Time of Day and Length of Antidepressant Drug Administration Influence Brain-Derived Neurotrophic Factor and TrkB Levels in Rat Brain

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Abstract: The expression of brain-derived neurotrophic factor (BDNF) mRNA and protein and its primary receptor, TrkB mRNA shows circadian oscillations in adult rats; however it has been unclear if juvenile rats also display a similar pattern in circadian oscillations. We determined the levels of BDNF and TrkB mRNA and of BDNF protein at four separate time points during a 24 hperiod in the hippocampus and frontal cortex. The expression of BDNF and TrkB undergoes diurnal oscillation in adult and postnatal day 21 rats, but no significant variation is present in postnatal day 13 rats. Antidepressant drug treatment also is known to influence BDNF and TrkB levels. However, the reported effects of antidepressant drug treatment on BDNF and TrkB are highly variable and may be influenced by multiple factors, including detection method, class of antidepressant drug, and length of administration. BDNF mRNA levels were decreased significantly in the hippocampus after acute desipramine (a tricyclic antidepressant) treatment compared to control. BDNF mRNA and protein levels, as well as TrkB mRNA levels, were unchanged in adult rats after subchronic and chronic treatment with either desipramine or escitalopram (a selective serotonin reuptake inhibitor) and treatment consistent with several reports in the literature. This study defines several important factors that must be taken into account when comparing BDNF and TrkB levels both within and among studies.

INTRODUCTION

In the brain, BDNF has been implicated in development, neural regeneration, synaptic transmission, synaptic plasticity and neurogenesis [1-5]. A role for BDNF and its primary receptor, TrkB, in the action of antidepressant treatments have emerged and are supported by several lines of evidence. BDNF mRNA expression in the hippocampus is decreased by stress and gluco-corticoids [6, 7], whereas many antidepressants increased BDNF and TrkB mRNA [8, 9]. Pretreatment with antidepressants chronically blocks the stress-induced decrease in BDNF mRNA expression in the hippocampus [8]. Behavioral studies have demonstrated that BDNF infused into the midbrain of rats produces an antidepressant-like effect in animal models of depression [10]. In addition, intracerebral infusion of BDNF stimulates serotonin turnover, synthesis and sprouting of serotonergic axons [11-14]. Decreased levels of BDNF may contribute to the atrophy of certain limbic structures including the hippocampus and cortex that has been observed in depressed patients. Collectively, these findings support that increased expression of BDNF and TrkB contribute to the neural adaptations necessary for the action of antidepressant treatment.

The expression of BDNF and its primary receptor, TrkB are known to depend on neuronal activity in the central nervous system. The expression of BDNF (mRNA and protein) and TrkB mRNA shows circadian oscillations in adult rats; however it is unclear if juvenile rats also display a similar pattern in circadian oscillations. The effects of pharmacological antidepressant drugs on BDNF gene expression have been extensively studied [9, 15, 16]. However, the reported effects of antidepressant drug treatment on BDNF and TrkB are highly variable and may be influenced by several factors, including detection method, brain region, age of animal, strain of animal, class of antidepressant drug, dose, dosing interval, route of administration, time interval after the last dose and time of sacrifice, time of day of sacrifice, and length of administration. We have evaluated several of these parameters on BDNF and TrkB mRNA levels and BDNF protein levels in rats: age of animal, brain region, circadian rhythm, class of antidepressant drug and length of administration.

MATERIALS AND METHODOLOGY

Animals

All animals were obtained from Harlan Industries (Indianapolis, IN) and juvenile rats were shipped in with the dam either on postnatal day (PND) 1 or one week prior to experiments. Pups were weaned from the dam at PND 21. All animals were group housed with food and water freely available and maintained on a 12 h light-dark cycle, on at 6:00 a.m. and off at 6:00 p.m. In many of the figures, the portion shaded in gray represents the time the rats spent in the dark. The animals were allowed to acclimate to their surrounding for one week prior to experiments and were also handled daily to become familiarized to the investigators. All treatments were according to standard protocols. Groups of rats were euthanized at 4 time points (07:00 a.m., 11:00 a.m., 7:00 p.m., and 11:00 p.m.), n = 4 per group for the circadian rhythm studies. The brains were dissected immediately and

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the hippocampi and frontal cortices were stored at -80ºC until and stored until ready to use.

Adult rats received twice daily intraperitoneal (i.p.) injections for 1, 5, 10, 15, or 20 days. The dose of drug given was 10 and 15 mg/kg/injection of ESC or DMI, respectively. Control rats in each age group were given saline i.p. injections. Rats were euthanized 12-14 h after the last injection and the brains were promptly removed. The cerebellum was isolated and saved at -80 ºC for determining concentrations of ESC, DMI and desmethyldesipramine, the major metabolite of DMI in rats. The hippocampi and frontal cortices were dissected and kept for analysis of BDNF and TrkB mRNA levels and BDNF protein levels. All procedures were done in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local Animal Care Committee.

Materials

Desipramine (DMI) was purchased from Sigma-Aldrich (Sigma, St. Louis, MO) and was dissolved in water. Escitalopram (ESC) was obtained from Toronto Research Chemicals (North York, ON. Canada) and dissolved in saline. All drugs were freshly prepared before use and injected i.p. Animals were weighed the morning of each injection day and then drug doses were calculated as milligrams per kilogram of body weight. Control animals were injected with saline vehicle.

