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Research report

Temporal changes in the expression of brain-derived neurotrophic factor mRNA in the ventromedial nucleus of the hypothalamus of the developing rat brain

Nobuhiro Sugiyama[a], Shigenobu Kanba[a], Jun Arita[b,*]

[a]*Department of Neuropsychiatry, University of Yamanashi Faculty of Medicine, Tamaho, Yamanashi 409-3898, Japan*
[b]*Department of Physiology, University of Yamanashi Faculty of Medicine, 1110 Shimokato, Tamaho, Nakakoma, Yamanashi 409-3898, Japan*

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Abstract

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family, which is important for the growth, differentiation, and survival of neurons during development. We have performed a detailed mapping of BDNF mRNA in the neonatal rat brain using a quantitative in situ hybridization technique. At postnatal day (PND) 4, hypothalamic structures showed only modest expression of BDNF mRNA, with the exception of the ventromedial nucleus (VMN), where expression was higher than that detected in the hippocampus. Abundant BDNF mRNA was also found in the bed nucleus of the anterior commissure, retrosplenial granular cortex, and the posteroventral part of the medial amygdaloid nucleus. Messenger RNAs encoding other neurotrophins, including nerve growth factor (NGF) and neurotrophin-3 (NT-3) and the BDNF receptor trkB, were not selectively localized in neonatal VMN. During subsequent developmental stages, BDNF mRNA expression in the VMN changed dynamically, peaking at PND 4 and falling to minimal levels in the adult brain. In contrast, the low levels of BDNF mRNA observed in the CA3 region of the hippocampus increased to adult levels following PND 10. As the VMN undergoes sexual differentiation, we compared BDNF, NGF, NT-3, and trkB mRNA expression in the VMN in males and females at embryonic day 20 and PND 4, but found no differences between them. These results suggest that localized and high level expression of BDNF mRNA in the neonatal VMN plays an important role in its neural organization and functional development.

Theme: Development and regeneration

Topic: Neurotrophic factors: expression and regulation

Keywords: Brain-derived neurotrophic factor; Hypothalamic ventromedial nucleus; Development; In situ hybridization; Hippocampus

1. Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family, which includes nerve growth factor (NGF), neurotrophin-3 (NT-3) and NT-4/5. This neurotrophin regulates the growth, differentiation, and survival of neurons in the central and peripheral nervous system by binding to the receptor tyrosine kinase trkB [8,10,15,23,35].

The distribution of mRNA for BDNF has been well studied in several brain areas, however most of the research has been conducted in the adult hippocampus, where the BDNF/trkB signaling pathway has been implicated in some types of learning and memory [29,36]. Research on the expression of mRNA for BDNF in other brain regions has been relatively neglected. For example, in hypothalamus the paraventricular and supraoptic nuclei contain BDNF mRNA-expressing neurons [1,11,21]. Expression of BDNF mRNA in these neurons depends on neural activity, including the response to osmotic stimuli [1,7] and immobilization stress [32]. In addition, a recent study of conditional deletion of BDNF indicated that BDNF deletion affects anxiety-related locomotor activity and food intake [30]. These findings suggest strongly that

*Corresponding author. Fax: +81-55-273-6730.
E-mail address: jarita@res.yamanashi-med.ac.jp (J. Arita).

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BDNF may play an essential role in the regulation of hypothalamic functions of the adult brain.

In order to better understand the role of BDNF in the development of the central nervous system (CNS), it is critical to determine the temporal and spatial patterns of BDNF mRNA expression during development. Expression of mRNA for BDNF has been quantified and localized in the developing rat hippocampus using the in situ hybridization technique [12]. Although some studies have examined BDNF in the adult hypothalamus, little is known concerning the spatiotemporal pattern of BDNF mRNA expression in the hypothalamus during development. The purpose of the present study was to examine developmental changes in BDNF mRNA expression in the rat hypothalamus using a quantitative in situ hybridization technique. We detected high levels of expression in the ventromedial nucleus (VMN) of the hypothalamus at perinatal ages. As the VMN is one of the nuclei that undergo sexual differentiation [22,25,26], we made a further comparison between males and females during the perinatal period for VMN expression of mRNAs encoding BDNF and its receptor, trkB, as well as two additional neurotrophins, NGF and NT-3.

2. Materials and methods

2.1. Animals

Timed pregnant Wistar rats were purchased from Japan SLC (Shizuoka, Japan) and maintained in a light- (lights on from 06:00 to 18:00 h) and temperature-controlled environment with free access to laboratory chow and water. Experiments were conducted in accordance with the guidelines of the Ethical Committee of Animal Experiments of Yamanashi Medical University. Offspring of the pregnant rats were decapitated on embryonic day (ED) 20 (the day before birth) and postnatal days (PNDs) 2, 4, 6, 10, 21, and 42 (the day of birth was designated as PND 1). Following decapitation, the brain was removed and frozen using dry ice powder, and stored at −80 °C until the time of sectioning. The sex of the perinatal animals was determined by inspection of the anogenital distance. In the experiment to examine sex differences of BDNF mRNA expression, vaginal smears were taken daily in the morning, and only rats showing two or more consecutive 4-day estrous cycles were killed at 12:00 h on diestrus day 1 ($n=5$) or proestrus ($n=5$).

