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Research report

Local infusion of brain-derived neurotrophic factor modifies the firing pattern of dorsal raphé serotonergic neurons

Pau Celada [a,*], Judith A. Siuciak [b], Tuan M. Tran [a], C. Anthony Altar [b], James M. Tepper [a]

[a] *Center for Molecular and Behavioral Neuroscience, Rutgers – The State University of New Jersey, University Heights, 197 University Avenue, Newark, NJ 07102, USA*

[b] *Regeneron Pharmaceuticals, 777 Old Saw Mill River Road, Tarrytown, NY 10536, USA*

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Abstract

Previous studies have reported a neuromodulatory effect of brain-derived neurotrophic factor (BDNF) on serotonin neurons in the central nervous system. In the present study, we examined the effects of local infusion of BDNF on the electrophysiological activity of serotonergic neurons in the rat dorsal raphé nucleus with extracellular single unit recording in vivo. Compared with vehicle-infused rats, chronic administration of BDNF (10–14 days) caused serotonergic neurons to fire in a significantly less regular pattern, without altering the mean firing rate or other measures of electrical activity. These results suggest that the ability of similar infusions of BDNF to produce behavioral effects (i.e. analgesia and an antidepressant-like effect) associated with elevated serotonin turnover may be in part the result of more irregular firing patterns of dorsal raphé neurons.

Keywords: Brain-derived neurotrophic factor; BDNF; Growth factor; Serotonin; Firing pattern

1. Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the nerve-growth factor family of neurotrophic factors (for review, see [20]). BDNF mRNA and protein immunoreactivity are widely distributed throughout the CNS [18,21,43,44,46]. Neuronal somata within the dorsal or median raphé nuclei contain the mRNA for the BDNF receptor, TrkB [24], bind BDNF with high affinity [8], and accumulate BDNF via retrograde transport after its infusion into the forebrain [25,45]. Accordingly, recent findings show that the intracerebral administration of BDNF produces neurochemical and behavioral effects that are consistent with a mediation by central serotonin (5-HT) neurons. Intracortical infusions of BDNF prevent the neurotoxin-induced loss of cortical 5-HT axons and promote a sprouting of uninjured 5-HT axons in intact rats [22]. Furthermore, infusion of BDNF in the midbrain, near the periaqueductal gray and dorsal raphé, elevates nociceptive thresholds [31,34] confers antidepressant-like effects [33], and increases 5-HT turnover in the forebrain and spinal cord, as determined by increases in 5-HIAA concentrations and the 5-HIAA/5-HT ratio [31,32]. The present study was undertaken to determine if the known effects of midbrain infusion of BDNF on serotonin metabolism and related behaviors could be linked to effects on the electrical activity of dorsal raphé serotonergic neurons.

2. Materials and methods

2.1. BDNF Infusions

Male Sprague-Dawley rats (Charles River) weighing 175–225 g were used and surgery was performed as previously described [31,34]. Briefly, rats were anesthetized with chloral hydrate (149 mg/kg) and sodium pentobarbital (30.8 mg/kg) and mounted in a stereotaxic apparatus. Rats were implanted with cannulae (Plastics One, Roanoke, VA, 6.8 mm length, 28 gauge) aimed at the midbrain, near the periaqueductal gray and dorsal raphé nucleus, at the following coordinates with respect to bregma: AP −7.6 mm, L 1.0 mm [27]. The cannulae were attached via a 2 cm length of tubing to an osmotic pump (Alzet 2002) which was implanted s.c. between the shoul-

* Corresponding author. Fax: (1) (201) 648-1588. E-mail: pau_celada@axon.rutgers.edu

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der blades. Animals received either phosphate-buffered saline (PBS) or BDNF (12 μg/day), a dose previously shown to produce stable behavioral effects when infused into the same locus [31,33,34]. All animals were treated in strict accordance with guidelines set forth in the PHS manual, *Guide for the Care and Use of Laboratory Animals*.

