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'Natural' and artificial monocular deprivation effects on thalamic soma sizes in pigeons

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THE dominance for visual pattern analysis of the left hemisphere in normal pigeons and the concomitant morphological asymmetries in the optic tectum can be attributed to a 'natural' prehatch monocular deprivation of the left eye resulting from an asymmetrical embryonic position within the egg. Using control animals and pigeons which were monocularly deprived for 10 days after hatching, the present study could show that the cellular soma sizes of the nucleus rotundus within the tectofugal visual pathway are modified by light experience depending on the timepoint and direction of lateralized stimulation. Although rotundal cell size is thus ontogenetically modified in an activity-dependent manner, a detailed comparison makes it likely that the mechanisms which govern developmental plasticity of visual pathways differ between birds and mammals. *NeuroReport* 10:3223–3228 © 1999 Lippincott Williams & Wilkins.

Key words: Lateralization; Morphological asymmetries; Plasticity; Tectofugal pathways

Introduction

Birds such as chicks or pigeons exhibit a dominance of the right eye/left hemisphere in visual pattern discrimination tasks [1,2]. This functional lateralization is accompanied by structural asymmetries in the two avian ascending visual pathways, the thalamo- and tectofugal visual system. In chicks, the right eye dominance is related to an asymmetry in the organization of the thalamofugal system ascending bilaterally from the diencephalic nucleus geniculatus lateralis, pars dorsalis (GLd) to the telencephalic visual wulst. In this pathway, the contralateral projection of the left GLd is more prominent than that of the right GLd [3,4]. In pigeons, on the contrary, morphological asymmetries are observed in the tectofugal system projecting to the forebrain via the mesencephalic tectum opticum and the diencephalic nucleus rotundus [1]. In addition to left-right differences in tectal soma size [5], the crossed portion of the tectorotundal projection is asymmetrically organized with more fibers ascending from the right tectum to the left rotundus than *vice versa* [6]. The resulting higher bilateral input to the left rotundus is in accordance with the dominance of the left hemisphere in visual discrimination tasks [7]. However, it is not known whether the asymmetrical visual input to the rotundus is associated with morphological asymmetries in this thalamic relay station.

The development of structural and functional asymmetries in birds is triggered by the epigenetic factor light. Avian embryos keep their head turned to the right so that the right eye is close to the translucent eggshell and the left eye is occluded by the body. This asymmetric position results in a stronger light stimulation of the right eye during the late embryonic phase. This natural monocular deprivation triggers the formation of lateralized visual circuits presumably by activity-dependent mechanisms [1,2,5]. During a sensitive period, which is confined to the embryonic phase in chicks [2] and which is extended into the post-hatching period in pigeons [8], manipulations of visual experience can modify functional as well as morphological asymmetries [2,8]. These monocular deprivation studies clearly show that lateralized visual input during a critical time span results in concomitantly altered asymmetries in the projections from both ascending visual pathways.

Monocular deprivation studies in mammals such as cats and monkeys have to some extent outlined the critical neural mechanisms which determine synaptic stabilization, perikaryal growth and subsequent visual performance of the geniculocortical system in response to environmental stimulation [9–11]. At the first glance, the general framework of the establishment of avian visual lateralization as a consequence of a 'natural' monocular deprivation resembles the mammalian pattern. However, many aspects of ontogenetic plasticity in the mammalian geniculocortical pathway are regarded as conse-

quences from competitive interactions between fibers carrying information by the two eyes [9,10]. Since the avian visual system is predominantly unilateral, a detailed analysis might reveal important points of deviation between avian and mammalian models. In this regard, the nucleus rotundus as the only station of the avian tectofugal pathway where bilateral visual input converges, represents a key structure for morphological comparisons. Therefore, this study represents an attempt to examine these basic questions regarding visual asymmetries in pigeons: is rotundal soma size asymmetric? Is the normal morphological rotundal pattern influenced by post-hatch monocular deprivation? Are the ontogenetic mechanisms which mediate light influences on maturation of visual pathways similar in birds and mammals? To find answers for these three questions we performed a morphometric analysis of rotundal soma size in normal and monocularly deprived pigeons.

