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Journal of Chemical Neuroanatomy 25 (2003) 249–259
Journal of CHEMICAL NEUROANATOMY
www.elsevier.com/locate/jchemneu

Light experience induces differential asymmetry pattern of GABA- and parvalbumin-positive cells in the pigeon's visual midbrain

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Received 3 October 2002; received in revised form 4 February 2003; accepted 19 March 2003

Abstract

The formation of functional and morphological asymmetries within the pigeon's tectofugal system depends on left–right differences in visual input during embryonic development. This asymmetric stimulation presumably affects activity-dependent differentiation processes within the optic tectum. Behavioral studies reveal that prehatch light stimulation asymmetry influences both left- and right-hemispheric processes in a differential way. Thus, we have to assume divergent effects on both hemispheres. This study represents an attempt to test the hypothesis that embryonic light asymmetry induces different, cell-type-specific effects in the left and the right optic midbrain. Since it is likely that inhibitory interneurons play a critical role in the establishment of asymmetries, we examined in both sides of the brain the soma sizes of GABA- and parvalbumin- (PV) immunoreactive (ir) cells of the tectum and the magnocellular isthmic nucleus in controls and in dark-incubated animals. No cell size asymmetries of magnocellular isthmic neurons were found in either dark-incubated or control birds. Dark-incubation also prevented the establishment of lateralized differences in GABAergic and PV-positive tectal cells. However, in control birds GABAergic cells displayed larger somata in the left tectum, whereas PV-ir neurons were enlarged within the right tectum. This complementary asymmetry pattern suggests that PV- and GABA-ir tectal cells represent different cellular populations which react differently to visual input. Thus, our data show that visual lateralization does not result from a mere growth promoting effect that enhances differentiation within the behaviorally dominant left side, but is constituted by different cell type-specific circuits which are divergently adjusted in the left and in the right tectum.

Keywords: Tectum opticum; Isthmic complex; GABA; Parvalbumin; Immunohistochemistry; Tectofugal; Lateralization

1. Introduction

A large number of studies show that birds demonstrate a behavioral lateralization of their visual system with a dominance of the left hemisphere for visual object analysis (Rogers and Andrew, 2002). Due to the complete decussation of the optic nerves in birds (Weidner et al., 1985) pigeons and chicks are more efficient in discriminating objects when using the right eye (Mench and Andrew, 1986; Güntürkün and Kischkel, 1992; Güntürkün et al., 2000). The conclusion that the left hemisphere is dominant for visual object processes is supported by numerous findings showing drastically reduced visual discrimination capabilities after left-sided forebrain lesions or biochemical manipulations (Güntürkün, 1997b; Rogers, 1996).

These functional asymmetries of the visual system can be associated with morphological left–right differences in the ascending visual systems (Güntürkün, 1997b; Rogers, 1996). In pigeons, visual lateralization is related to morphological asymmetries in the tectofugal pathway, transferring visual information mainly contralaterally via the mesencephalic optic tectum and the diencephalic nucleus rotundus to the forebrain. The optic tectum displays cell size asymmetries with the cells in layers 2–12 (Ramón y Cajal, 1911) having larger cell bodies within the left tectum, while neurons of layer 13

Abbreviations: GABA, γ-aminobutyric acid; Imc, nucleus isthmi magnocellularis; Ipc, nucleus isthmi parvocellularis; ir, immunoreactive; PV, parvalbumin; Slu, nucleus isthmi semilunaris.

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doi:10.1016/S0891-0618(03)00035-8

with projections to the rotundus are enlarged on the right (Güntürkün, 1997a). The rotundus projections of layer 13 neurons are bilateral with the crossed portion of this pathway being asymmetrically organized with more fibers ascending from the right tectum to the left rotundus than vice versa (Güntürkün et al., 1998). The higher bilateral input to the left rotundus is accompanied by larger cell bodies of rotundal relay neurons on this side (Manns and Güntürkün, 1999a).

The development of both functional and morphological lateralization depends on a minute asymmetry of prehatch visual stimulation (Rogers, 1996; Güntürkün, 1997b,c; Rogers and Deng, 1999; Güntürkün, 2002). Due to the asymmetrical head position of the embryo in the egg (Kuo, 1932), the left eye is covered by the embryo's body while the right eye is close to the egg shell. So, light shining through the shell stimulates the right eye while the left eye is visually deprived. The unbalanced photic input presumably induces a cascade of asymmetrical neuronal processes which culminate in the formation of lateralized visual circuits. Dark-incubation obliterates behavioral and morphological asymmetries (Güntürkün, 1993; Skiba et al., 2002) while monocular deprivation after hatching changes the normal lateralization pattern (Manns and Güntürkün, 1999a,b). The posthatch plasticity suggests that the final determination of visual asymmetries takes place after hatching. This implies that during posthatch development when visual circuits are known to mature under sensory control (Bagnoli et al., 1982, 1989, 1992; Fontanesi et al., 1993), the induced asymmetries must be stabilized despite a symmetric visual input.

A behavioral comparison of the visual performances of light and dark-incubated pigeons shows that light induces a left-hemispheric increase in visuoperceptual skills while it simultaneously decreases visuomotor speed within the right hemisphere. Thus, distinct aspects of visuomotor behavior are differentially adjusted within the hemispheres (Skiba et al., 2002). These functional consequences suggest that the effect of an asymmetrical embryonic light stimulation is not restricted to a simple left-hemispheric enhancement of visual processing. In contrast, specialized neuronal circuits seem to be differentially adjusted in both hemispheres (Skiba et al., 2002). A differential susceptibility of the hemispheres is supported by the observation that the presence and direction of receptor-binding asymmetries within some forebrain areas depends on the eye system which was exposed to light (Johnston et al., 1997).

Such a complex bihemispheric response could in principle result from an asymmetrical reactivity of distinct cellular populations to photic stimulation. This lateralized effect could be further enhanced by intra- and/or inter-hemispheric interactions. Evidence for such differential effects can already be observed at tectal level. A greater amount of light stimulation of the right eye not only induces larger neuronal somata in left retinorecipient tectal layers but also a reversed asymmetry of layer 13 neurons. Thus, light effects during ontogeny cannot be simply explained by growth promoting, trophic effect of light onto the left tectum. It is conceivable that inhibitory influences within or between the hemispheres additionally play an important role in the establishment of morphological left–right differences (Manns and Güntürkün, 1999a) and it is likely that these influences become critical during posthatch development.

