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ORIGINAL ARTICLE

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Distribution of BDNF, NT-3, trkB and trkC in the developing retino-tectal system of the pigeon (*Columba livia*)

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Abstract The distribution of the neurotrophins BDNF and NT-3 as well as their corresponding high-affinity receptors trkB and trkC was characterized by immunohistochemistry in the developing retino-tectal system of the pigeon. These neurotrophins are known to be important for survival and development of neuronal tissues, but also for activity-dependent neuronal plasticity. In pigeons visual asymmetry is established at the morphological and behavioral level due to a natural asymmetrical light input before hatch, which is followed by a posthatch period of consolidation with unbiased light stimulation. Since the retino-tectal system is the crucial entity of these events, we studied the retinal and the tectal distribution of these neurotrophins and their receptors during retino-tectal formation, to analyze the developmental sequences to which these neurotrophins are tuned. Here we demonstrate that in altricial pigeons no retinal immunolabeling of BDNF, NT-3 or their receptors could be detected before hatch, although a prominent tectal labeling pattern throughout most layers was evident. After hatch, both neurotrophins and their receptors showed a dramatic increase of retinal and tectal distribution. While the tectal and retinal protein synthesis of NT-3 vanished after 2 weeks, that of BDNF could still be revealed in adults. Therefore, the establishment of the retino-tectal system does not seem to depend on these neurotrophins before hatch, although they are probably utilized to shape the intratectal wiring pattern. In contrast, BDNF and NT-3 could play a prominent role in posthatch retino-tectal plasticity, as the consolidation of tectal asymmetries requires posthatch modifications of tectal circuits and proceeds within the first two posthatching weeks. These data are comparable with the distribution of neurotrophins in the retino-tectal system of chicks, although the onset of neurotrophin synthesis seems to be earlier in precocial chicks.

Keywords Neurotrophins · Retina · Optic tectum · Development · Lateralization

Abbreviations *BDNF* Brain-derived neurotrophic factor · *d* deep tectal layers (13–15) · *DAB* 3,3'-diaminobenzidine · *E* embryonic day · *GCL* ganglion cell layer · *im* intermediate layers (8–12) · *INL* inner nuclear layer · *IPL* inner plexiform layer · *NT-3* neurotrophin-3 · *ONL* outer nuclear layer · *OPL* outer plexiform layer · *OT* optic tectum · *PBS* phosphate buffered saline · *Ph* posthatching day · *RGC* retinal ganglion cells · *s* superficial layers (1–7) · *trkB* tyrosine-kinase receptor B · *trkC* tyrosine-kinase receptor C

Introduction

Neurotrophins are known to be transported retro- and anterogradely, to prevent apoptosis and to mediate plasticity in developing and adult neurons (Levi-Montalcini 1987; von Bartheld et al. 1996a, b; Cellerino and Maffei 1996). They exert their effects through binding to high-affinity tyrosine kinase receptors. TrkB is the high-affinity receptor for BDNF (Klein et al. 1991) and NT-4 (Ip et al. 1992), whereas NT-3 preferentially attaches to trkC (Lamballe et al. 1991). The visual system of chicks has since long served as a model system to analyze trophic influences in the development of connected neuronal structures (von Bartheld et al. 1996a, b; Herzog and von Bartheld 1998). It is well known that the retina requires trophic signals from the tectum (Hughes and McMoon 1979) and has itself trophic influences on innervated targets (Catsicas et al. 1992).

In the present study we performed investigations on the distribution of neurotrophins in the retino-tectal system of the pigeon, an altricial bird, in which the de-

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velopment of the visual pathways prolongs into post-hatching time (Manns and Güntürkün 1997). In pigeons approximately 90% of retinal axons terminate in the contralateral OT (Remy and Güntürkün 1991), whereby the retinal innervation of the OT starts at E14 and is not completed before Ph15. Electrophysiological studies demonstrated that full functionality of this system does not develop before the first weeks after hatching (Bagnoli et al. 1985, 1987).

Cytoarchitectonically, the OT contains fifteen distinguishable layers, in the present study grouped as s (layers 1–7), im (layers 8–12), and d (layers 13–15), based on their retinal input and output patterns. In pigeons, retinal input terminates in the s 2–7 of the contralateral OT (Hayes and Webster 1985). Visual information is then transmitted directly by axodendritic contacts or indirectly via horizontal or radial cells of the superficial and intermediate tectal layers to the multipolar neurons of the d (Hardy et al. 1985; Luksch et al. 1998; Hellmann and Güntürkün 1999), which are the major source for tectorotundal, tecto-triangular, or tectotegmental projections (Karten and Revzin 1966; Karten and Hodos 1970; Benowitz and Karten 1976; Hunt and Künzle 1976; Güntürkün et al. 1993).

In contrast to chicks, the retino-tectal system of pigeons displays morphological (Güntürkün 1997a) and connectional (Güntürkün et al. 1999) asymmetries that can be altered subsequent to dark-incubation (Güntürkün 1993) or post-hatch monocular deprivation (Manns and Güntürkün 1999a, b). Lateralization is displayed by a right eye – left hemisphere superiority in visual discriminations (Güntürkün 1997a). Up to now, data of neurotrophin expression in the visual system of birds mainly exist from precocial chicks. The present study was undertaken to describe the developmental distribution of BDNF, NT-3, trkB and trkC in the retino-tectal system of an altricial bird. The immunohistochemical characterization of these proteins in the retina and OT of the developing pigeon yield a hint for biochemical signals participating in the establishment of visual asymmetries in this species. Additionally, differences in the onset of a clearly visible neurotrophin distribution between precocial chicks and altricial pigeons are discussed.

