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Research report

Neonatal olfactory sensory deprivation decreases BDNF in the olfactory bulb of the rat

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Abstract

We hypothesized that brain-derived neurotrophic factor (BDNF) may be down-regulated in the olfactory bulb ipsilateral to experimental naris occlusion. Unilateral naris occlusion was performed on rats at postnatal day three (P3). On P10, P30, and P60 olfactory bulbs were weighed and assayed for tyrosine hydroxylase (TH), BDNF, and TrkB by Western blotting to determine the response of BDNF and its cognate receptor, TrkB, both during the acute phase of sensory loss (P10) and longer term. TH levels, which are highly dependent on intact input from the olfactory epithelium, were assayed as a means of determining the success of occlusion in each animal. At P10, BDNF protein expression was variable but most often increased ipsilateral to deprivation. In contrast, by P30 and P60 TH levels were found to be significantly decreased in the ipsilateral bulbs as were the levels of BDNF. TrkB protein levels changed little relative to the control side. Immunohistochemical localization of BDNF within the control-side olfactory bulb revealed small cells located mainly in the mitral cell layer and internal plexiform layer. Very few of the BDNF immunoreactive cells were visible in the bulb ipsilateral to the occlusion by P30. Given the roles of BDNF in survival of cells and plasticity during development, the decrease in BDNF expression subsequent to olfactory sensory deprivation may contribute to cellular and synaptic deficits observed by others following olfactory sensory deprivation.

Theme: Development and regeneration

Topic: Expression and regulation of trophic factors: neurotrophins

Keywords: Olfactory bulb; Sensory deprivation; Brain-derived neurotrophic factor; Tyrosine hydroxylase; TrkB; Western blots; Immunohistochemistry

1. Introduction

When naris occlusions are performed on rats before postnatal day 10 (P10), size differences of 25% are common between the two olfactory bulbs by P30 [5]. The sensory deprivation afforded by the occlusion provides loss of activity in the olfactory bulb due to decreased synaptic discharge of extant olfactory nerve axons. The fact that there is such a profound effect on development of the olfactory bulb with the deprivation suggests that developmental events are interrupted.

Previous studies have indicated that olfactory sensory deprivation in the neonate rat leads to cell death in the ipsilateral olfactory bulb [13,26]. A subsequent study found that the cell death is due to apoptosis mainly in the glomerular and granule cell layers of the bulb [29]. Thus, the interruption in development could be the result of a factor or factors that are essential to normal development.

Neurotrophins are widely regarded as having an important role in cell survival, differentiation and growth. Brain-derived neurotrophic factor (BDNF) has been found to have a number of developmental influences on the brain including cell differentiation and survival [18]. Brain survey studies using immunohistochemistry, in situ hybridization [8,36] and enzyme immunoassay [16] have revealed that BDNF is present in the olfactory bulb. In addition, immunohistochemistry has revealed that the high affinity receptor for BDNF, TrkB, is present in many granule cells [14], mitral cells [35] and periglomerular cells [14] or dendrites [14,35] within the bulb. Thus, TrkB would be expected to help mediate the signaling of BDNF within many cells of the bulb. Given the purported roles of

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BDNF, a change in BDNF expression within the bulb during development could produce profound changes in the olfactory bulb. The object of this study was to examine the short- and long-term effects of olfactory sensory loss in the neonate rat on olfactory bulb development, especially regarding the expression of BDNF.

2. Materials and methods

2.1. Animals

All procedures were approved by the Institutional Animal Care Committee at Memorial University of Newfoundland which follows the guidelines of the Canadian Council on Animal Care. The subjects were Sprague–Dawley rats housed at room temperature in animal care facilities of the medical school. The litters were culled to 12 pups/litter in this study.

2.2. Occlusions

Unilateral naris occlusion was performed on P3 rats (P1 being the day of birth). Hypothermia was used to anesthetize the pups before the occlusion was begun. Naris occlusion was performed using a surgical cauterizing instrument as described previously [25]. Only those animals with an obviously closed naris at the time of sacrifice were used for analysis. Naris occlusion inactivates, without destroying, the input from the olfactory epithelium to the bulb [9].

For Western blots, rats were sacrificed on P10, 30 and 60. The rats were rendered unconscious by placing them in a chamber filled with CO_2 and within 1 min were sacrificed by decapitation. The olfactory bulbs were separated from the brain by making a transverse cut through the caudal part of the bulb anterior to the frontal cortex. In Nissl-stained sections, this level contains olfactory bulb with no elements of the anterior olfactory nucleus. Each bulb was then quickly frozen by dry ice, weighed, and stored in microcentrifuge tubes at −70°C. For immunohistochemistry, animals were anaesthetized at P30 or P40 and sacrificed by transcardial perfusion as described previously [21,23].