Tectal activity is modulated by distinct inhibitory systems (Hunt and Künzle, 1976a,b). On the one hand, both tectal hemispheres are connected by mainly inhibitory tectotectal fibers (Hardy et al., 1984; Robert and Cuénod, 1969) whose origin is still unclear. In pigeons, this interaction is asymmetrically organized with a stronger influence of the left tectum onto the right one than vice versa (Keysers et al., 2000). Besides, the tectum is reciprocally connected with the isthmic complex which exerts excitatory and inhibitory influences on tectal cells (Felix et al., 1994; Wang et al., 1995, 2000). In birds, the isthmic complex consists of three nuclei: nucleus isthmi pars magnocellularis (Imc), nucleus isthmi pars parvocellularis (Ipc) and nucleus isthmi pars semilunaris (Slu). Only Imc has GABAergic neurons (Veenman and Reiner, 1994; Tömböl et al., 1995). Finally, the tectum itself bears a large number of GABAergic cells establishing radially and horizontally arranged intratectal connections (Hunt and Künzle, 1976a,b; Domenici et al., 1988). The maturation of these tectal GABAergic cells is influenced by retinal input (Bagnoli et al., 1989). Thus, asymmetrical visual stimulation might lead to morphological and/or functional left–right differences within this cellular population.

GABAergic cells are typically characterized by the expression of specific calcium-binding proteins like calbindin, calretinin or parvalbumin (PV) (for review Andressen et al., 1993). Plasticity studies showed that especially PV-immunoreactive (ir) neurons appear to be sensitive to alterations in visual input (Cellerino et al., 1992; Bonhoeffer, 1996; Lane et al., 1996). Since PV is expressed in the pigeon's tectum (Theiss et al., 1998) it is possible that particularly PV-ir cells are influenced by asymmetric visual stimulation and hence show asymmetric differentiation. As a consequence, these neurons might be critically involved in the establishment of asymmetrical visual circuits.

In order to elucidate the possible role of inhibitory neurons in the development of visual lateralization, we examined the asymmetry pattern of GABA- and PV-ir cells in two visual areas of normal and dark-incubated animals: (a) the optic tectum which, as a primary visual structure, directly interacts with retinal fibers and (b) the

Imc as a visual midbrain structure which receives visual information via tectal cells so that it is only indirectly influenced by visual stimulation. In parallel, we investigated the developmental pattern of the GABA- and PV-ir cellular populations in these midbrain areas.

2. Material and methods

For the present study, hatchlings of different ages, adult and adult dark-incubated homing pigeons (*Columba livia*) were used (Table 1). Adults were received from a local breeding stock. Thus, these animals developed under 'natural' daylight incubation conditions. For dark-incubation, fertilized eggs from lab-own breeding pairs were incubated in a still-air incubator kept in darkness at constant temperature (38.3 °C) throughout the entire period of incubation. After hatching, the nestlings were swapped with the artificial eggs the breeding birds were sitting on (Skiba et al., 2002). For the developmental immunohistochemical study, young animals were taken from lab-own breeding pairs which developed under a 12 h light/dark rhythm.

For immunohistochemical studies, animals received an injection of 50–200 units sodium heparin (Ratiopharm). After 15 min, the animals were deeply anesthetized with an overdose of equitesin (0.45 ml/100 g body weight) and perfused through the heart with 0.9% saline (40 °C) followed by 4% paraformaldehyde+0.2% glutardialdehyde (in 0.12 M phosphate buffered saline (PBS), pH 7.2, 4 °C). Brains were postfixed in the fixative +30% sucrose (w/v) for 2 h and cryoprotected overnight in 0.12 M PBS +30% sucrose (w/v) at 4 °C. On the following day, the brains were cryosectioned in frontal plane at 35 μm and the slices were collected in 0.12 M PBS containing 0.1% sodium azide. The left or right brain side was marked by a hole caused by insertion a small needle whereby labeling of the left or the right side was random between the preparates. The slices were stored at 4 °C until used for immunohistochemistry. Sections were immunolabeled with antibodies against GABA (polyclonal rabbit IgG, Sigma, Germany) or PV (monoclonal mouse IgG, Sigma). The experiments were carried out according to the specifications of the German law for the prevention of cruelty to animals.

Table 1
Number of experimental animals

Group	GABA	PV
PH 1	5	5
PH 7	5	5
PH 21	3	3
Adult controls	10	10
Adult dark-Incubated animals	11	11

2.1. Immunohistochemistry

Brain slices were reacted free-floating according to the ABC-technique. All steps of the immunohistochemical detection were performed on a shaker table at room temperature unless otherwise stated. Three washes at 10 min each with buffer (PBS) for PV- or 0.05M Tris-buffer for GABA-immunohistochemistry followed all incubation steps. After the first three washes, endogenous peroxidases were blocked with 0.3% H_2O_2 in 50% ethanol. Sections were incubated with primary antibody solution (PV 1/1000: antibody dissolved in 0.12M PBS+2% sodium chloride (w/v)+0.3% Triton X 100 (w/v)+0.1% sodium azide (w/v)+0.5% normal horse serum (w/v); GABA 1/10 000: antibody dissolved in 0.05M Tris +0.1% sodium azide (w/v) +0.5% normal rabbit serum (w/v)) for 72 h at 4 °C. The secondary antibody reaction was carried out with biotinylated goat anti-rabbit IgG (1/500; Vectastain Elite kit, Vector, Burlingame, CA) or horse anti-mouse IgG (1/200; Vectastain Elite kit, Vector; 1/200 0.12 M PBS+2% sodium chloride (w/v)+0.3% Triton X 100 (w/v)+0.5% normal serum), respectively, for 1 h at room temperature. Afterwards, the sections were incubated in an avidin-biotin-peroxidase solution (Vectastain ABC-Elite kit, 1/75 in 0.12 M PBS+2% sodium chloride (w/v)+0.3% Triton (w/v)). Peroxidase-activity was detected using a heavy metal intensified 3′3-diaminobenzidine (Sigma) reaction (Adams, 1981) which was modified by the use of 1% β-D-glucose-oxidase (Sigma) instead of hydrogen peroxidase (Shu et al., 1988). The sections were mounted on gelatinized slides, dehydrated and coverslipped with Permount (Fisher Scientific, New Jersey, USA). Some sections were counterstained with cresylviolet.