BDNF Enzyme-Linked Immunosorbent Assay

The animals were decapitated under isoflurane anesthesia, and the hippocampi and frontal cortices were dissected on an ice-cold glass plate and stored at -80 °C. The samples were thawed and processed with a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) measuring BDNF protein (ChemiKine BDNF Sandwich ELISA Kit CYT306; Chemicon International, Temecula, CA). In short, the tissue was homogenized with an Ultra Turrax Homogenizer in lysis buffer, 100 ml/g tissue (100 mM Tris-HCl, 1 M NaCl, 2 % bovine serum albumin, 4 mM ethylenediamine tetraacetic acid-Na^+, 0.1 % NaN_3, 2 % Triton X-100, 0.157 µg/ml benzamidine, 5 µg/ml aprotinin, 0.5 µg/ml antipain, 0.1 µg/ml pepstatin in dimethylsulfoxide, 17 µg/ml phenylmethylsulfonyl fluoride in 100% ethanol; all reagents from Sigma-Aldrich, St. Louis, MO). Samples were centrifuged at 14,000 x *g* for 30 min at 4 °C and supernatants collected and used for analysis. For the standard curve, a serial dilution of BDNF protein standard (included in the kit) was performed with lysis buffer (0-2,000 pg/ml). All samples and standards were prepared in triplicate. Incubation and washing were conducted according to the manufacturer's instructions with reagents from the kit, at room temperature, sealed and on a shaker. The optical density of the wells was analyzed in a Bio-tek Instruments, Inc. plate reader at 450 nm. A standard curve was generated from the serial BDNF standard dilutions, and BDNF protein concen-trations in the samples were extrapolated directly from the standard curve.

RNA Extraction, RT-PCR and TaqMan Real-time Reverse Transcription-PCR

Total RNA was isolated with TRIzol Reagent (Invitrogen) and purified with RNeasy columns (Qiagen, Valencia, CA, USA). RNA samples were subsequently treated with DNA-free DNAse (Ambion) for removal of trace amounts of DNA. RNA concentrations were determined by UV absorbance. Assays-on-Demand primers for BDNF (Rn00560868 m1) and TrkB (Ntrk2) (Rn00820626_m1) were purchased from Applied Biosystems Inc. (Foster City, CA). Real-time quantitative PCR was performed with cDNA using an ABI PRISM 7000 sequence detector (Applied Biosystems, Foster City, CA). BDNF and TrkB mRNA levels were determined and standardized with GAPDH (glyceraldehyde-3-phosphate dehydro-genase) internal control. Primers specific for GAPDH (Rn99999916_s1), a house keeping gene were used as a control. All data were normalized to GAPDH mRNA levels because of variation in RNA concentrations among samples. The threshold cycle for each sample was chosen from the linear range and converted to a starting quantity by interpolation from a standard curve run on the same plate for each set of primers.

Statistics

Data were analyzed by analysis of variance (ANOVA) followed by Tukey's test; $P<0.05$ was taken to be the level for significance.

RESULTS

Circadian Rhythm of BDNF and TrkB in the Hippocampus and Frontal Cortex of Adult Rats

Real-time PCR analysis showed a spontaneous oscillation of BDNF mRNA levels in the hippocampus during the 24-h period (Fig. **1**). The pattern of this variation was found to be similar to that observed in the frontal cortex (Fig. **2**), even though a wider range between maximum and minimum values occurred in the hippocampus. BDNF mRNA levels at 7 p.m. were the highest in both the hippocampus ($p<0.05$) and frontal cortex ($p<0.05$), one hour after the lights were turned off and the animals were in their active state.

In addition, real-time PCR assay with TrkB specific primers demonstrated that TrkB mRNA levels also undergoes significant variation during a 24-h cycle in both regions under investigation. As shown in Fig. (**1**), in the hippocampus the lowest TrkB mRNA was observed at 7 p.m., whereas the highest was reached at 7 a.m. ($p<0.05$). An even stronger variation was detected in the frontal cortex (Fig. **2**). The maximum level was obtained at 11 a.m. ($p<0.05$) and the minimum level at 11 p.m.

BDNF protein levels were also determined to undergo variation during a 24-hcycle. Hippocampal protein levels reached the maximal level at 7 a.m. ($p<0.05$), whereas the minimum level obtained in the frontal cortex was at 7 a.m. ($p<0.05$) (Fig. **3**).

Circadian Rhythm of BDNF and TrkB in the Hippocampus and Frontal Cortex of Postnatal Day 21 Rats

Real-time PCR analysis reveals that a spontaneous oscillation during the 24-h period was detected when BDNF mRNA level was quantified in the hippocampus (Fig. **4**) and frontal cortex (Fig. **5**) of postnatal day (PND) 21 pups. In both regions under investigation high BDNF mRNA levels were observed at 7 p.m. and then again at 7 a.m., whereas low levels were observed at 11 p.m. and then again at 11 a.m. Hippocampal and frontal cortical BDNF mRNA levels were significantly different from 7 a.m. (*$p<0.05$) at 11 p.m.

and 11 a.m. This circadian pattern is different from the pattern observed in adult rats, in which high levels are obtained only at 7 p.m. TrkB mRNA levels followed a similar pattern as that observed with BDNF mRNA expression in the hippocampus (Fig. **4**) and frontal cortex (Fig. **5**), although not to a significant degree.