2.2. Extracellular recording

The electrophysiological studies were made on day 11–14 after the onset of the BDNF infusion. This time point was chosen to allow comparison with previous neurochemical and behavioral studies of BNDF on the serotonergic system [31,32]. All recording experiments were performed with the investigators blind to the treatment groups. Rats were anesthetized with urethane (1.3 g/kg, i.p.) and placed in a stereotaxic frame. Small burr holes were drilled to allow placement of stimulating electrodes in the neostriatum (from bregma: AP 0.5, L 3.4, D −4.1 from cortical surface) and the medial forebrain bundle (AP −4.1, L 1.3, D −7.8) for antidromic identification of dorsal raphé serotonergic neurons [29]. For recording from the dorsal raphé a burr hole approximately 4 mm × 3 mm was drilled over lambda and the sagittal sinus was ligated, cut, and reflected.

Extracellular single units were recorded with glass micropipettes pulled from 2.0 mm capillary glass (WPI, Sarasota, FL) on a Narishige PE-2 pipette puller (Narishige Sci. Inst., Tokyo). Microelectrodes were filled with 1 M NaCl and possessed in vitro impedances of 4–10 MΩ. Electrical stimulation applied during the search for serotonergic neurons (5.3–5.7 mm below the cortical surface) consisted of monophasic square wave pulses (0.2–2.5 mA, 0.5 ms duration, 0.67/s). All data were recorded on magnetic tape for off-line analysis. Filter settings were 100 Hz for low pass and 30 kHz for high pass. Analyses of spike waveform and duration were made with a Nicolet 4094C (Nicolet Instruments Co., Madison, WI) oscilloscope interfaced to a Macintosh II computer by averaging 10 action potentials acquired at the filter settings described above. Spontaneous activity was analyzed for firing rate and pattern with first order interspike interval (ISI) histograms, calculation of the coefficient of variation of the first order interspike intervals, and autocorrelograms with a National Instruments MIO16L multifunction board and a Macintosh IIfx computer as previously described [39]. Firing rates, interspike intervals, and coefficients of variation of ISIs were analyzed with a two-tailed, unpaired *t*-test. Data are reported as mean ± S.E.M.

2.3. Neuronal identification

Serotonergic neurons were identified according to previously described electrophysiological criteria [26,29,41], which included a regular spontaneous firing rate (0.1–3 spikes/s), a 2–5 ms di- or triphasic extracellular waveform, and long latency antidromic responses to neostriatal or medial forebrain bundle stimulation. Some neurons representing typical recordings of presumed serotonergic and non-serotonergic neurons were tested for inhibition of spontaneous firing by intravenous injection of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), a selective 5-HT_{1A} autoreceptor agonist [12,17], and by the reversal of this inhibition by the antagonist, pindolol (1-[1H-indol-4-yloxy]-3-[isopropylamino]-2-propanol, 1–2 mg/kg i.v. [13]). 8-OH-DPAT was administered at 0.48 μg/kg/min until a total inhibition of firing was obtained.

2.4. Histology

At the end of each experiment the recording site was marked by a small lesion created by passing 20–30 μA through the recording electrode for 20–30 min. Rats were given a lethal overdose of urethane and perfused with 0.9% saline followed by 4% paraformaldehyde and 0.2% glutaraldehyde in 0.15 M phosphate buffer (pH 7.4) and the brains were removed.

3. Results

Extracellular recordings were obtained from a total of 99 dorsal raphé serotonergic neurons: 31 from vehicle-treated rats and 68 from BDNF-treated rats.

All neurons included in this report displayed waveforms typical of serotonergic neurons [29]. Two representative examples are shown in Fig. 1. All consisted of a 2–5 ms wide biphasic positive-negative (Fig. 1A and C) or triphasic positive-negative-small positive spike (not shown), that sometimes exhibited an inflection on the initial positive phase or in the negative component (Fig. 1A). There was

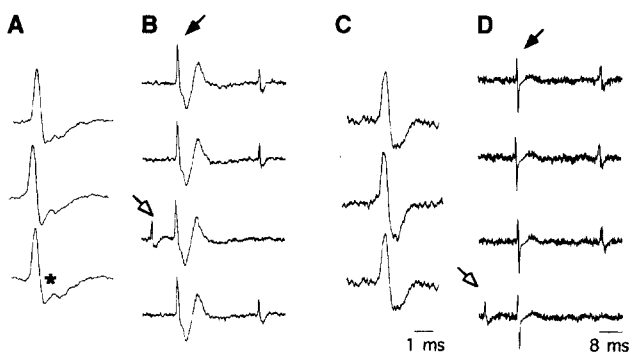

Fig. 1. Antidromic identification and waveforms of extracellularly recorded spontaneous spikes from two typical serotonergic neurons recorded in the DRN. A and C: biphasic waveforms. Note inflections on waveforms marked with asterisk in A. B, D: electrical stimulation of neostriatum evokes antidromic responses at long latencies for the neurons shown in A and C. In all figures, positivity is up. The stimulus artifact is labeled with a solid arrow and spontaneous spikes causing collision extinction are marked with an open arrow.