Materials and methods

Antibodies

The antibodies used were rabbit polyclonal to BDNF, NT-3, trkB and trkC (C-14) and purchased from Santa Cruz Biotechnology (USA). Previous studies showed that they recognize the same antigens in rat and pigeon (Vázquez et al. 1994; Hannestad et al. 1998). Immunolabeling was abolished when the primary antibody was omitted.

Tissue preparation

Thirty-one embryonic and hatched as well as five adult unsexed pigeons (*Columba livia*) from a local breeding stock were used for immunohistochemical examinations. Investigated ages were E14, E16, PH0, PH1, PH2, PH4, PH7, PH14 and adults (more than 5 months old), with four animals in each group. All experiments were carried out according to the specifications of the German law for the prevention of cruelty to animals.

Animals were injected with 1.000 units heparin 20 min before perfusion and anesthetized with equithesin (0.4 ml/100 mg body weight). Afterwards they were perfused transcardially with 100–200 ml 0.9% NaCl (4°C), followed by 200–500 ml fixative consisting of 4% paraformaldehyde and 0.2% glutaraldehyde in 0.12 M PBS (pH 7.4) at room temperature. The brains and eyes were removed and postfixed for 1 hour in the same fixative to which 30% sucrose was added. The materials were then stored in PBS with 30% sucrose for 24 h at 4°C for cryoprotection. The retinae were prepared and cross-sections were cut at 15µm on a freezing microtome. These sections were directly mounted on gelatinized slides. Frontal sections of the tectum were cut at 30 µm with a freezing microtome and collected free-floating in 0.12 M PBS.

DAB-Immunohistochemistry

All incubations were carried out on a shaker. Sections were first placed in 0.1% H_2O_2 for 30 min to inactivate endogenous peroxidase-activity, washed three times in PBS, incubated in 10% (w/v) normal goat serum in PBS for 30 min to block non-specific binding-sites in the tissue, and then incubated in the primary antibody in PBS overnight at 4°C. Antibodies were used in a concentration of 1/100 (diluted in 0.12 M PBS + 0.3% w/v Triton-X). Following thorough washing in PBS the material was incubated in the secondary antibody solution [vector biotinylated IgG goat-anti-rabbit antibodies (Burlingame, Calif., USA), 1/100 diluted in PBS + 0.3% Triton-X]. After three further washes a conventional ABC-peroxidase (Vector Elite-kit, Burlingame, Calif., USA) was performed. Afterwards, retinal cross-sections were immersed in 0.06% DAB and 0.3% hydrogen peroxide for 1 min, whereas with brain slices a heavy metal intensified DAB reaction, according to Adams (1981) and Shu et al. (1988) was performed, to obtain a better signal to background staining in the OT. Finally, retinae and brain-slices were washed in PBS, mounted, dehydrated and coverslipped.

Results

With the antibodies directed against BDNF, NT-3, trkB, and trkC an intensive immunoreaction of retinal and tectal neurons was observable. In general, BDNF, NT–3 and trkB showed perikaryal and neuropil staining of the proximal cell prolongations, whereas dense immunolabeling of trkC was restricted to the cell bodies.

Retina

BDNF distribution

No retinal BDNF-signal was detectable before hatch (not shown). From hatching day (Ph0) onwards scattered retinal ganglion cells were BNDF-labeled in the GCL, accompanied by a diffuse staining of the IPL (Fig. 1a–d). At later stages (Ph1 until adult) BDNF-like immunoreactivity increased in the GCL to constitute a well-stained band of cell somata. Additionally, at Ph2 scattered neurons in the INL displayed weak BDNF-labeling, as well as in the ONL. From Ph4 up to adulthood BDNF-like