2.3. Western blotting

2.3.1. Sample preparation

This was performed essentially as we described earlier [20,37]. Briefly, equal weights of protein (100 μg) from each sample were separated on the basis of gel mobility using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Minigels were prepared in acrylamide concentrations of 7.5% (w/v) for TrkB and TH or 15% (w/v) for BDNF. Stacking gels (4% w/v) were used in all cases.After the transfer of the protein from gels to nitrocellulose blots, the blots were processed for detection of specific proteins or placed in Ponceau S to visualize the total amount of protein transferred. Only those blots that showed equal protein levels for paired bulbs were subsequently analyzed.

2.3.2. Immunoblotting

Rabbit anti-BDNF (1/500 dilution, Santa Cruz Biotechnology) was raised against a peptide corresponding to residues 128–147 located at the C-terminus of human BDNF. We have shown in Western blots that this antibody is specific to denatured BDNF because the 14-kDa band is absent in BDNF knockout mice [11]. As a positive control, recombinant human BDNF (rhBDNF) was purchased from Alomone Labs (Israel).

To determine the success of sensory deprivation, expression of tyrosine hydroxylase (TH) was visualized with a rabbit antibody (diluted 1/2000, Eugene Technology) since sensory deprivation produces decreased expression of TH in dopaminergic periglomerular cells of the bulb [3,9]. Positive control for TH was obtained using cultured pheochromocytoma cell lysate (PC12, cell line expressing TH, provided by Dr Karen Mearow). Visualization of TrkB was obtained using an antibody (diluted 1/500, Transduction Laboratories) which recognizes residues 155–318, corresponding to a portion of the extracellular domain and is common to both the truncated ($TrkB_{trunc}$) and full length ($TrkB_{full}$) forms. The remaining processing of blots was as described previously [20,37].

2.3.3. Molecular weights of bands for BDNF, TH, $TrkB_{trunc}$ and $TrkB_{full}$

The specific protein bands were identified based on their molecular weights specified by the antibody manufacturer. The approximate band site for denatured BDNF was at 14 kDa, TH at 58 kDa, $TrkB_{full}$ at 145 kDa, and $TrkB_{trunc}$ at 95 kDa. The bands were verified as being the correct protein by comparison with the positive control run on each gel. A second antibody to the full length form of TrkB (Santa Cruz) was used in one experiment to confirm the band of the Transduction Labs antibody which marks $TrkB_{full}$. Both antibodies recognized the same 145 kDa band so the antibody made by Transduction Labs was used in subsequent experiments.

2.3.4. Image analysis

An image analysis system (Bioquant, R&M Biotechnology) was used to obtain optical density of the bands and surrounding background region in order to quantitate levels of proteins in the relevant bands. Relative optical density was calculated for each band [20,24,37]. Changes in protein were normalized by comparing the protein amount on the side ipsilateral to manipulation to the control side and presenting the comparison as a percentage of control. Analysis of each pair of bulbs was always performed using the same blot in order to keep exposure times using

enhanced chemiluminescence standardized for each pair. One sample *t*-tests were used to determine if proteins on the experimental side were significantly different from the control side.

2.4. Immunohistochemistry

Antibodies to BDNF (rabbit, 1/500 dilution, kindly provided by Dr Q. Yan, Amgen), TrkB (Santa Cruz, 1/500 dilution), TH (Eugene Technology, 1/1000 dilution), GFAP (DiaSorin, 1/200 dilution), GABA (DiaSorin, 1/1000 dilution), and microglia (OX-42, Cedar Lane Ltd. Inc., 1/500 dilution) were used for immunohistochemistry or immunofluorescence. Further processing of brain sections and visualization using diaminobenzidine or immunofluorescence were described previously [21,23]. It is important to note that the BDNF antibody from Amgen has been previously shown to be specific for BDNF as its staining was absent using immunohistochemistry in homozygote (−/−) BDNF knockout mice [36]

3. Results

Selection of olfactory bulbs to be utilized for Western blot analysis was based on qualitative analysis of naris occlusions so that only clearly naris-occluded rats were chosen for analysis. Weight measurements of olfactory bulbs helped confirm visual impressions of olfactory bulb atrophy on the side ipsilateral to occlusion in older animals. Selection criteria for analysis included the requirement that protein loading of each lane was equivalent as determined by comparing total protein content in nitrocellulose blots (Ponceau S visualization) for each control and experimental side pairing. In addition, only those blots with clear band quality were used. Decisions to accept or reject a blot for analysis were unbiased as the examiner did not know the origin of each lane. Examples of the Western blots for TH, BDNF and TrkB at P60 are shown in Fig. 1.

