EJ106199900073

Corticotropin-Releasing Factor Enhances Brain-Derived Neurotrophic Factor Gene Expression to Facilitate Memory Retention in Rats

Yun L. Ma, Kai Y. Chen, Chia L. Wei and Eminy H.Y. Lee

Institute of Biomedical Sciences
Academia Sinica
Taipei 115, Taiwan, ROC

Abstract

In the present study, we investigated the effects of corticotropin-releasing factor (CRF) injected into the dentate gyrus (DG) of the hippocampus on brain-derived neurotrophic factor (BDNF) mRNA expression and studied whether N-methyl-D-aspartate (NMDA) receptor mediates the effects of CRF on BDNF mRNA expression in the DG. Since both CRF and BDNF gene expressions are involved in memory processing in rats, we further investigated whether CRF facilitates memory retention through enhanced BDNF mRNA expression in the hippocampus. Effect of direct BDNF injection to the DG on retention performance in rats was also assessed. Results indicated that intra-DG CRF injection produced a dose-dependent (0.1 μg, 1.0 μg and 10 μg) increase in BDNF mRNA level, while intra-DG MK801 injection produced a dose-dependent (0.08 μg, 0.2 μg and 2.0 μg) decrease in BDNF mRNA expression in the DG. MK801, at a dose having no significant effect alone (0.08 μg), significantly antagonized the effect of CRF on BDNF mRNA expression. On the other hand, CRF (1.0 μg) consistently and markedly improved retention performance in rats in an inhibitory avoidance learning task. BDNF antisense oligonucleotide treatment, at a concentration which did not affect retention performance alone (0.5 mM), blocked the memory-enhancing effect of CRF. However, direct and chronic BDNF injection to the DG did not improve memory performance in rats. These results together suggest that at least one of the mechanisms responsible for the memory-facilitating effect of CRF is mediated through enhanced BDNF mRNA expression in the hippocampus. The lack of an effect of intra-DG BDNF injection on memory retention is also discussed.

Key Words: **corticotropin-releasing factor, brain-derived neurotrophic factor, MK801, hippocampus, gene expression, inhibitory avoidance learning, quantitative reverse-transcription polymerase chain reaction**

Introduction

Corticotropin-releasing factor (CRF) was found to produce various behavioral activations independent of its neuroendocrine function. For example, intraventricular (icv) injection of CRF increases locomotor activity (10), potentiates acoustic startle response (39), inhibits sexual behavior (50) and decreases food intake (31) in rats. More related to the present study, the same icv injection of CRF was found to improve acquisition/retention of a visual discrimination task in rats (28). We have previously also found that direct injections of CRF into the hippocampus (33), amygdala (38) and locus coeruleus (7) dose-dependently enhance memory retention in rats. Further, CRF mRNA level was increased in animals showing good retention performance (35) and CRF antisense oligonucleotide injection blocks the memory-facilitating effect of CRF (53). In line with these evidence, CRF was also found to produce a long-lasting enhancement of synaptic efficacy in hippocampal neurons (52). Evidence favoring the facilitatory role of CRF in

Corresponding author: Dr. Eminy H.Y. Lee, Ph.D. Institute of Biomedical Sciences, Academia Sinica, Taipei 115, Taiwan, ROC. Tel: 886-2-27899125, Fax: 886-2-27822835, E-mail: eminy@ibms.sinica.edu.tw
Recieved April 26, 1999; Revised May 15, 1999; Accepted May 18, 1999.

learning and memory also comes from other reports. For example, Tershner and Helmstetter have found that CRF injected into the periaqueductal gray facilitates retention performance in rats (51). Kumar and Karanth reported that CRF improves aversive memory in a conditioning learning task (32). Further, CRF-binding protein inhibitor, which indirectly elevates the level of free CRF in the cell, was shown to improve the acquisition of a visual discrimination task as well as the retention of an inhibitory avoidance task in rats (15). These results together suggest that CRF plays an important role in modulating the learning and memory processes in rats.

Brain-derived neurotrophic factor (BDNF) is a member of the nerve growth factor family. Other than its function in supporting the survival of dopamine neurons (20), acetylcholine neurons (2) and other types of neurons (16, 24, 49), accumulative evidence has suggested that BDNF also plays an important role in synaptic plasticity (42). For example, Kang and Schuman found that BDNF potentiates glutamatergic transmission and increases synaptic efficacy in rat hippocampal slice (25), while glutamatergic N-methyl-D-aspartate (NMDA) receptor blockade downregulates BDNF gene expression in the hippocampus (5, 14). On the other hand, induction of long-term potentiation (LTP) increases BDNF mRNA level in the dentate gyrus (DG) and CA layers of the hippocampus (4, 6, 46). Associated with these findings, we have recently found that the hippocampal BDNF mRNA level is higher in rats showing good retention performance and that BDNF antisense oligonucleotide treatment impairs memory retention and LTP in rats (43). Hippocampal BDNF mRNA level was also elevated upon spatial learning in rats (26). Moreover, in a mouse strain with selective reduction of BDNF in the cerebellum, impaired eyeblink conditioning was observed (48). Spatial learning ability in Morris water maze was also significantly impaired in BDNF mutant mice (40). These results together suggest that, other than the involvement in synaptic plasticity, BDNF is also involved in learning and memory functions.

Because the hippocampus contains both CRF (45) and CRF receptors (9) as well as high BDNF mRNA level, particularly in the DG (17), and the hippocampus is also implicated in modulating the learning and memory processes (23), the aim of the present study was to investigate whether CRF increases BDNF mRNA expression and whether CRF facilitates memory performance through enhanced BDNF mRNA expression in the DG. Due to the relatively small size of the DG tissue, the quantitative reverse-transcription polymerase chain reaction (RT-PCR) method was adopted for BDNF mRNA determination in the present study.