2.2. Preparation of the neural tissue for in situ hybridization

Frozen coronal sections of the brain were cut at a thickness of 20 μm and were thaw-mounted onto 3-aminopropyltriethoxysilane-coated glass slides, air dried, and then fixed in diethylpyrocarbonate (DEPC)-treated 4% paraformaldehyde in 0.1 M phosphate buffer for 15 min. The slides were washed twice for 10 min each time with DEPC-treated 0.3 M sodium chloride, 0.03 M sodium citrate (2× SSC). They were then dehydrated with ascending concentrations of ethanol, and dried in a vacuum desiccator overnight. The slides were stored in a plastic box containing desiccants at −80 °C until they were processed for in situ hybridization. In some experiments, every other section was used for Nissl staining with cresyl violet to determine precise neuroanatomical locations.

2.3. Complementary RNA (cRNA) probes for BDNF, NGF, NT-3, and trkB

To make cRNA probes, fragments of rat cDNAs for BDNF, β-NGF, NT-3, and trkB were obtained by reverse transcription and polymerase chain reaction (RT-PCR). Total RNA was extracted from samples of adult rat hippocampus by the acid guanidium–phenol–chloroform method using Trizol Reagent (Gibco-BRL, Rockville, MD, USA). Total RNA was reverse-transcribed and PCR was performed using Taq polymerase according to the manufacturer's instructions (GeneAmp® RNA PCR core kit, Applied Biosystems, Foster City, CA, USA). PCR consisted of an initial denature step at 94 °C for 105 s followed by 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. The forward and reverse primer sequences were (5′-AGTGGGTCACAGCGGCAGATA-3′) and (5′-CTTCCCCTTTTAATGGTCAGT-3′) for rat BDNF, (5′-CACATGGGGGAGTTTTCAGTG-3′) and (5′-CACGCAGGCTGTATCTATCCT-3′) for rat NGF, (5′-ATGCAGAGCATAAGAGTCACC-3′) and (5′-GCCTACGAGTTTGTTGTTTTC-3′) for rat NT-3, (5′-GATCAAGACTCTGTGAACCTC-3′), and (5′-TCTTGGCCATTAGGGTGTAGT-3′) for rat trkB. The lengths of the DNA fragments produced by these primers were 304, 307, 293, and 271 bases, corresponding to regions 515–818 [GenBank accession number M61175], 673–979 [M36589], 491–783 [M34643], and 1487–1757 [NM_012731] of each nucleotide sequence, respectively. The amplified PCR products were isolated by agarose gel electrophoresis, purified using the Qiaex II gel extraction kit (Qiagen, Hilden, Germany), and then subcloned to pPCR-Script Amp SK(+) plasmids (Stratagene, La Jolla, CA, USA). The sequences of the subcloned PCR products were determined using the BigDye terminator cycle sequencing kit (Applied Biosystems). For the production of antisense RNA probes, the plasmid was linearized with *Bam*HI for BDNF, NT-3, and trkB, and with *Not*I for NGF. Using T3 RNA polymerase for BDNF, NT-3, and trkB, or T7 RNA polymerase for NGF, radioactive cRNA probes were synthesized with [^{35}S]UTP from the linearized plasmids (Riboprobe in vitro transcription systems, Promega, Madison, WI, USA). For the production of the control sense probe for BDNF, the plasmid containing the BNDF cDNA sequence was linearized with *Not*I and transcribed

with T7 polymerase. Unincorporated nucleotides were removed with Mini Quick Spin columns (Boehringer, Indianapolis, IN, USA). The radioactivities of the probes used for hybridization were 4.95, 9.91, 4.95, and 0.50 μCi/ml for BDNF, NGF, NT-3, and trkB, respectively.

2.4. In situ hybridization histochemistry and film autoradiography

In situ hybridization was performed using standard procedures [31]. Briefly, tissue samples were permeabilized with a proteinase K treatment (0.02 U/ml) in 0.1 M Tris buffer containing 0.05 M EDTA at 37 °C for 30 min, followed by acetylation with 0.25% acetic anhydrate in 0.1 M triethanolamine and dehydration by ethanol. The [^{35}S]UTP-labeled cRNA probe was diluted in hybridization solution containing 50% formamide, 10% dextran sulfate, 600 mM NaCl, 2× Denhardt's solution, 10 mM Tris, 1 mM EDTA, 10 mM dithiothreitol (DTT) and 0.9 mg/ml tRNA. This was applied to each section, which was then hybridized at 54 °C for NGF or 60 °C for BDNF, NT-3, and TrkB in a DNA oven overnight. Following hybridization, the sections were treated with 4 μg/ml ribonuclease A for 30 min at 37 °C and washed through descending concentrations of SSC to a final stringency of 0.1× SSC at 60 °C for NGF, 70 °C for BDNF and NT-3, or 80 °C for trkB for 30 min. DTT was added to all washes at a final concentration of 1 mM. The sections were then dehydrated with ethanol, dried, and exposed to X-ray films (Kodak BioMax MR-1, Amersham, Piscataway, NJ, USA) for 10 days at room temperature.