Rats sacrificed on P30 and P60 displayed apparently normal tissue covering the occluded nares thereby preventing the entry of air. Because the results at P10 were somewhat different from that at P30 and P60, the descriptions below are grouped accordingly.

3.1. Postnatal day 10

3.1.1. Olfactory bulb morphology

Nares occlusions at P10 were inconsistent. Some nares became patent which is consistent with the literature [25]. Thus, the olfactory bulbs ipsilateral and contralateral to naris occlusion of rats sacrificed on P10 did not demonstrate significant difference in weight (Fig. 2A) although there was often a visual impression that the ipsilateral bulb was smaller.

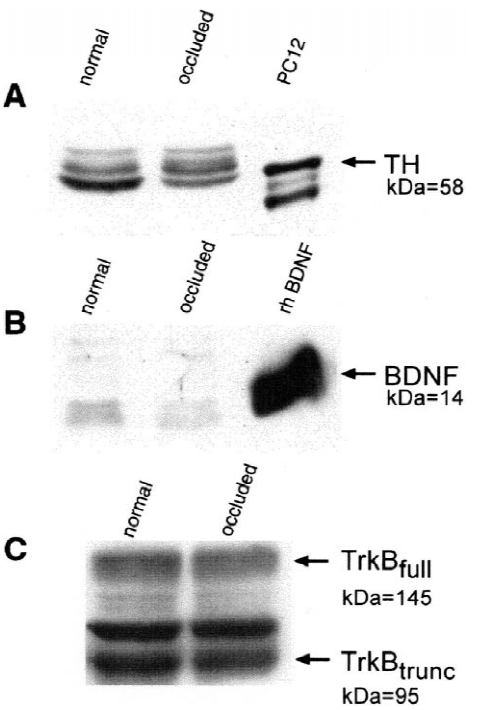

Fig. 1. Western blots. Visualization of Western blots using enhanced chemiluminescence to visualize (A) tyrosine hydroxylase (TH), (B) BDNF, and (C) full length ($TrkB_{full}$) or truncated ($TrkB_{trunc}$) forms of TrkB in P60 rats following unilateral naris occlusion in the neonate rat. Each lane represents the protein expression in the olfactory bulb from one animal. PC 12 cells were used as positive control for the TH expression while human recombinant BDNF was used as the positive control for BDNF. The bands corresponding to TH and BDNF (arrows) show decrease in optical density ipsilateral to the occlusion.

3.1.2. Protein levels of tyrosine hydroxylase (TH), brain-derived neurotrophic factor (BDNF) and TrkB at P10

TH levels measured by Western blots were not significantly decreased at P10 (Fig. 2A). Overall, there was no significant difference in BDNF content between the bulbs at P10 (Fig. 2A) but on an individual animal basis, six of eight comparisons showed the 14 kDa BDNF band ipsilateral to the occlusion to be increased 150–200% relative to the control side. The TrkB levels were not significantly changed although $TrkB_{full}$ tended to be increased ipsilateral to the naris occlusion (Fig. 2A).

3.2. Postnatal day 30 and postnatal day 60

3.2.1. Olfactory bulb morphology

Naris occlusion in rats with longer survival times (i.e. sacrifice at P30 and P60) was considerably more consistent than that observed in the rats sacrificed at P10. The unilateral closure of the naris in the older animals agreed with the significantly decreased weight of the ipsilateral bulb (Fig. 2).