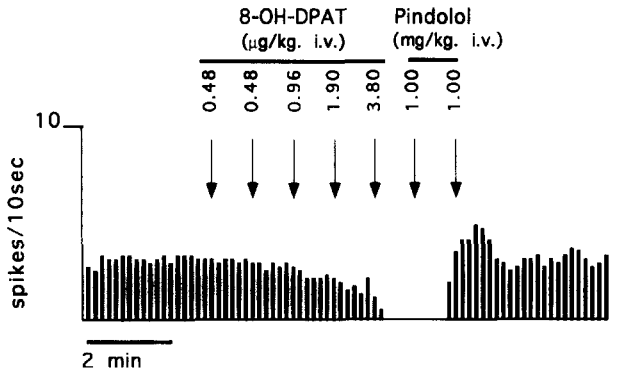

Fig. 2. The effect of intravenous administration of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, and antagonist pindolol, on the spontaneous firing of a representative serotonergic neuron. The concentration of 8-OH-DPAT was doubled each minute (0.48, 0.48, 0.96, 1.9, and 3.8 μg/kg), until total inhibition of spontaneous firing was achieved. Subsequent administration of the 5-HT antagonist, pindolol (2×1 mg/kg, i.v.) reversed the inhibition.

no difference in the frequency of these types of waveform encountered between the vehicle- and BDNF-treated neurons.

Fourteen of 99 neurons (14%) were antidromically activated by electrical stimulation from the neostriatum (25.6 ± 1.3 ms) and 6 of 99 neurons (6%) responded antidromically to stimulation of the medial forebrain bundle (7.4 ± 0.6 ms) with latencies similar to previously published values for serotonergic neurons [26,29] (Fig. 1B, D). The latencies for antidromic responses from neostriatum were similar for the neurons from vehicle-infused rats (25.5 ± 1.2; $n = 9$) and BDNF-infused rats (25.9 ± 3.0; $n = 5$).

Four antidromically activated neurons, presumed to be serotonergic on the basis of their waveform and firing pattern, and three additional neurons, presumed to be non-serotonergic, were checked for a dose-dependent inhibition of the spontaneous firing by intravenous injection of 8-OH-DPAT (increasing doses ranging from 0.48–3.8 μg/kg), and the reversal of this suppression by pindolol ($n = 2$). Fig. 2 shows a representative putative serotonergic neuron. Each of the four neurons presumed to be serotonergic that were tested was completely inhibited by 8-OH-DPAT, while the three presumed non-serotonergic neurons were unaffected (data not shown).

