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Photic inhibition of TrkB/Ras activity in the pigeon's tectum during development: impact on brain asymmetry formation

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Abstract

Asymmetric photic stimulation during embryonic or post-hatch development induces a functional lateralization of the pigeon's visual system, which is accompanied by left–right differences in tectal cell sizes. The intracellular membrane-anchored GTPase Ras can be activated by a number of upstream mechanisms including binding of brain-derived neurotrophic factor to its specific TrkB receptor. Ras activity plays an important morphogenetic role in neurons and therefore might also be involved in the asymmetric differentiation of tectal cells. To investigate the role of Ras, we determined the relative levels of activated Ras and of signalling active phospho-TrkB in tecta of light- and dark-incubated pigeons and combined this with an immunohistochemical detection of Ras-GTP and TrkB receptors. While Ras activation levels did not differ between light- and dark-incubated pigeons during embryonic development, directly after hatching Ras activity was significantly decreased in the stronger stimulated left tectum of light-incubated animals. This was accompanied by lower levels of TrkB phosphorylation. Immunohistochemical staining revealed Ras-GTP-positive cell bodies within the efferent cell layer. These cells were TrkB-positive and developed enlarged soma sizes within the right tectum during the first week after hatching. This association suggests asymmetric Ras activation to be involved in the asymmetric differentiation of the efferent cells as a result of asymmetric TrkB signalling. Because asymmetric light exposure occurs only during embryonic development, the observed transient asymmetric inhibition of TrkB/Ras activity after hatching may reflect differential embryonic maturation of tectal inhibitory circuits leading to a functional superiority of the right eye in the adult organism.

Introduction

Visual pathways in vertebrates have been established as models to examine how neuronal circuits adapt their differentiation to the environment. Developing neurons respond quickly to various epigenetic signals including sensory input, guidance molecules or trophic factors (Cohen-Cory, 2002; Vicario-Abejón *et al*., 2002). These factors regulate growth, retraction and stabilization of dendritic or axonal arbors and synapses (Holtmaat *et al*., 2005). Although some molecular mechanisms of axo-dendritic remodelling are evolving now (Wong & Ghosh, 2002; Li *et al*., 2004), their behavioural consequences are much less understood. The avian visual system is a suitable model to examine functional effects of altered visual pathways in response to modulations of the visual experience because it develops a behavioural lateralization with a superiority of the right eye/left hemisphere for detailed visual feature analysis that is dependent on asymmetric visual stimulation (Rogers, 1996; Güntürkün, 2002a). In pigeons, a functional lateralization is accompanied by morphological left–right differences in the tectofugal pathway (Güntürkün, 2002b).

Retinotectal development is regulated by visual stimulation (Ruthazer & Cline, 2004), and neurotrophic factors mediate different aspects of light influences. Visual stimulation adjusts the expression and/or release of neurotrophins and hence controls trophic support of target cells (Vicario-Abejón *et al*., 2002). In turn, neurotrophic factors control sprouting, branching and maintenance of axo-dendritic trees. Because brain-derived neurotrophic factor (BDNF), but not nerve growth factor, expression is regulated by light in the rat visual cortex (Castrén *et al*., 1992; Lindholm *et al*., 1994), it is assumed to be involved in the activity-dependent regulation of development in an antero- and retrograde fashion (McAllister *et al*., 1999; Huang & Reichardt, 2001; Menna *et al*., 2003). Trophic factors exert their biological role by activating their specific Trk tyrosine kinase receptors (Patapoutian & Reichardt, 2001; Teng & Hempstead, 2004), which activate members of the Ras superfamily of GTP-binding proteins to trigger several intracellular signalling cascades (Patapoutian & Reichardt, 2001; Huang & Reichard, 2003). The G protein Ras is one critical molecular switch for relaying neurotrophic actions (Borasio *et al*., 1989; Heumann, 1994; Kaplan & Miller, 2000). Constitutive neuronal Ras activation in a transgenic mouse model leads to neuronal hypertrophy (Heumann *et al*., 2000) accompanied by increased dendritic size and arborization, axonal calibre and density of afferent input (Arendt *et al*., 2004; Gärtner *et al*., 2005). This points to an important morphogenetic role of Ras-mediated signalling in the differentiation and maintenance of axo-dendritic complexity resulting in modulated synaptic connectivity (Heumann *et al*., 2000; Seeger *et al*., 2004). Because morphological asymmetries in the pigeon's tectofugal pathway become obvious as tectal cell size asymmetries, Ras signalling might be involved in their asymmetric differentiation. However, it is unclear if Ras activation is