The specificity of the antibody staining was confirmed in control studies omitting the primary antibody. In these cases only a faint background staining was observed.

2.2. Soma size measurements

In order to ensure a blind analysis, the slides were coded for experimental group and left and right brain side by a person who did not perform the morphometric analysis. Then, a detailed observation of the staining pattern was performed with the help of a Leica DMR microscope. A cell was considered to be immunopositive when the cell body exhibited a brown to black coloring omitting the nucleus. In the immunolabeled sections corresponding to the stereotaxic level A 3.75 (Karten and Hodos, 1967) of adult control and dark-incubated animals (Table 1), the cross sectional soma areas of 50 neurons in each layer containing immunopositive cells

were measured in each hemisphere by means of the image analyzing system 'analySIS' (Soft Imaging System, Münster, Germany). The boundaries of the selected cells were drawn by tracking the image displayed on the video screen with a computer mouse. The display was obtained with a JBC-TK camera attached to the microscope with a 40 × objective. The image analyzing system calculated the surface encircled. For intraspecific comparison, an index for the extent of soma size asymmetries between the hemispheres was calculated as the percent deviation from the mean value. Statistical analysis was performed with the PC-based statistic program Statistica (StatSoft, Tulsa, USA). We compared cell size differences between and within the experimental groups by using a multivariate analysis of variance (MANOVA) for repeated measures. Posthoc comparisons were performed by Tukey's HSD-tests or paired sample *t*-tests.

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). Contrast and brightness were adjusted to a variable extent to meet satisfying output results with the Fuji 'MediaLab' printer device.

3. Results

3.1. Adult labeling pattern

Both antibodies labeled a large number of cells within the pigeon's midbrain, but the staining pattern was substantially different. While the GABA-antibody gave rise to a somatic staining with occasionally visible stem dendrites, the PV-antibody labeled neurons and their processes up to finest dendritic ramifications (Fig. 1cFig. 8c).

As described in previous studies (Bagnoli et al., 1987; Domenici et al., 1988), GABA-ir cells could be detected in most retinorecipient tectal layers. GABA-immunoreactivity in layers 2–4 contained small to medium sized horizontal and multipolar cells. Layer 5 was characterized by larger horizontal cells and layers 7–10 by small radial neurons. Occasionally multipolar cells could be observed within layer 10. Apart from this somatic labeling, a diffuse neuropil staining could be detected in layers 4–8 which was most prominent in layer 4 (Fig. 1a).

In accordance with earlier studies (Pfeiffer and Britto, 1997; Theiss et al., 1998), PV-ir cells could be detected in layers 2–4, 6–7 and 9–10 while the whole optic tectum was filled with a network of PV-ir fibers (Fig. 1b and c). Dendrites of PV-ir cells within layers 2–4 mainly ramified within layer 4 while cells of layers 6–10 were radially arranged cells with long dendrites which traversed many layers (Fig. 1c). While the apical dendrites of layer 6 neurons ascended up to layer 3, those of layer 10 reached up to layer 6. Occasionally, some larger multipolar neurons could also be detected in layer 10. Apart from these neurons which reached with their dendrites into the superficial retinorecipient laminae, a small number of large cells at the border between layers 13 and 14 were also labeled (Fig. 1b, Figs. 8 and 9). These cells corresponded presumably to the solitary magnocellular neurons described by Martinez-de-la-Torre et al. (1987).

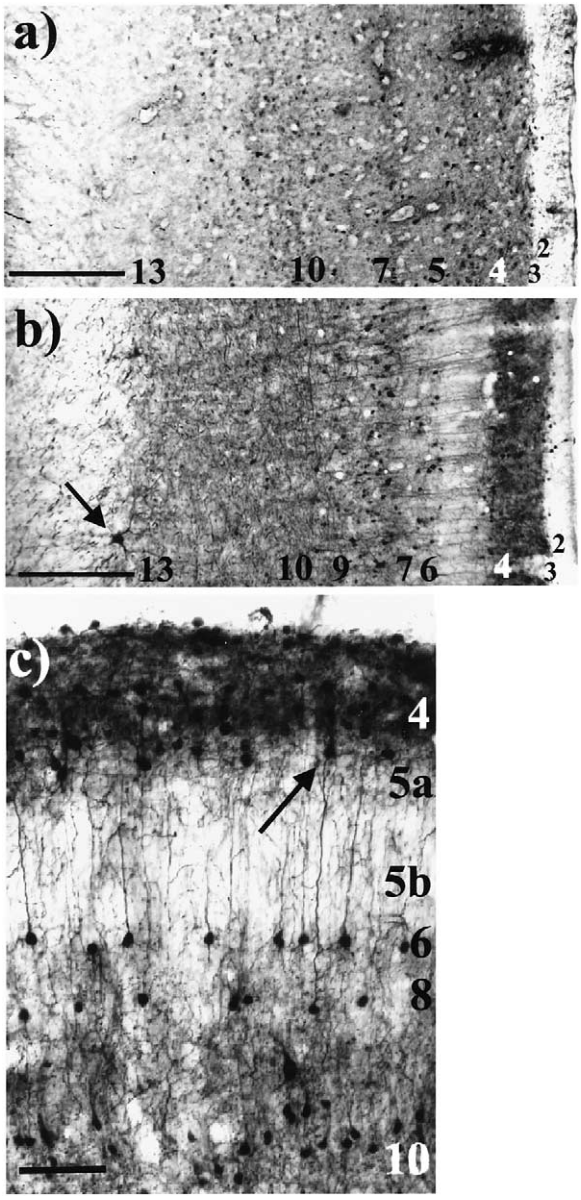


Fig. 1. GABA- (a) and PV- (b) immunoreactivity within the retinorecipient layers of the optic tectum. Arrow points to a multipolar cell at the border between layer 14 and 13. With PV (c), dendrites were labeled up to terminal arborizations (e.g. arrow). Bars indicate 200 μm in (a and b) and 50 μm in (c).