The mean firing rate of the serotonergic neurons was

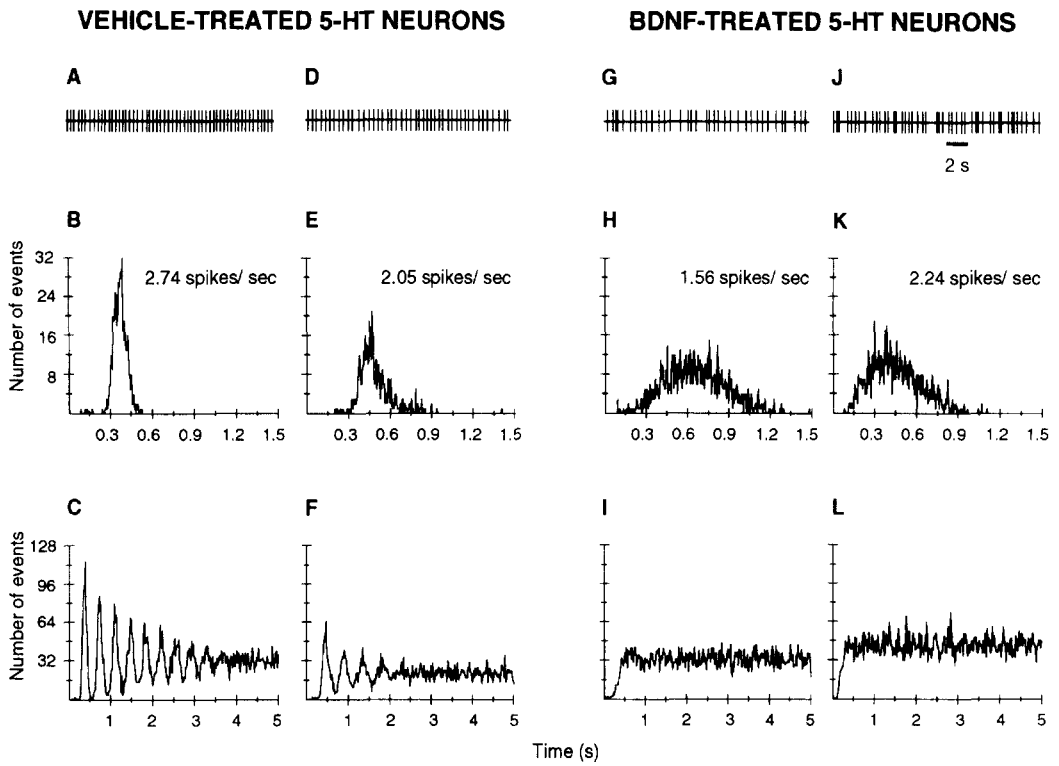

Fig. 3. Typical spontaneous activity for 5-HT neurons from rats which received 11–14 days of infusion of PBS vehicle (12 μl/day; A–C and D–F) or BDNF (12 μg/day; G–I and J–K). A and B: spike train from two representative vehicle-treated 5-HT neurons. G and J: spike train from two representative BDNF-treated 5-HT neurons. Note the less regular pattern of firing in the BDNF-treated neurons. B and E: first order interval histograms from the same vehicle-treated neurons. Mean interspike interval (ISI) (mean ± S.D.): 360 ± 50 and 487 ± 118 ms respectively. H and K: first order interval histograms from the same BDNF-treated neurons. Mean ISI: 643 ± 233 and 447 ± 233 ms respectively. For these neurons the coefficients of variation are 0.14 and 0.24 for the vehicle-treated 5-HT neurons and 0.36 and 0.41 for the BDNF-treated neurons. Bin width = 5 ms. Autocorrelation histograms from the same neurons. C and F: vehicle-treated neurons. I and L: BDNF-treated neurons. Note the regularly spaced peaks in histogram (C and F) which identify a pacemaker-like firing pattern. Bin width = 20 ms.

Table 1
Spontaneous serotonergic neuronal activity in dorsal raphé nucleus from rats which received 11–14 days of continuous infusion of PBS vehicle (12 μl/day) or BDNF (12 μg/day)

	Vehicle-treated 5-HT neurons	BDNF-treated 5-HT neurons
n	31	68
Firing rate (spikes/s)	1.93 ± 0.13	1.72 ± 0.09
Interspike interval (ISI) (ms)	606 ± 46	690 ± 35
Coefficient of variation of ISI	0.29 ± 0.01	0.38 ± 0.01 [a]

Values shown are mean ± S.E.M. The coefficient of variation of the interspike interval (ISI) is defined as standard deviation/mean.
[a] Signifies significantly ($t = -4.22$, df = 92, $P < 0.0001$) different from coeficient of variation of vehicle-treated 5-HT neurons.

similar in the vehicle- and BDNF-infused groups (Table 1). However, the spontaneous firing pattern of the BDNF-treated neurons (Fig. 3G and J) was significantly less regular than that of the vehicle-treated neurons (Fig. 3A and D), as determined by analysis of first order interval histograms and autocorrelograms of these neurons. The first order interval histograms show a tight distribution of the ISI around the mean of vehicle-infused rats (Fig. 3B and E). However, the coefficient of variation of the interspike interval was significantly greater in the BDNF-infused rats ($t = -4.22$ df = 92, $P < 0.0001$, Fig. 3H and K; Table 1). The rhythmic peaks in the autocorrelogram from the vehicle-infused rats (Fig. 3C and F) indicate a typical 5-HT neuron that fires in a pacemaker-like mode with very constant ISIs. The BDNF-treated neurons produced autocorrelograms that are typical of neurons firing in a more random mode (Fig. 3I and K).