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directly modulated by firing of stimulated sensory neurons or more indirectly by changes in BDNF levels. Thus, we examined if TrkB signalling and downstream Ras activity changes within the pigeon's tectum in response to photic stimulation and may hence display left–right differences due to asymmetric embryonic light exposure and combine this with an immunohistochemical analysis of the optic tectum.

Materials and methods

For the experiments, light- and dark-incubated pigeon embryos and hatchlings (*Columba livia*) were used (Table 1). Fertilized eggs from lab-own breeding pairs were incubated in a still-air incubator kept in darkness or under permanent illumination at constant temperature (38.3 °C) throughout the entire period of incubation (Skiba *et al*., 2002). Embryonic animals (E17) were taken out of the eggs and decapitated, while PH1 animals were decapitated directly after hatching. Some light-incubated hatchlings (Table 1) were swapped with artificial eggs the breeding pairs were sitting on and decapitated after 1 week (PH7). Brains were removed from the skull, and the tecta were snap-frozen in nitric oxygen and stored at −70 °C until biochemical analysis. Dark-incubated animals received light only during a very short time window of 1–2 min when the animals were decapitated, and PH7 animals were kept under a 12 h light : dark cycle from hatching onwards. For immunohistochemical examinations, young animals from lab-own breeding pairs or adult animals that developed under a 12 h light : dark rhythm were used (Table 1). The experiments were carried out according to the specifications of the German law for the prevention of cruelty to animals.

Pull-down assay and Western blot analysis

Homogenized tissue (in 50 mM Tris–HCl, pH 7.4, 100 mM NaCl, 2 mM $MgCl_2$, 10% glycerol, 1% Nonidet-P40) was centrifuged at 14,000 ***g***, 4 °C, and the supernatant was used for further experiments. Equal amounts of protein (15 μg/slot) were analysed with sodium dodecyl sulphate–polyacrylamide gel electrophoresis and Western blot [primary antibodies: mouse anti-pan Ras IgG, AB3, Chemicon, diluted 1 : 400 in Tris-buffered saline, anti-phospho Trk, pY490, NEB, 1 : 1000 and anti-TrkB, Santa Cruz, 1 : 1000; secondary antibody: anti-mouse-peroxidase diluted 1 : 10,000 and anti-rabbit peroxidase, 1 : 8000, Dianova, Germany]. Blots were analysed by densitometric measurement and quantified using the program TINA 2.09 (Raytest, Germany).

Protein (800 μg) from lysates was used for Ras pull-down experiments, which have been performed as described in detail by de Rooij & Bos (1997). For immunoprecipitation of TrkB, 800 μg protein from lysates was incubated with 1 μg/mL rabbit anti-TrkB antibody (Santa Cruz) and 15 μL anti-rabbit antibody coupled to agarose (Sigma) for 2 h at 4 °C. The precipitate was washed twice with homogenization buffer, twice with 100 mM Tris–HCl, 500 mM LiCl, pH 8.0, and twice with 10 mM Tris–HCl, pH 8.0. Proteins were eluted by boiling the precipitates in Laemmli loading buffer for 5 min and analysed by Western blotting. The ratio between optic densities of Ras-GTP- and total Ras-immunoblots served as an index for the relative levels of active Ras within the probes. Correspondingly, the ratio between optic densities of phospho-TrkB and total TrkB reflected the amount of activated TrkB. Inter- and intra-individual differences of data from the same blots were statistically analysed by non-parametric tests (Fig. 1).