3.2.2. Levels of TH, BDNF and $TrkB_{full}/TrkB_{trunc}$

In keeping with the consistency of the successful

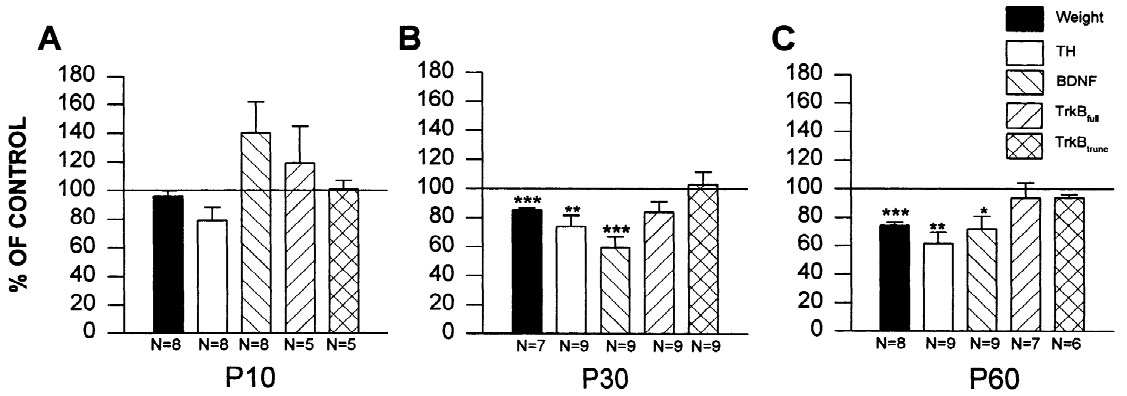

Fig. 2. Analysis of Western blots. The effects naris occlusion of the rat pup were analyzed by quantitating olfactory bulb weights, or expression of TH, BDNF, $TrkB_{full}$ or $TrkB_{trunc}$ at P10, 30 and 60. In each case the bulb on the experimental side was compared to that on the control side which was normalized to 100%. A one-sample *t*-test was used for statistical analysis. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

occlusions and comparatively smaller olfactory bulbs ipsilateral to naris closure, the TH levels in the bulb were significantly decreased on the occluded side at P30 ($t=3.875$, $P<0.01$) and P60 ($t=4.968$, $P<0.01$) (Fig. 2B, C). TH levels were either not changed or decreased in the ipsilateral bulbs in nine out of nine comparisons at P30 and eight of nine at P60.

BDNF was markedly affected by occlusions. At P30, BDNF protein was decreased in eight of nine ipsilateral bulbs which was extremely significant ($t=5.546$, $P<0.001$) (Fig. 2B). At P60, eight of nine pups exhibited decreased BDNF in the ipsilateral bulb ($t=3.155$, $P<0.05$) (Fig. 2C).

Despite the decrease in BDNF levels, the TrkB levels were not significantly affected by P30 and P60 (Fig. 2B,C). There was only a tendency for $TrkB_{full}$ to be decreased ipsilateral to occlusions.

3.3. Immunohistochemistry

The expression of BDNF was examined in seven rats that each had a naris occlusion at P1 or 2 until the time of sacrifice at P30–40. On the contralateral (control) side, in each case there was a predominant band of BDNF-immunoreactive cells located especially in the internal part the mitral cell layer of the bulb (Fig. 3B). A few lightly stained BDNF immunoreactive cells were also observed in the granule cell layer and other, less identifiable processes, were observed in the external plexiform and glomerular layers. No such label was observed when the primary antibody was omitted. Most of the labelled cells were lightly stained with few visible processes.

Several cells had a dendritic process oriented towards the external plexiform layer similar to granule cells while others were oriented horizontally. The cells ranged from 6 to 13 μm in long axis compared to 16–20 μm long mitral cells that were observed in adjacent Nissl sections. Judging by the size and position of the BDNF immunoreactive cells and comparisons to other Golgi impregnation studies [31] the BDNF immunoreactive cells appear to be Cajal, horizontal and granule cells.

On the side ipsilateral to naris occlusion, there was substantial decrease in the immunohistochemical expression of TH in periglomerular cells which is indicative of successful occlusion and is in keeping with numerous other studies. The BDNF cellular label was obviously diminished on the deprived side (Fig. 3A vs. B). TrkB immunohistochemistry revealed presence of the receptor both contralateral (Fig. 3D) and ipsilateral (Fig. 3C) to naris occlusion. The label was observed especially in granule cells and processes with the antibody we used.

In order to identify any other chemical phenotype of the BDNF immunoreactive cells, double-label immunohistochemistry was employed. We observed no BDNF colocalization with GABA (inhibitory interneurons), GFAP (astrocytes) or OX-42 (microglia). Thus, the BDNF immunoreactive cells had the shape characteristics of interneurons but their transmitter phenotype remains unknown.

4. Discussion

The results of this study show that BDNF expression in the olfactory bulb is substantially down-regulated by P30–P60 following occlusion of the naris in neonate rats. At the earliest age observed, P10, there was considerable variation of BDNF levels with a substantial trend towards increase on the occluded side. The quantitative results observed using Western blot analysis were elaborated qualitatively using immunohistochemistry which showed appreciable decrease of BDNF expression in small non-mitral cells located mainly within the mitral cell/internal

