated and housed individually in conventional Macrolon type III cages. The home cages were placed in a rack allowing visual, olfactory and auditory contact between animals.

The rats were 11 weeks old prior to the start of the CMS protocol. The rats had free access to food (Rodent low protein diet, B & K Universal AS, Norway) and water, except when the CMS procedure required deprivation. The ambient temperature was 22±1 °C with 52±2% humidity. Rats were kept on a reversed 12 h light/12 h dark schedule with gradually increasing lighting from 1800 h and lights fully on at 1900 h. The rats changed to the reversed L/D schedule 10 days before the start of

the experiment. Five to seven days are reported to be a sufficient time for establishing a new circadian rhythm in male SPD rats (Hillegaart and Ahlenius, 1994).

The same rats were also used for a separate study for which they were implanted with electroencephalogram (EEG)/electromyogram (EMG) electrodes for sleep recording and with a microdialysis cannula for sampling extracellular fluid. The cannula was implanted randomly in the left or right hippocampus, contralateral to the site used for BDNF and CREB analysis. At least 2 weeks were allowed for recovery and adaptation prior to start of the experiment. Sleep was recorded before and after the CMS protocol. Dialysates were acquired after CMS. The data relative to these procedures is considered in a separate paper (Gronli et al., submitted for publication).

2.3. Experimental procedure

The animals were divided into two groups. One group was exposed to chronic mild stress (CMS rats, $n=12$) for 5 weeks and the other group (control rats, $n=8$) was given ordinary daily care with daily supports of food and water and weekly change of bedding. The two groups of rats were housed separately in different rooms during the duration of the stress procedure.

Most of the CMS stressors were adapted from the procedure described by Willner and collaborators (Willner et al., 1987) and some stressors were included from Moreau and collaborators (e.g. empty bottle of water, restricted food) (Moreau et al., 1992). Each week included 2 h of paired caging, 3 h of tilted cage (45°), 18 h of food deprivation immediately followed by 1 h of restricted access to food (5 micropellets), 2 × 18 h of water deprivation immediately followed by 1 h exposure to an empty bottle, 21 h with wet cage (200 ml water in 100 g sawdust bedding), and 36 h of continuous light. Stressors were never presented simultaneously. Animals were exposed to stressors during the rats' active (dark) period and during the inactive (light) period. See (Gronli et al., 2005) for details of time and duration of the CMS protocol.

Intake or preference for sweet solutions have been the two measures of anhedonia most commonly used and accepted in the CMS literature (e.g. (Willner et al., 1987; Pucilowski et al., 1993; Willner, 1997; Benelli et al., 1999; Haidkind et al., 2003; Baker et al., 2006; Jayatissa et al., 2006). In the present study we used the one-bottle sucrose intake test (only the sucrose solution was available) to be consistent with previous CMS experiments carried out in our laboratory (Gronli et al., 2004, 2005). Before each test the animals were deprived of food and water for 4 h (10 a.m.–2 p.m., dark phase in the reversed L/D schedule). In no case were rats deprived of food or water prior to this 4 h period of deprivation. The rats were presented with a 1% sucrose solution during a one-hour window (2 p.m.–3 p.m., dark phase) and consumption was measured by comparing the bottle weight before and after the test window, as originally described by (Willner et al., 1987). As a pre-test condition, the rats were exposed twice (5 days and 1 day prior start of the CMS procedure) to the diluted sucrose solution, and the two groups showed a similar intake of sucrose (see Fig. 1). During the CMS protocol the sucrose intake and bodyweight were measured once a week, except for the last measurement that took place after 10 days instead of 7 days (see Fig. 1). To exclude possible confounding effect of the rats' consumption of sucrose associated to their bodyweight, we divided the animals' sucrose intake (in ml) by their body weight (in kg). The food and water deprivation period preceding sucrose intake measurement may be considered as a further stress applied on top of the CMS protocol. However, control rats were also exposed to the same procedure as a part of the sucrose test.