Table 1. Number of experimental animals in each group

	E17	PH1	PH7	Adult
Ras pull-down assay				
Light + dark	–	–	–	6
Light-incubated	6	13	7	
Dark-incubated	6	6	–	–
TrkB immunoprecipitation				
Light-incubated	–	5	–	–
Dark-incubated	–	5	–	–
Immunostaining				
Ras-GTP	–	4	–	3
TrkB	–	6	7	–

Immunohistochemistry

For immunohistochemical detection of Ras-GTP, a modified immunocytochemical protocol (Sherman & Ratner, 2001; Blöchl *et al*., 2004) was used. Animals were deeply anaesthetized 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 ice-cold 4% paraformaldehyde [in 0.12 M phosphate-buffered saline (PBS, pH 7.2) + 12 mM $MgCl_2$]. Brains were dissected out of the skull, post-fixed in the fixative + 30% sucrose (w/v) + 12 mM $MgCl_2$ for 2 h, cryoprotected overnight in 0.12 M PBS + 30% sucrose (w/v) + 12 mM $MgCl_2$ at 4 °C, and were cryosectioned in frontal plane at 35 μm.

After three washes with PBS + (PBS + 12 mM $MgCl_2$) the sections were incubated with 0.01 μg/μL RBD–GST (purified as described in de Rooij & Bos, 1997) overnight at 4 °C. Then the immunohistochemical detection of the RBD–GST complex was performed 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 5 min each with PBS + followed all incubation steps. Endogenous peroxidases were blocked with 0.3% H_2O_2 in A. dest. Sections were incubated with primary antibody solution [mouse anti-GST 1/100 (Cell Signalling Technology) dissolved in 0.12 M PBS + 0.1% Saponin 100 (w/v) + 0.5% normal horse serum (w/v)] overnight at 4 °C. The secondary antibody reaction was carried out with biotinylated horse anti-mouse IgG [Vectastain Elite kit, Vector, Burlingame, CA, USA; 1/100 0.12 M PBS + 0.3% Triton X-100 (w/v)] 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 + 0.3% Triton (w/v)]. Peroxidase activity was detected with diaminobenzidine according to Hellmann & Güntürkün (2001). Sections were mounted on gelatinized slides, dehydrated and coverslipped with Permount (Fisher Scientific, New Jersey, USA).

To verify the specificity of the staining, we performed several control experiments (Fig. 2). The specificity of the secondary antibody was confirmed by omitting the primary anti-GST antibody. To test specificity of the anti-GST antibody to the RBD–GST complex, staining was performed omitting incubation with RBD–GST (Fig. 2B). To test that RBD–GST binding was critically dependent on RBD, which in turn binds to Ras-GTP, sections were incubated with GST alone (data not shown). In order to confirm that the immunosignal depends on the specific RBD–GST binding to Ras-GTP, incubation was performed with a RBD–GST mutation that had lost its affinity to bind to Ras-GTP (kindly provided by C. Herrmann; Fig. 2C). In all these control experiments no or just a faint background staining was observed.