4. Discussion

The present study is the first demonstration of the effect of the neurotrophic factor, BDNF, on the electrical activity of serotonergic neurons. The electrophysiological properties of the presumed serotonergic neurons analyzed in this paper are consistent with the electrophysiological characteristics of serotonergic neurons reported in previous studies [5,10,29,40]. These characteristics include a slow spontaneous firing rate (0.1–3 spikes/s), an extremely regular pacemaker-like rhythm (in the control animals), an extracellular action potential waveform of 2–5 ms duration, long-latency antidromic responses to neostriatal and medial forebrain bundle stimulation, inhibition of spontaneous unit activity after intravenous injection of the serotonergic agonist 8-OH-DPAT [12,17], and the reversal of this inhibition by pindolol [13]. Dorsal raphé cells with these characteristics have been identified as serotonergic by combinations of electrophysiological, histochemical, and pharmacological techniques [1–4,26]. The types of waveforms observed belonged to neurons that could be antidromically activated by electrical stimulation from neostriatum, again with a latency in accord with previous reports [29].

Approximately one-third of the neurons in the dorsal raphé nucleus (DRN) are serotonergic [11,36] but the majority of dorsal raphé projections to the neostriatum are serotonergic [9,29]. It is important to note that non-serotonergic raphé-striatal projections have also been identified by anatomical and electrophysiological methods [29,35,42]. These non-serotonergic neurons can be discriminated from serotonergic neurons on the basis of their differing electrophysiological properties, for example, the opposite polarity of the extracellularly recorded waveforms [29,41]. The first component of the waveform of serotonergic neurons is positive, whereas that of the non-serotonergic neuron is negative. In addition, the spike duration of the non-serotonergic neurons is shorter than that of serotonergic neurons, and the spontaneous firing pattern of non-5-HT neurons is clearly more irregular than vehicle or BDNF-treated serotonergic neurons recorded in the present study. Thus, by all criteria available with extracellular recordings, the neurons we report on in the present study were serotonergic and the effect of BDNF on firing pattern cannot be explained by a preferential recording of non-5-HT neurons in BDNF versus vehicle-treated animals.

Previous studies have shown that administration of BDNF increases serotonin turnover following midbrain [31,32], intracerebroventricular [28,32], or supranigral delivery [7,23]. Central BDNF administration also produces analgesia and an antidepressant-like effect [31,33] which may be mediated by increases in the synaptic activity of 5-HT. However, microdialysis experiments have not detected an effect of midbrain-infused BDNF on extracellular 5-HT levels (M. Fritsche and C.A. Altar, unpublished observations). It is possible that techniques other than microdialysis may be required to observe the effects of BDNF on the ongoing activity of serotonergic or other neurotransmitter systems. For example, using microdialysis in the striatum, we have also not detected alterations in extracellular dopamine levels during chronic supranigral infusions of BDNF, even though these infusions elevate striatal dopamine metabolism, augment spontaneous behaviors and behavioral responses to (+)-amphetamine, and increase the accumulation of 3-methoxytyramine, a sensitive measure of dopamine release [6,7,23]. Likewise, microdialysis studies in the neostriatum fail to show significant increases in dopamine overflow when stimulation of the MFB is delivered in a burst pattern compared to equally spaced pulses [38]. Thus, measurements of electrical activity within identified nuclei may more readily identify neurotrophin effects on specific neurotransmitter populations than microdialyis measurements of extracellular neurotransmitter contents.

BDNF decreased the regularity of the spontaneous activity of serotonergic dorsal raphé neurons without concomitantly changing the mean firing rate or other measures

of electrical activity. Similar effects were observed in dopaminergic neurons following chronic infusions of BDNF above the substantia nigra [30]. However, in dopaminergic neurons, the tendency toward increased bursting was also accompanied by a 35% increase in the mean firing rate and a doubling of the number of cells encountered per track.