2.4. Tissue microdissection and sample preparation

Two days after the end of the CMS, the animals were anaesthetised with Isofluran gas (Isofluran Baxter, Norway) and decapitated. The brain was rapidly separated from the skull and divided into the two hemispheres. One hemisphere was rinsed with oxygenated ice-cold artificial cerebral spinal fluid and the dentate gyrus and CA region were rapidly dissected on an ice-cold glass dish, aliquoted into Eppendorf tubes, and stored at −80 °C until analysis. The other hemisphere included the site of the microdialysis probe. The side (left or right) for protein analysis or probe localisation was randomly assigned.

2.5. Antibodies

Primary antibodies used for immunoblotting were as follows BDNF (Sc-546 rabbit polyclonal IgG, 1:2000; Santa Cruz), total CREB rabbit polyclonal (1:2000, Upstate), Ser133 phospho-CREB rabbit polyclonal (1:2000, Santa Cruz), and β-actin (mouse monoclonal,1:5000, Sigma). The secondary antibody used was goat anti-rabbit IgG (1:5000, Calbiochem).

2.6. SDS-PAGE and Western blotting

Tissues were hand-homogenized with 15 strokes in 300 µl of Dynal lysis/binding buffer (Dynal Biotech ASA, Norway).

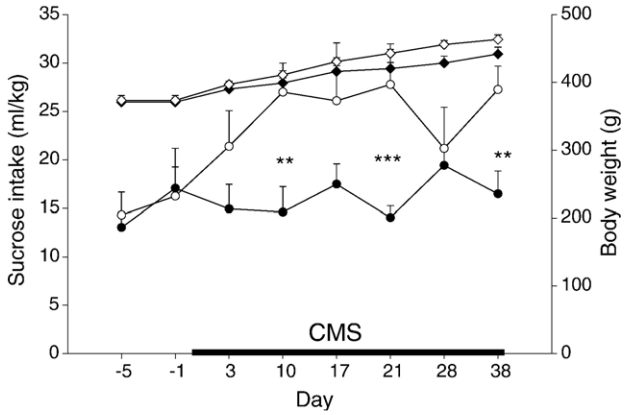

Fig. 1. Measure of sucrose intake (ml/kg) and bodyweight (g) before and during the CMS protocol. Circles indicate sucrose intake and diamonds indicate body weight. Open symbols indicate control rats and filled symbols rats exposed to CMS. Results are presented as mean±S.E.M. $^{**}p<0.01$, $^{***}p<0.001$ compared to control group.

Protein levels in homogenate samples were determined using the Lowry method. Equal amounts of protein (40 μg) were loaded onto sodium dodecyl sulfate polyacrylamide gel electrophoresis. Phosphorylated CREB and total CREB on 10% gels and BDNF on 12% gel were run overnight at constant 10 mA. Separated proteins were transferred onto a nitrocellulose membrane (Hybond-C Amersham) at constant voltage of 30 V overnight or 100 V for 1 h. Membranes were blocked on a gyro-rocker for 1 h at room temperature. Blocking buffer consisted of TBST (Tris-buffered saline/0.1% Tween-20) and 5% albumin from bovine serum (BSA). The primary antibodies were dissolved in blocking buffer containing 3% BSA and the blots for BDNF and total CREB were incubated for 2 h at room temperature and p-CREB was incubated at 4 °C overnight with constant shaking. Following three washes with TBST, blots were incubated for 1 h in horseradish peroxidase-conjugated secondary antibody dissolved in TBST. The blots were washed three times with TBST and proteins were visualized using enhanced chemiluminescence (Western Blotting Analysis System, Amersham pharmacia biotech, Norway). The membranes probed with BDNF and p-CREB antibody were also probed with β-actin antibody to check for equal loading. The molecular weight of the band that was analyzed for BDNF was 14 kDa which corresponds to the mature form of BDNF protein in the rat hippocampus. Autoradiographs were scanned on a densitometer and quantitated using Phoretics ID plus software.

2.7. Statistics

Some animals were excluded from statistical analysis because of technical complications related to the EEG/microdialysis implantation (3 animals before starting the CMS procedure and 3 more at the end of the CMS protocol).