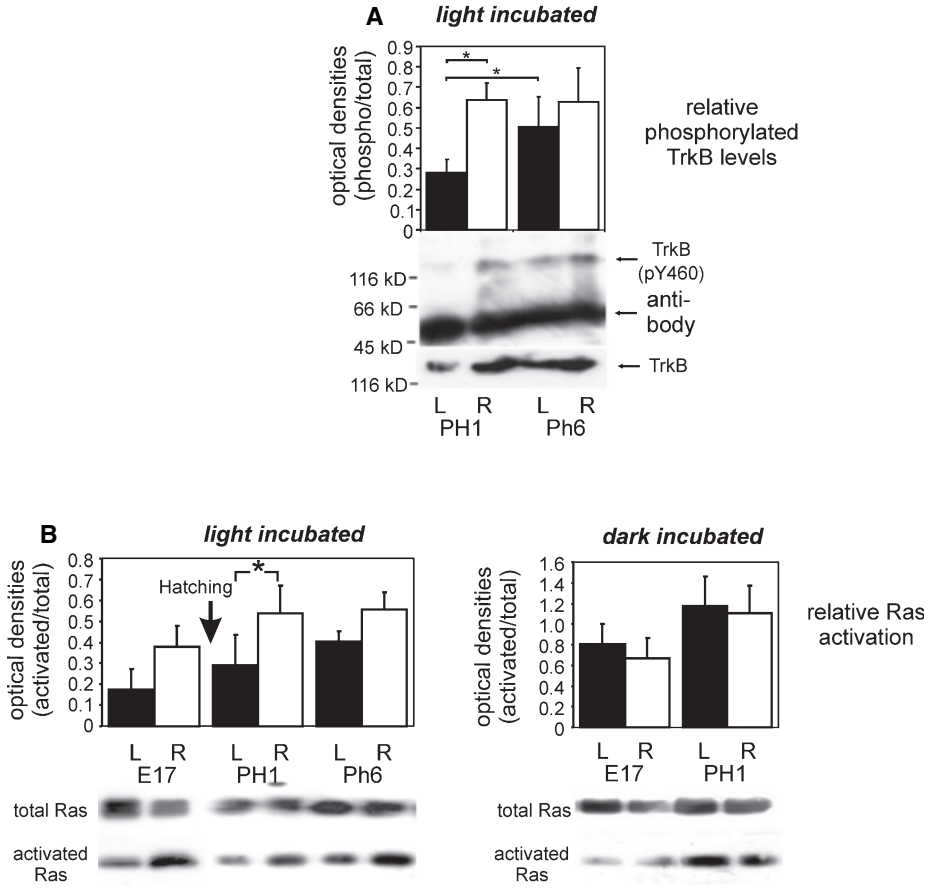


FIG. 1. Activation of Trk and Ras. The relative amount of phosphorylated Trk to total Trk (A) and activated Ras to total Ras (B) is analysed by semiquantitative optico-densitometrical evaluation of four (A) to six (B) blots. The histograms represent means ± SE, and are underlayed with a representative blot. *$P < 0.05$ according to Mann–Whitney U- or Wilcoxon matched-pairs test, respectively.

For TrkB receptor immunohistochemistry, sections were immunolabelled with rabbit anti-TrkB IgG (1/200; Santa Cruz). The secondary antibody reaction was carried out with biotinylated goat anti-rabbit IgG (1/200; Vectastain Elite kit, Vector) according to the ABC-technique (see above, Theiss & Güntürkün, 2001). The specificity of the antibody staining was confirmed in control studies omitting the primary antibodies.

Soma size measurements

Analysis of the staining pattern was performed with the help of a Leica DML microscope. In the immunolabelled sections (corresponding to the stereotaxic level A3.75; Karten & Hodos, 1967) of young pigeons (PH1, PH7; Table 1), the cross-sectional soma areas of 50 neurons in each layer containing immunopositive cells in each hemisphere were measured with the help of the image-analysing system 'analySIS' (Soft Imaging System, Münster, Germany; Manns & Güntürkün 1999a,b, 2003). For intra-specific comparison, an index for the extent of soma size asymmetries between the hemispheres was calculated as the percentage deviation from the mean value. Statistical analysis was performed with the PC-based statistic program Statistica (StatSoft, Tulsa, OK, USA). Photographic documentation was carried out with a digital camera (Zeiss AxioCam). Digital images were processed with Axiovision 3.0 (Zeiss, Germany). Contrast and brightness were adjusted with Photoshop 5.5 (Adobe, Mountain View, CA, USA).

Results

TrkB activation pattern

Analysis of TrkB immunoprecipitations revealed that the left and right optic tectum of light-incubated animals differed in TrkB activation levels during post-hatch development (Fig. 1A). Directly after hatching, the relative level of phosphorylated TrkB was significantly lower in the left tectum as compared with the right one (Wilcoxon matched-pairs test: $z = 2.022$; $P < 0.05$). This difference was levelled off at 1 week after hatching (Wilcoxon matched-pairs test: $z = 0.105$; $P = 0.917$) due to a selective increase in TrkB activation levels in the left tectum (Mann–Whitney U-test: $z = -2.008$; $P < 0.05$). In contrast, TrkB activation did not change in the right tectum that had been connected to the occluded eye in the embryo (Mann–Whitney U-test: $z = -0.365$; $P = 0.7$).

Ras activation pattern

We now asked if the observed differences in TrkB phosphorylation resulted in a similar activation pattern of Ras (Fig. 1B). In fact, directly after hatching, the relative Ras activities were significantly lower in the left tectum as compared with the right one (Wilcoxon matched-pairs test: $z = 2.366$; $P < 0.05$). Similar to TrkB activation, this difference in Ras activity was levelled off 1 week after hatching (Wilcoxon matched-pairs test: $z = 1.153$; $P = 0.248$). Adult animals also did not display left–right differences in Ras activity (data not shown).