2.5. Image analysis and statistics

Expression of mRNA for neurotrophins, as detected by in situ hybridization and film-based autoradiography, was quantified to examine developmental and sexual changes. We chose sections from individual animals that corresponded to Figs. 28–29 in the atlas of Paxinos and Watson [27] and contained the rostrocaudally middle part of the VMN and the dorsal hippocampus. The hybridization images appearing on the X-ray films were captured as 8-bit gray scale images using a scanner connected to a Macintosh computer. Adobe® Photoshop was used to reverse the gray levels, resulting in a pseudo-darkfield image. Nonlinearities in the film's response to the radioactive emissions were corrected using a series of calibrating sources of known radioactivity. They were made by spotting 0.5 μl of two-times serially diluted cRNA probe solutions on a nylon membrane (Hybond-N+), which were exposed to the film simultaneously with the tissue samples. Mean gray scale intensity levels were determined for an area of 5×5 pixels corresponding to 0.014 mm^2 in the CA3 region of the hippocampus and 97-pixel circle corresponding to 0.056 mm^2 in the VMN at PND 4. The locations of the areas chosen are shown in Fig. 1. In the developmental studies, the VMN area to be quantified ranged from 61 pixels corresponding to 0.035 mm^2 on ED 20 to 285 pixels corresponding to 0.164 mm^2 on PND 42, in proportion to growth in VMN at these ages. The locations of the brain areas were determined according to coordinates described in Paxinos and Watson [27]. Because of the large number of animals needed for the in situ hybridization assay and limitations in the number of sections that could be simultaneously subjected to an assay, one section from each animal was quantified in a given assay. Measures from each side in an individual animal were then averaged. We quantified sections from the same animal with at least three independent assays and obtained the same trends. The

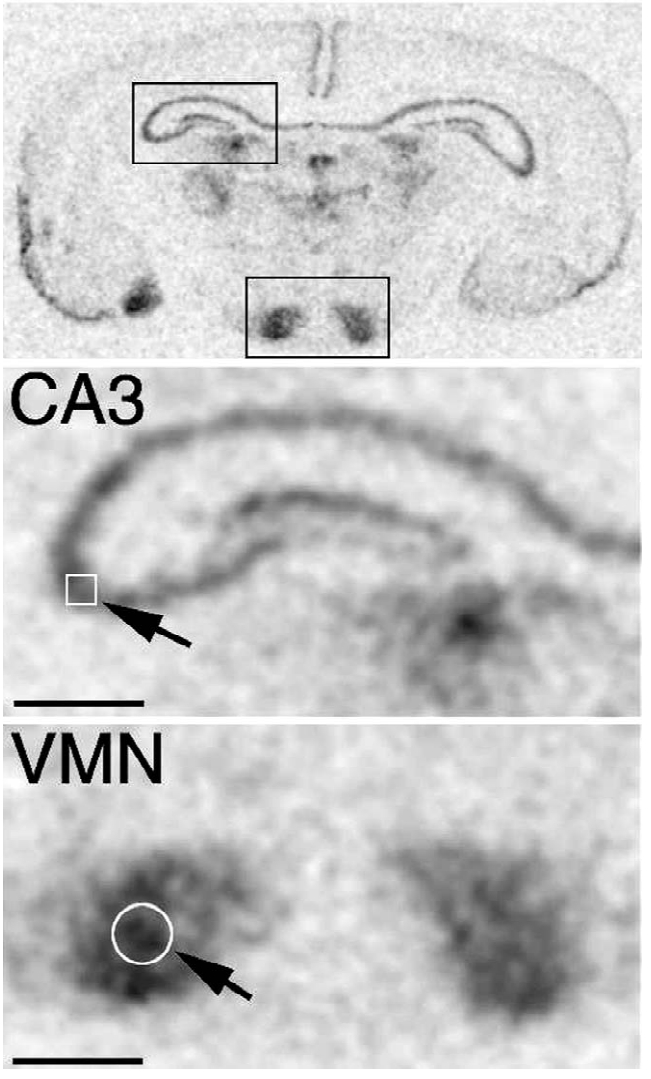

Fig. 1. Areas in which the relative amount of BDNF mRNA was quantified in the brain at postnatal day (PND) 4. The middle and lower panels show enlarged autoradiographs of hippocampal CA3 region and the hypothalamic ventromedial nucleus (VMN), respectively, corresponding to the anatomical areas indicated by the rectangles (black line) in the upper panel. A square (white line) and a circle in the middle and lower panels, respectively, indicate the areas used for quantitative image analysis. The scale bar represents 0.5 mm.

animal number for each group was five. One-way analysis of variance (ANOVA) was used to make comparisons between groups, followed by the Fisher's protected least significant difference post-hoc test.