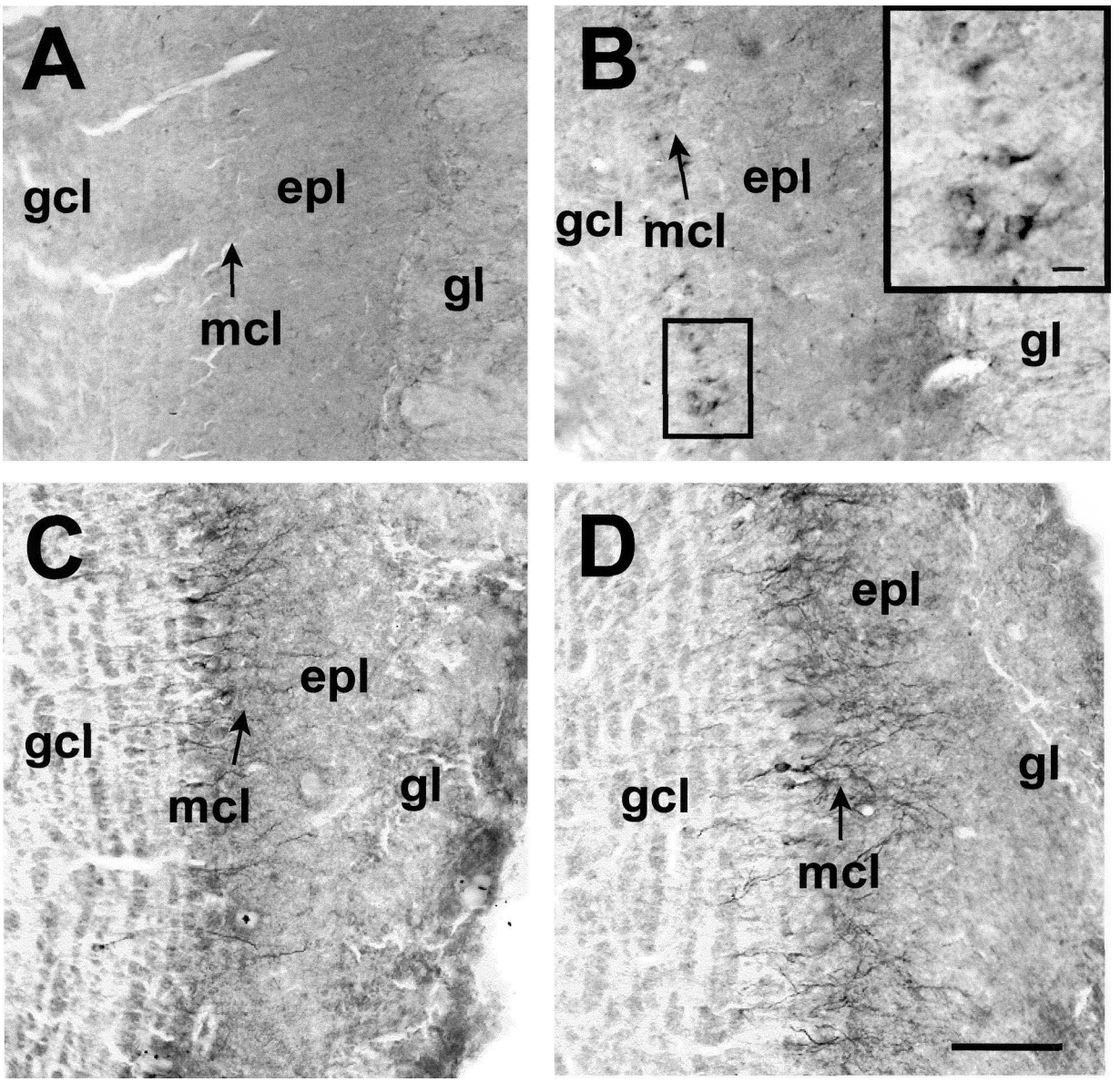

Fig. 3. Immunohistochemistry. Digitized brightfield images display the immunohistochemical localization of BDNF (A, B) and Trk B (C,D) ipsilateral (A,C) and contralateral (B, D) to a naris occlusion. The expression of BDNF immunoreactivity at P40 in small cells located in or near the mitral cell layer (mcl) was substantially reduced while TrkB immunoreactivity was not overtly changed after occlusion in the neonate. Bar in D, 100 μm. Bar in the inset of B, 10 μm. Abbreviations: gl, glomerular layer; epl, external plexiform layer; gcl, granule cell layer.

plexiform layer interface following neonatal naris occlusion.

4.1. Sensory deprivation and the olfactory bulb

4.1.1. Effect on morphology

Except at P10, the olfactory bulbs were consistently decreased in size ipsilateral to naris occlusion. This observation is consistent with other studies [5]. In contrast, occlusions performed on rats after P10 do not result in a significant decrease in the size of the olfactory bulb or constituent layers [6]. Thus, the sensory deprivation is most effective when performed on a neonate rat and, it may be argued, reflect interruption of developmental events.

4.1.2. Effect on tyrosine hydroxylase expression

TH expression in the olfactory bulb has been used for several years as an indicator of the integrity of the olfactory epithelium input to the bulb. Most studies in the past have used either biochemical (HPLC) analysis [2] or immunohistochemistry [1,22,28] to show decrease in TH following sensory deprivation or chemical deafferentation. This decrease has been shown to be in dopaminergic

periglomerular cells that receive input from the olfactory epithelium.