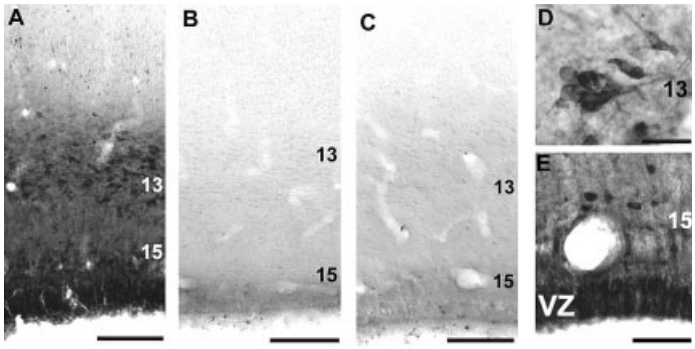


FIG. 2. Immunohistochemical detection of Ras-GTP within the tectum of a 1-day-old pigeon (A); omitting RBD–GST incubation (B) or incubation with a RBD–GST mutated molecule (C) prevented any immunostaining; while neurons within the efferent layer 13 displayed a somatic dot-like immunostaining (D), all cells within the ventricular neuroepithelium (VZ) were strongly labelled (E). Scale bars, 100 μm (A–C), 20 μm (D) and 50 μm (E).

Next we tested if the observed downregulation of Ras activity at PH1 in the left tectum resulted from asymmetric embryonic light exposure by comparing light- and dark-incubated animals. In distinct contrast to the light-incubated birds, dark-incubated pigeons did not develop any asymmetry in Ras activation between left and right tectum at PH1 (Wilcoxon matched-pairs test: $z = 0.674$; $P = 0.500$). There were also no differences in Ras activities between the left and right tectum before hatching either in dark- (Wilcoxon matched-pairs test: $z = 1.572$; $P = 0.116$) or light-incubated animals (Wilcoxon matched-pairs test: $z = 1.572$; $P = 0.116$; Fig. 1B).

Immunohistochemical detection of Ras-GTP

In order to identify major Ras activation sites at the cellular level, we performed an immunohistochemical detection of Ras-GTP. Directly after hatching, Ras-GTP-specific immunostaining was found within the ventricular zone (Fig. 2A and E). This signal disappeared in adult animals (not shown). Moreover, cells within the efferent cell layer 13 displayed a dot-like Ras-GTP immunosignal that was mainly confined to the cell bodies (Fig. 2D). In contrast, virtually no cells were labelled within the superficial retinorecipient laminae.

Asymmetry of TrkB-ir tectal cells

The coincidence of reduced Ras-GTP and phospho-TrkB in the left tectum suggests an involvement of the trkB-Ras signalling cascade in asymmetric tectal differentiation. Therefore, we used TrkB immunohistochemistry for a morphometric analysis of TrkB-immunoreactive tectal cells to identify cell populations displaying asymmetric differentiation during the first week after hatching.

In correspondence to a previous report (Theiss & Güntürkün, 2001), the immunohistochemical staining revealed several TrkB-positive cells within the optic tectum of 1- and 7-day-old pigeons. On the one hand, immunolabelled cells within the retinorecipient layers could be detected that were confined to spots spanning through the outer laminae (Fig. 3). These spots represented a mixed population of multipolar or horizontal neurons and smaller presumably immature cells. In these retinorecipient layers Ras-GTP was below the level of detectability in cell bodies (see above) and hence did not correspond to TrkB immunosignal. On the other hand, efferent layer 13 neurons were TrkB- as well as Ras-GTP immunopositive. The morphometric analysis showed that these cells increased cell body size from 1 day to 7 days after hatching (Fig. 4A). While directly after hatching, the soma sizes of TrkB-positive cells did not differ between the left and right tectum (Wilcoxon matched-pairs test: $z = 0.674$; $P = 0.500$), 1 week later cell bodies of the efferent layer 13 neurons were significantly enlarged within the right tectum (Wilcoxon matched-pairs test: $z = 2.197$; $P < 0.05$; Fig. 4A). Thus, these cells already displayed the adult asymmetry pattern. In contrast, the TrkB-positive cells within the retinorecipient layer did not establish asymmetric cell body sizes within the first week after hatching (Wilcoxon matched-pairs test: $z = 1.352$; $P = 0.176$; Fig. 4B).