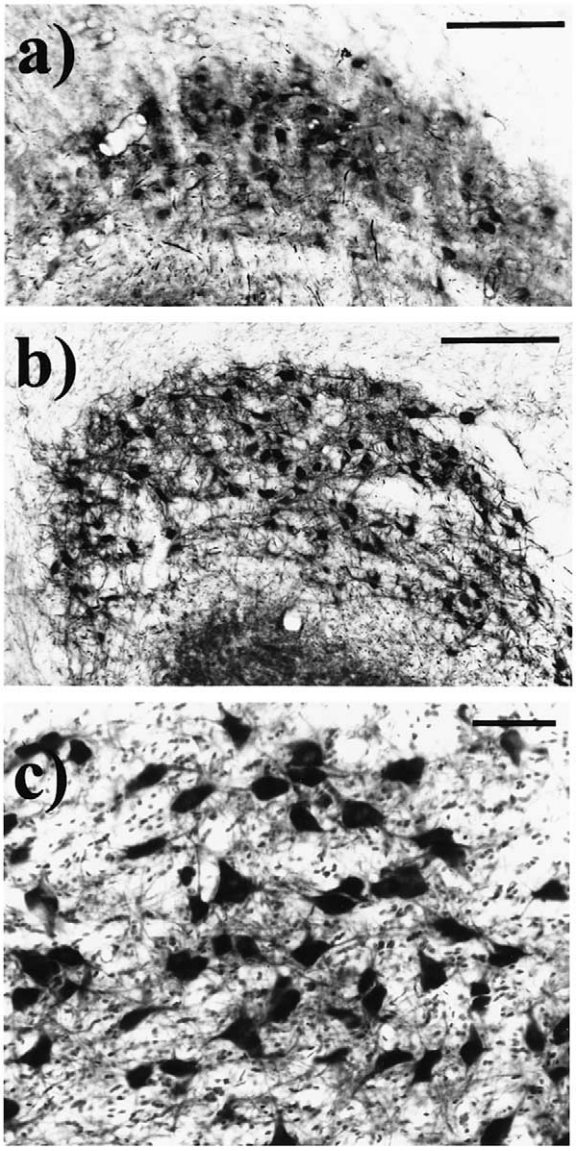


Fig. 2. GABA- (a) and PV- (b) immunoreactivity within the Imc. Counterstaining with cresylviolet indicated that virtually all magnocellular cells were PV-immunopositive (c). Bars indicate 200 μm in (a and b) and 100 μm in (c).

The Imc of the isthmic complex displayed somatic GABA- as well as PV-immunoreactivity (Fig. 2). Additionally, a dense network of ir fibers could be observed. Cresylviolet counterstaining revealed that most, if not all, Imc neurons exhibited GABA- as well as PV-immunoreactivity (Fig. 2c).

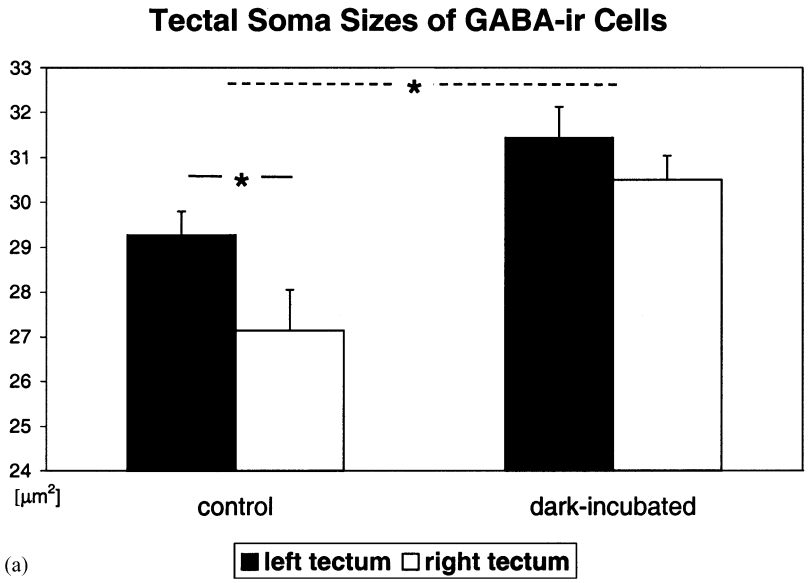
3.2. Cell size asymmetries

Since PV- and GABA-immunoreactivity was only partly colocalized within the tectal laminae, we performed separate morphometric analyses in PV- and in GABA-immunostained sections. In GABA-immunolabeled preparates, we selected layers 2, 3, 4, 5, 7 and 10. In PV-immunostained sections the selected layers were 2, 3, 4, 6, 9 and 10. The solitary magnocellular neurons were excluded from this analyses because their number was small. In the Imc, all neurons displayed GABA- as well as PV-immunoreactivity indicating a complete colocalization of the antigenes. Therefore, we measured cell sizes in PV-immunolabeled preparates only.