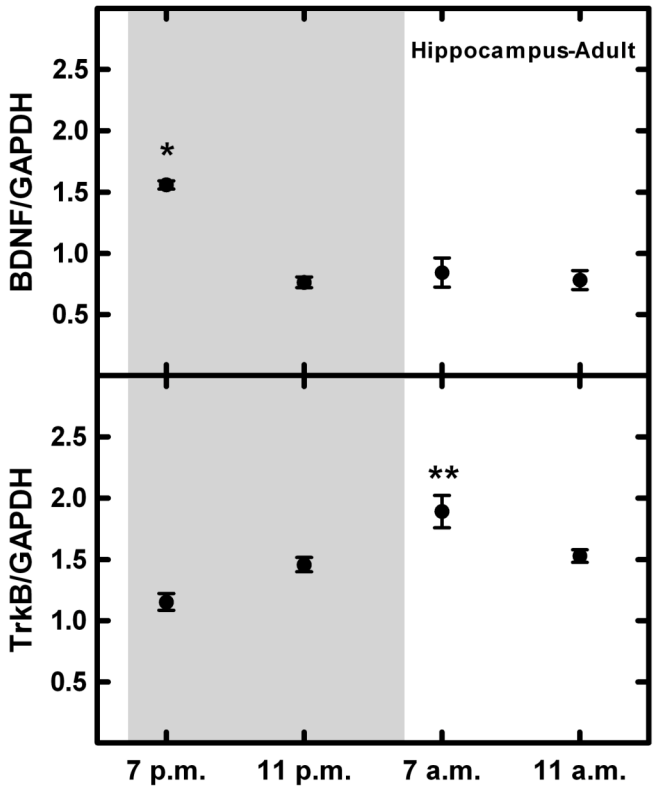

Fig. (1). Variations of BDNF and TrkB mRNA in the hippocampus of adult rats during a 24-h cycle. Symbols represent the mean values ± SEM of 4 rats euthanized at 7 p.m., 11 p.m., 7 a.m. and 11 a.m. BDNF and TrkB mRNA levels were determined using real-time PCR. Circadian times during which BDNF levels were significantly different (*p<0.05) in comparison with those observed at 11 p.m., 7 a.m., and 11 a.m.; **p<0.05 in comparison with 7 p.m., 11 p.m., and 11 a.m. The portion shaded in gray represents the portion of time the rats spent in the dark.

Hippocampal BDNF protein levels (Fig. **3**, middle left) also followed a similar oscillation pattern as mRNA levels in the hippocampus, although not to the same degree. Maximal protein levels were observed at 7 a.m. and minimal levels at 11 p.m. (*p<0.05 compared to 7 a.m.). No significant difference in BDNF protein levels was observed in the frontal cortex of PND 21 rats (Fig. **3**, middle right).

Lack of a Circadian Rhythm of BDNF and TrkB in the Hippocampus and Frontal Cortex of PND 13 Rats

Circadian oscillation variation was examined in the hippocampus and frontal cortex on PND 13 rats at four distinct time points. No significant pattern was observed in the hippocampus (Fig. **6**) and frontal cortex (Fig. **7**) of PND 13 rats. TrkB mRNA expression in the hippocampus (Fig. **6**) and frontal cortex (Fig. **7**) of PND 13 rats followed a similar pattern as BDNF expression, in which no significant difference was observed among the time points examined. Nevertheless, an oscillation variation was observed in BDNF levels in both the hippocampus (Fig. **3**, bottom left) and frontal cortex (Fig. **3**, bottom right) in PND 13 rats. BDNF protein levels were at a maximal level at 7 a.m. (*p<0.05 compared to 7 p.m., 11 p.m., and 11 a.m.) in the hippocampus and frontal cortex.

Acute Effects of ESC and DMI in the Hippocampus and Frontal Cortex of Adult Rats

One day of twice daily injections of ESC, a SSRI, in the hippocampus of adult rats caused a down-regulation ofBDNF mRNA levels in ESC-treated rats compared to controls (saline-treated) (Table **1**). A one-way ANOVA analysis revealed a significant effect of 10 mg/kg/injection of DMI (p<0.01) on BDNF mRNA levels in the hippocampus. Levels of BDNF mRNA in the hippocampus were unchanged following 10 mg/kg/injection, twice daily compared to control rats. Hippocampal BDNF protein levels were also unchanged after ESC and DMI (10 mg/kg/injection, twice daily) compared to saline treated rats (Table **1**). No change in BDNF and TrkB mRNA levels were observed after acute treatment with either ESC or DMI in the frontal cortex compared to control treated rats as well as no effect on cortical protein levels after antidepressant drug treatment (Table **1**).