Materials and Methods

Control (13 animals) and monocularly deprived animals (left eye deprived 10 animals, right eye deprived 12 animals) were used in the present study. Deprivation was performed by fixing a plastic cap on the right or left eye of young pigeons with a skin adhesive directly after hatching (Karaya paste, Hollister, Libertyville, USA). After 10 days, the caps were removed and the animals were raised until adulthood [8]. All birds were then deeply anesthetized with an overdose of equitesin (0.55 ml/100 g body weight) and perfused through the heart with 0.9% saline (40°C) followed by 4% paraformaldehyde (in 0.12 M phosphate buffer, 4°C, pH 7.2). The brains were removed and postfixed in 4% paraformaldehyde + 30% sucrose, cryoprotected in 0.12 M phosphate buffer + 30% sucrose at 4°C for 24 h and cryosectioned in frontal plane at 35 μm. Sections including nucleus rotundus were mounted on gelatinized slides and stained with cresylviolet.

In these preparates, the cross-sectional soma areas of 50 rotundal neurons in each diencephalic hemisphere were measured with the image analyzing system Analysis (SIS, Münster, Germany). The nucleus rotundus consists mainly of large and medium-sized multiangular principal neurons which constitute the efferent projection to the telencephalic ectostriatum. Very few interneurons of much smaller size are located between these principal neurons [12]. In order to examine a preferable homogenous cell population, only the principal neurons were analyzed in the present study and only cells which contained a clear nucleolus, a round and lightly colored nucleus and visible Nissl substance in the cytoplasm were included. The boundaries of these cells were drawn by tracking the image displayed on the video screen with a computer mouse. The display was obtained with a Kappa CF8 camera attached to an Olympus BH-2 microscope with a ×100 objective. The image analyzing system calculated the surface encircled. Measurements were performed at AP coordinates 6.0–6.25 (according to the atlas of Karten and Hodos [13]) in an area covering the whole mediolateral extent of the sagittal plane excluding nucleus triangularis. Left and right measurements were always performed at the same AP coordinate.

Results

The morphometric analysis of rotundal neurons revealed soma sizes ranging from 102 μm^2 to 590 μm^2. The mean values, varying between 242 μm^2 and 285 μm^2, differed between left and right rotundus and between the three groups (Fig. 1a). These differences were analyzed by means of a two-factorial analysis of variance for repeated measures (MANOVA: group × hemisphere). This analysis demonstrated a significant influence of the factor hemisphere ($F1/32 = 10.512$, $p < 0.01$) but not of group ($F2/32 = 0.061$, $p = 0.941$). The interaction between both factors was highly significant ($F1/32 = 20.905$, $p < 0.001$).

The three groups displayed a varying degree of differences between left and right rotundal soma size. While control animals exhibited larger neurons in the left hemisphere, left and right eye deprived birds displayed larger cells in the right hemisphere. *Post hoc* comparison (Tukeys HSD test for repeated measures) verified significant left–right differences in control ($p < 0.05$, Fig. 1a) and left eye deprived animals ($p < 0.001$, Fig. 1a). The rotundal cell sizes of right eye deprived birds did not differ significantly. These differences are illustrated in Fig. 1b, where the extent of soma size asymmetries was calculated as the percent deviation from mean soma size.

Beside differences between the left and right rotundus within one group, the rotundal soma sizes within one hemisphere varied between the groups. In both hemispheres, the rotundal soma sizes differed between control and deprived animals (Fig. 1b). In the right rotundus, largest neurons were found in left eye deprived birds while controls had the smallest cells. Contrary to this pattern, in the left rotundus, control animals exhibited the largest cells and left eye deprived birds had the smallest cells (Fig. 1a). Thus, in left as well as right eye deprived animals, the deprivation effects resulted in larger soma size values within the right rotundus.