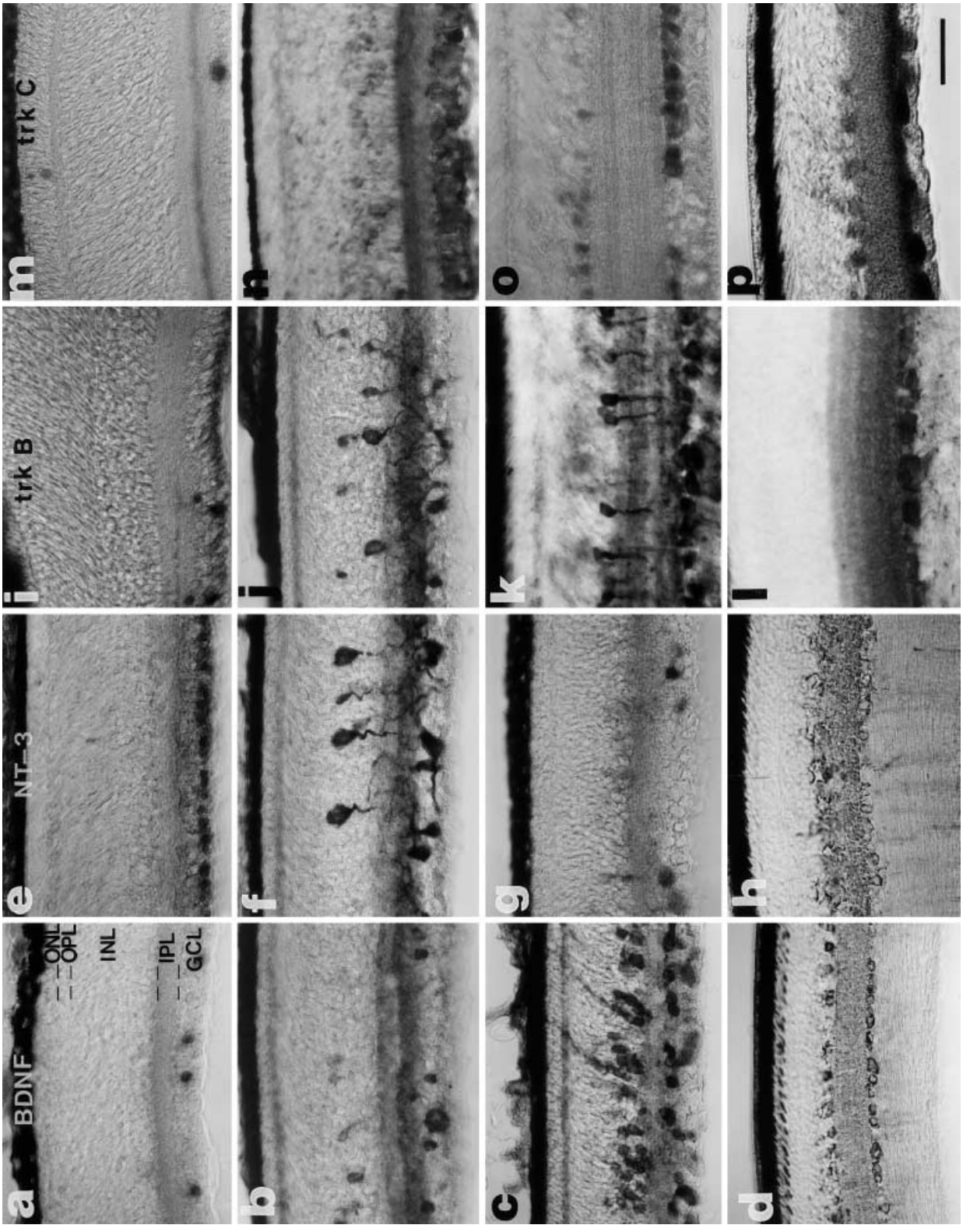


Fig. 1a–p Retinal BDNF, NT-3, trkB and trkC expression from hatching day onwards until adulthood. BDNF increased from the GCL (**a** Ph0) to the INL and the ONL (**b** Ph2; **c** Ph4; **d** adult). Immunoreactivity of NT-3 was disclosed from hatching day onwards in the GCL (**e**), with a peak of expression at Ph2 (**f**) in GCL and INL before fading (**g** Ph4; **h** adult). At hatching day scattered retinal ganglion cells started trkB (**i**) and trkC (**m**) expression, to still increase in GCL and INL until adult status (**j,n** Ph4; **k,o** Ph14; **l,p** adult). *Bar* 50µm

immunoreactivity was continuously salient in the GCL, along with BDNF staining of neurons of the INL and of the ONL. BDNF-positive neurons in the INL were mostly adjacent to the IPL, presumably representing amacrine cells. But also a number of bipolar cells showed BDNF-like immunoreactivity. BDNF-stained somata in the ONL probably represented photoreceptors.

NT-3 immunolabeling

As with BDNF, NT-3 also evinced no prehatching labeling in the retina (not shown). However, in contrast to BDNF, NT-3 immunolabeling started extensively at Ph0 in retinal ganglion cells, with nearly all of them synthesizing this neurotrophin (Fig. 1e–h). This labeling pattern was accompanied by a diffuse IPL staining. At Ph2 prominent NT–3 positive neurons with long processes were visible in an intermediate INL sublamina, presumably representing bipolar cells. From Ph4 onwards the NT-3 immunosignal decreased in the GCL, although moderately stained retinal ganglion cells could still be identified. Additionally, weak NT-3-positive neurons, probably representing bipolar and amacrine cells were found in the INL. In adult animals, the NT-3-like immunoreactivity was still weak in retinal ganglion cells and in amacrine cells. Besides the perikaryal staining of retinal neurons from Ph0 onwards, a moderate NT-3 like immunoreactivity of the IPL was obvious.

TrkB immunoreactivity

At E16 a weak trkB-like immunoreactivity was detectable in the optic nerve layer, although no labeling was evident within the retina (not shown). The first few trkB-positive retinal ganglion cells and INL-neurons were found at Ph0 (Fig. 1i–l). At this stage also a moderate neuropil staining of the IPL was visible. In the following days (Ph1 up to Ph14) the density of trkB-positive neurons increased in the GCL and the INL, reaching their maximum at Ph14. TrkB-labeled somata with processes in the INL were mostly positioned adjacent to the IPL, and were therefore presumably amacrine cells. However, also neurons in the center of this layer, probably bipolar cells, disclosed trkB-like immunoreactivity. Besides this labeling pattern a light and diffuse staining of the somata of photoreceptors in the ONL was detectable between Ph1 and Ph14. In adult pigeons, trkB-stained somata were restricted to the GCL, accompanied by a neuropil staining of the IPL.