Discussion

Here we show that asymmetric photic stimulation during embryonic development reduces Ras activation in the stronger stimulated left tectum of freshly hatched pigeons. This is associated with lower phosphorylation of TrkB receptors and indicates for the first time a modulation of Ras by visual input *in vivo* as a secondary consequence of asymmetrical embryonic light input.

Differences in the activation of the TrkB-Ras signalling cascade between the left and right eye system are likely to be related to the development of the lateralized visual system in pigeons. The asymmetric position of the avian embryo in the egg leads to a stronger activation of the right eye/left hemisphere by light shining through the egg shell. Conceivably, this induces asymmetric differentiation processes within left- and right-sided visual circuits culminating in the functional dominance of the left hemisphere for feature-based object analysis (Güntürkün, 2002a, b; Prior *et al*., 2004).

Light-dependent developmental regulation of Ras activation

With the exception of PH1, the relative activation of Ras in the developing optic tectum of pigeons did not differ between light- and dark-incubated animals. This independence indicates that Ras activation is not directly related to retinotectal neurotransmission. This is presumably not caused by missing retinal innervation of the optic tectum because large tectal areas are already invaded by retinal fibres even before hatching, suggesting the presence of retinotectal synapses in embryonic pigeons (Manns & Güntürkün, 1997).

Although no direct light effects were detectable before hatching, embryonic light conditions affected the relative levels of Ras activation after hatching with differential significance for the left and right eye system. While Ras activation did not change in the right tectum, Ras activity was reduced within the left tectum and hence

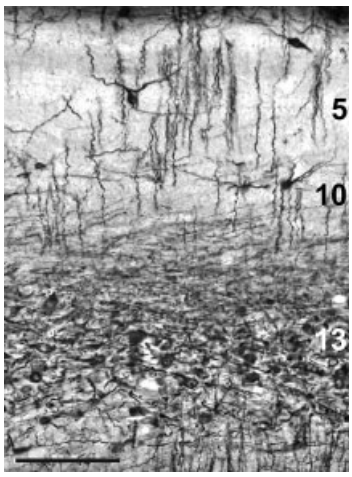
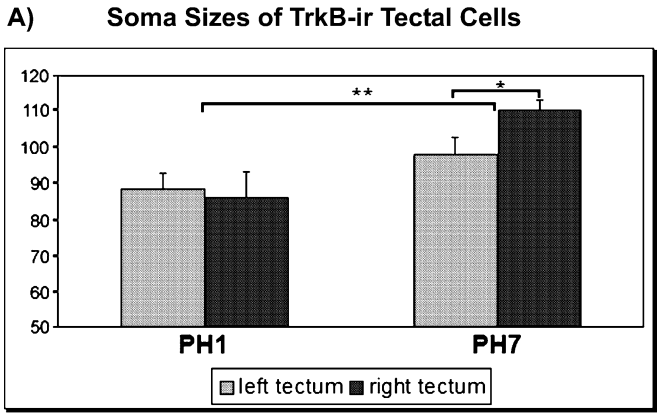


FIG. 3. Immunohistochemical staining of TrkB-positive cells within the tectum of a 7-day-old pigeon – apart from a staining of layer 13 neurons several multipolar and small cells in the superficial laminae are labelled; Scale bar: 100 μm.



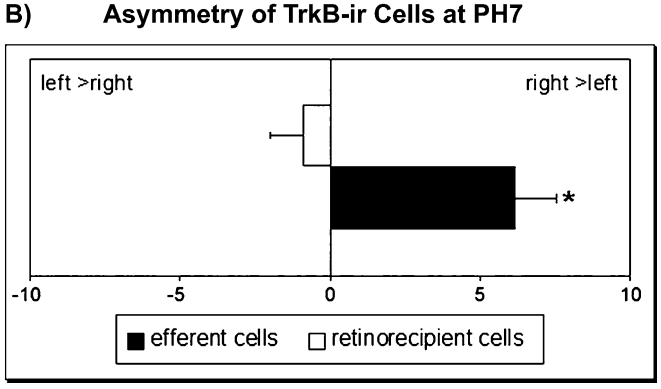


FIG. 4. Development of soma size asymmetries in TrkB-positive tectal cells; in contrast to cells in the superficial laminae, layer 13 neurons increase in size during the first week after hatching (A; soma size in μm^2); asymmetry is expressed as the percentage deviation from mean value (B); bars represent standard error; $*P < 0.05$; $**P < 0.01$ according to Wilcoxon matched-pairs test.