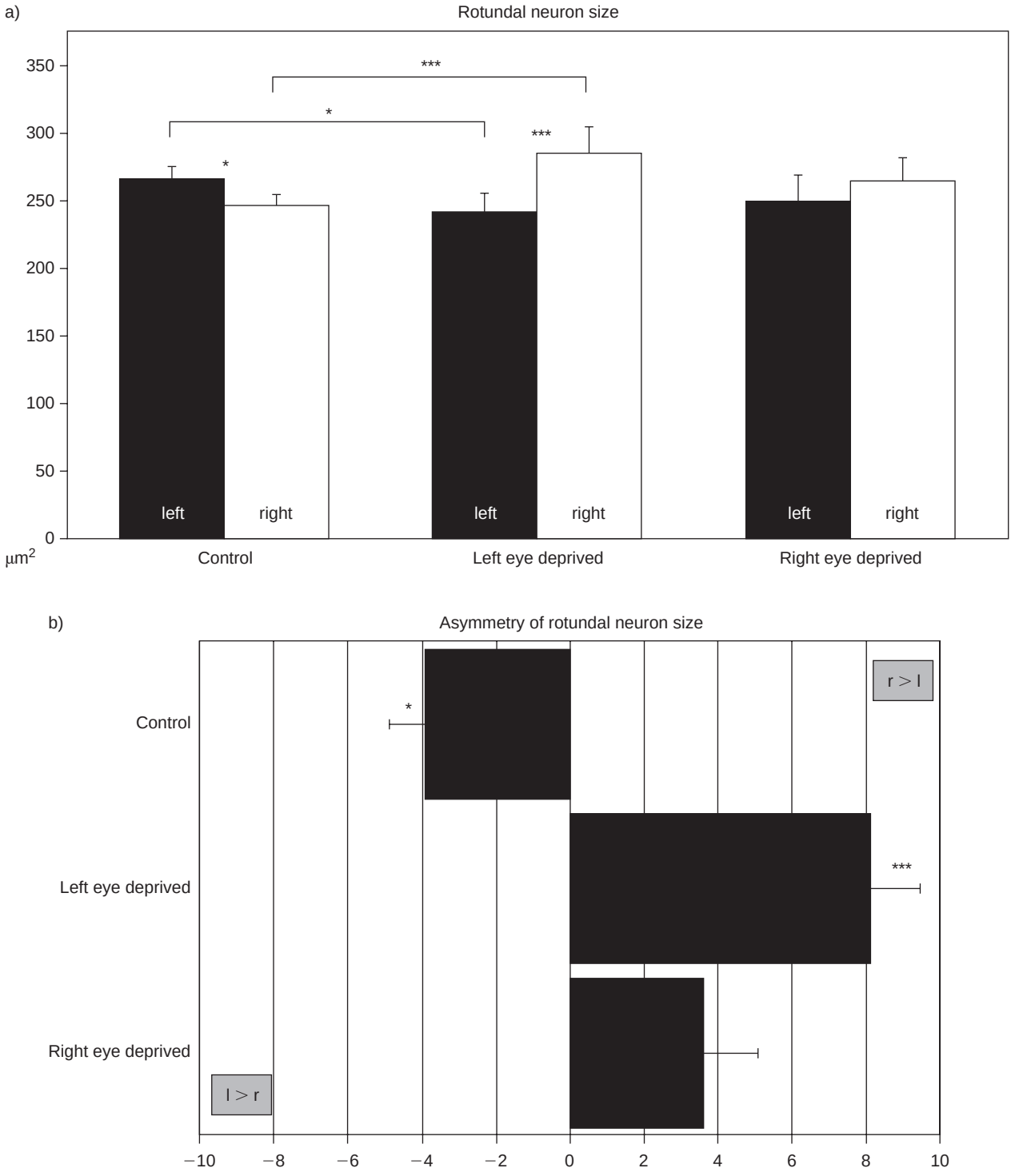

FIG. 1. Rotundal soma size differences. (**a**) rotundal soma sizes in the three experimental groups ($^{*}\ p < 0.05$; $^{***}\ p < 0.001$, *post hoc* comparisons). Bars represent standard errors (controls $n = 13$; left eye deprived $n = 10$; right eye deprived $n = 12$); (**b**) rotundal soma size asymmetry in the experimental groups expressed as percentage deviation from mean soma size which was calculated as [(mean soma size of left and right rotundus) − (soma size left rotundus)] × 100/(mean soma size of left and right rotundus). Positive values imply greater somata in the right (r) rotundus, negative values in the left (l) rotundus (bars represent s.e., asterisks as in (a).

This effect was more pronounced in left eye deprived birds. *Post hoc* comparison between control and left eye deprived animals confirmed the significant enlargement of neurons in the right rotundus of left eye deprived birds (HSD: $p < 0.001$, Fig. 1a), indicating a hypertrophy of neurons in the deprived hemisphere. The decrease of soma sizes in the non-deprived left rotundus of left eye deprived animals relative to controls was likewise significant (HSD: $p < 0.05$, Fig. 1a). *Post hoc* comparisons between

right eye deprived and control or left eye deprived animals did not reveal significant differences.