The present study showed that Western blot analysis is also an efficient means of determining the overall expression of TH in the olfactory bulb.

4.2. Regulation of BDNF levels

There was considerable variation in BDNF levels at P10 in pups with naris occlusions. This variation may reflect the fact that the naris has not been closed very long or that at P10 there may still be some air reaching the olfactory epithelium through an incomplete naris occlusion. The variability of BDNF levels in naris-occluded pups at P10 could mean that there is a transient period when BDNF is increasing in some animals in an effort to overcome the negative effects of olfactory deprivation. In summary, there appears to be an unknown element in some animals with naris closure that produces a substantial transient increase (up to 200%) in BDNF in the olfactory bulb.

In the present study, the decrease in BDNF in the bulb with longer survival is tied to an environmental factor such as sensory deprivation. The decreased olfactory stimulation would obviously decrease neurotransmitter activity in the bulb with subsequent decrease in intracellular signaling pathways. Evidence in the hippocampus suggests that neuronal activity mediated by non-NMDA glutamate receptors regulates synthesis of BDNF [38]. It is noteworthy that the olfactory nerve mediates its effect on mitral cells of the olfactory bulb via NMDA and non-NMDA receptors [10]. Thus, changes in neurotransmitter activation could have consequences for BDNF expression although the precise mechanisms in the bulb need to be determined.

In other sensory systems, BDNF expression is also regulated by the integrity of sensory input in the developing brain. In the visual system, monocular deprivation leads to reduced BDNF mRNA expression in the visual cortex [4,17,32]. Similarly, in the somatosensory system, vibrissae removal, which affects somatosensory input to cortical barrel fields of rodents, decreases BDNF mRNA expression [33]. Thus, the down regulation of BDNF following different types of sensory deprivation may be a universal occurrence in the developing animal.

4.3. Potential roles of BDNF and TrkB

Sensory deprivation initiated before P10 results in substantial loss of neurons in the bulb [26], probably due to cell death [13] by apoptosis of periglomerular and granule cells [29]. In other neuronal systems, BDNF is involved in cell survival, differentiation and plasticity. Therefore, the increased apoptotic cell death in olfactory bulbs ipsilateral to occlusion could be the result of a down-regulation of BDNF. It is intriguing that if the naris occlusion is reversed in the juvenile rat after sensory deprivation of the neonate, there is a recovery of olfactory bulb size due to increased cell proliferation in the first days after reopening of the naris [9]. A enticing follow-up study would be to determine if BDNF is up-regulated during this phase of recovery of the bulb since a factor that enhances cell proliferation would be expected to be increased during that time period.

More direct evidence of a role for BDNF in cell survival in the olfactory bulb can be gleaned from BDNF infusion studies. In other cortical regions, BDNF can prevent cell shrinkage [15] or cell death following axonal injury [15,34]. Thus there is a good probability that BDNF plays a similar role in the sensory-deprived olfactory bulb. In a different vein, unilateral infusion of BDNF into the lateral ventricle increases the number of newly generated neurons in the olfactory bulb [39]. Thus, besides cell survival, another role of BDNF in the olfactory bulb may be in the genesis of new neurons.

An unexpected observation of this study was that there was no consistent change in TrkB expression following olfactory deprivation. Prolonged BDNF infusion into the olfactory bulb of the adult rat produces decreased expression of TrkB protein [12]. From this, one might have speculated that the decreased BDNF observed in the present study would have lead to increased TrkB protein levels in the bulb but this was not observed. Nevertheless, our results are in keeping with those observed in the visual system where monocular deprivation in the neonate results in decreased BDNF mRNA without a change in TrkB mRNA [4]. Although the TrkB protein or messenger RNA levels do not appear to change with sensory deprivation, it is possible that other components of TrkB signal transduction such as phosphorylation of TrkB [40] could be affected by the decreased BDNF.

In summary, BDNF levels were most often reduced in the ipsilateral bulbs of naris-occluded rats when compared with their contralateral counterparts. This difference was significant when observations were made in P30 and P60 rats. These observations provide indirect support to the hypothesis that BDNF levels are tied to developmental events of the olfactory bulb. The hypothesis that BDNF decrease causes the gross morphological and cellular decrease in the bulb requires more study. The observed changes in the present study of BDNF within the olfactory bulb following sensory deprivation in the neonate could provide a model for sensory deprivation in the CNS during early periods of critical development. In humans, early global sensory deprivation leads to subtle, yet measurable, behavioral and cognitive problems in children [7,27,30]. Study of neurotrophins in animal sensory deprivation models could, as suggested by others, lead to methods of treating learning disorders and other developmental disorders in children [19].