TrkC immunosignal

Pigeon embryos at E16 evinced no retinal trkC-like signal (not shown), whereas at Ph0 scattered retinal ganglion cells and a weak staining of the IPL was evident (Fig. 1m–p). From Ph1 up to the adult status, trkC immunolabeling increased in the GCL, and from Ph4 onwards neurons of the INL, adjacent to the IPL, were labeled with the antibody. Furthermore at Ph0 a diffuse labeling of the IPL was obvious. In adult pigeons a moderate trkC-like immunoreactivity was observed in retinal ganglion cells. Additionally, weakly labeled amacrine cells of the INL, close to the IPL, were seen.

Optic tectum

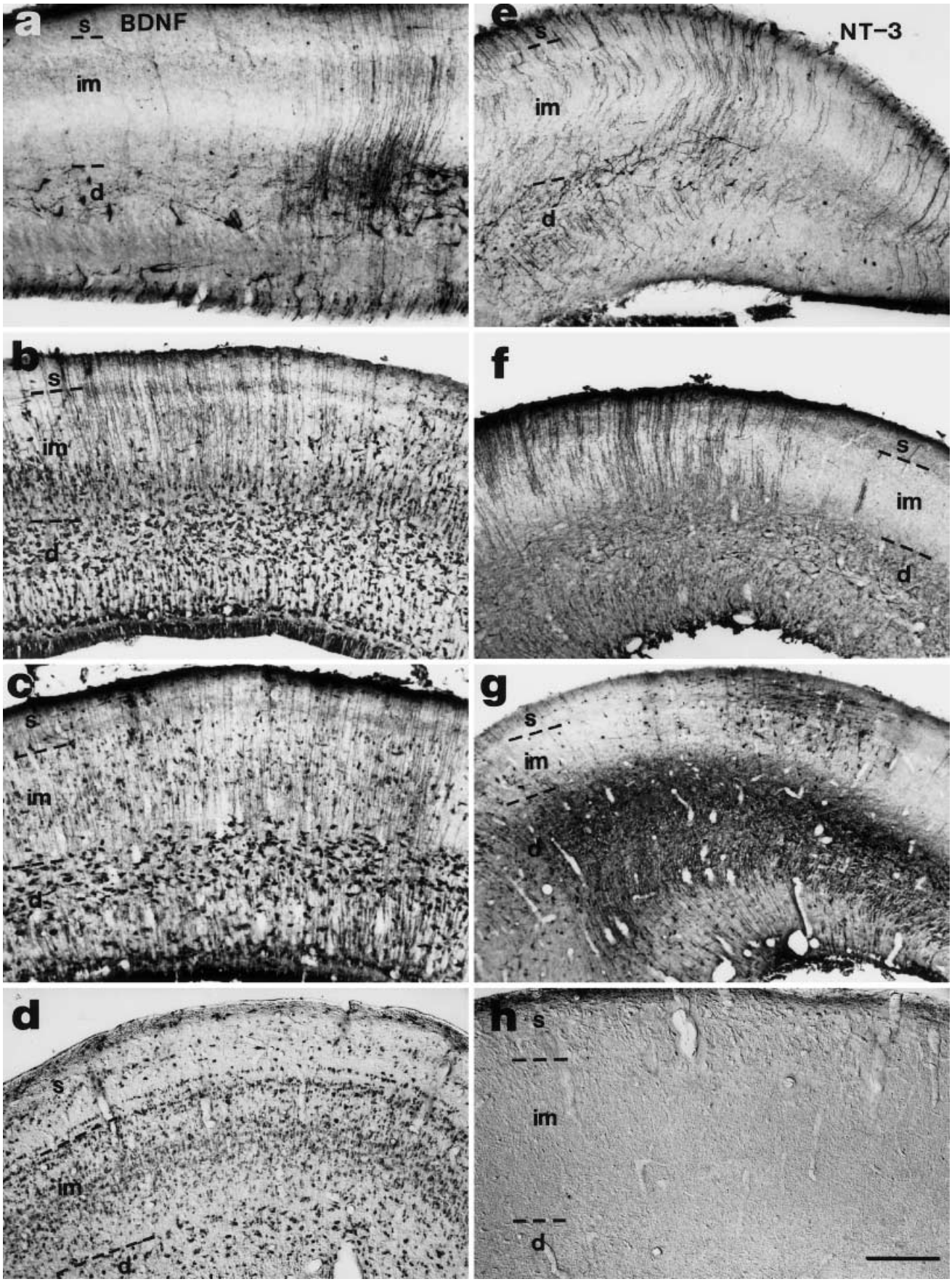
Tectal BDNF distribution

In developing and adult pigeons BDNF showed a pronounced somatic staining of tectal neurons in different layers (Fig. 2a–d). Many BDNF positive neurons were large with labeled proximal dendritic processes, but also some small cells were identifiable. At E14 BDNF positive neurons were detected in the deep layers of the rostro-lateral tectum (not shown). Two days later (E16) BDNF-like immunoreactivity was expanded to the whole rostro-caudal, as well as to the ventro-dorsal extension of neurons in the d. Scattered neurons in the intermediate tectal laminae also showed BDNF-like immunoreactivity. From hatching day onwards all tectal laminae showed a prominent, somatic BDNF-like immunoreactivity, in which BDNF-labeled neurons were especially evident between Ph0 and Ph2. In these stages a uniform BDNF-labeling was visible in the deep tectal laminae and in layer 10, whereas in the other intermediate and superficial layers a staining pattern with expanded columns of neurons was observable (Fig. 4a). Besides this somatic staining between stages E16 and Ph14 radially oriented fibres reaching from the ventricle up to the superficial layers could be observed (Fig. 2a–c). This fibre staining was also heterogeneous in the developing tectum, as it first appeared in the rostral tectum at E16, to be visible throughout the whole tectum between Ph0 and Ph2, before it disappeared in the caudo-ventral tectum at Ph14 (not shown).