3. Results

3.1. Distribution of BDNF mRNA in the neonatal rat brain

The distribution of BDNF mRNA expression in the male rat brain at PND 4 is shown in Fig. 2. High levels of BDNF expression can be seen throughout the hippocampus, including the CA1–CA4 regions and the suprapyramidal blade of the dentate gyrus (DG) (Fig. 2J–L). However, the infrapyramidal blade showed no signal above background. This was likely due to its underdevelopment at this age. Moderately positive signals for BDNF mRNA were found in the parasagittal region of cerebral cortex, corresponding to the retrosplenial granular cortex (Fig. 2J–L). Among the amygdaloid structures, only the posteroventral part of the medial amygdaloid nucleus (MePV) expressed substantial amounts of BDNF mRNA. The remaining parts of the amygdala did not show signal for BDNF mRNA (Fig. 2K, L). Although BDNF mRNA was detected in the midline thalamic nuclei, including the anterodorsal nucleus and the paraventricular nucleus, the lateral thalamic nuclei did not appear to express detectable amounts of mRNA for BDNF (Fig. 2J–L).

Throughout the preoptic–hypothalamic region, BDNF mRNA signal was confined to just two regions. First, the bed nucleus of the anterior commissure (BAC), which is located immediately lateral to the fornix as it crosses behind the anterior commissure, showed moderate levels of mRNA (Fig. 2H). Second, mRNA for BDNF was abundant in the VMN throughout its rostrocaudal extent (Fig. 2K, L). Surprisingly, these levels were much higher than those found in the hippocampus at the same PND. This distribution of in situ hybridization signals was shown to be specific for BDNF mRNA, as hybridization with a sense BDNF probe showed almost undetectable signals in both adult and neonatal brains (Fig. 3). We also examined expression of mRNAs encoding other neurotrophins, including NGF and NT-3, and mRNA encoding the BDNF receptor trkB in the VMN at PND 4 (Fig. 4). Strong mRNA signals for NT-3 were found exclusively in the retrosplenial granular cortex and in the CA1 region and the

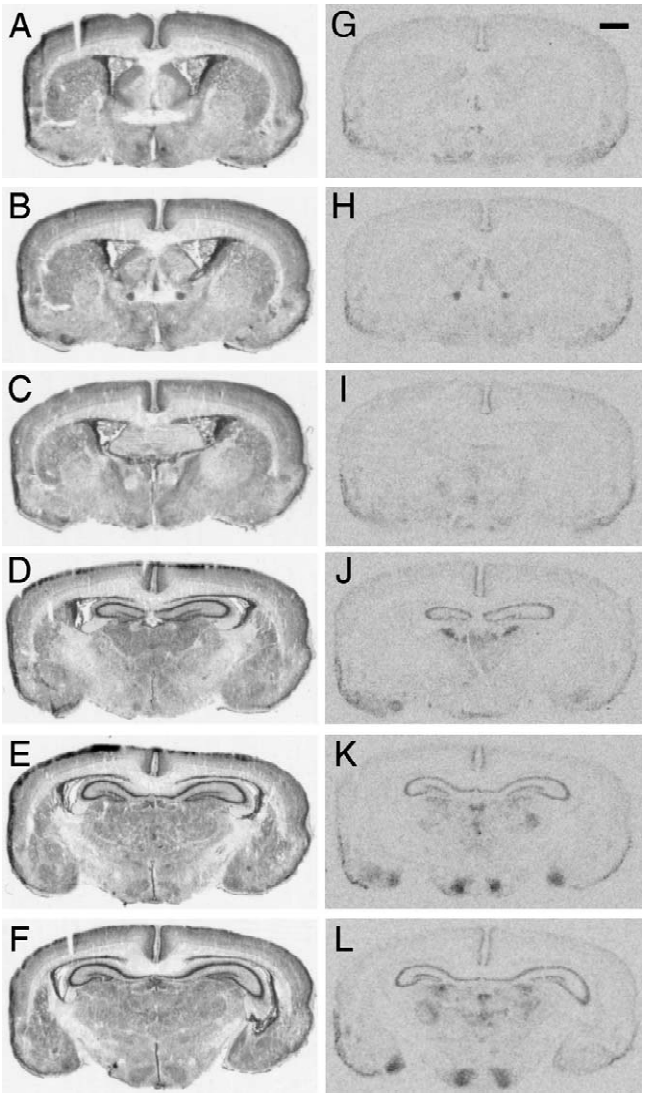

Fig. 2. Distribution of BDNF mRNA in male rats at PND 4. The right panels (G–L) show a rostrocaudal series of sections that were processed for in situ hybridization histochemistry using a [^{35}S]-labeled cRNA probe for BDNF. The left panels (A–F) are photomicrographs of the Nissl-stained adjacent sections. The scale bar represents 1.0 mm.