It is possible that the failure to note a significant change in the mean firing rate of dorsal raphé serotonergic neurons following BDNF treatment, despite the fact that similar treatment produced an increase in serotonin turnover [31,32], was due to the fact that the recordings were obtained from anesthetized rats. However, the slow and regular firing pattern of central serotonergic neurons is ubiquitous among neurons recorded from the dorsal raphé nucleus in mice, cats and rats, whether they are freely moving, unanesthetized but immobilized, or anesthetized with urethane, chloral hydrate or chloralose [19]. In one study, dorsal raphé serotonergic neurons were recorded in cat and monitored during the induction of chloral hydrate anesthesia [16]. Although anesthesia produces significant changes in the responsivity of serotonergic neurons to external and pharmacological stimuli [37] there was only a very modest decrease in firing rate and no apparent change in the firing pattern of dorsal raphé serotonergic neurons after anesthesia was induced [16]. Thus, although we cannot rule out the possibility that anesthesia masked an increase in spontaneous firing rate caused by chronic administration of BDNF, it does not appear likely.

The relationship of the changes in firing pattern but not frequency to the release of serotonin remains uncertain. However, a growing body of recent literature suggests that increases in neurotransmitter release can be correlated with an increase in the bursting pattern of monoamine neurons. Thus, the pattern of firing might be as significant a variable as the mean firing frequency in the presynaptic modulation of monoamine release [14,15]. Thus, the results of the present study suggest that the less regular firing pattern and increased tendency towards bursting caused by BDNF may contribute to the elevation in serotonin turnover previously reported [29,30] as well as the elevated nociceptive thresholds and antidepressant-like effects associated with midbrain infusions of BDNF [31,33,34].

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References

[1] Aghajanian, G.K., Foote, W.E. and Sheard, M.H., Action of psychotogenic drugs on single midbrain raphé neurons, *J. Pharmacol. Exp. Ther.*, 171 (1970) 178–187.

[2] Aghajanian, G.K. and Haigler, H.J., L-Tryptophan as a selective histochemical marker for serotonergic neurons in single-cell recording studies, *Brain Res.*, 81 (1974) 364–372

[3] Aghajanian, G.K. and VanderMaelen, C.P., Intracellular recordings from serotonergic dorsal raphé neurons: pacemarker potentials and the effect of LSD, *Brain Res.*, 238 (1982) 463–469.

[4] Aghajanian, G.K. and VanderMaelen, C.P., Intracellular identification of central noradrenergic and serotonergic neurons by a new double labeling procedure, *J. Neurosci.*, 2 (1982) 1786–1792.

[5] Aghajanian, G.K., Wang, R.Y. and Baraban, J., Serotonergic and non-serotonergic nerons of the dorsal raphé: reciprocal changes in firing induced by peripheral nerve stimulation, *Brain Res.*, 153 (1978) 169–175.

[6] Altar, C.A., Boylan, C.B., Jackson, C., Lindsay, R.M. and Hyman, C., Brain-derived neurotrophic factor augments rotational behavior and nigrostriatal dopamine turnover in vivo, *Proc. Natl. Acad. Sci. USA*, 89 (1992) 11347–11351.

[7] Altar, C.A., Boylan, C.B., Fritsche, M., Jackson, C., Lindsay, R.M. and Hyman, C., The neurotrophins NT-4/5 and BDNF augment serotonin, dopamine, and GABAergic systems during behaviorally effective infusions to the substantia nigra, *Exp. Neurol.*, 130 (1995) 31–40.

[8] Altar, C.A., Criden, M.R., Lindsay, R.M. and DiStefano, P.S., Characterization and topography of high affinity [^{125}I] neurotrophin-3 binding to mammalian brain, *J. Neurosci.*, 13 (1993) 733–743.

[9] Azmitia, E.C. and Segal, M., An autoradiographic analysis of the differential ascending projections of the dorsal and median raphé nuclei in the rat, *J. Comp. Neurol.*, 179 (1978) 641–668.

[10] Bramwell, G.J., Factors affecting the activity of 5-HT-containing neurones, *Brain Res.*, 79 (1974) 515–519.

[11] Descarries, L., Watkins, K.C., Garcia, S., Beaudet, A., The serotonin neurons in nucleus raphé dorsalis of adult rat: a light and electron microscope radioautographic study, *J. Comp. Neurol.*, 207 (1982) 239–254.

[12] Fletcher, A., Bill, D.J., Bill, S.J., Cliffe, I.A., Dover, G.M., Forster, E.A., Haskins, J.T., Jones, D., Mansell, H.L. and Reilly, Y., WAY100135: a novel, selective antagonist at presynaptic and postsynaptic 5-HT$_{1A}$ receptors, *Eur. J. Pharmacol.*, 237 (1993) 283–291.