Tectal NT-3 immunosignal

NT-3-synthesizing neurons could be detected during the whole developmental period of tectal laminae, but not in adults (Fig. 2e–h). At E14 cell somata of the deep and intermediate tectal layers showed a moderate NT-3-like immunosignal (not shown), increasing in intensity and quantity up to E16. From Ph0 up to Ph4 NT-3-positive neurons were also detected in the superficial layers, sometimes with a columnar fashion (Fig. 4b). Especially

Fig. 2a–h Immunoreactivity to BDNF and NT-3 in the optic tectum during development. While BDNF expression increased from E16 (**a**) throughout juvenile ages (**b** Ph0; **c** Ph2) until adult status (**d**), NT-3 staining was restricted to embryonic and juvenile developmental stages (**e** E16; **f** Ph0; **g** Ph2; **h** adult). Orientations of the images are with their pial surface to the top. *Bar* 200µm ▶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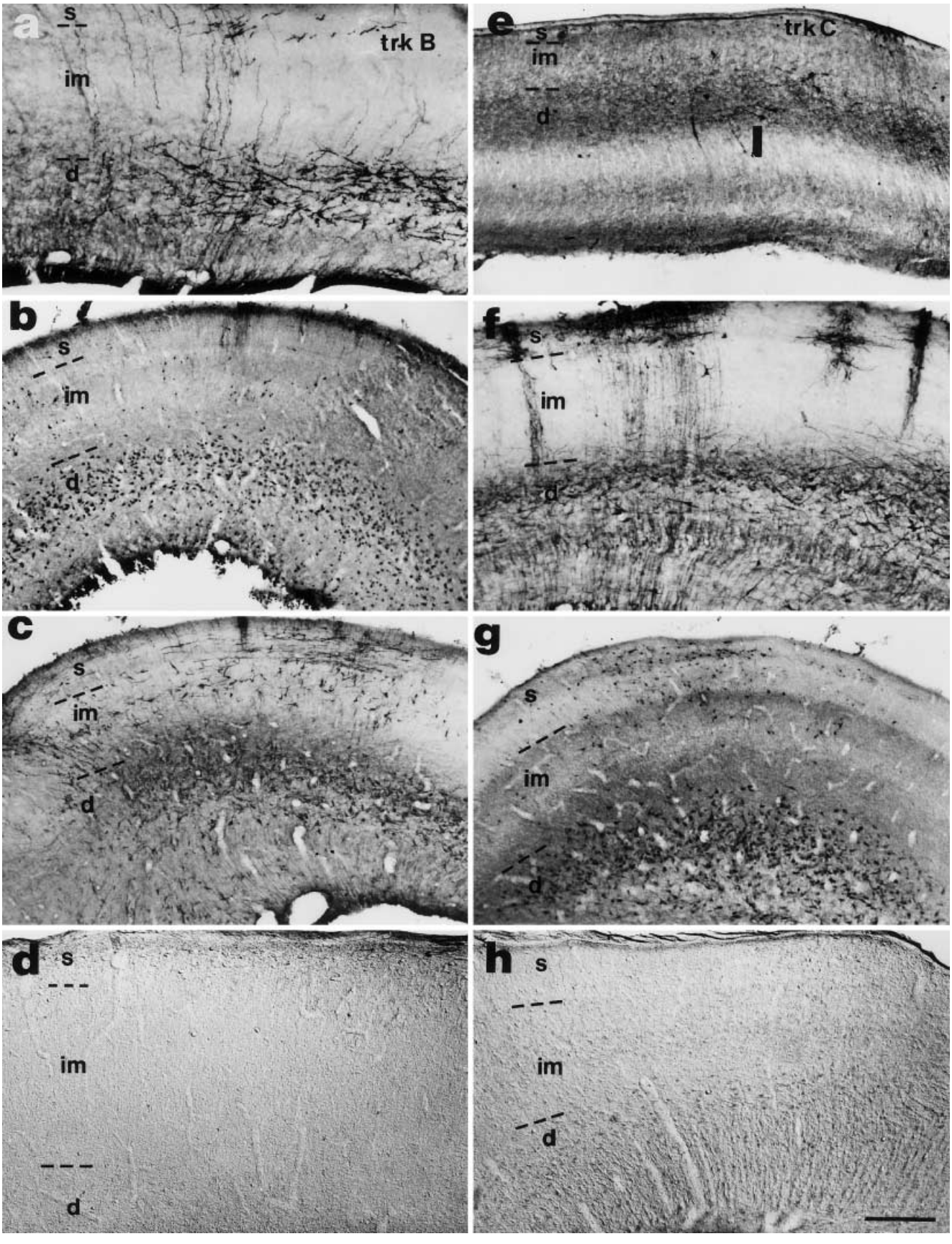

Fig. 3a–h Tectal distribution of trkB and trkC from E16 until adulthood. TrkB immunoreactivity (**a** E16; **b** Ph0; **c** Ph2; **d** adult), as well as trkC labeling (**e** E16; **f** Ph0; **g** Ph2; **h** adult) were prominent during the embryonic and juvenile development of the optic tectum. *Bar* 200µm