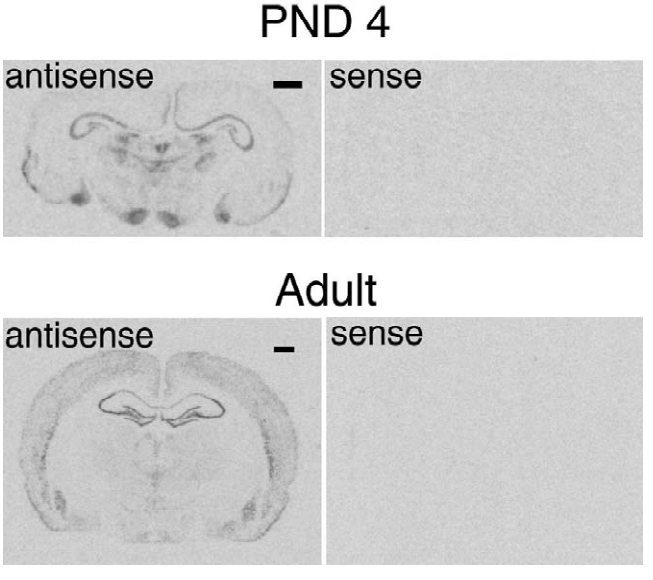

Fig. 3. Autoradiographs of in situ hybridization histochemistry with a BDNF antisense probe (left panels) or a sense control probe (right panels) at PND 4 and adult. The scale bar represents 1.0 mm.

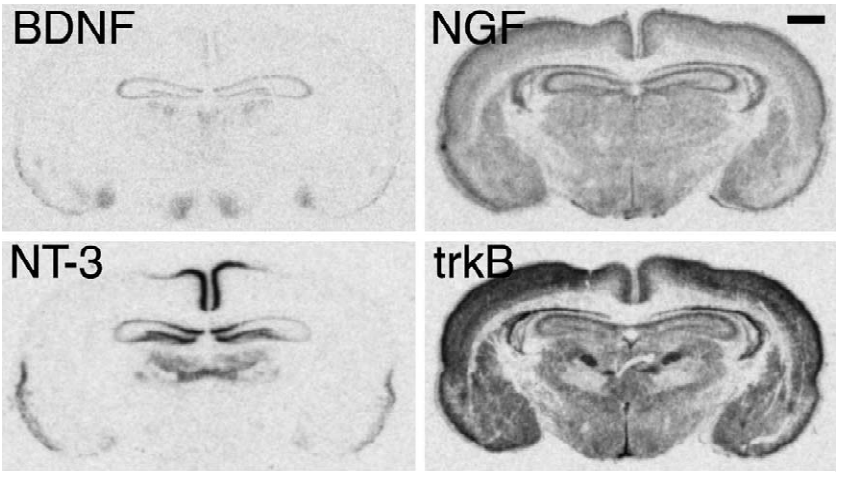

Fig. 4. Autoradiographs of BDNF, NGF, NT-3, and trkB in situ hybridization histochemistry at the rostrocaudal level containing the hippocampus and the VMN at PND 4. The scale bar represents 1.0 mm.

DG of the hippocampus. This is consistent with the distribution of NT-3 mRNA in neonatal rats reported elsewhere [18]. However, there was no signal for NT-3 in the VMN. Messenger RNA for NGF and trkB was distributed in a number of neural structures at relatively high levels. Within the VMN, expression was moderate, though slightly higher than that in surrounding areas.

3.2. Developmental changes in BDNF mRNA expression

In the mapping study made at PND 4 described above, high-level expression of BDNF mRNA was observed in the VMN and hippocampus. Therefore, we focused on developmental changes in BDNF mRNA expression in the VMN and compared them with measures in the hippocampus, which have been well studied [12,13,20]. Representative autoradiographs of BDNF mRNA in the VMN and the hippocampus from ED 20 to PND 42 are shown in Fig. 5. At ED 20, the VMN already expressed substantial amounts of BDNF mRNA and contained more abundant BDNF mRNA in neonatal rats than adult rats. Note that the reverse was true in the hippocampus.

Quantitative analysis of temporal changes in BDNF mRNA revealed that the relative amount of BDNF mRNA in the VMN peaked at PND 4 (Fig. 6, upper panel). The peak level was almost two-fold higher than that in the CA3 region of the hippocampus at PND 2–6. Expression levels of BDNF mRNA in the VMN gradually decreased thereafter and reached a minimal level at PND 42. Expression levels at PND 21 and 42 were significantly lower than those at PND 4 ($P<0.01$). In contrast, levels of BDNF mRNA in the CA3 region remained low during the first postnatal week and rapidly increased immediately prior to PND 10, at which point it achieved levels seen in the adult (Fig. 6, lower panel). Signals in the CA3 at PND 10 were significantly higher than those at PND 6 ($P<0.01$).