Materials and Methods

Animals

Male Sprague-Dawley rats (weighing 200-250g) bred in the Institute of Biomedical Sciences, Academia Sinica in Taiwan, were housed three per cage in a temperature-regulated room (23±2 °C) and maintained on a 12/12 hr light/dark cycle (light on at 6:30 am) with food and water continuously available. All behavioral experiments were conducted during the light phase of the diurnal rhythm.

Drugs and Reagents

CRF was purchased from Peninsula Laboratory Inc. (CA, USA). MK801 was purchased from Research Biochemical Inc. (MA, USA). BDNF sense and antisense oligonucleotides were synthesized from Oligos Etc. (Oregon, USA). BDNF protein was purchased from Promega (WI, USA). Primer pairs were synthesized by Genosys Biotechnologies, Inc. (TX, USA). RNA isolation kit was purchased from Biotecx Laboratories, Inc. (TX, USA). DNase, RNase inhibitor (RNasin), dNTP, avian myeloblastosis virus reverse transcriptase (AMVRT) and *Taq* polymerase were purchased from Promega. All other chemical reagents were purchased from Sigma (MO, USA) of the highest grade.

Inhibitory Avoidance Learning Task

Approximately one week after the surgery, rats were trained in a one-way inhibitory avoidance learning task. The apparatus consisted of a trough-shaped alley divided by a sliding door into an illuminated safe compartment and a dark compartment facing away from the door. As the rat turned around, the door was opened. After the rat entered the dark compartment, the door was closed and a 1.0 mA/1 sec footshock was administered. The rat was removed from the alley after receiving the shock, administered the appropriate posttraining treatments and returned to its home cage. On the retention test given 24 hr later, the rat was again placed into the illuminated compartment and the latency to step into the dark compartment was recorded as a measure of retention performance. Rats that did not enter the dark compartment within 600 sec were removed from the alley and assigned a ceiling score of 600.

Surgery and Intra-DG Drug Administration

All animals were subjected to stereotaxic surgery under sodium pentobarbital anesthesia (40 mg/kg, ip). The 23-gauge stainless steel thin wall cannulae

(10 mm long) were implanted bilaterally into the dorsal surface of the DG in the hippocampus at the following coordinates: A.P. -3.6 mm from bregma, M.L.±2.4 mm from midline and D.V. -3.0 mm below the skull surface. Two small stainless steel screws serving as anchors were implanted over the right frontal and left posterior cortices. The cannulae were affixed to the skull with dental cement. A stylet was inserted into each cannula to maintain patency. After recovery from the surgery, animals received drug infusions while they were awake and gently held by the experimenter. The injection was administered through a 30-gauge injection needle connected to a 10 μl Hamilton syringe by 0.5 m polyethylene tubing (PE-20). The injection needle was bent at a length such that, when inserted into the cannula, the needle tip would protrude 1.5 mm beyond the tip of the cannula. Drug solutions were introduced into the PE tubing and were delivered into the DG manually at a rate of 0.5 μl/min. A volume of 0.8 μl was injected into each DG bilaterally throughout all experiments. Figure 1 illustrates the area of the DG tissue been punched out for BDNF mRNA determination.

Total RNA Extraction

Total RNA was prepared according to the method of Chomczynski and Sacchi (8). Briefly, tissue was homogenized with Ultraspec™ RNA isolation kit. The homogenate was kept on ice at 4 °C for 5 min, added with 0.2 ml chloroform, and centrifuged at 14, 500 × g for 15 min. The aqueous phase was added with equal volume of isopropranol and kept on ice for 10 min. The reaction mixture was centrifuged at 14, 500 × g for 10 min. The pellet RNA was washed twice with 75% ethanol and precipitated by subsequent centrifugation at 11,500 × g for 5 min. The pellet was briefly dried under vacuum and dissolved in DEPC-treated water. To avoid DNA contamination, total RNA (20 μl) was further treated with DNase (0.1 U/μl) in the presence of RNase inhibitor (0.2 U/μl) for 30 min at 37 °C in *Taq* buffer. After phenol/chloroform (3:1, v/v) extraction, the aqueous phase was recovered by centrifugation (14,500 × g for 15 min). It was then added with 1/10 volume of 2.5 M sodium acetate and 2 volume of 95% ethanol. The mixed solution was kept in a freezer (-20 °C) for at least 4 hr and centrifuged at 14,500 × g for 15 min. The pellet was then washed with 75% ethanol and dried.

Quantitative RT-PCR Analysis of BDNF mRNA Level

Because of the small size of the DG tissue and because BDNF belongs to the category of low-copy gene expression (36), the quantitative RT-PCR method was adopted for the present study as described previously (19). Briefly, 0.05 μg of total RNA was reverse transcribed by avian myeloblastosis virus reverse transcriptase (AMVRT) (8U) at 42 °C with oligo-dT (0.5 μg/μl) as primers in a 20 μl reaction buffer containing 50 mM Tris-HCl (pH 8.3), 50 mM KCl, 10 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM spermidine, 1 mM dNTPs and 1 U/μl of RNasin. After 1 hr, the AMVRT was heat-inactivated at 95 °C for 5 min. For PCR quantification, the endogenously expressed mRNA for hypoxanthine phosphoribosyltransferase (HPRT) (22) was used as the internal control which was coamplified with the BDNF mRNA (17). One set of PCR primers was used for BDNF and HPRT, respectively. The oligonucleotide primer pairs for PCR were designed by using a computer program (41). The sequences for primer pairs of BDNF were 5' (5'-CACTCCGACCCTGCCCGCCG-3') and 3'(5'-TCCACTATCTTCCCCTTTTA-3') and that for HPRT were 5'(5'-CTCTGTGTGCTGAAGGGGGG-3') and 3'(5'-GGGACGCAGCAACAGACATT-3') which flanked a region of 364 bp and 625 bp for BDNF and HPRT, respectively. The RT product was incubated with 0.4 μM of primer sets and 1U of *Taq* polymerase in a 20 μl reaction mixture containing *Taq* buffer, 1. 5 mM $MgCl_2$, 200 μM each of dATP, dTTP and dGTP, 100 μM dCTP and 5 μCi [^{35}S] dCTP. To increase the specificity of PCR amplification, a touch down program was designed for the conditions of denaturing (94 °C, 1 min), annealing (3 cycles each at 65 °C, 62 °C and 59 °C in order and then 21 cycles at 55 °C) and polymerization (72 °C, 2 min). A final 10 min incubation at 72 °C was carried out after these 30 cycles of PCR. Aliquots from PCR reaction were electrophoresised through a 8% polyacrylamide gel. The gel was then exposed to the X-ray film and quantified by a phosphoimage analyzer (Phosphoimager, Molecular Dynamics, CA, USA). Pilot experiments were conducted to determine the range of PCR cycle which amplification efficiency remained constant and the amount of amplified PCR product

