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Differential effects of ocular BDNF-injections onto the development of tectal cells characterized by calcium-binding proteins in pigeons

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Abstract

The optic tectum of vertebrates bears a set of visual neurons which can be differentiated by the expression of distinct calcium-binding proteins (CaBPs). Using immunohistochemistry, we mapped the distribution of the CaBPs calbindin (CB) and parvalbumin (PV) in the pigeon's optic tectum and examined if their differentiation is affected by retinal brain-derived neurotrophic factor (BDNF)-injections. CB-immunoreactive (ir) and PV-ir cells displayed a lamination pattern which differed from other birds. While PV-ir cells were present in several retinorecipient tectal laminae, CB-ir cells were confined to layer 3 and 5 and – as a specialization of pigeons – were also detected in a subpopulation of layer 13 neurons. Comparison of saline- and BDNF-injected animals revealed that this general expression pattern was not affected by ocular BDNF-injections. In contrast, the size of tectal cells was differentially modulated. While CB-ir cells in layers 3 and 13 were unaffected by retinal BDNF, cells in layer 5 developed enlarged cell bodies. The PV-ir cells displayed smaller soma sizes within both tectal hemispheres suggesting also an indirect effect of retinal BDNF. These data indicate a differential sensitivity of tectal cell types to retinal BDNF, which might be one mechanism by which retinal input modulates tectal circuitries.

Keywords: Asymmetry; Birds; Calbindin; Neurotrophic factor; Parvalbumin

1. Introduction

The optic tectum of vertebrates is an essential relay station for visuomotor control and it bears a conserved set of cell types comprising topographically ordered input from the eyes and an output that reaches, among other targets, premotor hindbrain regions [17]. Due to differential dendritic arborization within retinorecipient layers 2 up to 8 [5], subclasses of tectal neurons process different visual information [5] and are hence, integrated into different functional circuits [6]. Little is known about the mechanisms regulating this specialization. Apart from endogenous factors, retinal input which is known to be involved in the maturation of visual neurons [18] might be involved in these processes. Calcium-binding proteins (CaBPs) like parvalbumin (PV) or calbindin (CB) characterize neuronal subpopulations within the tectum [15], delineating functionally distinct neuronal subcircuits [3]. Thus, their expression pattern can help to investigate the differentiation of distinct tectal cell types.

Tectal expression of PV and CB is partly regulated by retinal input. Retinal ablation depletes PV and CB in the pigeon's tectum [2] and light reduces cell body size of PV-immunoreactive (PV-ir) tectal cells [12]. Different aspects of light-dependent neuronal differentiation are mediated by the neurotrophic factor brain-derived neurotrophic factor (BDNF) [21]. Visual stimulation adjusts the expression and/or release of BDNF and hence, regulates the trophic support of target cells even in an anterograde manner [1,14]. Accordingly, retinal BDNF could play a prominent role in tectal differentiation. This can be tested in the altricial pigeon which hatches with an immature visual system [11]. BNDF and its high-affinity receptor TrkB is present in the developing retinotectal system with a dramatic increase in BDNF expression directly after hatching [20] and at least differentiation of PV-ir cells occurs only after hatching [12]. Thus, we examined in how far retinal BDNF-injections affect cell body sizes of tectal cell types which are characterized by the expression of PV and CB.

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2. Material and methods

For the immunohistochemical characterization of PV- and CB-ir cells, adult pigeons (*Columba livia*) were perfused with icecold 4% paraformaldehyde. Brains were postfixed for 2 h and cryoprotected overnight at 4 °C. The brains were cryosectioned in frontal plane at 40 μm and sections were immunolabeled with antibodies against PV (monoclonal mouse IgG, Sigma; 1/1000) or CB (monoclonal mouse IgG, Swant; 1/2000) according to the ABC-method [12].

In a first step to test the role of BDNF in the regulation of the differentiation of PV- and CB-ir tectal cells, pigeon embryos were incubated in complete darkness. After hatching, 50 ng BDNF dissolved in 5 μl saline ($n = 6$) or 5 μl saline for control ($n = 6$) were injected into the right eye of pigeon hatchlings for three consecutive days. As adults (12–18 month after birth), the animals were perfused to perform a morphometric analysis of PV- and CB-ir tectal cells [12].