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References

[1] H. Baker, T. Kawano, V. Albert, T.H. Joh, D.J. Reis, F.L. Margolis, Olfactory bulb dopamine neurons survive deafferentation-induced loss of tyrosine hydroxylase, Neuroscience 11 (1984) 605–615.

[2] H. Baker, T. Kawano, F.L. Margolis, T.H. Joh, Transneuronal regulation of tyrosine hydroxylase expression in olfactory bulb of mouse and rat, J. Neurosci. 3 (1983) 69–78.

[3] H. Baker, K. Morel, D.M. Stone, J.A. Maruniak, Adult naris closure profoundly reduces tyrosine hydroxylase expression in mouse olfactory bulb, Brain Res. 614 (1993) 109–116.

[4] Y. Bozzi, T. Pizzorusso, F. Cremisi, F.M. Rossi, G. Barsacchi, L. Maffei, Monocular deprivation decreases the expression of messenger RNA for brain-derived neurotrophic factor in the rat visual cortex, Neuroscience 69 (1995) 1133–1144.

[5] P.C. Brunjes, Unilateral naris closure and olfactory system development, Brain Res. Rev. 19 (1994) 146–160.

[6] P.C. Brunjes, M.J. Borror, Unilateral odor deprivation: Differential effects due to time of treatment, Brain Res. Bull. 11 (1983) 501–503.

[7] S.A. Cermak, L.A. Daunhauer, Sensory processing in the postinstitutionalized child Sensory processing in the postinstitutionalized child, Am. J. Occup. Ther. 51 (1997) 500–507.

[8] J.M. Conner, J.C. Lauterborn, Q. Yan, C.M. Gall, S. Varon, Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: Evidence for anterograde axonal transport, J. Neurosci. 17 (1997) 2295–2313.

[9] D.M. Cummings, H.E. Henning, P.C. Brunjes, Olfactory bulb recovery after early sensory deprivation, J. Neurosci. 17 (1997) 7433–7440.

[10] M. Ennis, L.A. Zimmer, M.T. Shipley, Olfactory nerve stimulation activates rat mitral cells via NMDA and non-NMDA receptors in vitro, NeuroReport 7 (1996) 989–992.

[11] J.P. Fawcett, R. Aloyz, J.H. McLean, S. Pareek, F.D. Miller, P.S. McPherson, R.A. Murphy, Detection of brain-derived neurotrophic factor in a vesicular fraction of brain synaptosomes, J. Biol. Chem. 272 (1997) 8837–8840.

[12] L. Frank, S.J. Wiegand, J.A. Siuciak, R.M. Lindsay, J.S. Rudge, Effects of BDNF infusion on the regulation of TrkB protein and message in adult rat brain, Exp. Neurol. 145 (1997) 62–70.

[13] L.L. Frazier-Cierpail, P.C. Brunjes, Early postnatal cellular proliferation and survival in the olfactory bulb and rostral migratory stream of normal and unilaterally odor-deprived rats, J. Comp. Neurol. 289 (1989) 481–492.

[14] R.H. Fryer, D.R. Kaplan, S.C. Feinstein, M.J. Radeke, D.R. Grayson, L.F. Kromer, Developmental and mature expression of full-length and truncated trkB receptors in the rat forebrain, J. Comp. Neurol. 374 (1996) 21–40.

[15] K.M. Giehl, W. Tetzlaff, BDNF and NT-3, but not NGF, prevent axotomy-induced death of rat corticospinal neurons in vivo, Eur. J. Neurosci. 8 (1996) 1167–1175.

[16] R. Katoh Semba, I.K. Takeuchi, R. Semba, K. Kato, Distribution of brain-derived neurotrophic factor in rats and its changes with development in the brain, J. Neurochem. 69 (1997) 34–42.

[17] E.S. Lein, C.J. Shatz, Rapid regulation of brain-derived neurotrophic factor mRNA within eye-specific circuits during ocular dominance column formation, J. Neurosci. 20 (2000) 1470–1483.

[18] G.R. Lewin, Y.-A. Barde, Physiology of the neurotrophins, Annu. Rev. Neurosci. 19 (1996) 289–317.

[19] B. Lu, A. Figurov, Role of neurotrophins in synapse development and plasticity, Rev. Neurosci. 8 (1997) 1–12.

[20] J.H. McLean, C.W. Harley, A. Darby-King, Q. Yuan, pCREB in the neonate rat olfactory bulb is selectively and transiently increased by odor preference-conditioned training, Learn. Mem. 6 (1999) 608–618.