Sucrose and body weight: Analysis of Variance (ANOVA) for repeated measures was performed on sucrose intake and bodyweight with group as independent factor and time as repeated measure in 17 animals (CMS, $n=11$ and control, $n=6$). Subsequently, the effect of CMS or control treatment on sucrose intake over days was further analysed with one-way repeated measure ANOVA. Differences between groups were assessed by multiple comparisons performed by least significant deviation post hoc test. Comparison between the expressions of BDNF, p-CREB, total CREB and the β-actin in the CMS group and in the control group were made using two sample Student's *t*-test assuming equal variance. The number of animals for protein analysis were 14 (CMS, $n=9$ and control, $n=5$). Correlation analysis between sucrose intake (obtained on Day 38) and BDNF, p-CREB or total CREB was performed on pooled data, from both control and CMS-exposed rats ($n=14$) using Pearson's correlation analysis. Statistica 5.0 (StatSoft, Inc.) was used for all statistical analyses. Significance was accepted at $p<0.05$, two-tailed. All values are presented as mean±S.E.M.

3. Results

3.1. Rats exposed to CMS consume less sucrose than controls

The rats exposed to CMS drank less sucrose solution than the controls during the CMS period (Day 5 to Day 38; $F_{(1,14)}=8.05$, $p=0.01$). The control rats increased their consumption ($F_{(5,25)}=2.65$, $p=0.047$), while the rats exposed to CMS maintained the same level throughout the study ($F_{(5,45)}=1.01$, $p=0.42$). The pattern of an increased consumption in controls (sucrose intake greater than baseline intake) was not observed in any rat exposed to CMS.

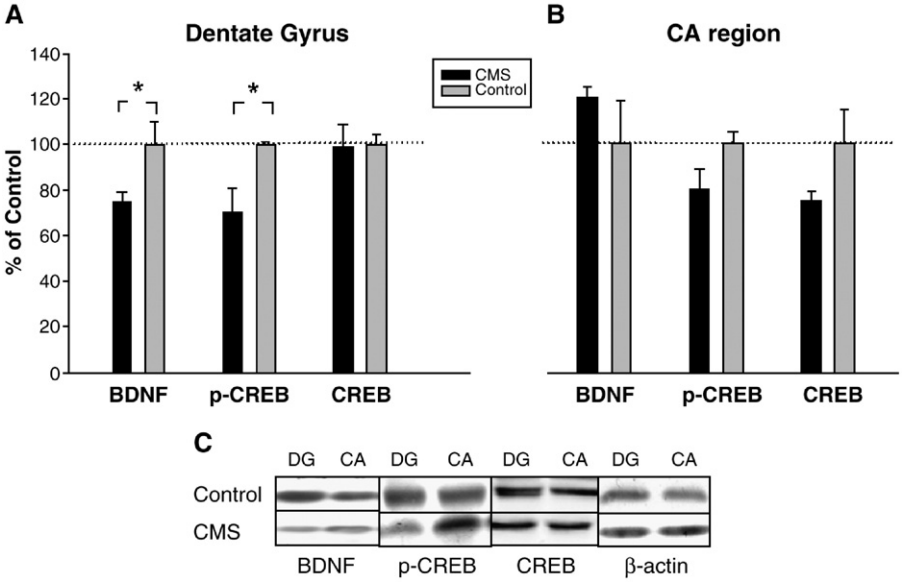

Fig. 2. The expression of BDNF, p-CREB and CREB in the dentate gyrus (A) and the CA region (B) after CMS and control condition. Data are shown as per cent of control group mean±S.E.M. *indicate $p<0.05$ compared to the control group. C shows a representative band intensity of Western Blot for one animal.

No change in body weight was observed ($F_{(1,15)}=1.45$, $p=0.25$) suggesting that rats exposed to CMS gained weight at the same rate as their controls during the CMS period. Sucrose consumption and body weight in the control and CMS groups are shown in Fig. 1.