Discussion

The present study shows that the nucleus rotundus in pigeons is characterized by left–right differences of soma sizes with larger neurons in the left rotundus. This morphological asymmetry could be related to the functional left hemisphere dominance for visual pattern analysis in birds. The neuronal asymmetry was modified by monocular deprivation after hatching with left eye deprivation leading to a reversal of soma size asymmetries and right eye deprivation abolishing left–right differences. These data support the decisive role of light in the activity-dependent development of structural lateralization in the pigeon's tectofugal pathway. Since, however, monocular deprivation has fundamentally different effects on tectal [8] and rotundal cell sizes, asymmetries of light input seem to initiate different neuronal processes at different levels of the tectorotundal pathway depending on the timepoint and direction of monocular deprivation.

Avian embryos keep their head turned to the right so that the right eye is exposed to light which is shining through the translucent egg shell while the left eye is occluded by the body. Due to the complete decussation of the optic nerves, the visual system of the left hemisphere is therefore stimulated to a larger degree by light before hatching [1,2,14]. It is conceivable that the morphological cell size asymmetry with larger perikarya in the left rotundus of control animals results from this lateralized prehatching light stimulation. This would then closely resemble the condition in the retinorecipient layers of the optic tectum which also display larger cells in the stronger stimulated left hemisphere. Prehatching light stimulation asymmetry therefore probably determines morphological asymmetries at tectal and rotundal level [5,8]. These results are in accordance with findings from monocular deprivation studies in mammals [9] and zebra finches [15] which all reported smaller soma sizes of neurons receiving afferents from the deprived eye. Thus, at first glance, the present results might point to close similarities of the mechanisms which govern natural monocular deprivation due to the asymmetrical embryonic posture in birds and postbirth monocular deprivation in mammals. However, although seemingly similar, different mechanisms have to be involved. A first hint is provided when comparing the anatomical locations where the effects of deprivation occur. Morphological soma size effects of monocular deprivation in mammals are restricted to the binocular portion of the lateral geniculate nucleus and are absent in the retina and visual cortex [9]. These effects are regarded as secondary consequences from synaptic competition at cortical level between geniculate fibers representing the deprived and the non-deprived eye [10,16].

While effects of embryonic lateralized light input or post-hatch monocular deprivation are also absent in the retina of birds [5,17], they can be encountered in the optic tectum [5,8], the nucleus rotundus [15] (present study), and in the ectostriatum [18]. Thus, pre- or post-hatch monocular deprivation affects perikaryal sizes along the whole tectofugal system. While inputs of both eyes could compete at rotundal level [6], a comparable competition is absent in the tectum and unlikely in the ectostriatum [19]. This suggests that visual deprivation effects in birds are mediated through activity-correlated and eventually trophic deprivation effects within one hemisphere which possibly operate without direct synaptic competition between neurons representing deprived and non-deprived eyes.

A further clue for differences between mammalian and avian visual deprivation mechanisms results from the fact that in the mammalian geniculocortical system, only the unilateral absence of contoured visual patterns induce significant deprivation effects. Asymmetries of luminance alone do not lead to alterations [16]. This supports the assumption that fiber competition is mediated by a Hebbian mechanism which requires correlated activity of pre- and postsynaptic cells for stabilization or retraction of synapses [10,16,20]. In chicks and pigeons, the situation must be different since light has to shine through the eggshell and the closed lid of the embryo to induce cerebral asymmetries [14,21]. Therefore natural monocular deprivation in pigeons has to be induced by brightness and not by contoured visual pattern differences. Brightness differences are probably coded by mere activity differences between the eyes and could induce asymmetries by the release of neurotrophins between the stimulated and the deprived hemisphere [1]. Such activity-dependent trophic effects could generate the morphological left–right differences between rotundal cells observed in the present study.

At this point it is important to clarify that the avian tectofugal system is not the equivalent of the geniculocortical pathway in mammals. However, the tectofugal system in pigeons is morphologically and functionally [8,14] modified by lateralized visual input during a critical ontogenetic time span and additionally guides visual performance in normal animals [7]. The same holds for the mammalian geniculocortical pathway. Therefore this comparison is guided by a systemic perspective and aims to

clarify whether the epigenetic factor light acts along universal mechanisms to govern ontogenetic plasticity in different visual systems.