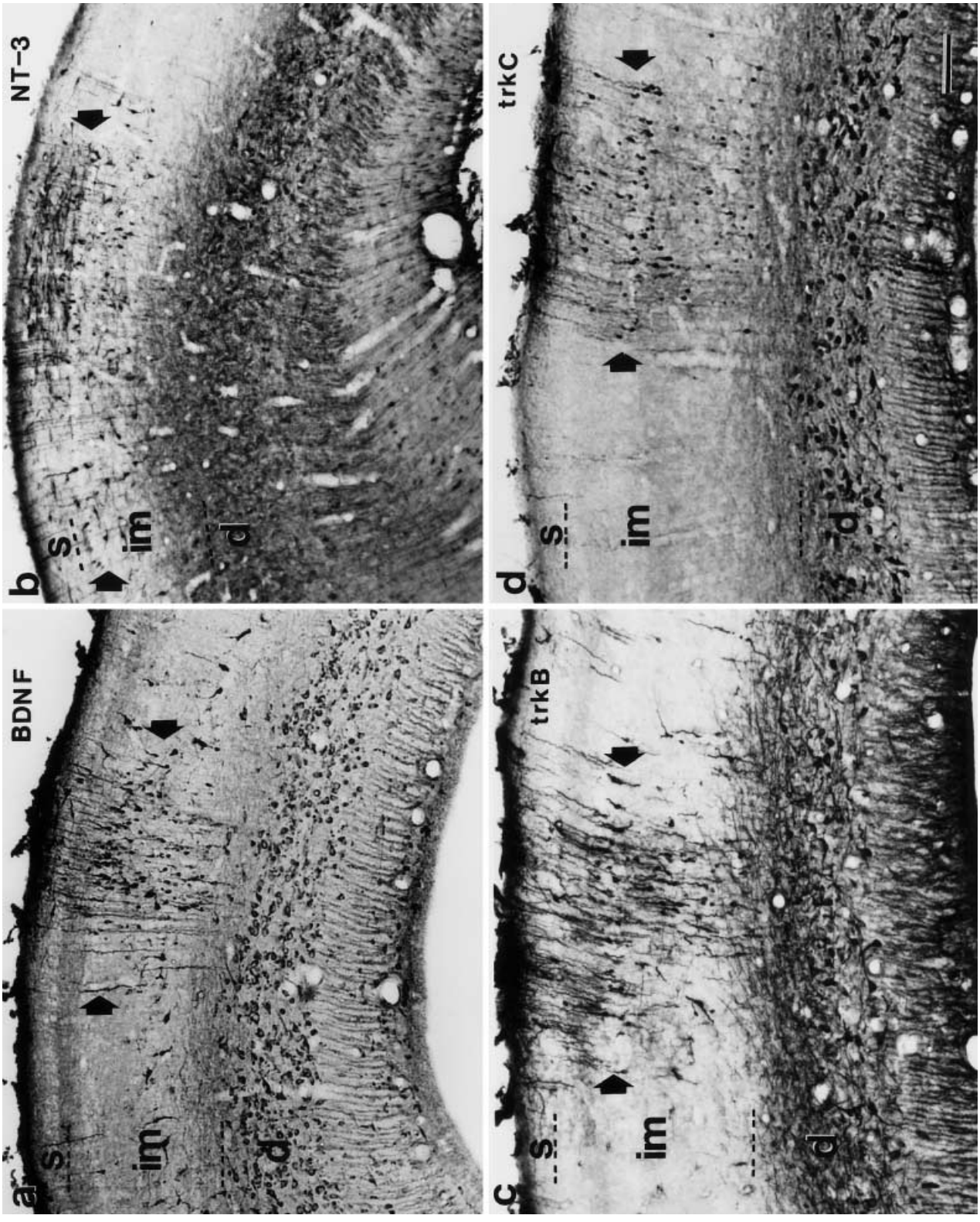

Fig. 4 Tectal BDNF (**a**), NT-3 (**b**), trkB (**c**) and trkC expression (**d**) at PH1 (**a,c,d**) and PH2 (**b**). *Arrows* indicate columnar staining patterns in the superficial (*s*) and intermediate layers (*im*), but not in the deep layers (*d*). *Bar* 100µm

horizontal cells in these layers showed a strong NT-3 immunoreactive signal of somata and proximal dendrites. After Ph4 NT-3 distribution was restricted to the deep tectal layers (not shown). At Ph14 and in adult pigeons no NT-3 positive neurons were observable any longer. Furthermore, radially oriented fibres expanding through

all tectal laminae without somatic staining could be observed at E16 up to Ph4 (Fig. 2e–h). They could be observed, even slightly longer, only in the ventro-caudal tectum (not shown).

Tectal trkB immunoreactivity

The distribution pattern of trkB-positive neurons comprised a time-dependent course during tectal development (Fig. 3a–d). Between E14 (not shown) and E16 only neurons in deep and intermediate laminae of the rostral tectum were labeled. From Ph0 until Ph4 also deep, intermediate and superficial tectal layers of the whole tectum started to show a prominent trkB immunosignal, sometimes with a columnar fashion restricted to intermediate and superficial layers (Fig. 4c). Many cells showed well-stained proximal dendritic processes, so that they could be further classified as radial and horizontal cells. After Ph4, trkB receptor localization was mostly restricted to layer 13, with a few cells in intermediate and superficial layers of the caudo-ventral tectum being labeled up to Ph7 (not shown). In adult pigeons the trkB antibody labeled no tectal neurons. Radially oriented fibres expanding from the inner tectal surface up to the superficial layers were trkB positive from E14 up to Ph14, with a peak between Ph0 and Ph7 (Fig. 3a, c).