3.2. Selective reduction of BDNF protein and p-CREB in the dentate gyrus after CMS

Rats subjected to CMS exhibited a specific inhibition of BDNF protein and p-CREB levels in the dentate gyrus (Fig. 2). BDNF protein levels were downregulated 25.0%±3.5% ($p=0.014$) and p-CREB was inhibited 29.7%±7.3% ($p=0.013$), relative to control rats. No difference was present in the CA region ($p=0.23$ and $p=0.11$, respectively). The expression of total CREB did not differ between CMS and control groups, neither in the dentate gyrus nor in the CA region ($p=0.88$ and $p=0.60$, respectively). The protein β-actin, used as a loading control for this study, showed no difference between the two groups of animals in dentate gyrus or CA regions ($p=0.98$ and $p=0.97$, respectively).

3.3. Expression of p-CREB in the dentate gyrus correlates with sucrose intake

We used a correlation analysis to investigate the relationship between the animals' expression of BDNF protein, p-CREB and total CREB in the dentate gyrus and the CA region and their sucrose intake at the end of the experiment (Day 38). A positive correlation was found in the expression of p-CREB in dentate gyrus ($r=0.64$; $p=0.013$). No correlation was found for p-CREB in the hippocampus proper ($r=0.09$; $p=0.75$). The expression of BDNF protein or total CREB did not correlate with sucrose intake in the dentate gyrus ($r=0.54$; $p=0.14$, $r=-0.17$; $p=0.67$, respectively) or hippocampus proper ($r=0.13$; $p=0.76$, $r=-0.27$; $p=0.47$, respectively). Thus, the rats that consumed less sucrose solution were those having lowest expression of p-CREB specifically in the dentate gyrus, see Fig. 3.

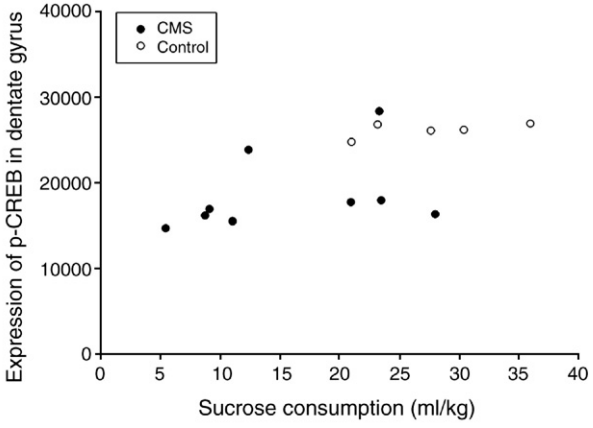

Fig. 3. Correlation between sucrose intake (ml/kg) and expression of p-CREB in dentate gyrus ($r=0.64$; $p=0.013$; $n=14$). Open symbols indicate control rats and filled symbols rats exposed to CMS.

4. Discussion

In the present study we investigated the effects of chronic mild stress on the hippocampal BDNF-CREB system. CMS resulted in a significant decrease in the expression of BDNF protein and CREB activation in the dentate gyrus but not in the CA region. The expression of total CREB and the control protein β-actin were unaffected by CMS. During the CMS protocol, rats consumed less sucrose solution compared to a control group. This change correlated with p-CREB down-regulation in the dentate gyrus.

The chronic mild stress paradigm (originally described by (Willner et al., 1987)) is a validated model of depression obtained by using mild stressors. The protocol is regarded as being close to model the human situation, consisting more of daily hassles than traumatic events (see review in (Willner, 2005)). In the CMS model, both consumption of and preference for palatable solutions as well as decreased intracranial self-stimulation behavior have served as markers of generalized decrease in sensitivity to reward (anhedonia) (Willner et al., 1987; Papp et al., 1991; Moreau et al., 1992; Muscat and Willner, 1992; Pucilowski et al., 1993; Willner, 1997; Benelli et al., 1999; Haidkind et al., 2003; Gronli et al., 2004, 2005; Baker et al., 2006; Jayatissa et al., 2006), although, contrasting results have also been reported (Matthews et al., 1995; Forbes et al., 1996; Nielsen et al., 2000). The present data confirm that rats exposed to CMS consume less sucrose compared to a control group, consistent with our earlier findings (Gronli et al., 2004, 2005), and their consumption is lower throughout the protocol. We interpret the results as an indication of CMS-induced anhedonia (a core symptom of depression). Earlier observations of other depression-like effects occurring after CMS (e.g. alterations in sleep and sexual activity) (Gronli et al., 2004, 2005) support this view.