within that tectum that is stronger photically stimulated during the embryonic phase. Thus, as a consequence of asymmetric embryonic visual experience, Ras activation became unbalanced. However, this process happened at a time-point when retinal input was in fact symmetrical. Although we cannot exclude subtle left–right differences in the Ras activation pattern at other time points, our data suggest that neuronal processes are triggered with hatching that show sufficient asymmetry to be detectable with our method. This might result from differential maturation of a neuronal system that starts to control Ras activity only after hatching in response to biased embryonic light experience.

Ras activation at cellular levels during tectal development

Ras signalling transduces a variety of cellular responses like proliferation, survival or differentiation in a strictly cell type-specific manner (Borasio *et al*., 1993; Heumann *et al*., 2000; Takai *et al*., 2001; Malumbres & Barbacid, 2003). Accordingly, the immunohistochemical staining identified Ras-GTP within different tectal laminae-bearing cells of diverse developmental stages. Ras-GTP was detected within the tectal neuroepithelium presumably indicating its mitotic activity in young animals. Accordingly, this signal disappeared in adult animals (data not shown). In addition, Ras-GTP was detected within the efferent cell layer 13. In these cells, Ras activation might reflect BDNF/TrkB signalling.

Because reduced Ras activity in the left tectum is accompanied by lower TrkB activation on this side and because several cells within the developing optic tectum express BDNF and its TrkB receptors (Theiss & Güntürkün, 2001; present results), it is conceivable that reduced Ras activation is caused by a decreased activation of TrkB signalling in response to an enhanced embryonic light stimulation. Correspondingly, preliminary ELISA data show reduced levels of BDNF in the left tectum of 1-day-old light-incubated pigeons (data not shown). Because immunohistochemical data demonstrated a substantial increase in BDNF/TrkB after hatching (Theiss & Güntürkün, 2001), a dependence on the BDNF/TrkB cascade might also explain why light affects Ras signalling only after hatching. However, a Ras-independent neurotrophin/Trk receptor-induced regulation of sodium channels was previously observed in transformed PC12 cells (D'Arcangelo & Halegoua, 1993), and therefore a causal relationship between activation of Trk receptor and Ras cannot be unequivocally claimed from our data. In this case, an enhanced embryonic light trigger leads to a decreased phosphorylation of TrkB, which is associated with a reduced level of signalling active Ras protein in the left tectum.

Nevertheless, this effect is in contrast to results from the visual cortex of the rat where dark rearing leads to a decrease in BDNF mRNA and in phosphorylation of TrkB receptors (Tropea *et al*., 2001; Viegi *et al*., 2002). This discrepancy could point to the possibility that reduced Ras activation in the left tectum is not a direct light effect but results from altered intratectal differentiation processes.

Inhibitory tectal pathways during development

Experience-dependent plasticity in the developing visual cortex is critically regulated by local γ-aminobutyric acid (GABA) circuits (Hensch *et al*., 1998). Thus, embryonic light might primarily affect inhibitory systems, which in turn switch to enhanced inhibitory control onto neurons in the left tectum during the post-hatching phase. Tectal processing is regulated by several inhibitory pathways (Hunt & Künzle, 1976a,b). GABAergic tectal interneurons establish radially and horizontally arranged connections (Hunt & Künzle, 1976b; Domenici *et al*., 1988). The maturation of these cells is influenced by retinal input (Bagnoli *et al*., 1989), and GABAergic cells develop larger cell bodies within the left tectum in response to asymmetric

photic stimulation (Manns & Güntürkün, 2003). Ocular BDNF injections enlarge calbindin-positive and presumably GABAergic cells, indicating that these cells are BDNF responsive (Luksch & Golz, 2003; Manns & Güntürkün, 2005). Although subpopulations of layer 13 neurons receive direct retinal input (Luksch *et al*., 1998; Hellmann & Güntürkün, 2001), they are also connected with tectal interneurons, e.g. the major class of layer 13 neurons with dendritic arborizations within retinorecipient layer 5 develop synaptic glomeruli that combine synaptic contacts between their ascending dendrites, their retinal afferents and neurites of GABAergic interneurons (Luksch & Golz, 2003). Those tight interactions might regulate the post-hatch differentiation of efferent cells.