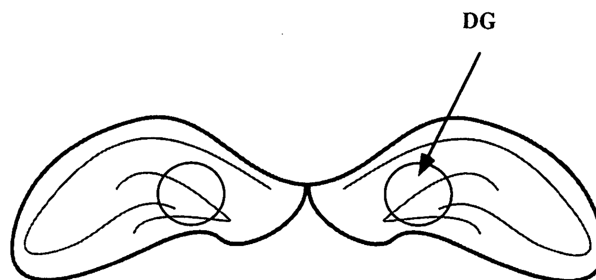


Fig. 1. A schematic diagram showing the relative size and position of the dentate gyrus (DG) tissue which was punched out for BDNF mRNA determination.

was proportional to the amount of input RNA, as described previously (19).

Statistics

The results of BDNF mRNA level were analyzed with one-way analysis of variance (ANOVA). Specific comparisons between each experimental group and a common control group were made with the Dunnett's *t*-test. Because the distribution of retention score was uneven and was truncated at 600, nonparametric Mann-Whitney U test was used to analyze the data for retention performance.

Experiment 1

This experiment was designed to examine the dose-response effects of CRF injected into the DG on BDNF mRNA level in this area. Animals were randomly divided into four groups to receive different doses of CRF infusions. Group I received intra-DG saline infusion; Group II received intra-DG CRF (0.1 μg) infusion; Group III received intra-DG CRF (1.0 μg) infusion and Group IV received intra-DG CRF (10 μg) infusion. Animals were sacrificed 30 min after the infusion. Their brains were removed and the DG was punched out from the hippocampal slices and subjected to BDNF mRNA determination.

Experiment 2

This experiment examined the effects of NMDA receptor blockade on BDNF mRNA expression in the DG and the interactions between CRF and NMDA receptor on BDNF mRNA expression in the same area. In the first part, rats were randomly distributed to four groups. Group I received intra-DG saline infusion; Group II received intra-DG MK801 (0.08 μg) infusion; Group III received intra-DG MK801 (0.2 μg) infusion and Group IV received intra-DG MK801 (2 μg) infusion. Animals were sacrificed 45 min following the infusion and the DG was similarly punched out. In the second part, different rats were also divided into four groups. Group I received intra-DG saline + saline infusions; Group II received intra-DG saline + CRF (0.1 μg) infusions; Group III received intra-DG MK801 (0.08 μg) + saline infusions and Group IV received intra-DG MK801 (0.08 μg) + CRF (0.1 μg) infusions. The interval between the two infusions was 15 min and rats were sacrificed 30 min after the second infusion. The DG was also punched out and subjected to BDNF mRNA determination.

Experiment 3

This experiment investigated whether CRF facilitates memory retention through enhanced BDNF mRNA expression in the DG. Rats were randomly distributed to four groups. Group I received intra-DG BDNF sense oligonucleotide (0.5 mM) + saline infusions; Group II received intra-DG BDNF sense oligonucleotide (0.5 mM) + CRF (0.1 μg) infusions; Group III received intra-DG BDNF antisense oligonucleotide (0.5 mM) + saline infusions and Group IV received intra-DG BDNF antisense oligonucleotide (0.5 mM) + CRF (0.1 μg) infusions. This concentration of BDNF antisense oligonucleotide was used because we have previously found that at a higher concentration (1 mM), BDNF antisense itself impairs retention performance in rats (43). The oligonucleotide sequence for BDNF sense was 5'-TGAGAAGAGTGATGACAA-3' and that for BDNF antisense was 5'-TTGTCATCACTCTTCTCA-3'. BDNF sense and antisense oligonucleotides (0.5 mM) were given to rats repeatedly for five times with 12 hr apart between two injections (at 9:00 am and 9:00 pm, respectively). The last infusion was given in between behavioral training and testing. CRF was administered immediately after training and retention measured 24 hr later. After the retention test, animals were sacrificed and the DG was dissected out for BDNF mRNA determination.

Experiment 4

This experiment examined the effects of direct BDNF infusions to the DG on retention performance in rats. Animals were randomly assigned to two groups. Group I received chronic intra-DG saline infusions (one injection per day for 6 days); Group II received chronic intra-DG BDNF infusions (0.5 μg, one injection per day for 6 days). The last infusion was given immediately after training, and the retention test was conducted 24 hr later.

