Post-hatch activity-dependent modulation of visual asymmetry formation in pigeons

H. Prior, B. Diekamp,[I] O. Güntürkün and M. Manns[CA]

Biopsychology, Institute of Cognitive Neuroscience; Faculty of Psychology, Ruhr-University Bochum, 44780 Bochum, Germany; [I]Department of Psychological and Brain Sciences, Ames Hall, Johns Hopkins University, Baltimore, MD 21218-2686, USA

[CA]Corresponding Author: Martina.Manns@ruhr-uni-bochum.de

Received 2 March 2004; accepted 31 March 2004

DOI: 10.1097/01.wnr.0000129575.43925.10

The embryonically induced visual lateralization in pigeons can be modified by occlusion of one eye after hatching. Here we show that this deprivation effect could be also attained by short-term blocking of retinal activity with tetrodotoxin (TTX), leading to a dominance of the ipsilateral hemisphere in a visual discrimination task. This lateralization pattern resulted from a performance increase conveyed by the non-deprived hemisphere, while performance with the TTX-injected eye did not differ from that of saline-injected controls. Thus, post-hatch modulation of visual lateralization is mediated by TTX-sensitive, activity-dependent neuronal mechanisms. The transient silencing of one visual input alters the activity balance between the left and right eye system, enhancing visuoperceptive skills in the relatively higher active hemisphere. *NeuroReport* 15:1311–1314 © 2004 Lippincott Williams & Wilkins.

Key words: Birds; Lateralization; Plasticity; Tetrodotoxin; Visual system

INTRODUCTION

The visual system of birds has been established as an outstanding model to gain insights into the neuronal mechanisms underlying the development of functional and morphological asymmetries in the brain [1,2]. Birds like chickens and pigeons display a right eye/left hemisphere dominance for detailed visual feature analysis which is accompanied by morphological asymmetries in the ascending visual pathways [1]. While chicks exhibit transient left-right differences in the thalamofugal but not in the tectofugal projection [3], in pigeons the tectofugal system displays lifelong morphological asymmetries. This pathway starts with the projection from the retina to the contralateral optic tectum and proceeds via the diencephalic nucleus rotundus to the forebrain [4]. Apart from tectal [5,6] and rotundal [7] cell size differences, the connectivities within the tectorotundal projection are also organized asymmetrically [8].

The formation of both behavioural and anatomical asymmetries is triggered by a lateralized light exposure of the embryo in the egg resulting from an asymmetrical embryonal head turning that places the right eye close to the semitranslucent eggshell [1,2]. Dark incubation prevents the development of a right eye/left hemispheric dominance for visual object analysis [9] and inhibits the generation of tectal cell size asymmetries [6,9]. Comparison of light- and dark-incubated animals shows that the stronger stimulated left hemisphere exhibits enhanced visuoperceptual skills, while visuomotor abilities are reduced in the right hemisphere [9]. These data suggest a differential sensitivity of visual circuits to photic stimulation. However, pigeons hatch with closed eyes and an immature retinotectal system [10–12]. Thus, despite the critical role of skewed embryonic light input, the final stabilization of a visual lateralization occurs in post-hatching days when visual circuits are known to mature under sensory control. During this period, the asymmetry pattern can be modified by the occlusion of one eye [7,13]. Right eye deprivation leads to a reversal of the functional and morphological asymmetry pattern, while left-eye deprivation increases right-eye superiority [7,13]. Since it is known that monocular deprivation affects neuronal cell size in the tectofugal pathway [7,13–15] and since anatomical asymmetries are present within this system, the unbalanced visual stimulation can directly influence the differentiation of the tectofugal pathway. The development of this pathway is well known to be controlled by afferent activity [16] and neurotrophic factors are key mediators of this regulation [17]. Visual stimulation adjusts the expression and/or release of neurotrophic factors and hence regulates the trophic support of target cells [16,17]. While many actions are dependent on neurotransmission, the neurotrophic factor BDNF can also promote synapse development in the absence of neuronal activity [18]. Accordingly, the effects of an asymmetric photic stimulation onto visual pathways can be mediated by different neuronal mechanisms. On the one hand, asymmetric retinal activity can result in asymmetric retinofugal neurotransmission and hence leads to activity differences between the left- and right-eye systems which are finally responsible for the establishment of visual lateralization. On the other hand,