Furthermore, tectal processing is modulated by different inhibitory afferents. While the asymmetrically organized intertectal commissures (Robert & Cuénod, 1969; Hardy *et al*., 1984; Keysers *et al*., 2000) are more likely to be involved in the preservation of asymmetric processing in adults (Güntürkün & Böhringer, 1987), forebrain influences arising from the visual Wulst as the telencephalic target of the thalamofugal pathway (Bagnoli *et al*., 1980; Miceli *et al*., 1987; Güntürkün, 2000) might be involved in asymmetric tectal TrkB/Ras activation. Recently it has been shown that only the left visual Wulst controls bilateral tectofugal processing, indicating that the telencephalic output of the thalamofugal system is lateralized in pigeons (Folta *et al*., 2004). In chicks, the thalamofugal system displays asymmetric developmental speed in response to biased photic stimulation (Rogers & Bolden, 1991; Rogers, 1996). Thus, the observed different developmental pattern of Ras activation in the left and right tectum might reflect asymmetries in the maturation of tectofugal forebrain input, which in turn modulates tectal maturation and/or processing.

Interestingly, it has been previously reported that GABAergic stimulation switches from enhancing to repressing BDNF expression during maturation in hippocampal neurons (Berninger *et al*., 1995). Thus, stimulation of the GABAergic system or blockade of the glutamatergic system leads to decrease in BDNF synthesis (Zafra *et al*., 1991). The asymmetric reduction of TrkB activation described here is consistent with a light-induced enhancement of developmental maturation of GABAergic systems in the left tectum providing a reciprocal regulation between neurotrophin expression and neural activity.

Morphogenetic Ras effects

Differential activation of Ras in neurons of transgenic synRas mice during postnatal development shows that Ras activation leads to a MAP-kinase-dependent enlargement of cell body sizes, which are accompanied by more complex dendritic arbors, larger axo-dendritic calibres and enhanced afferent input (Heumann *et al*., 2000; Arendt *et al*., 2004; Gärtner *et al*., 2005). The pigeon's optic tectum exhibits a complex soma size asymmetry pattern, with the vast majority of cells larger in the left tectum (Güntürkün, 1997; Skiba *et al*., 2002; Manns & Güntürkün, 2003). Only parvalbumin-positive cells located in the superficial retinorecipient layers 2–10 (Manns & Güntürkün, 2003) and efferent layer 13 cells (Güntürkün, 1997; Skiba *et al*., 2002) are enlarged on the right side, and hence in that tectum that displays higher TrkB/Ras activation directly after hatching.

However, superficially located TrkB-positive cells show Ras-GTP levels that are not detectable with our immunohistochemical method and these cells do not develop asymmetric cell body sizes during the first post-hatching week. In contrast, the efferent cells within layer 13 display strong TrkB- as well as Ras-GTP-immunoreactivity. These cells develop smaller cell bodies in the left tectum at PH7 probably in response to decreased TrkB/Ras activation at PH1 on this side. In fact, compared with dark-incubated animals, the size asymmetry of layer 13 neurons results from a decrease of cell body size in the left tectum and not from enhanced growth of neurons in the right tectum (Skiba *et al*., 2002). This delayed morphogenetic effect is compatible with the slow time course of neurotrophin- or Ras-mediated effects on cell body volume (Heumann *et al*., 1983, 2000). This observation is again compatible with a local inhibition of TrkB signalling selectively in layer 13 cells of the left tectum (Manns & Güntürkün, 1999a). Thus, in sum, cell size development within the efferent TrkB-positive population is likely to be a result of the observed asymmetric Ras activation pattern.

In summary, the present data show that TrkB-Ras signalling corresponds to developmental processes leading to an asymmetric functional architecture of the pigeon's visual system that is likely to be mediated by the photic enhancement of inhibitory pathways. These processes are dependent on asymmetric embryonic light input but gain significance only after hatching.

Acknowledgements

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Abbreviations

BDNF, brain-derived neurotrophic factor; GABA, γ-aminobutyric acid; PBS, phosphate-buffered saline.

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