Results

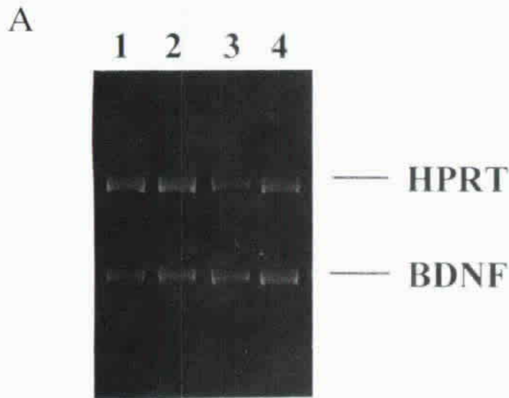
Experiment 1

The dose-response effects of intra-DG CRF infusion on BDNF mRNA level are shown in Figure 2. One-way ANOVA revealed that there was an overall significant effect of CRF on BDNF mRNA expression in the DG ($F_{(3,25)}$= 3.53, $p<0.05$). Further analyses indicated that the main effect was contributed by CRF at 1.0 μg and 10 μg which both significantly increased BDNF mRNA level in the DG (tD= 2.08 and 2.32, respectively, $p<0.05$).

Experiment 2

The dose-response effects of intra-DG MK801

A



B

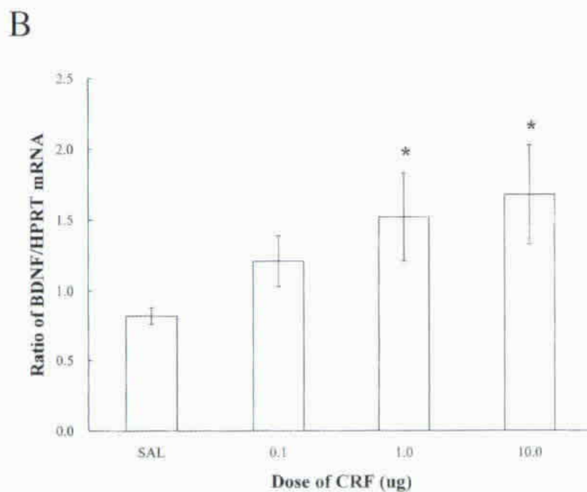
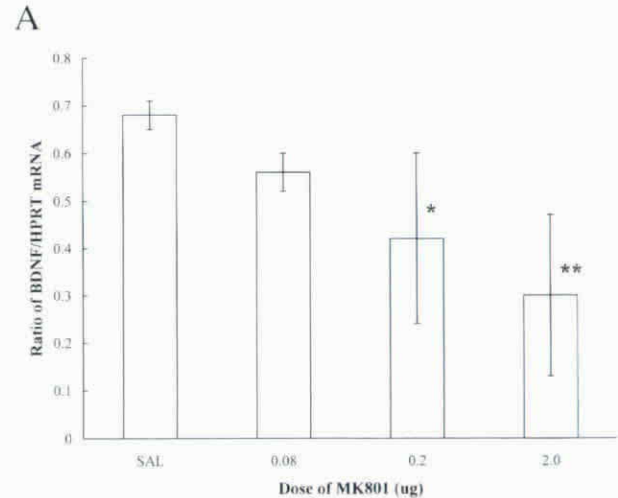


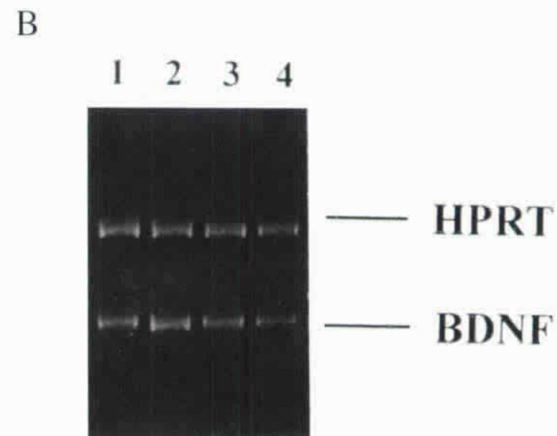
Fig. 2. (A) A representative gel pattern illustrates the HPRT and BDNF cDNA bands upon various doses of intra-DG CRF injections. Lane 1: saline, lane 2: CRF (0.1 μg), lane 3: CRF (1.0 μg) and lane 4: CRF (10.0 μg) (B) Dose-response effects of intra-DG CRF injections on BDNF mRNA level in the DG. n=6~9 in each group. Data are expressed as mean±SEM. *p<0.05.

infusion on BDNF mRNA level in the DG are shown in Figure 3A. Results indicated that MK801 dose-dependently decreased BDNF mRNA level in the DG ($F_{(3,16)}$=7.21, $p<0.01$). Further analyses with Dunnett's *t*-test revealed that MK801 at 0.2 μg and 2 μg both significantly decreased BDNF mRNA level (tD= 2.61 and 4.16, $p<0.05$ and $p<0.01$, respectively). The interactive effects between CRF and MK801 on BDNF mRNA expression are illustrated in Figures 3B and 3C. Statistical analyses revealed that CRF (1.0 μg) consistently and markedly elevated BDNF mRNA level in the DG (tD= 2.37, $p<0.05$). MK801, at a dose having no significant effect on BDNF mRNA alone (0.08 μg) (tD=0.08, $p>0.05$), markedly prevented the enhancing effect of CRF on BDNF mRNA measure (tD=0.57, $p>0.05$ when comparing the MK801 + CRF group with the control group).

A



B



C

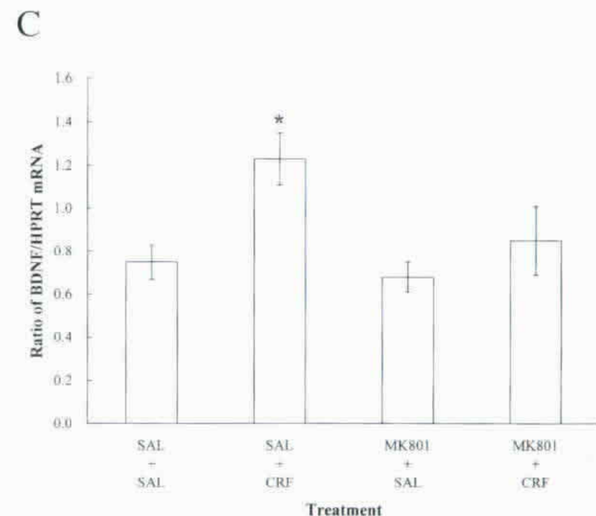


Fig. 3. (A) Dose-response effects of intra-DG MK801 injections on BDNF mRNA level in the DG. (B) A representative gel pattern illustrates the HPRT and BDNF cDNA bands upon MK801 and CRF injections. Lane 1: saline, lane 2: CRF (1.0 μg), lane 3: MK801 (0.08 μg) and lane 4: MK801 (0.08 μg) + CRF (1.0 μg). (C) Interactive effects between CRF (1.0 μg) and MK801 (0.08 μg) on BDNF mRNA level in the DG. n=5 each group. Data are expressed as in Fig. 2. *p<0.05 and **p<0.01.