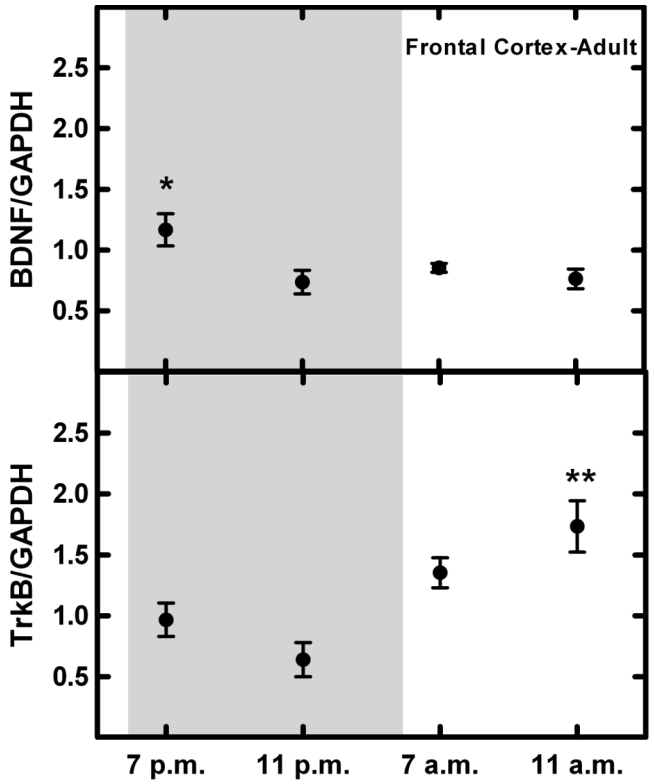

Fig. (2). Variations of BDNF and TrkB mRNA in the frontal cortex of adult rats during a 24-h cycle. Symbols represent the mean values ± SEM of 4 rats. Circadian times during which BDNF levels were significantly different (*p<0.05) in comparison with 11 p.m. and (**p<0.05) in comparison with 7 p.m. and 11 p.m.

Subchronic and Chronic Effects of ESC and DMI in the Hippocampus and Frontal Cortex of Adult Rats

Subchronic (5 - 10 days) treatment of twice daily i.p. injections failed to cause a significant effect on BDNF and