3. Results

Tectal layers displayed a sublamination with respect to immunoreactivity against PV and CB (Fig. 1). According to previous reports [12,19], PV-ir cells could be detected within layers 2–4, 6–10, while the whole optic tectum was filled with a network of PV-ir fibers. Radial dendrites of PV-ir cells could be observed ascending into the superficial layers 2–4 (Fig. 1A). CB-ir cells were present within layers 3, 5 and 13. Apart from the somatic staining, a dense fibre network could be detected within layers 3 and 5, primarily representing the horizontally oriented dendritic arbors of the intrinsic CB-ir cells since at least the CB positive cells in layer 5 have no axonal structures [10]. Within the efferent layer 13, a subpopulation of multipolar neurons was CB positive, whose dendrites were confined to layers 12 and 13 (Fig. 1B).

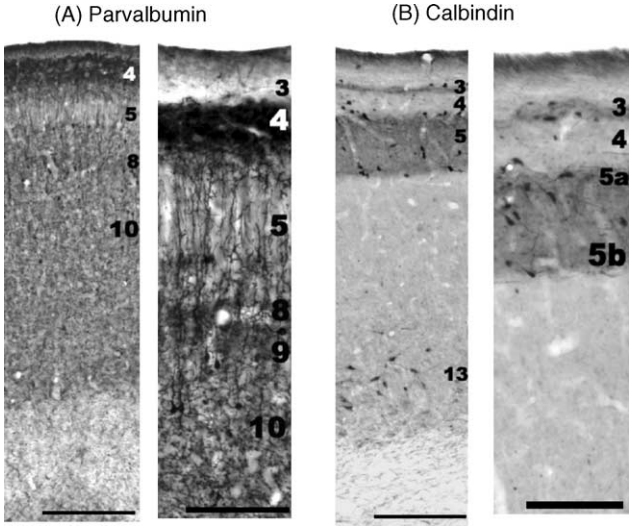

Fig. 1. (A) PV-ir cells were labelled within layers 2–4, 6, 8–10. (B) CB-ir cells could be detected within layers 3, 5 and 13. Bars represent 200 μm.

The immunohistochemical examination of BDNF- and saline-injected animals evinced no differences in the laminar organization of PV- and CB-ir cells. However, morphometric analysis demonstrated differential effects of intraocular BDNF-injections onto cell body sizes (Fig. 2). Compared to saline controls, the soma sizes of PV-ir cells in all tectal layers were reduced in both tectal hemispheres (Fig. 2A; Mann–Whitney U test: $Z = 2.355$, $p < 0.05$). In contrast, the cell bodies of CB-ir were enlarged in layer 5 but only in the BDNF-enriched left tectum (Fig. 2C; Mann–Whitney U tests: left tectum: $Z = -2.008$, $p < 0.05$; right tectum: $Z = -1.095$, $p = 0.273$) while the soma sizes of the layer 3 (Fig. 2B; Mann–Whitney U test: $Z = 0.104$, $p = 0.917$) and layer 13 (Fig. 2D; Mann–Whitney U test: $Z = -0.365$, $p = 0.715$) CB-ir cells were not affected.

4. Discussion

Consistent with studies in other vertebrates, e.g. [4,13,16], the pigeon's optic tectum displays a sublamination with respect to the localization of PV- and CB-ir cells. This pattern exhibits some deviations compared to other birds. So, PV-ir neurons are present in more layers than in chicken [16] or quail [7]. In contrast, the expression of CB in the retinorecipient tectal layers seems to be similar between these birds, except the CB-ir subpopulation of multipolar layer 13 neurons, which is only present in pigeons. Due to the restriction of their dendrites to layers 12 and 13, these cells do not receive direct retinal input and hence, might represent a subclass of the ascending population (type II; [5]) or a descending cell type [6]. Since the expression of CaBPs is assumed to be linked to the modulation of neuronal firing pattern [8], CB expression indicates a specialized functional role of these cells.

This principal laminar organization seems to be independent from retinal BDNF support. But comparison of saline- and BDNF-injected animals disclosed modulated soma sizes of PV- and CB-ir tectal cells. While the CB-ir efferent cells in layer 13 were not affected, neurons within the retinorecipient layers reacted differentially to intraocular BDNF although all of them are likely to represent tectal interneurons with direct retinal input. This suggests differential sensitivity to retinal BDNF, what might be related to a divergent embedding into functional tectal circuits. CB-ir cells in layer 3 did not display changed soma sizes. In contrast, CB-ir cells within layer 5 which presumably represent GABAergic neurons [10] developed larger cell bodies within the BDNF-enriched left tectum. Due to their direct synaptic contact with retinal fibers [10], this is likely to be a direct result of the growth-promoting effect of BDNF onto GABAergic cells [21]. On the other hand, all PV-ir tectal cells displayed decreased cell sizes in response to ocular BDNF-injections. This shrinkage provides evidence for an inhibitory influence of BDNF onto this cell population and is consistent with a suppressive effect of light onto PV expression in the mammalian superior colliculus [9].