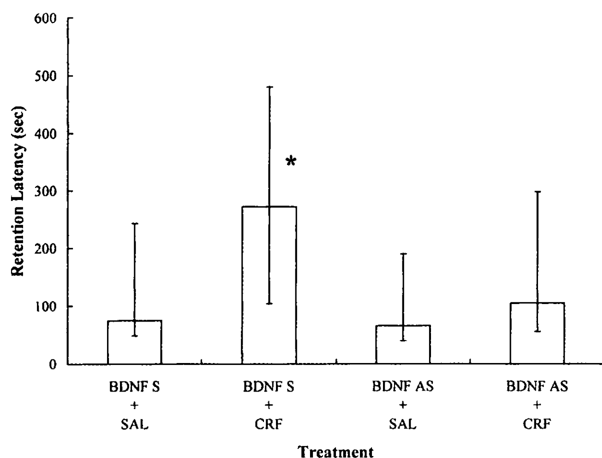


Fig. 4. Interactive effects between CRF (1.0 μg) and BDNF antisense oligonucleotide (0.5 mM) injections to the DG on retention performance in rats. n=10 in each group. Data are expressed as median ± interquartile range. BDNF S: BDNF sense oligonucleotide, BDNF AS: BDNF antisense oligonucleotide. *p<0.05.

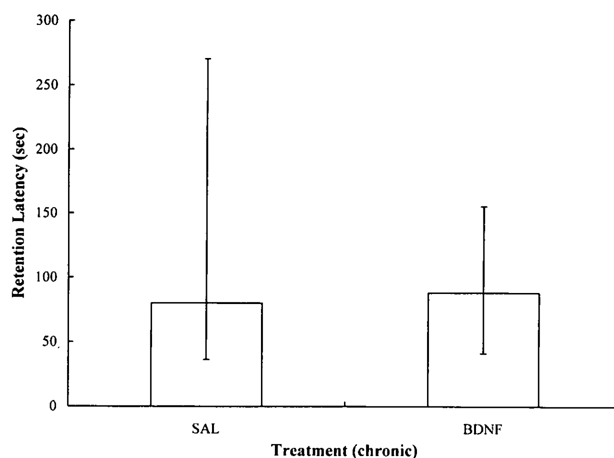


Fig. 5. Effect of chronic BDNF injection to the DG (0.5 μg each side, one injection per day, 6 days in all) on retention performance in rats. Data are expressed as median ± interquartile range. n=10 in each group. 26

Experiment 3

Whether CRF facilitates memory retention through enhanced BDNF mRNA expression in the DG is examined in this experiment and the results are shown in Figure 4. Results indicated that intra-DG CRF administration markedly improved retention performance in rats (Mann-Whitney U test, U=21, Z=2.20, p<0.05). BDNF antisense oligonucleotide infusion, at a dose which did not affect retention performance alone (0.5 mM) (U= 47.5, Z=0.19, p>0. 05), significantly impaired the memory-enhancing effect of CRF (U=34.5, Z=1.17, p>0.05 when comparing the BDNF antisense + CRF group with the control group).

Experiment 4

Effects of chronic BDNF infusion to the DG on retention performance in rats are shown in Figure 5. Mann-Whitney U test analysis revealed that chronic BDNF treatment did not markedly affect retention performance in rats (U= 23, Z=0.19, p>0.05).

Discussion

Results of the present study revealed that intra-DG injections of CRF dose-dependently increased BDNF mRNA level in the DG. This effect of CRF appears to be mediated through the glutamate NMDA receptors since MK801 pretreatment blocked the effect of CRF on BDNF mRNA expression. Further, inhibition of BDNF expression by BDNF antisense treatment prevented the memory-enhancing effect of CRF, while direct BDNF injection to the hippocampus did not improve memory retention in rats. These results are consistent with previous findings that both CRF (35, 53) and BDNF (26, 43) gene expressions in the hippocampus play important roles in learning and memory processes. Further, we have presently found that CRF may enhance retention performance through increased BDNF mRNA expression in the hippocampus. This is demonstrated because inhibition of BDNF expression significantly impaired CRF-induced memory facilitation in rats. We have previously shown that blockade of NMDA receptor prevents the memory-facilitating effect of CRF in the hippocampus (34). These results are consistent with the report of Castren et al. that MK801 decreases BDNF gene expression in the hippocampus (5) and the report of Gwag and Springer that NMDA receptor activation increases BDNF mRNA expression in the hippocampal formation (14). However, these results are not consistent with the report of Hughes et al. that MK801 induces BDNF mRNA expression in rat cortical neurons (18). This is probably due to different doses of MK801 and different routes of administration used in these studies. One possible synaptic relationship underlying these observations is that CRF afferents may terminate on glutamatergic terminals in the hippocampus; therefore, activation of CRF receptors located on glutamatergic terminals may facilitate glutamate release and NMDA receptor activation, as well as the subsequent upregulation of BDNF mRNA expression. However, the cellular mechanisms underlying NMDA receptor activation-mediated BDNF mRNA expression requires further examination.