Tectal trkC expression

With the antibody against trkC a clear and age-dependent somatic staining of tectal neurons could be observed (Fig. 3e–h). At E14 trkC-labeled neurons were seen in d (not shown), extending to the intermediate tectal layers at E16. In the period of time from hatching till Ph2 a prominent trkC-synthesis in neurons of all tectal layers was observed. TrkC immunolabeling was uniform in the d along the ventro-dorsal extension, however in the intermediate and superficial layers the antibody against trkC marked neurons in a columnar fashion (Fig. 4d). From Ph4 onward, trkC immunoreactivity of tectal cells decreased mainly in the intermediate and superficial layers, and after Ph7 somatic trkC signal was restricted to the d. In adults no labeling of tectal neurons was detectable. Radially oriented fibres without somatic staining were observable in stages E16 (Fig. 3e) to Ph1 (not shown).

Discussion

The present data show that the distribution of neurotrophins and their corresponding trk receptors in retina and tectum is in close correspondence with the development of the retino-tectal system in the pigeon. In the retina, the first immunosignal of BDNF and NT-3 is detectable at hatching day, whereas in the tectum it is initially visible at E14. Moreover NT-3-synthesis seems to vanish in the OT after retino-tectal development is completed, while BDNF is synthesized until adulthood. Therefore neurotrophins very likely play an important role in the establishment of the retino-tectal system, but probably also act as autocrine or paracrine factors in the retina and tectum itself. Since their onset of retinal synthesis starts after hatch, they do not seem to play an important role in prehatch light-stimulation induced asymmetry, but could be a decisive factor in establishing posthatch tectal asymmetries.

Local action of neurotrophins

In pigeons as in other species the retinal distribution of BDNF and NT-3 as well as their corresponding high-affinity receptors make locally restricted intraretinal functions of these neurotrophic factors likely. In earlier investigations a coexpression of neurotrophins and corresponding receptors was demonstrated in chick dorsal root ganglia, suggesting local circuits of trophic action (Schecterson and Bothwell 1992). The same seems to hold for the retina (NT-3: Bovolenta et al. 1996; BDNF: Liu et al. 1997). Indeed, BDNF of retinal ganglion cells seems mostly to stem from intraretinal sources, whereas the tectally-derived amount of BDNF is small (Herzog and von Bartheld 1998). This accords with our data showing a BDNF and NT-3 immunosignal in bipolar and amacrine cells, while trkB and trkC receptors were present in ganglion cells, as well as in the INL and trk B also slightly in the ONL.

These data are comparable with the neurotrophin expression pattern in chicks, where BDNF mRNA and BDNF immunolabeling was demonstrated in photoreceptors, in neurons of the INL, and in RGC, with expression peaks around E12–E15 (Hallböök et al. 1996; Herzog and von Bartheld 1998). NT-3 expression was shown in the pigment epithelium since E5 (Bovolenta et al. 1996), and in amacrine cells and RGC between E9 and E11 (de la Rosa et al. 1994). Besides this, trkB- and trkC-synthesis was also demonstrated in photoreceptors, in bipolar cells and amacrine cells (Garner et al. 1996; Hallböök et al. 1996), and also in RGC with peaks around E12–E15 (Hallböök et al. 1996; von Bartheld et al. 1996a). Therefore in principle the pattern of retinal neurotrophin distribution is comparable between altricial pigeons and precocial chicks, even though the onset of neurotrophin synthesis seems to be earlier in chicks.

Due to the observed relation of BDNF and NT-3 to that of trkB and trkC distribution, also intratectal neurotrophic mechanisms are comparable between pigeons and chicks. The present study demonstrates, that trkB and trkC are present in tectal neurons before the first retinal fibres innervate this structure in the pigeon. In chicks, BDNF expression was demonstrated from E4 onward (Herzog et al. 1994), 2 days before retinal innervation starts (Crossland et al. 1975). NT-3 mRNA was shown in the superior colliculus of juvenile rats, but not in the OT of chicken (von Bartheld 1998). Also trkB is expressed in tectal neurons of chicks since E6 (Garner et

al. 1996), as well as trkC mRNA at E15 (Escandón et al. 1994; von Bartheld et al. 1996a).

As the differentiation of tectal neurons in chicks begins at E3/E4 (Goldberg 1974; Puelles and Bendala 1978), and as these neurons were shown to express the corresponding trkB receptor (Biffo et al. 1994), BDNF could regulate tectal neurite development in chicks (Herzog et al. 1994). The distribution pattern of BDNF and NT-3 and the corresponding trk-receptors in the developing pigeon tectum makes also in this altricial bird intrinsic trophic mechanisms conceivable. They probably might regulate the development of the tectal morphology by stimulating the migration of neurons as well as their dendritic ramification.

However, corresponding with the altricial status of this animal, the same developmental sequences of the retinotectal system as in chicks seem to take place in the retina and the OT of pigeons with a delay of up to ten days. Additionally, in both species intraretinal as well as intratectal neurotrophic mechanisms of BDNF and NT-3 are likely.