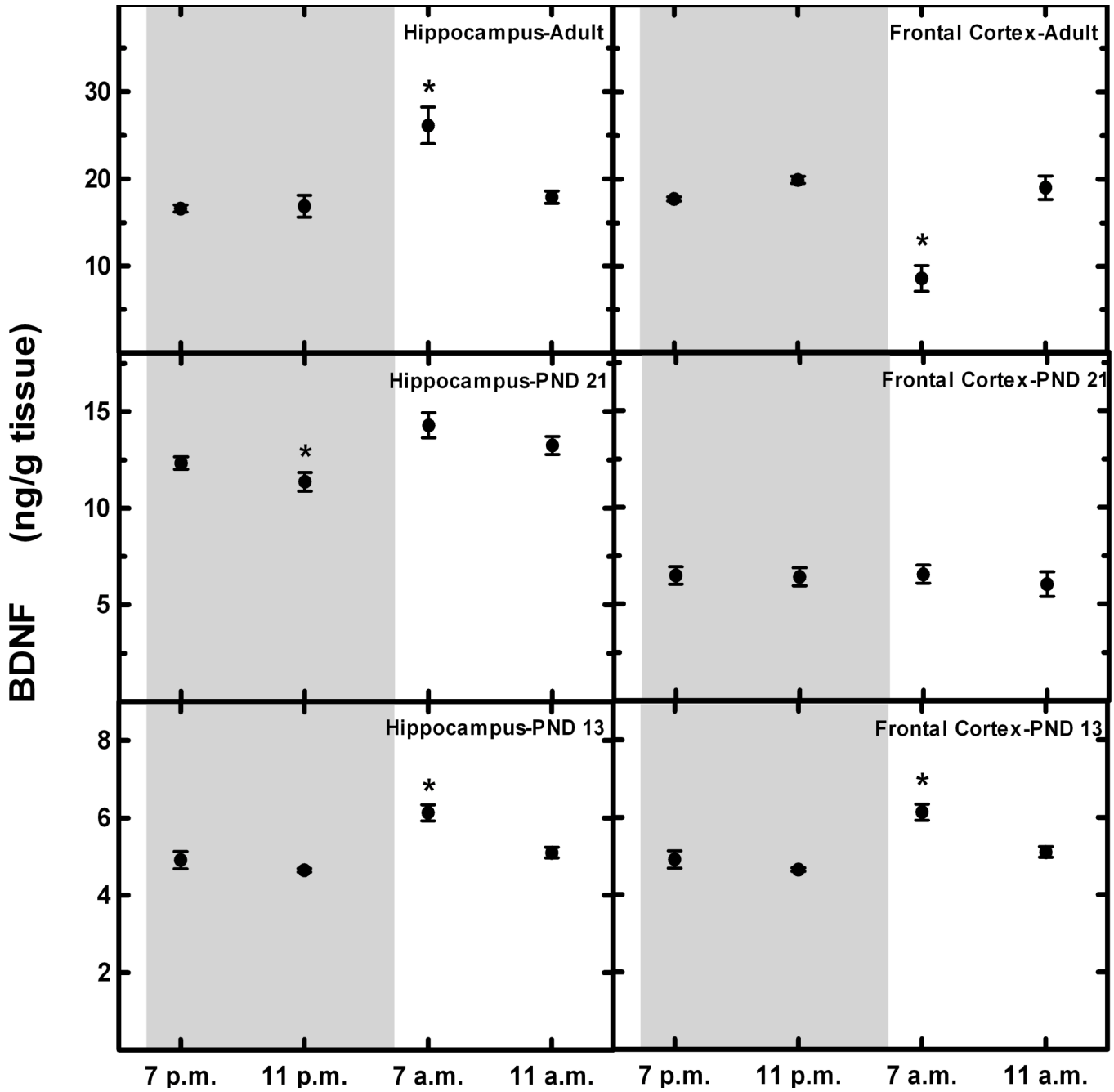

Fig. (3). Variations of BDNF protein in the adult rat hippocampus (top left) and adult rat frontal cortex (top right), PND 21 rat hippocampus (middle left) and PND 21 rat frontal cortex (middle right), and PND 13 rat hippocampus (bottom left) and PND 13 rat frontal cortex (bottom right) rats during a 24-h cycle. Symbols represent the mean values ± SEM of 4 rats. BDNF protein levels were determined using a Chemicon ELISA assay. Top left: circadian times during which BDNF levels were significantly different (*$p<0.05$) in comparison with those observed at 7 p.m., 11 p.m., and 11 a.m. in adult rat hippocampus. Top right: circadian times during which BDNF levels were significantly different (*$p<0.05$) in comparison with those observed at 7 p.m., 11 p.m., and 11 a.m. in adult frontal cortex. Middle left: circadian times during which BDNF levels were significantly different (*$p<0.05$) in comparison with those observed at 7 a.m. in PND 21 hippocampus. Bottom left: PND 13: circadian times during which BDNF levels were significantly different (*$p<0.05$) in comparison with those observed at 7 p.m., 11 p.m., and 11 a.m in PND 13 hippocampus. Bottom right: circadian times during PND 13 rats: circadian times during which BDNF levels were significantly different (*$p<0.05$) in comparison with those observed at 11 p.m., 7 a.m., and 11 a.m.in PND 13 frontal cortex.

TrkB mRNA levels after treatment with ESC and DMI at 10 mg/kg/injection in both the hippocampus and frontal cortex (Table **1**). In addition, no changes in BDNF protein levels were also observed after ESC and DMI in the hippocampus and frontal cortex (Table **1**). Chronic (15 – 20 days) treatment of twice daily i.p. injections of DMI and ESC also failed to produce a significant effect on BDNF and TrkB mRNA levels in the hippocampus and frontal cortex (Table **1**). Furthermore, no changes in BDNF protein levels were observed after ESC and DMI in the hippocampus and frontal cortex (Table **1**).

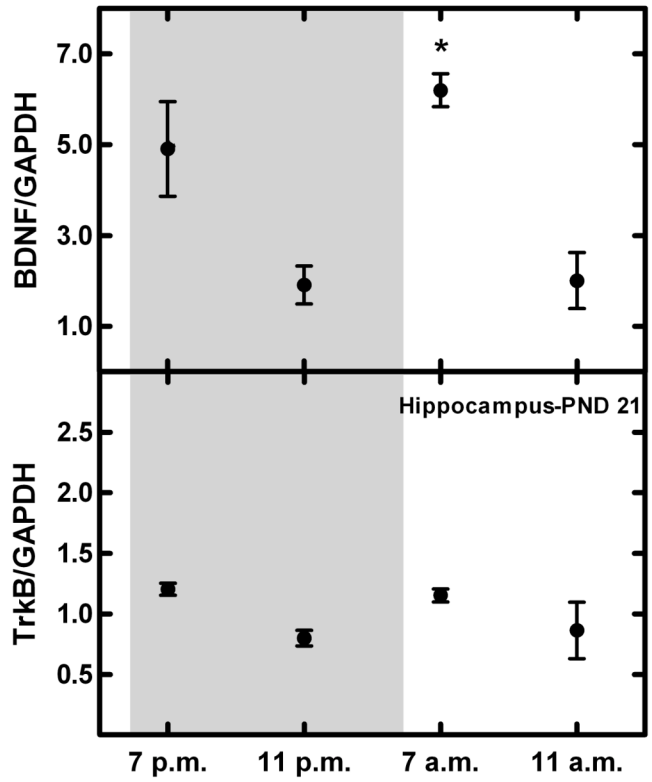

Fig. (4). Variations of BDNF and TrkB mRNA in the hippocampus of PND 21 rats during a 24-h cycle. Symbols represent the mean values ± SEM of 4 rats. Circadian times during which BDNF levels were significantly different (*p<05) in comparison with those observed at 11 p.m. and 11a.m.