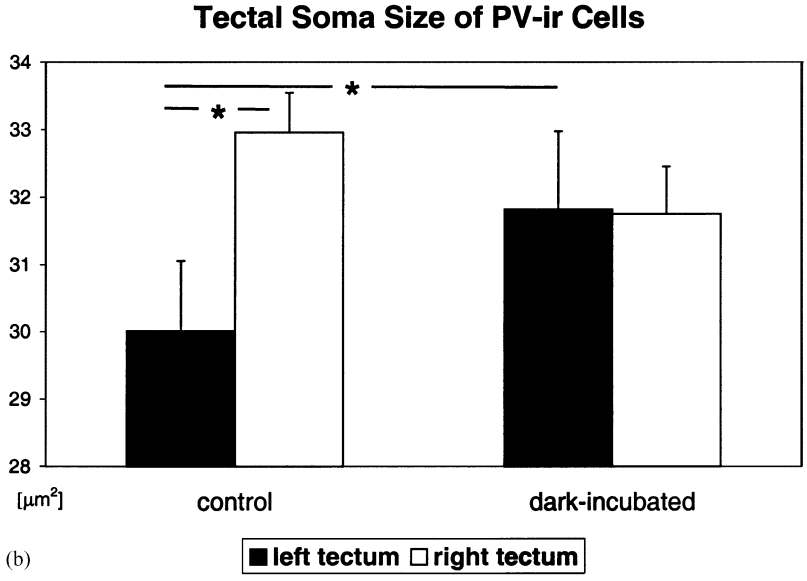
In general, comparison of cell sizes in single layers of the left and the right tectum revealed soma size asymmetries in controls but not in dark-incubated-animals. While GABA-ir cells displayed larger cell bodies within the left tectum, PV-ir neurons were enlarged within the right tectum (Figs. 3 and 4). We analyzed these size differences by performing three-factorial MANOVAs (Group × Brain side × Layer).

The average cell size of GABA-ir neurons was significantly larger in dark-incubated animals than in controls ('Group' effect: $F(1, 18) = 8.68$, $P < 0.01$; Fig. 3a). In general, cells were larger within the left tectum ('Brain side' effect: $F(1, 18) = 6.38$, $P < 0.05$) and cell sizes differed between laminae ('Layer' effect: $F(4, 72) = 389.86$, $P < 0.001$). The actual cell size of a cell within a given layer was influenced by the developmental light conditions ('Group × Layer' interaction: $F(4, 72) = 5.054$, $P < 0.01$) and by the side in which the cell was located (Brain side × Layer interaction: $F(4, 72) = 3.518$, $P < 0.05$). A planned comparison showed that only the tecta of control animals established asymmetrical soma sizes with larger cells on the left ($F(1, 18) = 6.9$, $P < 0.05$; Fig. 3a). According to posthoc paired sample t tests, significant asymmetries were confined to layers 4, 7 and 10 ($P < 0.05$; Fig. 4a and c). A planned comparison revealed that the emergence of size differences between the tecta in controls mainly resulted from a significant decrease of cell sizes within the right tectum of controls compared to dark-incubated animals, $F(1, 18) = 11.829$, $P < 0.01$, (Fig. 3a). Cell sizes within the left tectum did not differ, $F(1, 18) = 2.389$, $P = 0.14$ (Fig. 3a).

In contrast to the GABA-ir cellular population, PV-ir neurons displayed equal average cell sizes in control and dark-incubated animals ('Group' effect: $F(1, 21 = 0.06$, ns; Fig. 3b). But cell sizes differed significantly between the hemispheres with larger cells on the right side ('Brain side' effect: $F(1, 21) = 14.303$, $P < 0.01$) and the extent of these differences depended on the embryonic light condition ('Group × Brain side' interaction: $F(1, 21) = 15.737$, $P < 0.001$). A planned comparison demonstrated that cell sizes varied between the tecta solely in controls ($F(1, 16) = 9.036$, $P < 0.01$; Fig. 3b, Fig. 4b and d). According to posthoc paired sample t-tests, cells of all tectal layers were enlarged in the right tectum ($P < 0.01$ for each lamina; Fig. 4b). This asymmetry pattern resulted from a significant cell size decrease of PV-ir neurons within the left tectum of controls compared to that of dark-incubated animals ($P < 0.05$; Fig. 3b).



(a)



(b)

Fig. 3. Tectal soma sizes of GABA- (a) and PV- (b) ir cells within controls and dark-incubated animals; bars represent standard error, $*P < 0.05$ according to MANOVA; $*P < 0.05$ according to posthoc comparison.

In contrast to the tectum, PV-ir cells of the Imc did not exhibit soma size asymmetries neither in controls nor in dark-incubated animals. Statistical analysis evinced no significant influences of any factor confirming that no size differences between control and dark-incubated animals or between the brain sides existed (Fig. 5).

3.3. Developmental pattern

While the multipolar neurons of the Imc displayed a strong GABA- as well as PV-immunoreactivity from the first day after hatching onwards (Fig. 6), the GABA- and PV-ir tectal cells exhibited a divergent posthatch developmental pattern. Although a prominent number of GABA-ir cells could be identified directly after hatching in all tectal layers (Fig. 7), PV-immunoreactivity was only scarce. Apart from few magnocellular neurons in prospective layers 13–14, only some spots of faintly labeled PV-ir cell bodies in the retinorecipient layers could be identified (Fig. 8a). During the first week after hatching, the number and staining-intensity of PV-ir cell bodies within spots of the retinorecipient layers increased. Beside this somatic labeling, a diffuse neuropil staining emerged within the outermost laminae (prospective layers 2–4) presumably constituted by the developing dendritic network (Fig. 8b). During the following weeks, tectal PV-immunoreactivity further increased, until 3 weeks after hatching the dendritic and somatic staining pattern obtained an adult-like