[13] Gehlbach, G. and VanderMaelen, C.P., Pindolol blocks the inhibitory effect of Gepirone, a 5-HT$_{1A}$ agonist, on the firing of serotonergic dorsal raphé neurons in the rat brain slice, *Soc. Neurosci. Abstr.*, 13 (1987) 1649.

[14] Gonon, F.G., Non linear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry, *Neuroscience*, 24 (1988) 19–28.

[15] Gonon, F.G., Suaud-Chagny, M.F., Mermet, C.C. and Buda, M., Relation between impulse flow and extracellular catecholamine levels as studied by in vivo electrochemistry in CNS. In K. Fuxe and L.F. Agnati (Eds) *Volume Transmission in the Brain : Novel Mechanisms for Neural Transmission*, Raven Press, New York, 1991, pp. 337–350.

[16] Heym, J., Steinfels, G.F. and Jacobs, B.L., Chloral hydrate anesthesia alters the responsiveness of central serotonergic neurons in the cat, *Brain Res.*, 291 (1984) 63–72.

[17] Hjorth, S.A. and Magnusson, T., The 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT, preferentially activates cell body 5-HT autoreceptors in rat brain in vivo, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 338 (1988) 463–471.

[18] Hofer, M., Pagliusi, S.R., Hohn, A., Leibrock, J. and Barde, Y.A., Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain, *EMBO J.*, 9 (1990) 2459–2464.

[19] Jacobs, B.L., Heym, J. and Steinfels, G.F., Physiological and behavioral analysis of raphe unit activity, In L.L. Iversen, S.D. Iversen and S.H. Snyder (Eds.), *Handbook of Psychopharmacology Vol. 18*, Plenum Press, New York, 1984, pp. 343–395.

[20] Lindsay, R.M., Weigand, S.J., Altar, C.A. and DiStefano, P.S., Neurotrophic factors: From molecules to man, *Trends Neurosci.*, 17 (1994) 182–190.

[21] Maisonpierre, P.C., Belluscio, L., Friedman, B., Alderson, R.F., Wiegand, S.J., Furth, M.E., Lindsay, R.M. and Yancopoulos, G.D., NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression, *Neuron*, 5 (1990) 501–509.

[22] Mamounas, L.A., Blue, M.E., Siuciak, J.A. and Altar, C.A., BDNF promotes the survival and sprouting of serotonergic axons in rat brain, *J. Neurosci.*, 15 (1995) 7929–7939.

[23] Martin-Iverson, M.T., Todd, K.G. and Altar, A., Brain-derived neurotrophic factor and neurotrophin-3 activate striatal dopamine and serotonin metabolism and related behaviors: interactions with amphetamine, *J. Neurosci.*, 14 (1994) 1262–1270.

[24] Merlio, J.P., Ernfors, P., Jaber, M. and Persson , H., Molecular cloning of rat *trk*C and distribution of cells expressing messenger RNAs for members of the *trk* family in the rat central nervous system, *Neuroscience*, 51 (1992) 513–532.

[25] Mufson, E.J., Kroin, J.S., Sobreviela, T., Burke, M.A., Kordower, J.H., Penn, R.D. and Miller, J.A., Intrastriatal infusions of BDNF: Regrograde transport and colocalization with dopamine containing substantia nigra neurons in rat, *Exp. Neurol.*, 129 (1994) 15–26.

[26] Park, M. Imai, R. and Kitai, H., Morphology and intracellular responses of an identified dorsal raphe projection neuron, *Brain Res.*, 240 (1982) 321–326.

[27] Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, Academic Press, Sydney, 1982.

[28] Pelleymounter, M.A., Cullen, M.J. and Wellman, C.L., Characteristics of BDNF-induced weight loss, *Exp. Neurol.*, 131 (1995) 229-238.

[29] Sawyer, S.F., Tepper, J.M., Young, S.J. and Groves, P.M., Antidromic activation of dorsal raphé neurons from neostriatum: physiological characterization and effects of terminal autoreceptor activation, *Brain Res.*, 332 (1985) 15–28.

