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Research report

A morphological study of the nucleus subpretectalis of the pigeon

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Abstract

In pigeons, the tectofugal system is functionally as well as structurally lateralized. So for example the right nucleus rotundus is less modulated by right forebrain influences than the left nucleus rotundus by the left ones. This functional lateralization pattern may depend on a dynamic balance between left and right tectal processing. Apart from inhibitory interactions at tectal level, suppressive influences might directly affect rotundal neurons by GABAergic input from a cluster of nuclei, the bed nuclei of the tecto-thalamic tract. A major afferent of these nuclei is the side branch of the tectorotundal projection which is of bilateral origin and which is involved in the regulation of ipsilateral as well as bilateral visual processing. Hence, an important role of the bed nuclei could be the interhemispheric communication and in turn the mediation of functional asymmetries. In a first step to unravel asymmetric influences of these nuclei, the present study investigated if the largest of the bed nuclei, the nucleus subpretectalis displays morphological asymmetries in the pigeon. We found that the nucleus subpretectalis in fact exhibits asymmetric cell sizes with larger cell bodies on the left side. This asymmetrical pattern was not present in dark-incubated animals indicating that cell size asymmetries within nucleus subpretectalis are induced by asymmetric photic stimulation during embryonic development.

Keywords: Visual asymmetries; Lateralization; Tectofugal system; Light incubation

1. Introduction

Pigeons are visually lateralized with a dominance of the right eye/left hemisphere for visual object analysis [5]. This functional asymmetry may be associated with morphological left–right differences in the tectofugal pathway, which transfers visual information via the mesencephalic optic tectum and the diencephalic nucleus rotundus to the forebrain [6,7]. The optic tectum displays cell size asymmetries with larger cell bodies in layers 2–12 on the left side [17], while neurons of the efferent layer 13 are enlarged on the right [5]. These efferent cells project bilaterally onto the nucleus rotundus whereby the crossed portion of this pathway is asymmetrically organized with more fibers ascending from the right optic tectum to the left nucleus rotundus than vice versa [9,10]. The higher bilateral input to the left nucleus rotundus is accompanied by larger cell bodies of rotundal relay neurons on this side [15]. These visual asymmetries develop in response to lateralized photic stimulation during embryonic development and thus, are not present in animals incubated in darkness [19].

Electrophysiological data show that the left as well as the right nucleus rotundus are more strongly affected by the left visual wulst than by the right one [4,22]. It is conceivable that such a weak right hemispheric modulation is the result of suppressive subtelencephalic interactions. Reversed lateralization patterns after lesions of the tectotectal commissure [8] indicate that the actual functional lateralization pattern depends on a dynamic balance between left and right tectal processing [18]. Another modulator of the lateralized processing could be the tectorotundal system, which is modulated by the bed nuclei of the tecto-thalamic tract, the largest of which is the SP (nucleus subpretectalis) [2,21] (Fig. 1). During integration of bilateral visual input at rotundal level, information from the ipsilateral eye is selectively inhibited by GABAergic fibers from this cluster of nuclei, thereby modulating the shift of attention from one eye to the other [23]. As such an attention shift may play a critical role in modulating functional lateralization, the present study is a first step to investigate if the SP is involved in the mediation

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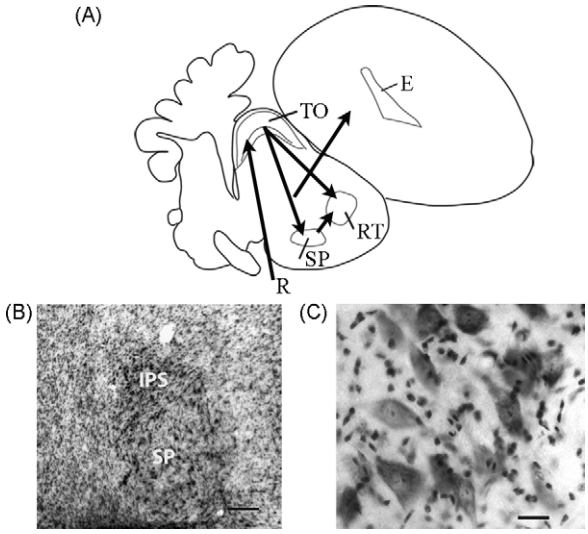

Fig. 1. (A) Schematic view of the tectofugal pathway showing the afferent and efferent projections of the nucleus subpretectalis (SP). E, entopallium; TO, optic tectum; R, retina; RT, nucleus rotundus; SP, nucleus subpretectalis. (B) Cresyl violet staining of nucleus subpretectalis (SP) and nucleus interstitio-pretecto-subpretectalis (IPS). Scale bar = 200 μm. (C) Cresyl violet stained neurons within nucleus subpretectalis. Scale bar = 5 μm.