Direct CRF injections to the brain were shown to improve learning and memory performance in

various behavioral tasks (32, 33, 51). BDNF gene expression was also shown to be important during memory consolidation (43). Our present results demonstrated that one of the cellular mechanisms responsible for CRF-induced memory facilitation is mediated through enhanced BDNF gene expression since BDNF antisense treatment abolished the memory-enhancing effect of CRF. Whether BDNF mRNA expression also mediates other central effects of CRF requires further investigation.

In the present study we have also found that chronic BDNF injection to the DG did not enhance memory retention in rats. We do not know what are the appropriate explanations for this finding yet; however, in another study, Fischer et al. have similarly reported that intraventricular injection of BDNF did not improve memory impairment in aged rats in a spatial learning task (12). Meanwhile, Pelleymounter et al. have reported that intra-hippocampal BDNF injection did not improve spatial learning in rats (47). One explanation for this could be that BDNF injected to the DG is preferentially been retrogradely transported to the cholinergic cell body area, the septum, or transported to other cells within the hippocampus, such as the CA3 area. Therefore, we may expect to see a memory-facilitating effect if BDNF is directly injected into the septum or the CA3 region. Otherwise, BDNF receptors may be located on inhibitory interneurons in the hippocampus, such as the GABA neurons (13). Therefore, the synaptic transmission upon exogenously applied BDNF is inhibited. In the study of Fischer et al., they have attributed the lack of an effect of BDNF on spatial memory to body weight loss in these animals due to chronic BDNF injections (12). In the present study, we have also found a significant loss of body weight in BDNF-treated rats when compared with the control rats (unpublished observations). However, whether this 20 % decrease in body weight contributes to memory impairment awaits further investigation. On the other hand, our results are not congruent with the observations examining the role of BDNF in LTP, a cellular model for studying long-term memory (3). In the study of Kang and Schuman, they found that BDNF produces a relatively long-lasting change in synaptic activity of hippocampal slice (25). Mice lacking the BDNF gene also showed impaired LTP (29) and that transfection of the BDNF gene restores LTP in BDNF mutant mice (30). We do not know the possible differential roles that BDNF may play in memory processing and LTP yet, but all the above LTP studies were done in hippocampal slices *in vitro* in an acute injection paradigm, while the bahavioral studies were carried out *in vivo* in a chronic injection regimen. Levine et al. have also reported that BDNF only produces short-term, but not long-term enahncement of synaptic transmission in hippocampal slices (37). Further, limbic seizure and kindling epilepsy were also shown to increase BDNF mRNA level in the hippocampus (11, 21, 44), while there has no report showing that BDNF injection produces seizure and/or epilepsy in animals. Indeed, in the present study we have found that rats injected with chronic BDNF did not show any sign of seizure or epilepsy (unpublished observations). Although a BDNF autocrine loop has been proposed in sensory neurons (1), and BDNF as well as BDNF receptor mRNAs were found to coexist in hippocampal neurons (27), the above results together suggest that the physiological manifestation of endogenously expressed BDNF may not always parallel with that of exogenously applied BDNF. The exact mechanisms underlying this discrepancy require further investigation.

In the present study, we have also found that the locomotor activity level was in general lower in BDNF-treated rats than that of controls (unpublished observations). In another study, we have similarly found that chronic BDNF injection to the substantia nigra markedly decreased locomotor activity in mice (Hung and Lee, unpublished observations). Since BDNF was found to support the survival of DA neurons in the mesencephalon culture (20), the unexpected result of a decreased locomotor activity upon BDNF injection to rats and mice suggests that there are other unknown physiological effects when BDNF is directly injected into the living brain. However, the decreased locomotor activity of BDNF-treated rats in the present study should not affect animal's performance on retention measure because the inhibitory avoidance paradigm requires the animals not to move to represent good retention performance, which should, consequently, increase their retention scores, but it is not what we have observed.

In summary, we have presently demonstrated that hippocampal BDNF mRNA expression can be upregulated by the neuropeptide CRF in a dose-dependent manner, while BDNF mRNA expression was dose-dependently downregulated by the NMDA receptor antagonist MK801. MK801 also effectively prevented the effect of CRF on BDNF mRNA measure. Further, intra-DG CRF was found to improve retention performance in rats and this effect was significantly prevented by BDNF antisense pretreatment. However, direct BDNF injection to the DG did not enhance memory retention in rats. These results together with earlier findings support the role of endogenous BDNF mRNA expression in memory processing. It further suggests that at least one of the mechanisms underlying the memory-facilitating effect of CRF is mediated through enhanced BDNF mRNA expression in the hippocampus. Yet, the lack of an effect of exogenously

applied BDNF on memory performance awaits further investigation.

Acknowledgment

This work was supported by research fund from the Institute of Biomedical Sciences, Academia Sinica, Taiwan, the Republic of China.