A stress-induced downregulation of BDNF production and signalling in the neocortex and limbic structures (including the hippocampus and dentate gyrus) are likely implicated in the pathophysiology of depression (Duman et al., 1997; Altar, 1999; Hashimoto et al., 2004). This possibility is supported by human data showing a correlation between depression/depressive personality with a decreased level of serum BDNF (Karege et al., 2002; Lang et al., 2004). The present study also argues in favor of this hypothesis. Exposure to chronic mild stress decreases the expression of BDNF protein and the activated form of the transcription factor CREB in the rat dentate gyrus. Kuroda and McEwen (1998) showed no difference in dentate gyrus BDNF mRNA levels following chronic immobilisation stress. The discrepancy with our results may be due to the fact that BDNF undergoes post-translational modifications and thus, the mRNA levels do not directly correlate to the protein level (Lesch and Manji, 1992; Jacobsen and Mork, 2004). Accordingly, mRNA and protein are not expected to necessarily change in the same way in response to stress. A recent study provides insight into the molecular mechanism controlling BDNF transcription during a form of social chronic stress in mice (Tsankova et al., 2006). Tsankova and collaborators' results strongly implicate BDNF

synthesis in the dentate gyrus in stress-induced depression and antidepressant action.

In the present study we found that p-CREB in dentate gyrus was positively correlated with the animals' sucrose intake. The issue of whether hippocampal and dentate gyrus neurons are sensitive to reward behaviour is controversial. However, a connection between the expression of reward and dentate gyrus function has been reported (Collier and Routtenberg, 1984; Tabuchi et al., 2003; Jayatissa et al., 2006) and a concept of a hippocampal–ventral tegmental area (VTA) loop has recently been developed (Lisman and Grace, 2005). It has been shown that firing of hippocampal neurons varies in response to reward. In fact, neurons in ventral hippocampus show high incidence of irregular bursts as a consequence of liquid intake reward (Tabuchi et al., 2003). Consistent with our results showing that rats consuming less sucrose have the lowest expression of p-CREB in dentate gyrus, Jayatissa and collaborators reported a correlation between the recovery from anhedonia, measured by sucrose intake, and an increase in the dentate gyrus' cytogenesis after antidepressant treatment (Jayatissa et al., 2006). Taken together, these findings may indicate that an anhedonia-like effect is linked to altered synaptic function and cellular survival in the dentate gyrus.

The dentate gyrus seems to be an area more vulnerable to prolonged mild stress exposure. The existence of specific stress-sensitive brain circuits has been previously suggested (Herman and Cullinan, 1997). However, the computational function of the dentate gyrus remains enigmatic. Recent work suggests that the dentate gyrus performs fine spatiotemporal separation of novel and complex cues (Kesner et al., 2004; Lee et al., 2005). In so doing, the dentate gyrus may disambiguate stimuli to allow sparse encoding of information. Among the unique features of the dentate gyrus is the robust neurogenesis that continues into adulthood (Altman and Das, 1965; Altman and Bayer, 1990) and exposure to CMS appears to have an important controlling influence on neurogenesis. It has been shown that mice subjected to CMS exhibited a 53% reduction of granule cell neurogenesis 30 days after the end of a 7-week stress period (Alonso et al., 2004). BDNF signalling favours the survival of newborn granule cells (Kirschenbaum and Goldman, 1995; Sairanen et al., 2005) and is a major regulator of activity-dependent synaptic plasticity represented by long-term potentiation (LTP) in the dentate gyrus (Bramham and Messaoudi, 2005). A downregulation of dentate gyrus BDNF protein expression during CMS, as in our findings, may therefore be expected to impair the ability of the dentate gyrus to process and store information through mechanisms of neurogenesis, synapse maturation and synaptic plasticity (Golarai and Sutula, 1996; Becker, 2005; Kuipers and Bramham, 2006). Failure in dentate gyrus function are compatible with altered emotionality (Jaako-Movits and Zharkovsky, 2005), cognitive impairment (Witgen et al., 2005), social isolation and impaired memory (Bartesaghi, 2004), all common symptoms of depression. However, BDNF protein was not significantly correlated with the animals' sucrose intake. In depressed unmedicated patients, levels of serum BDNF are decreased and negatively correlated with the Montgomery-Asberg-Depression Rating Scale score for depression (Karege et al., 2002). In healthy unrelated volunteers, a low level of serum BDNF is correlated to vulnerability to develop mood disorders (Lang et al., 2004).