unbalanced retinal activity can induce an asymmetric expression of neurotrophic factors like BDNF. This can lead to an asymmetrical trophic support of retinal targets which might be independent from neurotransmission. Tectal cell populations are indeed differentially sensitive to afferent input. While the survival of superficial tectal cells is regulated by trophic retinal support, the deep efferent cells depend on retinotectal neurotransmission [19]. This differential sensitivity suggests mechanisms dependent as well as independent from neurotransmission to be critically involved in post-hatch plasticity of asymmetry formation, and these differences might function as the structural basis for distinct effects onto visuomotor circuits. In a first step to unravel the decisive neuronal mechanisms, we wanted to know if the transient blockade of retinal activity modulates the establishment of visual asymmetries. These data would provide evidence for the dependence of asymmetry formation on asymmetric neurotransmission. Therefore, we injected tetrodotoxin (TTX) or saline into the left or right eye of 1-day old pigeon hatchlings. The birds were tested as adults in a grit-grain discrimination task to estimate the degree and direction of visual lateralization.

MATERIALS AND METHODS

Animals were taken from breeding pairs in our own laboratory in Bochum kept under a 12:12 h light:dark cycle. After hatching, animals received a single injection of a 0.5 mM tetrodotoxin solution (TTX, Sigma) dissolved in saline (0.9% NaCl) with 0.2 ng TTX/g body weight (right eye injection 6 animals; left eye injection 3 animals). Control animals were injected with equivalent volumes of saline (right eye injection 6 animals; left eye injection 5 animals). Injections were performed with a sterilized insulin syringe after the left or right eye was locally anaesthetized with Xylocaine. The needle penetrated about 2.5 mm deep within the caudodorsal eye ball in order to avoid lesions of the optic apparatus.

When adult, the bi- and monocular vision of the birds was tested in a grit-grain discrimination task to estimate the degree and direction of visual lateralization. For monocular behavioral tests, one eye was occluded by a cardboard cap which was fixed around the eye with a Velcro band. The animals had to peck 30 white Dari-grains from a translucent trough which was positioned in front of the pigeon's home cage and filled with small pebbles of varying size but resembling the seeds in color and shape.

A pilot study revealed that the TTX-injected animals tended to peck more slowly than control birds. In the original protocol [20], the pecking time was restricted to 30 s and the percentage of pecks leading to consumption served as a measure of discrimination accuracy. Since former experiments had shown that pecking speed does not contribute to visual lateralization in this task [9,13,20], we restricted the number of pecks to 30 without any time limit to obtain a measure of discrimination accuracy that is unaffected by motor speed. Thus, the trough was removed after 30 pecks and the time needed for one trial as well as the number of grains swallowed was noted. Discrimination accuracy was then calculated as the percentage of grains eaten relative to the 30 pecks. To compare performances with the left and right eye, the extent of functional asymmetry was estimated as the percent deviation from the mean discrimination accuracy.

During the entire testing period, the animals were food deprived and maintained at 80% of their free-feeding weight. The animals were trained under each seeing condition until they achieved a stable performance score and the subsequent ten tests under each seeing condition were included into the behavioral analysis. Performances of the animals were analyzed by means of a two-factor ANOVA with TTX treatment as a fixed factor and vision as a repeated measure. In case of significant main effects, between-group effects were evaluated by *post hoc* Tukey HSD tests and within-subject factors by *post hoc* paired sample *t*-tests.

All experiments were carried out according to the specifications of the German law for the prevention of cruelty to animals.