References

1. Acheson, A., Conover, J. C., Fandl, J. P., DeChiara, T. M., Russel, M., Thadani, A., Squinto, S. P., Yancopoulos, G. D. and Lindsay, R. M. A BDNF autocrine loop in adult sensory neurons prevents cell death. *Nature* 374: 450-453, 1995.
2. Alderson, R. F., Alterman, A. L., Barde, Y. A. and Lindsay, R. M. Brain-derived neurotrophic factor increases survival and differentiated functions of rat septal cholinergic neurons in culture. *Neuron* 5: 297-306, 1990.
3. Bliss, T. V. and Collingridge, G. L. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361: 31-9, 1993.
4. Bramham, C. R., Southard, T., Sarvey, J. M., Herkenham, M. and Brady, L. S. Unilateral LTP triggers bilateral increases in hippocampal neurotrophin and trk receptor mRNA expression in behaving rats: evidence for interhemispheric communication. *J. Comp. Neurol.* 368: 371-82, 1996.
5. Castren, E., Penha, B. M., Lindholm, D. and Thoenen, H. Differential effects of MK-801 on brain-derived neurotrophic factor mRNA levels in different regions of the rat brain. *Exp. Neurol.* 122: 244-252, 1993.
6. Castren, A., Pitkanen, J. S., Sirvio, J., Parsadanian, D. L., Lindholm, D., Thoenen, H. and Riekkinen, P. J. The induction of LTP increases BDNF and NGF mRNAs but decreases NT-3 mRNA in the dentate gyrus. *Neuroreport* 4: 895-898, 1993.
7. Chen, M. F., Chiu, T. H. and Lee, E. H. Y. Noradrenergic mediation of the memory-enhancing effect of corticotropin-releasing factor in the locus coeruleus in rats. *Psychoneuroendocrinology* 17: 113-124, 1992.
8. Chomczynski, P. and Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162: 156-159, 1987.
9. DeSouza, E. B. Corticotropin-releasing factor receptors in the rat central nervous system: Characterization and regional distribution. *J. Neurosci.* 7: 88-100, 1987.
10. Eaves, M., Britton, T., Rivier, J., Vale, W. and Koob, G. Effects of corticotropin releasing factor on locomotor activity in hypophysectomized rats. *Peptides* 6: 923-926, 1985.
11. Ernfors, P., Bengzon, J., Kokaia, Z., Persson, H. and Lindvall, O. Increased levels of messenger RNAs for neurotrophic factors in the brain during kindling epileptogenesis. *Neuron* 7: 165-76, 1991.
12. Fischer, W., Sirevaag, A., Wiegand, S. J., Lindsay, R. M. and Bjorklund, A. Reversal of spatial memory impairments in aged rats by nerve growth factor and neurotrophin 3 and 4/5 but not by brain-derived neurotrophic factor. *Proc. Natl. Acad. Sci.* 91: 8607-8611, 1994.
13. Freund, T. F. and Buzsaki, G. Interneurons of the hippocampus. *Hippocampus* 6: 347-470, 1996.
14. Gwag, B. J. and Springer, J. E. Activation of NMDA receptors increases brain-derived neurotrophic factor (BDNF) mRNA expression in the hippocampal formation. *Neuroreport* 5: 125-128, 1993.
15. Heinrichs, S. C., Vale, E. A., Lapsansky, J., Behan, D. P., McClure, L. V., Ling, N., De, S. E. B. and Schulteis, G. Enhancement of performance in multiple learning tasks by corticotropin-releasing factor-binding protein ligand inhibitors. *Peptides* 18: 711-6, 1997.
16. Hofer, M. and Barde, Y. A. BDNF prevents neuronal death *in vivo*. *Nature* 331:262-262, 1988.
17. Hofer, M., Pagliusi, S., Hohn, A., Leibrock, J. and Barde, Y. A. Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. *EMBO J.* 9: 2459-2464, 1990.
18. Hughes, P., Dragunow, M., Beilharz, E., Lawlor, P. and Gluckman, P. MK801 induces immediate-early gene proteins and BDNF mRNA in rat cerebrocortical neurons. *Neuroreport* 4: 183-186, 1993.
19. Hung, H. C. and Lee, E. H. Y. The mesolimbic dopaminergic pathway is more resistant than the nigrostriatal dopaminergic pathway to MPTP and MPP^+ toxicity:role of BDNF gene expression. *Mol. Brain Res.* 41: 16-26, 1996.
20. Hyman, C., Hofer, M., Barde, Y. A., Juhasz, M., Yancopoulos, G., Squinto, S. and Lindsay, R. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature* 350: 230-232, 1991.
21. Isackson, P., Huntsman, M. M., Murray, K. D. and Gall, C. M. BDNF mRNA expression is increased in adult rat forebrain after limbic seizures:temporal patterns of induction distinct from NGF. *Neuron* 6: 937-948, 1991.
22. Jansen, J. G., Vrieling, H. V., Zeeland, A. and Mohn, G. The gene encoding hypoxanthinne-guanine phosphoribosyltransferase as target for mutational analysis:PCR cloning and sequencing of the cDNA from the rat. *Mutation Res.* 266: 105-116, 1992.
23. Jarrard, L. E. On the role of the hippocampus in learning and memory in the rat. *Behav. Neural. Biol.* 60: 9-26, 1993.
24. Johnson, J. E., Barde, Y. A., Schwab, M. and Thoenen, H. Brain-deriverd neurotrophic factor supports the survival of cultured rat retinal ganglion cells. *J. Neurosci.* 6: 3031-3038, 1986.
25. Kang, H. and Schuman, M. Long-lasting neurotrophin-inducing enhancement of synaptic transmission in the adult hippocampus. *Science* 267: 1658-1662, 1995.
26. Kesslak, J. P., So, V., Choi, J., Cotman, C. W. and Gomez, P.-F. Learning upregulates brain-derived neurotrophic factor messenger ribonucleic acid: a mechanism to facilitate encoding and circuit maintenance? *Behav. Neurosci.* 112: 1012-9, 1998.
27. Kokaia, Z., Bengzon, J., Metsis, M., Kokaia, M., Persson, H. and Lindvall, O. Coexpression of neurotrophins and their receptors in neurons of the central nervous system. *Proc. Natl. Acad. Sci.* USA, 90: 6711-6715, 1993.
28. Koob, G. F. and Bloom, F. E. Corticotropin-releasing factor and behavior. *Fed. Proc.* 44: 259-263, 1985.
29. Korte, M., Carroll, P., Wolf, E., Brem, G., Thoenen, H. and Bonhoeffer, T. Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc. Natl. Acad. Sci.* USA, 92: 8856-60, 1995.
30. Korte, M., Griesbeck, O., Gravel, C., Carroll, P., Staiger, V., Thoenen, H. and Bonhoeffer, T. Virus-mediated gene transfer into hippocampal CA1 region restores long-term potentiation in brain-derived neurotrophic factor mutant mice. *Proc. Natl. Acad. Sci.* USA, 93: 12547-52, 1996.
31. Krahn, D. D., Gosnell, A., Grace, M. and Levine, A. S. CRF antagonist partially reverse CRF and stress-induced effects on feeding. *Brain Res. Bull.* 17: 285-289, 1986.
32. Kumar, K. B. and Karanth, K. S. Alpha-helical CRF blocks differential influence of corticotropin releasing factor (CRF) on appetitive and aversive memory retrieval in rats. *J. Neural. Transm.* 103: 1117-26, 1996.
33. Lee, E. H. Y., Hung, H. C., Lu, K. T., Chen, W. H. and Chen, H. Y. Protein synthesis in the hippocampus associated with memory facilitation by corticotropin-releasing factor in rats. *Peptides* 13: 927-37, 1992.
34. Lee, E. H. Y., Lee, C. P., Wang, H. I. and Lin, W. R. Hippocampal CRF, NE, and NMDA system interactions in memory processing in the rat. *Synapse* 14: 144-153, 1993.
35. Lee, E. H. Y., Huang, A. M., Tsuei, K. S. and Lee, W. Y. Enhanced