Finally, we observed two remarkable differences between neonatal and adult hippocampus: (1) during the first postnatal week the suprapyramidal blade of the DG showed much stronger signal intensities than the infrapyramidal blade, whereas both blades expressed similarly high levels of BDNF mRNA in the adult; and (2) CA1 and CA2 regions showed very strong signals, comparable to those in the CA3 region during the first postnatal week, whereas CA1 and CA2 expressed modest levels of BDNF mRNA in the adult.

3.3. Comparison between male and female neonatal rats in their expression of mRNA encoding BDNF, NGF, NT-3, and trkB in the hypothalamic VMN

Since the VMN is one of the nuclei that undergo sexual differentiation during the perinatal period, it is possible that differential expression of BDNF mRNA in the neonatal VMN in males and females may play a role in sexual differentiation. Therefore, we compared BDNF, NGF, NT-3, and trkB mRNA expression in the VMN in males and females. We chose to make this comparison at PND 4, as this is the age at which expression of mRNA for BDNF reached its peak. No differences were found between the sexes at this age (Fig. 7). Furthermore, we determined BDNF mRNA expression in male and female rats at four different developmental stages, namely, ED 20, PND 4, PND 21, and at 7–8 weeks old. However, we did not find sex differences in BDNF mRNA expression in the VMN at any developmental stage (Fig. 8).

4. Discussion

Our observations concerning BDNF mRNA expression in the neonatal hippocampus are consistent with findings from previous studies [12]. Compared to the hippocampus, little is known about BDNF mRNA in the neonatal rat

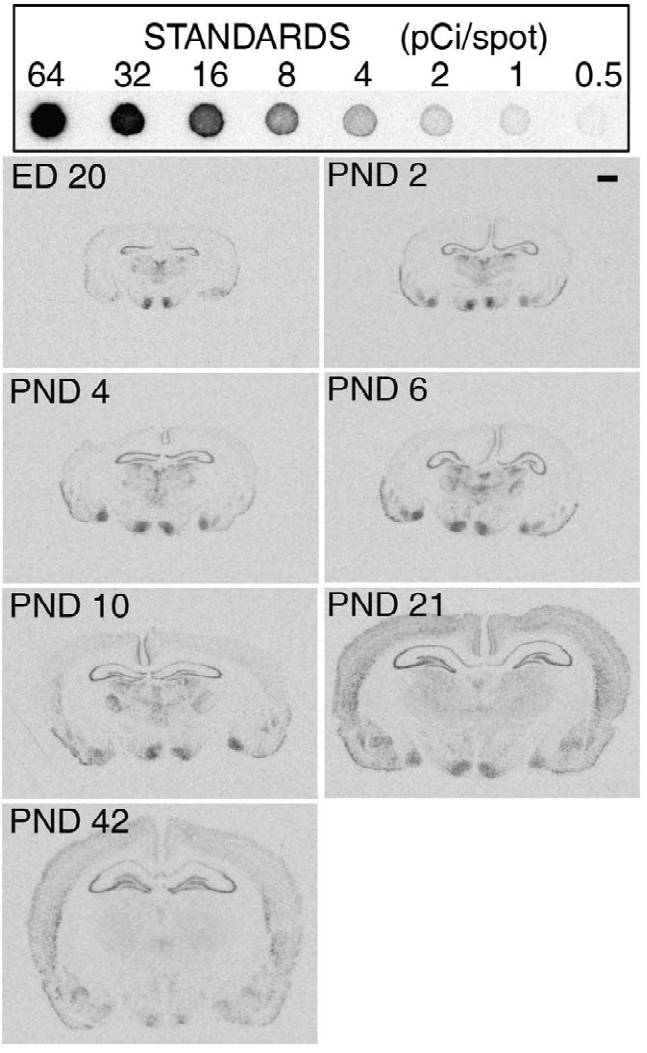

Fig. 5. Representative autoradiographs of BDNF in situ hybridization histochemistry in the hippocampus and the VMN from embryonic day (ED) 20 to PND 42. Standards were made by spotting two-times serially diluted cRNA probe solutions ranging between 0.5 and 64 pCi/spot. The scale bar represents 1.0 mm.

hypothalamus. A previous qualitative study using double in situ hybridization histochemistry described very strong or strong coexpression of BDNF and trkB in developing hypothalamic structures, including the lateral hypothalamus, the arcuate nucleus, and the paraventricular nucleus. However, coexpression in the VMN was described as moderate [24]. To our knowledge, this is the first study to demonstrate that BDNF mRNA expression is confined to the neonatal VMN, at levels substantially higher than that found in the hippocampus. Little, if any, expression was found in the remaining preoptic–hypothalamic structures, with the exception of the BAC. On the other hand, mRNA distributions for NGF and NT-3, which are members of the same neurotrophin family, were quite dissimilar to that of BDNF at neonatal stages. There was no signal of NT-3 mRNA in the VMN, and NGF mRNA signals in the VMN showed a similar level to that detected in the