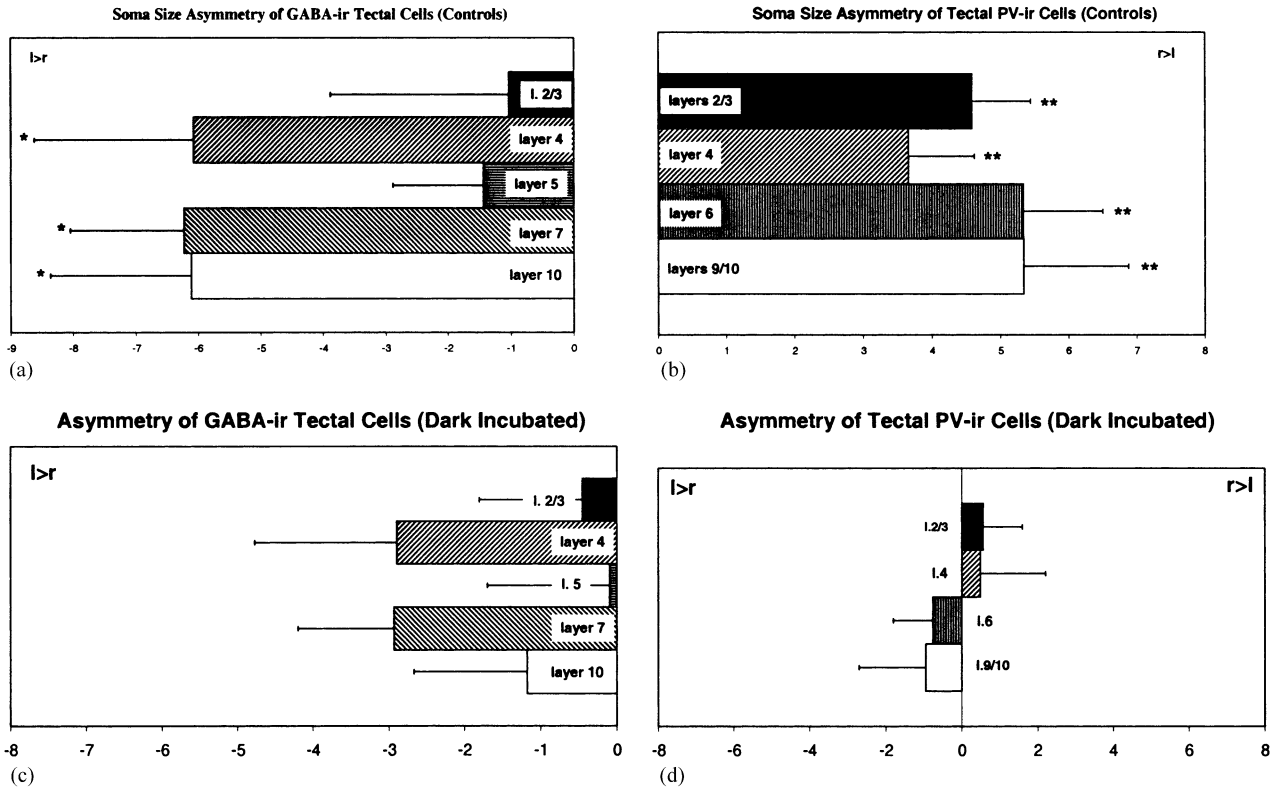


Fig. 4. Soma size asymmetries of tectal GABA- (a and c) and PV- (b and d) ir cells within control and dark-incubated animals; the extent of asymmetry, expressed as the percent deviation from mean value, differs between laminae and cellular populations; bars represent standard error; $*P < 0.05$; $**P < 0.01$ according to posthoc comparison.

morphology (Fig. 8c). Due to the less detailed morphological staining pattern, comparable assertions about the maturation of GABA-ir cells could not be made.

Within the deeper tectal layers, at posthatching day 1 GABA- as well as PV-ir fibers could be observed running within the efferent fiber layer (prospective layer 14), occasionally ascending up to prospective layer 10 (Fig. 8a, Fig. 9). The origin of these fibers remained unclear but it is possible that they represent a mixture of efferent and afferent axons. Some of the PV-ir fibers are possibly axons of the solitary magnocellular neurons. Since the Imc and the nucleus spiriformis lateralis both display GABA- and PV-immunoreactivity from hatching onwards (Fig. 9) and since both of them innervate the tectum (Reiner et al., 1982), some of the remaining labeled fibers were probably afferents from these nuclei.

4. Discussion

The present study reveals cell size asymmetries of specific cellular populations within the optic tectum with larger GABA-ir cells in the left and larger PV-ir neurons in the right tectum. This indicates that these cells constitute distinct cellular populations which react differently to visual input. The formation of left–right differences in both cell types obviously depends on an asymmetrical visual stimulation during the embryonic development since dark-incubated animals do not develop tectal soma size asymmetries. The absence of corresponding left–right differences in the Imc suggests that not all visual structures develop cell size asymmetries in response to an unbalanced visual input. This might point to specific developmental processes which are responsible for the establishment of left–right differences in the optic tectum and which might be related to differential connectivity patterns of the neuronal populations.

It is generally assumed that PV can be used as a marker for GABA since Celio (1984) reported that cortical PV-expression is exclusively confined to GABAergic cells. However, several subsequent reports also mentioned colocalization of PV with other neurotransmitters like glutamate (Nitsch et al., 1994) or glycine (Aoki et al., 1990). Although an analysis of colocalization was not the focus of the present study, our data make it likely that PV and GABA are, at least in part, not colocalized in the tectum, while there seems to be a complete match of GABA- and PV-expression in the Imc.

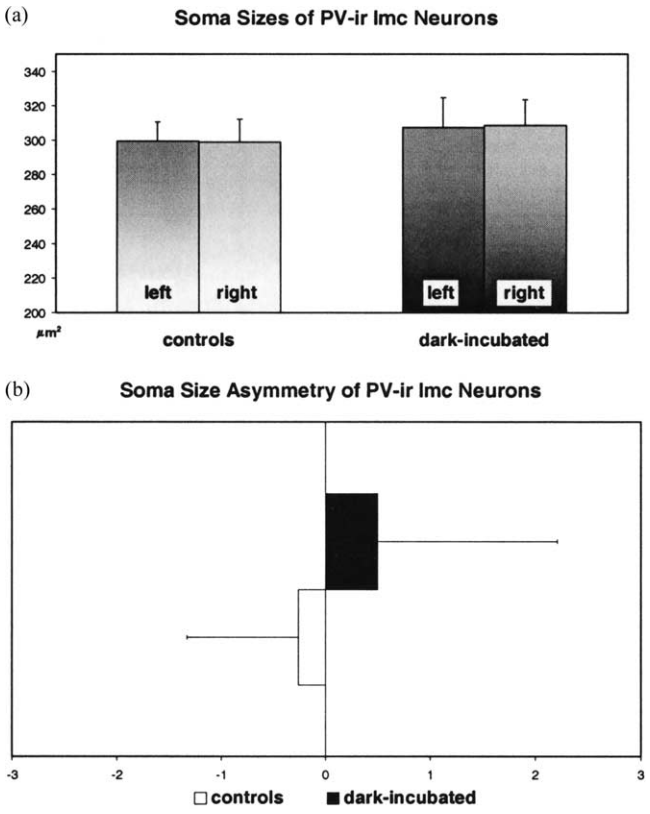


Fig. 5. (a) Soma sizes of PV-ir Imc neurons within controls and dark-incubated animals. (b) Soma sizes of PV-ir cells expressed as the percent deviation from mean value within the Imc of control and dark-incubated animals; bars represent standard error.