The present study also reveals important differences of the effects of monocular deprivation depending on the neural structure under analysis. According to a previous study [8], post-hatch monocular deprivation reduces neuronal cell sizes in the retinorecipient layers of the contralateral tectum resulting in larger tectal neurons on the side of the deprived eye. Therefore left eye deprivation (natural left eye deprivation + post-hatch left eye occlusion) results in an increase of left-skewed tectal asymmetry, while right eye deprivation (natural left eye deprivation + post-hatch right eye occlusion) leads to a reversal of tectal lateralization. Surprisingly the present study reveals a completely different set of effects for the rotundus. Here, right eye deprivation abolished left–right rotundal asymmetries while left eye deprivation reversed the asymmetry pattern of the rotundus. Thus, although control (naturally left eye deprived) and left eye deprived animals were exposed to a synergistic monocular deprivation during development, the effects on rotundal soma size were opposing. Additionally, although left and right eye deprived birds received a contrasting asymmetrical light stimulation after hatching, the effects on rotundal soma size were by no means opposing but gradual.

On rotundal level, these opposing effects before and after hatching might be explained by influences from components which gain functional significance only after hatching.

GABAergic immunoreactivity in the rotundus evolves during post-hatch development [22,23]. Therefore the inhibitory rotundal innervation by GABAergic fibers from pretectal nuclei like nucleus subpretectalis and nucleus interstitio-pretecto-subpretectalis, which form side pathways of the tectorotundal projections [24,25], mature only after hatching. For the rotundal activity level, modulatory inputs from these pretectal nuclei are supposed to play an important role [24,25]. This implies that rotundal activity is probably differently modulated before and after hatching depending on the developmental stage of the GABAergic input. Thus, asymmetric light stimulation could generate different rotundal activity levels depending on timepoint and direction of monocular deprivation. Due to this scenario, control birds might develop smaller neurons in the deprived right rotundus due to the smaller degree of visual activation in the right side of the visual system. In contrast, the right rotundus of posthatch left eye deprived animals would after hatching receive a lower photic stimulation by tectofugal fibers on the left side, but probably also a lower inhibitory input from pretectal nuclei. The differential effect of these two variables might lead to the paradoxical effect that the deprived hemisphere develops larger cells while the stronger stimulated left hemisphere develops reduced cell sizes due to its concomitantly higher level of inhibition. The possible details of the excitatory and the inhibitory interactions at rotundal level and their role in the morphological growth of somata are far from being clear. However, it is likely that the final size of rotundal cells is determined by three factors: the prehatch natural deprivation which is transmitted by excitatory tectorotundal fibers; the post-hatch stimulation pattern which represents a mixture of excitatory tectorotundal and inhibitory pretectal inputs; and the asymmetry of tectorotundal projections which provide the left rotundus with a more bilateral input [6]. A final understanding of the partly paradoxical results of the present study will depend on a deep understanding of the complex pattern of interactions of these three factors.

Morphological asymmetries are very likely powerful indicators for a lateralization of information processing [3,4,8,9,14]. Since, however, as shown in the present study, differential timepoints can result in divergent effects depending on the studied structure, morphological and functional asymmetries can take different directions. Posthatch monocular deprivation of the left eye results in a strong right eye dominance and in larger retinorecipient tectal neurons on the left side [8]. The same procedure, however, leads to a significant hypertrophy of right rotundal cells (present study). Thus, the functional dominance of one hemisphere seems to emerge from a complex patchwork of structural asymmetries which can be differently adjusted during ontogeny.

Conclusion

The aim of the present study was to clarify whether cells of the nucleus rotundus in pigeons are modified by natural prehatch and artificial post-hatch monocular deprivation in a lateralized manner. Control animals, which receive a higher light stimulation of the right eye due to an asymmetrical position of the embryo within the egg, indeed showed significant morphological asymmetries with larger neurons in the left rotundus. Posthatch monocular deprivation resulted in larger right sided rotundus neurons after left eye deprivation and in an abolishment of morphological rotundus asymmetries after right eye deprivation. Combined with evidences from other studies, these data make it likely that the formation of morphological asymmetries in pigeon depends on an activity-dependent process, is differently modu-

lated at different ontogenetic timepoints and in different structures, and diverges from the general maturational scenario encountered in mammals.

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