RESULTS

Discrimination performance differed between the experimental groups (mean $\pm$ s.e.m. results: TTX-right eye $68.33 \pm 2.66\%$; TTX-left eye $68.33 \pm 5.05\%$; saline-right eye $58.8 \pm 3.29\%$; saline-left eye $63.5 \pm 2.56\%$; $F(3,16)=3.382$, $p<0.05$) with the TTX-injected groups achieving higher discrimination scores than the saline-injected birds (TTX-injected animals $68.33 \pm 2.39\%$; saline-injected animals $60.9 \pm 2.15\%$; planned comparison: $F(1,16)=7.105$, $p<0.05$; Fig. 1). Discrimination accuracy depended on the seeing conditions (binocular vision $73.35 \pm 1.91\%$; left-eye vision $60.55 \pm 2.84\%$; right-eye vision $58.85 \pm 2.68\%$; $F(2,32)=14.123$, $p<0.0001$). In agreement with previous studies [8,12,19], all animals consumed the highest number of grains under binocular conditions ($p<0.001$). There was a significant group $\times$ vision interaction ($F(6,32)=3.447$, $p<0.01$). Both saline-injected groups showed no differences in their discrimination performance seeing with the left or right eye (Fig. 1, Fig. 2), possibly due to the different method of testing than used in previous studies [9,13,20]. However, the fact that these animals achieved slightly better results seeing with the injected eye (Fig. 1) indicates that the injections as such did not cause impairments of the injected eye. Animals which received a right eye TTX-injection displayed better results with the left eye than the right ($p<0.01$). Animals which received a TTX injection in the left eye achieved better discrimination scores seeing with the right eye, although this last difference was not significant due to the

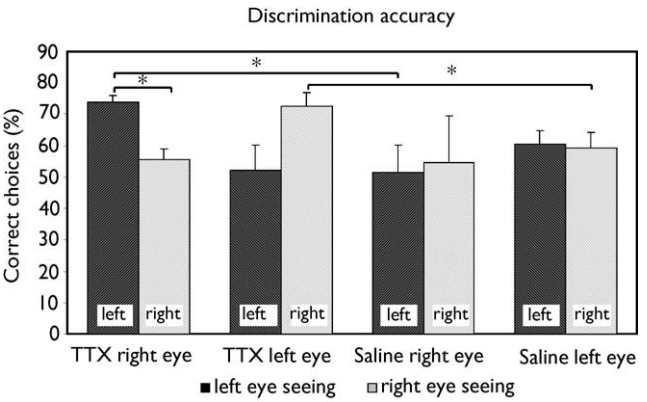

Fig. 1. Mean monocular performances of the experimental groups expressed as percentage discrimination success in the grit grain discrimination task. $*p<0.05$. Bars represent s.e.

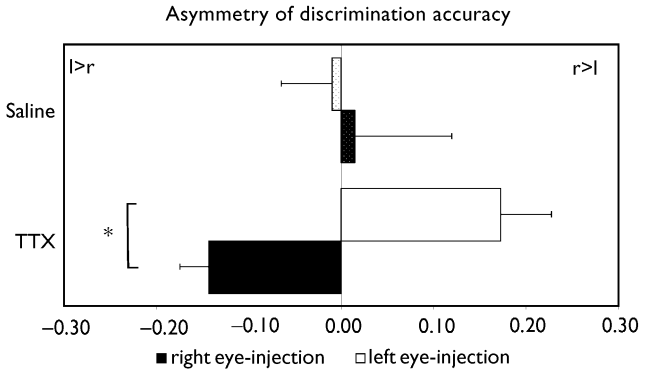

Fig. 2. Asymmetry of discrimination performances in the experimental groups estimated as the percent deviation from the mean discrimination accuracy. Positive values imply better vision with the left eye, negative values better discrimination scores when seeing with the right eye. $*p < 0.05$. Bars represent s.e.

low number of experimental animals (Fig. 1, Fig. 2). Thus, the TTX injection determined the functional dominance of the contralateral eye. The injections resulted in different functional asymmetry patterns whose significance was confirmed by an ANOVA with discrimination asymmetry as a dependent variable ($F(3,16)=4.581$, $p<0.05$; Fig. 2). *Post hoc* comparison verified that the contrasting performance asymmetry of left- and right-eye TTX-injected animals was significant ($p<0.05$; Fig. 2).

Comparing the results of the TTX- and saline-injected animals, the performance with the TTX-injected eye did not differ from that of the saline-injected eyes, while the contralateral non-deprived eye displayed significantly higher discrimination scores ($p<0.01$; Fig. 1). These data indicate that the lateralization pattern observed in the TTX-injected animals did not result from a decrease in the performance with the injected eye but from an enhancement of the performance with the non-injected eye.