4.1. Developmental pattern

The present study shows that GABA- and PV-ir immunoreactivity in the optic tectum displays distinct developmental patterns. A large number of GABA-ir cells could be detected immediately after hatching. In contrast, PV-immunoreactivity was very scarce and only after 1 week the first ramifying dendrites and some cell bodies could be observed. The dendritic maturation with

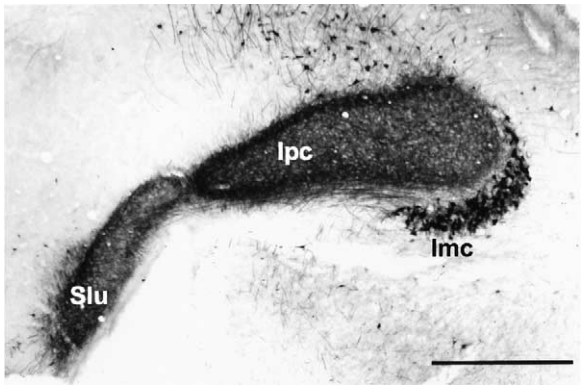


Fig. 6. PV-immunoreactivity within the isthmic complex of a 7-day old pigeon. While the Imc exhibited a strong somatic labeling, Ipc and Slu were filled with a dense fiber netting. Bar indicates 500 µm.

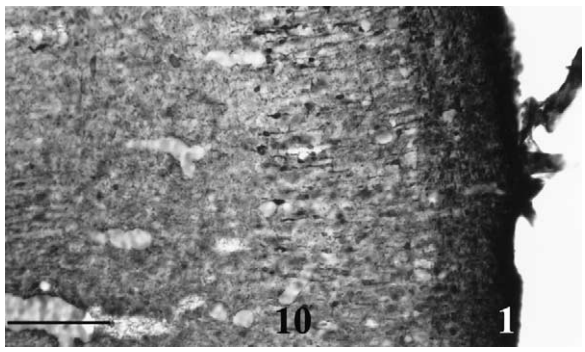


Fig. 7. GABA-immunoreactivity within the tectum of an 1-day old pigeon. Several ir neurons with dendrites ascending into the superficial layers could be detected within prospective layer 10; apart from a diffuse neuropil staining, the superficial layers contained many labeled cells. Bar indicates 100 µm.

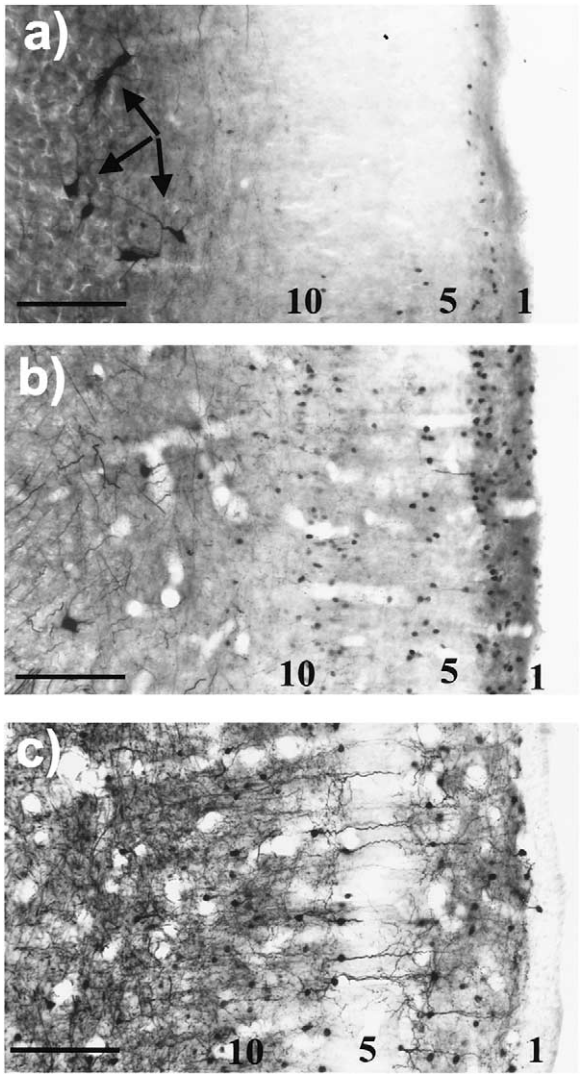


Fig. 8. Development of PV-immunoreactivity within the tectum of 1-day old (a), 7-day old (b) and 21-days old (c) pigeons—while directly after hatching only some somata within the superficial layers were labeled, the large multipolar neurons (arrows, a) within prospective layer 13/14 were mature. Labeling of the laminae corresponds to the prospective adult layers. Bars indicate 100 µm.

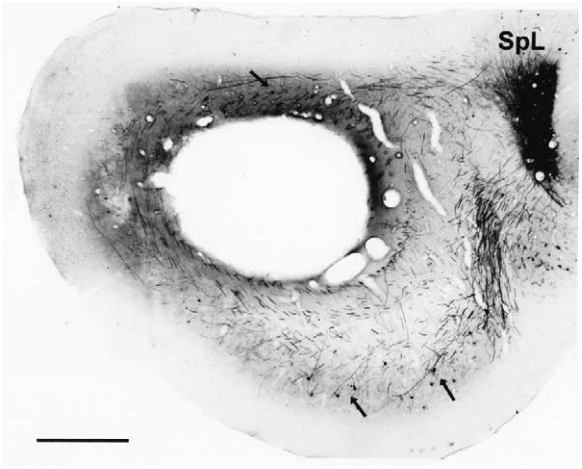


Fig. 9. PV-immunoreactivity within the rostral tectum of an 1-day old pigeon: within the deeper layers, PV-ir fibers and several multipolar neurons (arrows) could be observed while no superficial labeling was apparent. Note the strong labeling of the nucleus spiriformis lateralis (SpL). Bar indicates 500 μm.

an adult-like lamination pattern was not finished before 3 weeks after hatching. This sequence suggests that the establishment and maturation of the synaptic connections of PV-cells is established predominantly during the posthatching period. Due to the poor resolution of the GABA-immunostained neurites, comparable assertions about the dendritic development of GABAergic neurons can not be made. However, it is likely that the development of GABA cells might start earlier but is not finished at hatch since Bagnoli et al. (1989) demonstrated that GABA cell densities decreased while neuropil staining intensity increased during the posthatching period.