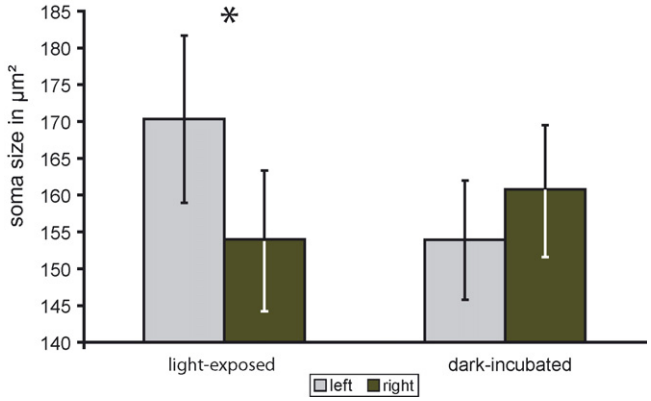

Fig. 2. Mean values for soma size differ in the light-exposed group with the left hemisphere showing larger soma size. Bars represent standard error. $^{*}p < 0.05$ according to post hoc test.

of functional asymmetries. Moreover, we investigate if SP cell sizes are affected by embryonic light stimulation by comparing cell sizes of light- and dark-incubated animals.

2. Materials and methods

Eighteen adult pigeons (*Columba livia*) of unknown sex were used in this study. The age of the animals differed between 2 and 5 years. Nine animals were obtained from local breeders. As pigeon pairs abandon their clutch from time to time these animals have been exposed to light before hatching [1]. The other nine animals were dark-incubated animals with parents coming from the same breeder as the light-exposed animals [16,19].

For morphometric analysis, animals were trascardially perfused with 4% paraformaldehyde. Brains were postfixed and subsequently cut in frontal plane at 40 μm on a freezing microtome and the slices were collected in PBS containing 0.1% sodium azide. After mounting, every second slice was stained with cresyl violet. For soma size measurements, the slices were encoded for experimental group and left and right hemisphere to ensure a blind analysis. In one section, the cross-sectional soma areas of 50 neurons within the SP along the complete dorsoventral dimension (stereotaxic level about A 4.75 [13]) (Fig. 1B and C) were measured in each hemisphere by means of the image analyzing system "analySIS" (Soft Imaging System, Münster, Germany) with 20× objective of a Leica DMR microscope [16].

Statistical analysis was performed with the statistic program Statistica 7.1 (StatSoft, Tulsa, USA). We compared left–right differences within and between the two groups by using a two factorial multivariate analysis of variance for repeated measurements (MANOVA: group × hemisphere). In case of a significant result of an overall analysis, we used Tukey's honestly significant difference (HSD) test for single comparisons.

Photographic documentation was carried out with a digital camera (Zeiss AxioCam). Digital images were processed with Axiovision 3.0 (Zeiss, FRG) and Photoshop 5.5 (Adobe, Mountain View, CA) where contrast and brightness were adjusted.

3. Results

The morphometric analysis of SP neurons revealed soma sizes with mean values varying between 108.71 μm^2 and 237.3 μm^2 for light-exposed group and between 110 μm^2 and 203.4 μm^2 for dark-incubated animals (Fig. 1C). The light-exposed animals showed a mean soma size of $170.35 \pm 11.32\ \mu m^2$ on the left side and $154 \pm 9.32\ \mu m^2$ on the right side. Mean soma size of the dark-incubated group was $153.94 \pm 8.03\ \mu m^2$ on the left side and $160.78 \pm 8.72\ \mu m^2$ on the right side (Fig. 2).

Cell size differences were analyzed by means of a two-factorial ANOVA for repeated measures. This analysis demonstrated no significant influence of either the factor hemisphere ($F = 1.616$; $p = 0.222$) or the factor group ($F = 0.142$; $p = 0.712$) but a significant interaction between both factors ($F = 9.629$; $p = 0.007$). Post hoc comparisons (Tukeys HSD test for repeated measurements) verified significant left–right differences in light-exposed animals ($p = 0.03$), with the right hemisphere showing 10% smaller cell sizes than the left hemisphere.

4. Discussion

The results of the present study clearly reveal morphological left–right differences in the SP of pigeons, which might be related to functional visual asymmetries.

The SP shows larger neurons in the left hemisphere than in the right one hence, displaying the same cell size asymmetry as the nucleus rotundus [15]. Both nuclei receive their major input from the bilateral projection of the optic tectum [11,12]. It is this projection that might induce morphological asymmetries of the nucleus rotundus since the left nucleus rotundus receives stronger bilateral input than the right one [9]. Although putative left–right differences in SP afferents have not yet been investigated, the parallel asymmetry in nucleus rotundus and SP suggest a similar asymmetry of tectal input.

In contrast, dark-incubated animals did not display cell size asymmetries between the left and right SP indicating that SP asymmetries are induced by asymmetric light stimulation during embryonic development. This is in accordance with previous results, which demonstrate light triggered functional [19] as well

as morphological asymmetries within optic tectum and nucleus rotundus [14–16].

Physiological and lesion studies show that functional lateralization patterns not only depend on static, structural asymmetries but also on a dynamic balance between left- and right-hemispheric processing [20]. The SP is in a position to play a key role in mediating this balance given its proposed involvement in the shift of attention between the eyes [3,18]. This shift might be mediated by inhibiting the neuronal input from the ipsilateral eye at rotundal level when the contralateral eye becomes activated [15,3]. Since cell size is regarded as an indicator for the complexity of cellular connections enlarged left hemispheric SP cells suggest that the left SP might lead to an increased suppression of input from the left eye and in turn to a functional strengthening of the right eye/left hemisphere.

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