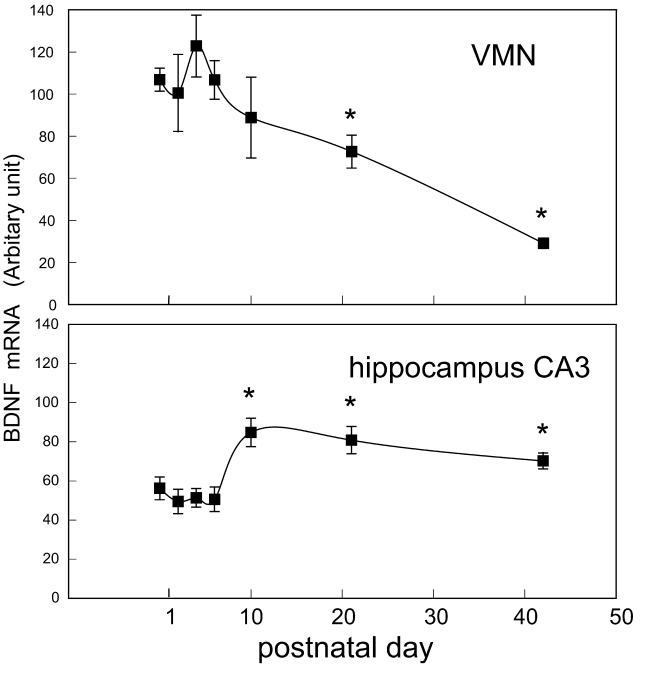

Fig. 6. Developmental changes in BDNF mRNA expression in the VMN (upper panel) and in hippocampal CA3 (lower panel), as analyzed using quantitative in situ hybridization histochemistry. The day of birth is designated as postnatal day 1 (PND 1). Values represent the mean and S.E.M. ($n=5$). The difference from the peak observed in the VMN at PND 4 or the minima observed in the hippocampal CA3 at PND 6 was significant ($P<0.05$).

surrounding areas. This indicates a preferential expression of BDNF mRNA in the VMN among the neurotrophins. Consistent with the BDNF expression in the VMN, we found abundant simultaneous mRNA expression of trkB, which serves as the receptor for BDNF and mediates its actions. These results suggest that BDNF may have a critical and specific role in the VMN during development.

The most detailed time-course data available on BDNF mRNA levels are confined to the hippocampus. To our knowledge, there are no quantitative studies that have used

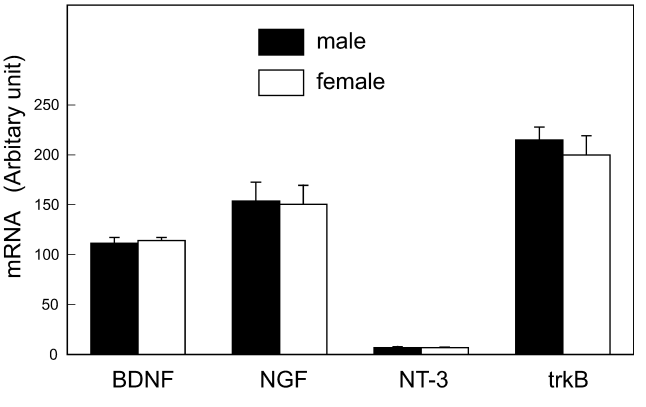

Fig. 7. VMN expression of mRNA encoding BDNF, NGF, NT-3, and trkB in male and female hypothalamus at PND 4. Values represent the mean and S.E.M. ($n=5$).

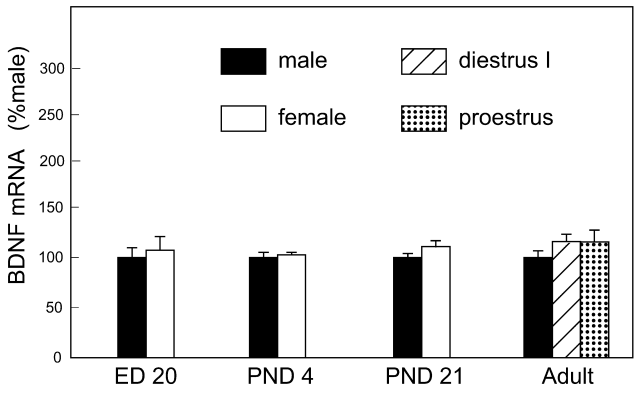

Fig. 8. VMN expression of BDNF mRNA in male and female rats at varying developmental stages. Regarding adult female rats, two groups in which female rats were killed at 12:00 h on diestrus day 1 and proestrus were used for mRNA quantification. BDNF mRNA expression is indicated relative to male rats at corresponding developmental stages. Values represent the mean and S.E.M (n=5).