[21] J.H. McLean, M.T. Shipley, Serotonergic afferents to the rat olfactory bulb: I. Origins and laminar specificity of serotonergic inputs in the adult rat, J. Neurosci. 7 (1987) 3016–3028.

[22] J.H. McLean, M.T. Shipley, Postmitotic, postmigrational expression of tyrosine hydroxylase in olfactory bulb dopaminergic neurons, J. Neurosci. 8 (1988) 3658–3669.

[23] J.H. McLean, M.T. Shipley, D.I. Bernstein, Golgi-like, transneuronal retrograde labelling with CNS injections of herpes simplex virus type 1, Brain Res. Bull. 22 (1989) 867–881.

[24] J.H. McLean, M.T. Shipley, D.I. Bernstein, D. Corbett, Selective lesions of neural pathways following viral inoculation of the olfactory bulb, Exp. Neurol. 122 (1993) 209–222.

[25] E. Meisami, Effects of olfactory deprivation on postnatal growth of the rat olfactory bulb utilizing a new method for production of neonatal unilateral anosmia, Brain Res. 107 (1976) 437–444.

[26] E. Meisami, R. Mousavi, Lasting effects of early olfactory deprivation on the growth, DNA, RNA and protein content and Na–K-ATPase and AChE activity of the rat olfactory bulb, Dev. Brain Res. 2 (1982) 217–229.

[27] S.J. Morison, E.W. Ames, K. Chisholm, The development of children adopted from Romanian orphanages, Merrill-Palmer Q. 41 (1995) 411–430.

[28] N.S. Nadi, R. Head, M. Grillo, J. Hempstead, N. Grannot-Reisfeld, F.L. Margolis, Chemical deafferentiation of the olfactory bulb: Plasticity of the levels of tyrosine hydroxylase, dopamine and norepinephrine, Brain Res. 213 (1981) 365–377.

[29] J. Najbauer, M. Leon, Olfactory experience modulates apoptosis in the developing olfactory bulb, Brain Res. 674 (1995) 245–251.

[30] The English and Romanian Adoptees (ERA) Study Team, M. Rutter, Developmental catch-up, and deficit, following adoption after severe global early privation, J. Child Psychol. Psychiatry 39 (1998) 465–476.

[31] S.P. Schneider, F. Macrides, Laminar distributions of interneurons in the main olfactory bulb of the adult hamster, Brain Res. Bull. 3 (1978) 73–82.

[32] A.A. Schoups, R.C. Elliott, W.J. Friedman, I.B. Black, NGF and BDNF are differentially modulated by visual experience in the developing geniculocortical pathway, Dev. Brain Res. 86 (1995) 326–334.

[33] T.D. Singh, K. Mizuno, T. Kohno, S. Nakamura, BDNF and trkB mRNA expression in neurons of the neonatal mouse barrel field cortex: normal development and plasticity after cauterizing facial vibrissae, Neurochem. Res. 22 (1997) 791–797.

[34] Q. Yan, C. Matheson, O.T. Lopez, J.A. Miller, The biological responses of axotomized adult motoneurons to brain-derived neurotrophic factor, J. Neurosci. 14 (1994) 5281–5291.

[35] Q. Yan, M.J. Radeke, C.R. Matheson, J. Talvenheimo, A.A. Welcher, S.C. Feinstein, Immunocytochemical localization of TrkB in the central nervous system of the adult rat, J. Comp. Neurol. 378 (1997) 135–157.

[36] Q. Yan, R.D. Rosenfeld, C.R. Matheson, N. Hawkins, O.T. Lopez, L. Bennett, A.A. Welcher, Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system, Neuroscience 78 (1997) 431–448.

[37] Q. Yuan, C.W. Harley, J. Bruce, A. Darby-King, J.H. McLean, Isoproterenol increases CREB phosphorylation and olfactory nerve-evoked potentials in normal and 5-HT-depleted olfactory bulbs in rat pups only at doses that produce odor preference learning, Learn. Mem. 7 (2000) 413–421.

[38] F. Zafra, B. Hengerer, J. Leibrock, H. Thoenen, D. Lindholm, Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors, EMBO J. 9 (1990) 3545–3550.

[39] T. Zigova, V. Pencea, S.J. Wiegand, M.B. Luskin, Intraventricular

administration of BDNF increases the number of newly generated neurons in the adult olfactory bulb, Mol. Cell Neurosci. 11 (1998) 234–245.

[40] U. Zirrgiebel, Y. Ohga, B. Carter, B. Berninger, N. Inagaki, H. Thoenen, D. Lindholm, Characterization of TrkB receptor-mediated signaling pathways in rat cerebellar granule neurons: involvement of protein kinase C in neuronal survival, J. Neurochem. 65 (1995) 2241–2250.