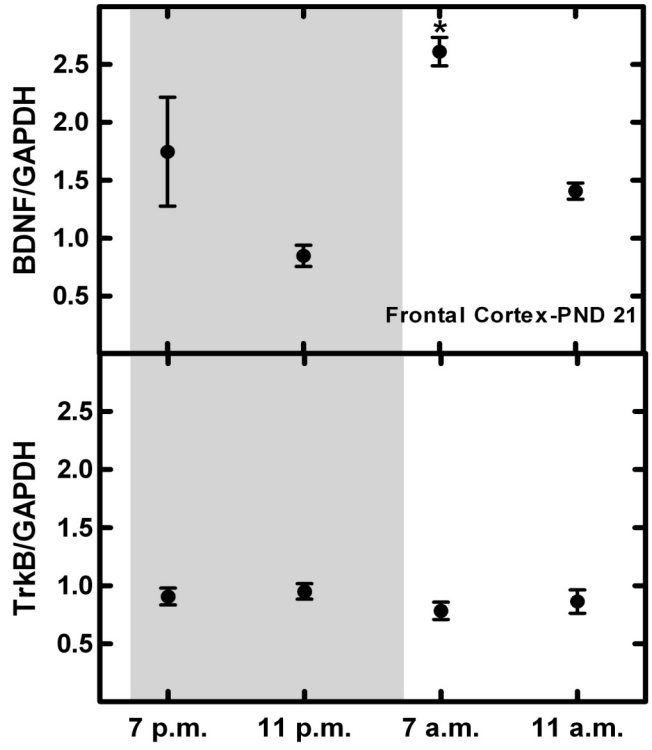

Fig. (5). Variations of BDNF and TrkB mRNA in the frontal cortex of PND 21 rats during a 24-h cycle. Symbols represent the mean values ± SEM of 4 rats. Circadian times during which BDNF levels were significantly different (*p<0.05) in comparison with those observed at 11 p.m. and 11 a.m.

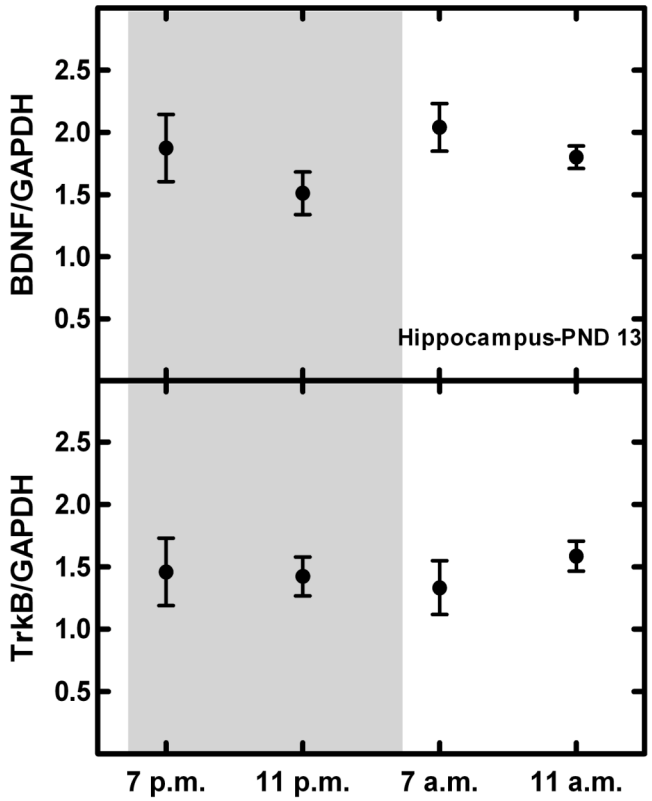

Fig. (6). Variations of BDNF and TrkB mRNA in the hippocampus of PND 13 rats during a 24-h cycle. Symbols represent the mean values ± SEM of 4 rats.

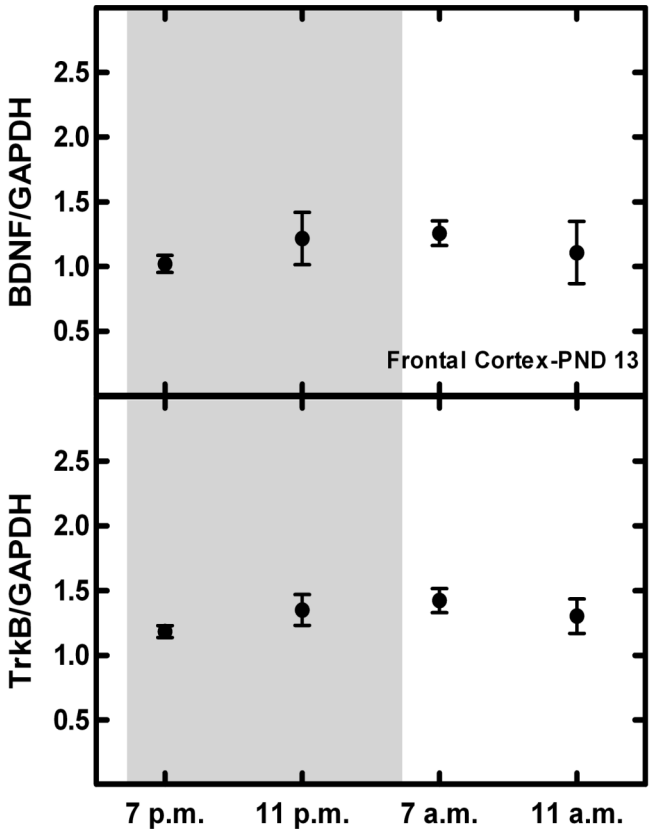

Fig. (7). Variations of BDNF and TrkB mRNA in the frontal cortex of PND 13 rats during a 24-h cycle. Symbols represent the mean values ± SEM of 4 rats.