[30] Shen, R., Altar, C.A. and Chiodo, L.A., Chronic supranigral infusion of BDNF increases the electrophysiological activity of pars compacta dopamine neurons, *Proc. Natl. Acad. Sci. USA*, 91 (1994) 8920–8924.

[31] Siuciak, J.A., Altar, C.A., Wiegand, S.J. and Lindsay, R.M., Antinociceptive effect of brain-derived neurotrophic factor and neurotrophin-3, *Brain Res.*, 633 (1994) 326–330.

[32] Siuciak, J.A., Boylan, C.B., Fritsche, M. Altar, C.A. and Lindsay, R.M., BDNF increases monoaminergic activity in rat brain following intracerebroventricular or intraparenchymal administration, *Brain Res.*, in press.

[33] Siuciak, J.A., Wiegand, S.J. and Lindsay, R.M., Antidepressant-like effect of brain-derived neurotrophic factor (BDNF), *Neuropsychopharmacology*, 10 Suppl. (1994) 187S.

[34] Siuciak, J.A., Wong, V., Pearsall, D., Wiegand, S.J. and Lindsay, R.M., BDNF produces analgesia in the formalin test and modifies neuropeptide levels in rat brain and spinal cord areas associated with nociception, *Eur. J. Neurosci.*, 7 (1995) 663–670.

[35] Steinbusch, H.W.M., van der Kooy, D., Verhofstad, A.A.J. and Pellegrino, A., Serotonergic and non-serotonergic prejections from the nucleus raphé dorsalis to the caudate-putamen complex in the rat, studied by a combined immunofluorescence and fluorescent retrograde axonal labeling technique, *Neurosci. Lett.*, 19 (1980) 137–142.

[36] Steinbusch, H.W.M. and Nieuwenhuys, R., The raphé nuclei of the rat brainstem: a cytoarchitectonic and immunohistochemical study. In P.C. Emson (Ed.), *Chemical Neuroanatomy*, Raven Press, New York, 1984, pp. 131–207.

[37] Tao, R. and Auerbach, S.B., Anesthetics block morphine-induced increases in serotonin release in rat CNS, *Synapse*, 18 (1994) 307–314.

[38] Tepper, J.M., Creese, I. and Schartz, D.H., Stimulus-evoked changes in neostriatal dopamine levels in awake and anesthetized rats measured by microdialysis, *Brain Res.*, 559 (1991) 283–292.

[39] Tepper, J.M., Trent, F. and Nakamura, S., Postnatal development of the electrical activity of rat nigrostriatal dopaminergic neurons, *Dev. Brain Res.*, 54 (1990) 21–33.

[40] VanderMaelen, C.P. and Aghajanian, G.K, Electrophysiological and pharmacological characterization of serotonergic dorsal raphé neurons recorded extracellularly and intracellularly in rat brain slices, *Brain Res.*, 289 (1983) 109–119.

[41] Wang, R.Y. and Aghajanian, G.K., Antidromically identified serotonergic neurons in the rat midbrain raphé: evidence for collateral inhibition, *Brain Res.*, 132 (1977) 186–193.

[42] Wang, Q.-P. and Nakai, Y., The dorsal raphé: An important nucleus in Pain modulation, *Brain Res. Bull.*, 34 (1994) 575–585.

[43] Wetmore, C., Cao, Y., Pettersson, R.F. and Olson, L., BDNF: Subcellular compartmentalization and internuronal tranfer as visualized with anti-peptide antibodies, *Proc. Natl. Acad. Sci. USA*, 88 (1991) 9843–9847.

[44] Wetmore, C., Ernfors, P., Persson, H. and Olson, L., Localization of brain derived neurotrophic factor mRNA to neurons in the brain by in situ hybridization, *Exp. Neurol.*, 109 (1990) 141–152.

[45] Wiegand, S.J., Alexander, C., Lindsay, R.M. and DiStefano, P.S., Axonal transport of [^{125}I]-labeled neurotrophins in the central nervous system, *Soc. Neurosci. Abstr.*, 17 (1991) 1121.

[46] Yan, Q., Matheson, C., Sun, J., Radeke, M.J., Feinstein, S.C. and Miller, J.A., Distribution of intracerebroventricularly administratered neurotrophins in rat brain and its correlation with Trk receptors expression, *Exp. Neurol.*, 127 (1994) 23–36.