in situ hybridization techniques to examine changes in BDNF mRNA expression during development. The present study shows that by PND 10, expression of BDNF mRNA in the CA3 region increases to levels that are found in the adult, in good agreement with previous studies using the Northern blotting technique [20], RNase protection assays [13], and in situ hybridization [12]. Thus, our quantitative in situ hybridization system seems to correctly identify changes in BDNF mRNA expression during development without the loss of spatial information that is typically seen when using the technique. We showed a high level of expression of BDNF in the VMN during the first postnatal week and even in the fetus at ED 20, the day before birth. Interestingly, there was a dramatic change in BDNF mRNA expression in the VMN with a peak at PND 4, which decreased gradually from the second postnatal week and attained minimal levels by PND 42. This is in contrast to the developmental change observed in the hippocampus, where expression of BDNF mRNA reaches stable, elevated levels at PND 10. BDNF has neurotrophic actions, leading to dendritic growth, nerve branching and remodeling, network development, and neuronal differentiation [8,10,15,23,35]. Based on the neurotrophic actions, the differences in BDNF mRNA expression between the VMN and the hippocampus during development suggest that maturation of the hypothalamic VMN may precede maturation of the hippocampus.

A major obstacle in elucidating the role of BDNF has been early postnatal lethality of homozygous $BDNF^{-/-}$ mice. Despite this obstacle, several studies have demonstrated remarkable changes in hypothalamic functions among BDNF-deleted animals. Heterozygous $BDNF^{+/-}$ mice develop increased locomotor activity, age-dependent hyperphagia and obesity, and increased serum insulin levels [17,19]. In addition, a recent study of conditional deletion of BDNF in the postnatal brain using the cre-loxP recombinant system also demonstrated that BDNF-deleted mice showed mature onset obesity and higher levels of locomotor activity following exposure to a novel environment [30]. Consistent with these findings, Pelleymounter et al. [28] demonstrated severe appetite suppression and weight loss following infusion of BDNF into the lateral ventricle. These findings strongly suggest that BDNF plays an important role in the regulation of anxiety-related locomotor activity and of feeding behavior. The VMN is important in the regulation of weight and feeding behavior. Lesions to the VMN cause hyperphagia, obesity, and hyperinsulinemia [4,16]. Moreover, there is substantial evidence that the VMN is involved in the regulation of locomotor activity [4,9,37]. On the basis of these findings, it has been suggested that BDNF expression in the VMN is involved in the regulation of feeding behavior and locomotor activity. In this regard, it is possible that: (1) BDNF expression during the perinatal period may have a neurotrophic action on the organization of networks involved in the regulation of feeding behavior and locomotor activity; or (2) BDNF expression in the adult may have a non-neurotrophic action, modifying these behaviors. Our results showing abundant expression of BDNF in the neonatal VMN seem to substantiate the former possibility. Note, however, that there is evidence that infusion of BDNF into heterozygous $BDNF^{+/-}$ mice produce weight loss, even following the development of obesity [17]. This argues in favor of the second possibility. Therefore, we assume that BDNF expressed during the perinatal period also exerts a non-neurotrophic action on the VMN, modifying the regulation of feeding behavior and locomotor activity that is required during this phase of development.

The VMN is one of the nuclei that undergo sexual differentiation during the perinatal period [22,25,26]. Recently, McCarthy and co-workers [2,3] reported that there was more phosphorylated form of the transcription factor cAMP response element-binding protein (CREB) in the VMN in neonatal males or testosterone-treated females than in neonatal females. They also showed that $GABA_A$-receptor activation by muscimol increased phosphorylated CREB in the VMN of neonatal males, while decreasing it in females. These findings suggest that phosphorylated CREB may act as a mediator of sexual differentiation in the VMN. Since BDNF, which contains a cAMP response element site within its promoter region [34], is not only downstream of CREB, but also upstream of CREB [14], we would expect to find sex differences in BDNF expression in the VMN during this critical period for sexual differentiation. However, we did not detect significant sex difference either at ED 20 and PND 4 or during prepubital and adult periods, consistent with the results of a previous study by Solum et al. [33]. These results suggest that neonatal BDNF expression in the VMN is not correlated with phosphorylation of CREB. It may be that CREB phosphorylation involves alternative, as yet unidentified mechanisms to induce sexual differentiation in the VMN.

We have found that during the neonatal period BDNF

mRNA expression in the amygdala was confined to the MePV while in the adult it was observed primarily in the lateral nucleus. These developmental changes in the MePV took place in parallel with changes in the VMN. The VMN receives dense projections from the MePV [6], and these two nuclei form a functional neural circuit [5]. We speculate that neural development in the MePV and the VMN may both be under the control of a common neurotrophic factor, BDNF.

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