The present study shows that rats exposed to CMS lack any effect in the hippocampal CA region. There has been reported a more marked down-regulation of BDNF in the subregion CA3 compared to CA1 after acute restraint stress (mRNA: Smith et al., 1995; protein: Franklin and Perrot-Sinal, 2006), a difference which was no longer significant after chronic stress exposure (Smith et al., 1995). We cannot rule out any subregional CMS-effect of the CA region since the CA1 and CA3 regions in the present study were not distinguished. A dysregulation of BDNF has been found in several brain regions after exposure to stressors more traumatic than the ones employed in the CMS model (e.g. footshocks, cold swim, immobilization stress) (Smith et al., 1995; Russo-Neustadt et al., 2001; Rasmusson et al., 2002; Franklin and Perrot-Sinal, 2006). However, there are a few studies that do not support a decreased expression of brain BDNF (mRNA: Kuroda and McEwen, 1998; protein: Angelucci et al., 2000). Angelucci et al. (2000) reported higher concentrations of BDNF protein in frontal cortex and occipital cortex of Flinders Sensitive-Line rats compared to the controls, a finding, however, never replicated (Angelucci et al., 2003). The dysregulation of BDNF observed in animal models of depression is consistent with the evidence suggesting that BDNF may play a role in the mechanisms underlying antidepressant action. In patients treated with antidepressant medication at the time of death, BDNF protein expression was increased in hippocampus compared to untreated patients (Chen et al., 2001a,b). In rats, chronic (but not acute) treatment with antidepressants and electroconvulsive shock treatment increases BDNF mRNA in the hippocampus (Nibuya et al., 1995; Russo-Neustadt et al., 2000). Moreover, infusion of BDNF into the midbrain (Siuciak et al., 1997) and bilaterally into the dentate gyrus (Shirayama et al., 2002) produces an antidepressant-like effect in learned helplessness rats and in the forced swim test.

The present study shows a marked reduction of p-CREB in dentate gyrus in CMS compared to control rats. Activation (phosphorylation) of the transcription factor CREB is strongly implicated in synaptic plasticity and regulates the transcription of specific target genes encoding proteins including BDNF (Tao et al., 1998; Grewal et al., 1999). Thus, by down-regulating p-CREB expression, CMS may ultimately influence hippocampal plasticity. CREB expression and function are upregulated by chronic antidepressant treatment in rodents and humans (Nibuya et al., 1996; Dowlatshahi et al., 1998; Thome et al., 2000). Using CREB deficient mice, Conti et al. (2002) showed that CREB acts upstream of BDNF in response to antidepressant treatment. Induced over-expression of CREB in the dentate gyrus and not in the CA1 or CA3 regions is associated with an antidepressant effect (Chen et al., 2001a,b). Changes in neural plasticity mediate the ability to produce an appropriate adaptive responses to environmental stressors disorders and have been suggested to contribute to the pathophysiology of psychiatric disorders (Duman et al.,

1994). Our results indicate that p-CREB may be a sensitive measure of synaptic plasticity alterations.

In conclusion, this study demonstrates a dysfunction of the BDNF-CREB system specifically in the dentate gyrus following chronic mild stress. The significant correlation between activated CREB and sucrose intake may indicate that an anhedonia-like effect is linked to altered CREB-related synaptic function. Hence, CREB may be a more sensitive measure of depression-related synaptic alterations. CMS appears to be a useful model to investigate the function of the dentate gyrus in stress-related depression.

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