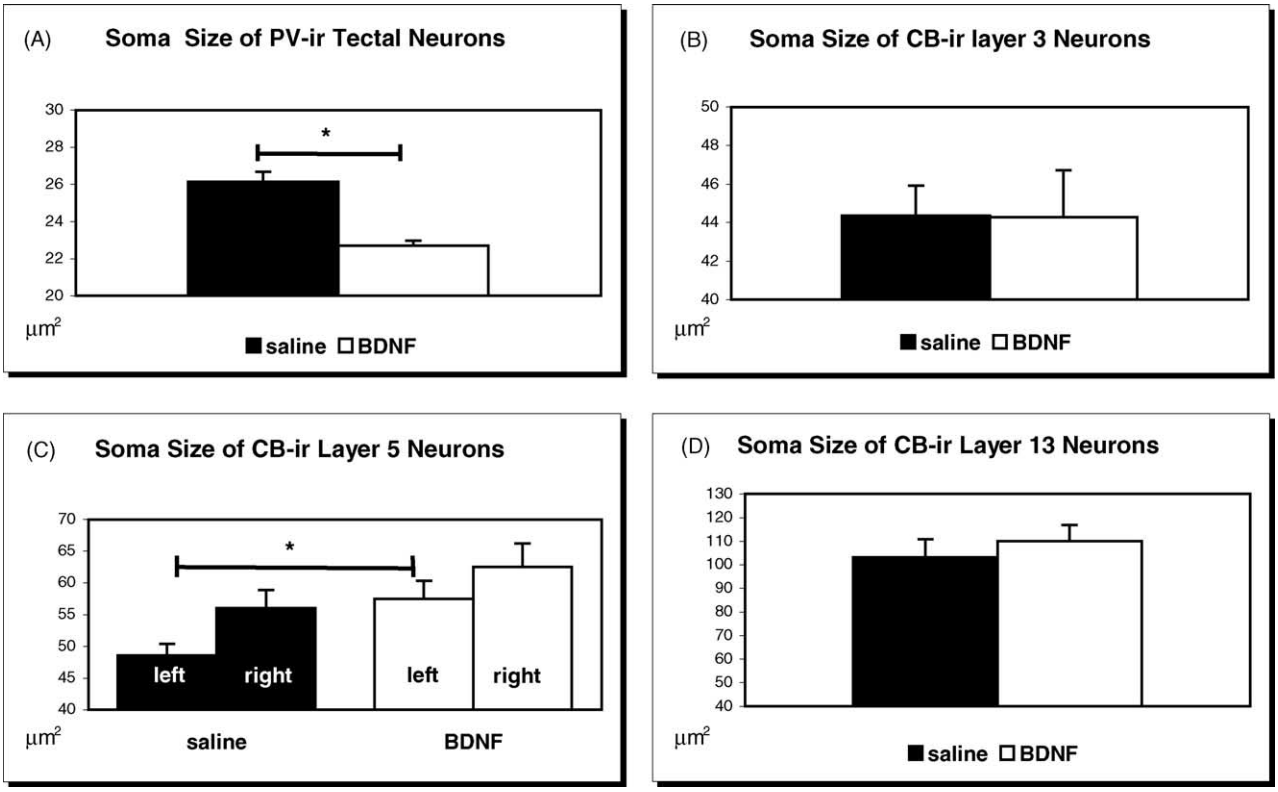

Fig. 2. Cell body size of PV-ir (A) and CB-ir (B–D) tectal cells. Bars represent standard error ($^{*}p < 0.05$ according to Mann–Whitney *U* tests).

However, inhibition was not confined to the BDNF enriched left tectum, but affected both tectal hemispheres. Bilateral effects were also observed after asymmetric embryonic light stimulation that led to smaller PV-ir cells within both tecta [12]. Thus, a direct influence of retinal input is not sufficient to explain the effect on differentiation of the PV-ir cell population. The bilaterality of light as well as BDNF effects suggests that intra- and/or interhemispheric inputs may play a critical role in the differentiation of PV-ir tectal cells.

In conclusion, the present data indicate that ocular BDNF-injections affect maturation of distinct subpopulations of tectal cells among those characterized by expression of PV and CB. Possible similar effects on other retinorecipient tectal cells were not investigated. Since the soma size of a neuron can be regarded as a marker for the extent of its axo-dendritic arborizations, different cell sizes indicate differential connectivity pattern. Thus, the differential sensitivity of tectal neurons to afferent trophic support might be one mechanism by which retinal input can shape the functional architecture of tectal circuits.

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