DISCUSSION

The present data show that the transient inhibition of retinal activity with TTX leads to the formation of a behavioural lateralization of the visual system in pigeons. Comparisons with saline-injected animals revealed that this lateralization pattern resulted from a significant enhancement of the discrimination accuracy with the non-injected eye and not from a suppression of the performance with the injected one. Thus, the effects of monocular deprivation are not confined to the deprived brain side but affect both hemispheres. Such a bilateral deprivation effect has also been shown in zebra finches in which closure of one eye changes tectofugal cell sizes in both brain halves [14,15]. Besides, these results demonstrate that the TTX treatment did not perturb mechanisms of visual discrimination which might have been responsible for asymmetric performances. This is further supported by the fact that TTX-injected animals achieved in general higher discrimination scores than the saline-injected controls. However, according to our testing design, the saline-injected animals displayed no lateralization at all. This result was in contrast to previous results showing that an asymmetric photic stimulation during embryonic development is sufficient to induce a visual lateralization [6,9,20]. It is conceivable that the absence of a time restriction masked a visual lateralization in these birds.

Comparable with monocular deprivation after hatching by occluding one eye with an eye cap for 10 days [13], single intraocular TTX injections modulate the visual lateralization pattern of pigeons. This result verifies the activity dependency of asymmetry formation and demonstrates that the mediating neuronal mechanisms depend on TTX-sensitive neurotransmission. TTX blocks action potentials in retinal afferents, diminishing retinofugal transmission and hence modulating the development of visual pathways up to forebrain levels [21]. Since morphological asymmetries in the pigeon's visual system are present within the tectofugal pathway it is very likely that deprivation effects are directly manifested within the retinotectal projection [5]. Differentiation of the retinotectal system is characterized by a highly dynamic phase of dendritic and axonal arbor growth, followed by retraction and stabilization. During this phase, the cells react very quickly to changes of the afferent input [16]. Silencing retinal activity with TTX induces an increase in the number of dying tectal cells [19,22]. Since such increased cell death is compensated by a subsequent phase of enhanced cell survival when activity recovers, the net total of tectal cells is relatively unaltered [22]. While TTX prevents the refinement of retinal topography leading to reduced visual acuity after long-term application [21], even short term inhibition of retinal activity affects the dynamics of axo-dendritic arbor development [18,23,24]. Retinal activity recovers within 8–24 h after TTX injections [22]. All in all, this short-term inhibition of the retinal activity is sufficient to induce subtle developmental modifications, but does not severely disrupt retinotopic refinement. Accordingly, the discrimination scores of the TTX-injected eyes did not differ from the saline-injected eyes.

The TTX-induced asymmetry pattern can be attributed to an increased discrimination accuracy of the non-injected eye. This result confirms that it is the stronger stimulated hemisphere which enhances visuoperceptual skills in response to an asymmetric stimulation [9], but this effect does not just result from a growth-promoting effect within the higher active brain side. The non-deprived hemispheres of the TTX-injected animals and the saline-injected controls were equally stimulated by retinal input. Nevertheless the non-deprived hemisphere of TTX-injected animals developed the superior discrimination abilities. Thus, the decisive determinant of asymmetry formation seems to be the relative activity difference between the left- and right-eye seeing system and not the absolute amount of activation. This effect is also exemplified in the soma size of tectal GABAergic cells. While light stimulation decreases GABAergic cell sizes in both tectal hemispheres, the stronger stimulated tectum develops larger cell bodies [6]. The necessary integration of activity from the left and the right side might be mediated by interhemispheric connections [25] which finally stabilize induced asymmetries. Nevertheless, the present data further substantiate the key role of asymmetrical retinal activity before and after hatching.

CONCLUSIONS

The present study shows that in pigeons, the transient blockade of retinal activity after hatching is sufficient to induce a dominance of the eye that was not temporarily deprived of neural activity. Therefore the relative

balance of activity on the left and the right side determines the direction of functional lateralization with enhanced visuoperceptual skills in the stronger activated hemisphere. Thus, the present data support the crucial role of photic stimulation for the determination of a lateralized brain architecture.

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Acknowledgement: This research was supported by a grant from the SFB Neurovision of the Deutsche Forschungsgemeinschaft.