hippocampal corticotropin-releasing factor gene expression associated with memory consolidation and memory storage in rats. *Chin. J. Physiol.* 39: 197-203, 1996.

36. Leibrock, J., Lottspeich, F., Hohn, A., Hofer, M., Hengerer, B., Masiakowski, P., Thoenen, H. and Barde, Y. A. Molecular cloning and expression of brain-derived neurotrophic factor. *Nature* 341: 149-152, 1989.
37. Levine, E. S., Dreyfus, C. F., Black, I. B. and Plummer, M. R. Brain-derived neurotrophic factor rapidly enhances synaptic transmission in hippocampal neurons via postsynaptic tyrosine kinase receptors. *Proc. Natl. Acad. Sci.* 92: 8074-8077, 1995.
38. Liang, K. C. and Lee, E. H. Y. Intra-amygdala injections of corticotropin-releasing factor facilitates inhibitory avoidance learning and reduce exploratory behavior in rats. *Psychopharmacology* 96: 232-236, 1988.
39. Liang, K. C., Melia, K. R., Miserendino, M. J., Falls, W. A., Campeau, S. and Davis, M. Corticotropin-releasing factor: long-lasting facilitation of the acoustic startle reflex. *J. Neurosci.* 12: 2303-12, 1992.
40. Linnarsson, S., Bjorklund, A. and Ernfors, P. Learning deficit in BDNF mutant mice. *Eur. J. Neurosci.* 9: 2581-7, 1997.
41. Lowe, T., Sharefkin, J., Yang, S. Q. and Dieffenbach, C. W. A computer program for selection of oligonucleotide primers for polymerase chain reactions. *Nucl. Acid Res.* 18: 1757-1761, 1990.
42. Lu, B. and Figurov, A. Role of neurotrophins in synapse development and plasticity. *Rev. in Neurosci.* 8: 1-12, 1997.
43. Ma, Y. L., Wang, H. L., Wu, H. C. and Lee, E. H. Y. Brain-derived neurotrophic factor antisense oligonucleotide impairs memory retention and inhibits long-term potentiation in rats. *Neuroscience* 82: 957-967, 1998.
44. Morimoto, K., Sato, K., Sato, S., Yamada, N. and Hayabara, T. Time-dependent changes in neurotrophic factor mRNA expression after kindling and long-term potentiation in rats. *Brain. Res. Bull.* 45: 599-605, 1998.
45. Palkovits, M., Brownstein, M., Spiess, J., Rivier, J. and Vale, W. Distribution of corticotropin-releasing factor in rat brain. *Fed. Proc.* 44: 215-220, 1985.
46. Patterson, S. L., Grover, L. M., Schwartzkroin, P. A. and Bothwell, M. Neurotrophin expression in rat hippocampal slices:a stimulus paradigm inducing LTP in CA1 evokes increases in BDNF and NT-3 mRNAs. *Neuron* 9: 10801-1088, 1992.
47. Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Gollub, M. and Wellman, C. The effects of intrahippocampal BDNF and NGF on spatial learning in aged Long Evans rats. *Mol. Chem. Neuropathol.* 29: 211-26, 1996.
48. Qiao, X., Chen, L., Gao, H., Bao, S., Hefti, F., Thompson, R. F. and Knusel, B. Cerebellar brain-derived neurotrophic factor TrkB defect associated with impairment of eyeblink conditioning in Stargazer mutant mice. *J. Neurosci.* 18: 6990-9, 1998.
49. Sendtner, M., Holtmann, B., Kolbeck, R., Thoenen, H. and Barde, Y. A. Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. *Nature* 360: 757-759, 1992.
50. Sirinathsinghji, D. J. S., Rees, L. I. I., Rivier, J. and Vale, W. Corticotropin-releasing factor (CRF) is a potent inhibitor of sexual receptivity in the female rat. *Nature* 305: 230-235, 1983.
51. Tershner, S. A. and Helmstetter, F. J. Injections of corticotropin-releasing factor into the periaqueductal gray enhance Pavlovian fear conditioning. *Psychobiology* 24: 49-56, 1996.
52. Wang, H. L., Wayner, M. J., Chai, C. Y. and Lee, E. H. Corticotrophin-releasing factor produces a long-lasting enhancement of synaptic efficacy in the hippocampus. *Eur. J. Neurosci.* 10: 3428-37, 1998.
53. Wu, H. C., Chen, K. Y., Lee, W. Y. and Lee, E. H. Y. Antisense oligonucleotides to corticotropin releasing factor impair memory retention and increase exploration in rats. *Neuroscience* 78: 147-153, 1997.