This developmental time course is typical for inhibitory cells. Several studies show that the onset of PV expression lags behind that of GABA immunoreactivity (Solbach and Celio, 1991; Mize et al., 1996; Vogt Weisenhorn et al., 1998). The delayed PV-maturation led to the assumption that the onset of PV expression starts with the functional maturation of a specific system but does not necessarily coincide with the beginning of inhibitory activity (Andressen et al., 1993; Okada and Kudo, 1997). In this regard, the late expression of PV in the pigeon's optic tectum correlates well with the electrophysiological maturation of the retinotectal system which becomes functional during the first week after hatching (Bagnoli et al., 1987). Since Imc neurons display PV-immunoreactivity directly after hatching, this structure seems to have a more rapid temporal pattern of maturation without posthatch alterations which were visible with our methods.

4.2. Light influences on cell size development

Since the eyes of the pigeon's embryo are asymmetrically stimulated during the late embryonic phase, prehatch tectal development is asymmetrically affected by retinal input (Güntürkün, 1993). The induced lateralized differentiation processes can be characterized as a primary asymmetry. Since the retinotectal system of pigeons is immature at hatch (Bagnoli et al., 1985, 1987, 1989, 1992; Manns and Güntürkün, 1997) its final maturation occurs after hatching under visual control and is hence epigenetically determined. As a consequence, the final stabilization of the primary asymmetries should occur after hatching when morphological left–right differences in tectal soma sizes become apparent (Güntürkün, 1997a). Thus, the posthatching period represents a highly critical timepoint in which structural and functional asymmetry patterns can be changed by monocular deprivation (Manns and Güntürkün, 1999a,b). During this period, tectal interneurons might be critically involved in processes which transfer primary asymmetries into a stable asymmetrical functional architecture of the optic tectum and which concomitantly establish their own soma size asymmetry pattern.

The results of the present study show that the average cell size of tectal GABA- and PV-ir neurons depends on the embryonic light conditions and hence differs between the left and the right tectum. Both cellular populations only develop asymmetrical soma sizes after embryonic light stimulation but not under dark conditions. However, light has differential effects on the two cell types: While the size of GABA-ir cells decreases in the right (light-deprived) tectum, those of PV-ir cells are reduced on the left (stimulated) side. As a consequence, the GABA- and PV-ir neurons establish opposing cell size asymmetries.

At first glance, the formation of larger GABAergic cells within the left tectum might point to a growth promoting effect of light. However, the comparison with dark-incubated animals shows that the size asymmetry is not caused by an accelerated growth of GABA-ir cells within the left tectum, but is induced by a shrinkage of cells within the right. This also explains why dark-incubated birds had on average larger GABA-ir cell bodies. Thus, it is more likely that the size asymmetry of GABA-ir cells is governed by indirect and presumably competitive processes within or between the tecta in response to an unbalanced visual input.

The response of PV-ir tectal neurons to visual stimulation is different. The decrease of cell sizes within the left tectum of controls provides evidence for an inhibitory effect of light onto PV-ir cells. This effect is in accordance with reports showing that visual input suppresses the expression of PV in the mammalian superior colliculus during a specific sensitive phase (Lane et al., 1996). However, this inhibitory effect possibly does not result from a direct light effect on PV-ir cells since the mean cell sizes between control and dark-incubated animals did not differ. A suppressive

effect only becomes obvious in response to asymmetric visual stimulation. Again, these conditions provide evidence for indirect competitive intra- or inter-hemispheric processes similar as for the GABA-ir cells. These processes probably mainly act posthatching since PV-cells slowly differentiate within the first 3 weeks after hatch. Altogether, these facts imply that synaptogenetic interactions during posthatch tectal maturation are involved in the suppression of PV-immunoreactivity within the left tectum as a secondary consequence of embryonic asymmetric light input.

The decisive role of specific tectal connections for the formation of asymmetries is confirmed by the fact that the PV-cells of the Imc do not develop soma size differences between the brain halves. Since Imc receives visual input via the optic tectum, the cells can react only indirectly to visual stimulation by interactions with tectal neurons. It is conceivable that the absence of asymmetry in Imc is due to its precocious developmental pattern which shields this structure from the tectal processes which occur during the stabilization of asymmetry.

Visual stimulation modulates axonal and dendritic processes of target areas over a prolonged period of time (Sin et al., 2002). These effects not only promote growth and stabilization but are also able to induce pruning of dendritic arbors (Cantallops et al., 2000). Consequently, dark-incubation can result in an enlargement of tectal dendritic trees in amphibia (Cohen-Cory, 1999; Sin et al., 2002). Thus, assuming that soma size is a marker for the extent of neurite arbors, larger cell bodies might not inevitably indicate more complexly differentiated cells but can also result from reduced pruning of redundant dendritic branches. The present data, which demonstrate a stimulation-dependent establishment of GABA- and PV-ir cell size asymmetries provide evidences for both effects. While the larger cell bodies of PV-ir cells within the deprived right tectum can be explained by reduced pruning, the effects onto GABAergic cells seem to be more complex. The enlarged cells in dark-incubated animals point to a decreased pruning of GABAergic cells, while the establishment of larger cell bodies within the stronger stimulated tectum provides evidence for enhanced dendritic complexity. Altogether, these effects modulate visual circuits within both tectal sides and are hence able to induce hemisphere-specific modes of visually-guided behavior (Skiba et al., 2002).

Acknowledgements

This research was supported by the Sonderforschungsbereich 509 NEUROVISION of the Deutsche Forschungsgemeinschaft.

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