Table 1. The Effects of 1, 5, 10, 15, and 20 Days of Twice Daily i.p. Escitalopram or Desipramine Injections in Adult Rats on BDNF (mRNA and Protein) and TrkB mRNA Levels. All Animals were Euthanized Twelve to Fourteen Hours After the Last Injection, Corresponding to 6 – 8 a.m. BDNF and TrkB mRNA Levels were Examined Using Real-Time PCR, Whereas BDNF Protein was Measured Using the Chemicon ELISA Assay. Data are Means of the Fold of Saline Control (±S.E.M.) of 4 Rats per Group

Drug	Days of Injections (2x Daily i.p.)	BDNF in Hippocampus		BDNF in Frontal Cortex		TrkB in Hippocampus	TrkB in Frontal Cortex
		mRNA	Protein	mRNA	Protein	mRNA	mRNA
Escitalopram	1	0.95 ±0.07	0.96 ±0.06	1.05 ±0.10	1.13 ±0.06	1.05 ±0.04	1.06 ±0.03
Desipramine	1	0.70 ±0.05*	0.88 ±0.05	1.00 ±0.09	0.93 ±0.07	1.06 ±0.06	0.99 ±0.04
Escitalopram	5	0.81 ±0.08	1.08 ±0.12	1.02 ±0.14	1.03±0.08	0.96 ±0.06	0.83 ±0.06
Desipramine	5	0.84 ±0.07	0.99 ±0.02	0.90 ±0.08	0.98 ±0.05	0.92 ±0.02	0.96 ±0.06
Escitalopram	10	0.99±0.08	1.15 ±0.04	0.94±0.05	1.20 ±0.02	1.01 ±0.06	0.95 ±0.03
Desipramine	10	0.85 ±0.06	1.21 ±0.09	1.10±0.12	1.08 ±0.06	1.00 ±0.05	0.93 ±0.05
Escitalopram	15	1.17±0.10	1.02 ±0.04	0.90 ±0.08	1.12 ±0.06	0.85 ±0.05	1.00 ±0.06
Desipramine	15	0.91 ±0.08	1.16 ±0.04	0.87±0.10	1.07 ±0.03	0.87 ±0.06	1.11 ±0.07
Escitalopram	20	0.88±0.08	1.01±0.10	1.15 ±0.19	1.05±0.04	0.91±0.05	0.88 ±0.04
Desipramine	20	0.79 ±0.04	0.96 ±0.06	1.19±0.10	0.97 ±0.06	1.00±0.06	0.87±0.06

* $p<0.0$.

DISCUSSION

BDNF expression, as well as the expression of other neurotrophins, is well known to be regulated by neuronal activity. The results demonstrate that the expression of BDNF undergoes diurnal changes in the absence of any experimental manipulation. The observation that BDNF mRNA induction in adults occurs concurrently with the beginning of the activity period suggests a possible association between levels of BDNF mRNA and the rest/activity cycle of the rat. In this study, TrkB, the primary receptor for BDNF was also shown to undergo significant variation in both the hippocampus and frontal cortex during a 24-h period. The increase in TrkB mRNA level is delayed in comparison to the BDNF mRNA induction, possibly suggesting that unlike BDNF, the induction of TrkB transcription may require intervening protein synthesis.

Pollock and colleagues have demonstrated that BDNF protein circadian variations are observed in the visual cortex, superior colliculus, hippocampus and cerebellum homogenates [17], however in this study a 14/10 h light/dark cycle was used, which may be a potential problem when comparing between studies. In the adult hippocampus the regulation of the oscillation variation of BDNF protein is different than that of BDNF message. High levels of protein are observed at 7 a.m., possibly indicating that the length of time required after BDNF mRNA induction occurs to produce protein. However in the frontal cortex this same pattern was not observed in respect to protein levels, indicating that the hippocampus and frontal cortex may not undergo the same pattern of circadian oscillation in regards to BDNF protein levels.

High levels of BDNF mRNA in the hippocampus and frontal cortex were observed in PND 21 rats at 7 a.m. and 7 p.m., respectively. This pattern of oscillation variation is different in adult rats, as maximal levels of BDNF mRNA were observed at the 7 p.m. time. This may indicate that in juvenile animals a higher amount of BDNF message is present and multiple induction times are needed. This corresponds to the development of BDNF mRNA in rats in which peak levels are reached around post-natal day 20 [18]. A similar trend in regulation of TrkB was also observed in the hippocampus and frontal cortex of PND 21, although the changes were not significant, indicating that TrkB mRNA levels may undergo different circadian rhythm regulation.

Human infants are born without a circadian rhythm in cortisol and they acquire it during their first few years of life [19], which is consistent with the lack of a significant variation in BDNF or TrkB mRNA levels in PND 13 rats. However, BDNF protein levels did show variation and were at maximal levels at 7 a.m., in both the hippocampus and frontal cortex in PND 13 rats.

In our experiments, the levels of mRNA and protein of BDNF in hippocampus and frontal cortex of adult, PND 21 and PND 13 are not parallel with each other. For example, BDNF protein is produced 12 hour after increase in BDNF mRNA in hippocampus. In other experiments BDNF protein is decreased 12 hour after increase in BDNF mRNA in frontal cortex in adult. Although our experiments were not designed to address this issue, it is clear that the relationship between mRNA and protein levels is complex, particularly in the central nervous system where proteins may be transported some distance from where they are synthesized. In addition, protein synthesis may be differentially regulated in various brain regions. Finally, the mechanisms regulating both mRNA and protein synthesis may undergo alterations as the central nervous system matures.

The effects of antidepressant drug treatment on BDNF gene expression in adult rats have been extensively studied [15, 20]. However, the effect of antidepressant drug treatment on BDNF is highly variable and may be influenced by numerous factors, including detection method, age of animal, class of antidepressant drug, dose, method of drug delivery, how often the antidepressant is administered, time interval between the last dose and sacrifice, time of day of sacrifice,

and length of administration [9]. Studies examining the effects of acute 10 mg/kg DMI treatment on BDNF mRNA levels have shown a significant down-regulation of mRNA levels in the hippocampus when animals are sacrificed 4 hours after the injection [21]. No change in hippocampal and cortical levels have also been reported after acute 10 mg/kg DMI treatment 4-24 post drug [22, 23]. Acute 15 mg/kg DMI treatment on BDNF mRNA levels also follows the same pattern as 10 mg/kg DMI treatment. No change in BDNF mRNA levels in the hippocampus 2-3 hours post drug [8, 24] whereas, a significant reduction of mRNA levels in the hippocampus and frontal cortex has also been reported at the same time points, 2-3 hours post drug [25, 26]. From these few studies it is clear that the regulation of BDNF is highly variable.

In our experiments, the levels of mRNA and protein of BDNF in hippocampus and frontal cortex of adult, PND 21 and PND 13 are not parallel with each other as one might expect that they would be. For example, BDNF protein is produced 12 h after increase in BDNF mRNA in hippocampus, whereas in other experiments BDNF protein is decreased 12 h after increase in BDNF mRNA in frontal cortex in adult. Although our experiments were not designed to address this issue directly, it is clear that there is a complex relationship between mRNA levels and the levels of the resulting proteins, especially in the brain where proteins may be transported within neurons. In addition, protein synthesis may be differentially regulated in different brain regions. Finally, the regulatory mechanisms for both mRNA and protein synthesis is likely to change as the brain matures.

We observed a down-regulation of BDNF mRNA levels after acute DMI (10 mg/kg/injection, twice daily) 12-14 hours post drug in the hippocampus similar to what others have also reported [21, 25, 26]. Hippocampal and cortical BDNF mRNA and protein levels after acute ESC treatment were unchanged. To date no previous studies have examined the acute effects of ESC on BDNF or TrkB mRNA and BDNF protein levels in rats. No change in TrkB mRNA levels was also observed after acute DMI [8] and ESC treatment in the hippocampus and frontal cortex. An important distinction between the current study and others in the literature is that our rats receive twice daily injections so the total dose for one day is 20 mg/kg, instead of 10 mg/kg. This was done to maintain more constant drug levels in the brain.

Several studies report that long-term treatment with classical antidepressants, lithium and electro-convulsive shock increase mRNA and/or protein levels of BDNF in the rodent hippocampus and/or prefrontal cortex [22, 25, 27-33]. However, two weeks of DMI (10 mg/kg/day) treatment fails to induce an up-regulation of BDNF in the hippocampus and frontal cortex [23]. Coppell and colleagues have also used a similar treatment paradigm in which the animals were injected twice daily for 14 days with 10 mg/kg/injection and BDNF mRNA levels were examined. No significant effect on BDNF mRNA levels after DMI treatment in the hippocampus and frontal cortex were observed, similar to our findings with subchronic and chronic DMI treatment [22]. However, one study reports a significant up-regulation in BDNF mRNA levels in the hippocampus and cerebral cortex after two weeks of DMI (10 mg/kg/day) treatment [34]. A similar trend is observed when the dose of DMI is increased to 15 mg/kg/day for two weeks. One study reports an up-regulation of BDNF levels in the hippocampus [23], whereas another reports no significant effect on BDNF mRNA levels in the hippocampus [24].

A consistent finding is that three weeks of DMI (10 and 15 mg/kg/day) induces an up-regulation of BDNF mRNA in the hippocampus and frontal cortex [21, 23, 31, 35], although no increase is observed in BDNF protein [27, 31]. Only one study has examined the effects of chronic treatment on BDNF mRNA and protein levels. Jacobsen and Mork report that chronic ESC (21 days *via* osmotic minipump) failed to affect BDNF gene expression in the hippocampus and frontal cortex, but decreased BDNF protein in both regions [31].

CONCLUSION

Our results demonstrate that the expression of BDNF and TrkB mRNA is influenced by several factors. The factors of time of day and age of animal affected levels of BDNF and TrkB. In adult and PND 21 rat brain undergoes diurnal regulation in basal conditions, although the pattern of regulation observed is different for BDNF and TrkB. Furthermore, a circadian oscillation pattern was also observed in BDNF protein levels in both adult and PND 21 rats. However, no significant regulation was observed in PND 13 rats for BDNF or TrkB mRNA levels, indicating that young animals are different than adult animals suggesting that neurodevelopmental processes may also be different and regulated in a dissimilar fashion.

In adults, depression can be effectively treated with several classes of antidepressant drugs, including SSRIs and TCAs, whereas in children the TCAs have not been shown to be effective [36]. However antidepressant regulation of BDNF and TrkB is highly variable and is influenced by several factors and as a consequence inconsistent findings are observed. Furthermore, the lack of significant changes in antidepressant-induced BDNF synthesis observed in some [27, 31] may be related to the use of healthy animals in most studies. BDNF has been hypothesized to play a vital role in action of antidepressant treatment and pathology of depression. The exact mechanism by which increased BDNF expression could be therapeutic in depression is not known and appears to be affected by several factors. This study defines several important factors that must be taken into account when comparing BDNF and TrkB levels both within and among studies.

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