BDNF and NT-3 as target derived and as efferent trophic factors

In several studies RGC were shown to depend on their target, as tectal ablation or optic stalk transsection result in their death (Vanselow et al. 1990). Since BDNF mRNA could be detected in the tectum of various species (Leibrock et al. 1989; Cohen-Cory and Fraser 1994; Herzog et al. 1994; Herzog and von Bartheld 1998), and since Fournier et al. (1997) demonstrated the retrograde transport of microinjected BDNF from the tectum to the retina in rats, it is likely that BDNF is a trophic factor for retinal neurons (Cohen-Cory and Fraser 1995). In the pigeon tectum, BDNF as well as NT-3 immunosignal was first shown at E14. This is approximately the day when retinal fibres start innervating this structure (Manns and Güntürkün 1997). However, a clearly visible prehatch retinal immuno-positive signal of the corresponding trkB and trkC receptors was not detected. Although minor receptor quantities might remain undetected with immunohistochemical techniques, these data make it likely that RGC of pigeons do not depend to an important degree on tectal BDNF or NT-3 before hatch. However, as horizontal and radial cells in the superficial and intermediate tectal layers showed a BDNF- and NT-3-like immunosignal during the whole retino-tectal development, and as trkB- and trkC-labeling was prominent from hatching day onwards in the retina, these neurotrophins could be important candidates in shaping the posthatch retino-tectal system. These BDNF- and NT-3-dependent posthatch processes seem at least initially to be independent of patterned light input, since pigeons open their eyes only about 1 week after hatching.

In recent studies neurotrophins were discussed to also fulfill trophic influences in an anterograde direction (Fawcett et al. 1998; Herzog and von Bartheld 1998), as anterograde transport of neurotrophins could be demonstrated in several investigations (von Bartheld et al. 1996a; Zhou and Rush 1996). It is therefore likely that BDNF and NT-3, which are both prominently expressed in the pigeon retina from hatching day onwards, are anterogradely transported and released in the superficial tectal layers. After hatch, they probably regulate the outgrowth and neuronal circuitry of tectal neurons, that do express trkB and trkC receptors. In principle, these trophic influences of retinal origin could not only shape tectal neurons in the retinorecipient layers 2–7, but also neurons in the intermediate laminae that reach with their radially oriented dendrites into superficial layers. Even a large part of the tectorotundal neurons in lamina 13 have dendrites in retinorecipient laminae and receive monosynaptic retinal input (Hardy et al. 1985; Luksch et al. 1998; Hellmann and Güntürkün 1999). Thus, the posthatch anterograde release of retinally derived BDNF and NT-3 could shape virtually the complete tectal circuitry.

Visual asymmetry and neurotrophic mechanisms

The synaptic release of neurotrophins seems to be at least in part activity-dependent and regulated by the activation of glutamate receptors (Lindholm et al. 1994; Thoenen 1995) that have been revealed in the tectum (Theiss et al. 1998, Huang et al. 1998). In the chick retina trkB and trkC receptor expression is similarly up-regulated by light exposure and down-regulated by darkness (Okazawa et al. 1994). Thus, light stimulation is likely to result in a high activity level of retinal ganglion cells, which then could influence the activity-dependent secretion of trophic molecules in the tectum. Due to the late start of retinal BDNF and NT-3 labeling at hatching day, these neurotrophins do not seem to play an important role in prehatch light-stimulation induced asymmetry. However, they could be an important contributor to the posthatch stabilization of visual lateralization.

Due to the slow maturation of the visual system in the altricial pigeon, the retinorecipient layers of only some tectal areas are innervated by retinal fibers at hatch (Manns and Güntürkün 1997). While at Ph0 retinal fibers already exhibit their adult lamination pattern in the rostral tectum, the prospective retinorecipient layers 2–7 of the caudoventral tectum are not innervated at all. Since tectal morphological asymmetries depend on prehatch light input (Güntürkün 1997b), the retinorecipient rostral tectum already displays asymmetries with larger somata on the left side, while no left-right differences are present in the caudo-ventral tectum (Manns 1998). However, 2 weeks after hatch the complete OT, including the caudo-ventral portion, displays morphological asymmetries (Manns 1998). Since after hatching light input is unbiased, the transfer of asymmetries to the caudo-ventral tectum might result either from horizontal intratectal mechanisms or from asymmetrical interactions with other structures, as recently shown to exist (Keysers et al. 2000).

Whatever the mechanisms that spread morphological asymmetries throughout the tectum within the first two posthatching weeks, they have to depend on a high level of posthatch plasticity. Indeed, 10 days of lateralized posthatch photic stimulation are able to fundamentally modify tectal asymmetries as measured in soma sizes (Manns and Güntürkün 1999a). In this period of time BDNF and NT-3 are massively labeled in the retina. These neurotrophins exert morphological effects like an increase in the complexity of retinal arbors within the tectum (Inoue and Sanes 1997) and an enlargement of soma size (Ventimiglia et al. 1995). At the same time, a strong immunosignal of the corresponding receptors trkB and trkC was observed throughout the tectum. Both trk-receptors displayed a columnar expression, making it likely that neuronal circuits that traverse laminar boundaries from superficial-to-deep are established. Since NT-3 is selectively expressed in a short posthatch period within the tectum, it might be especially tuned to these morphological processes. Indeed, although BDNF and NT-3 have synergistic effects, NT-3 especially seems to promote the proliferation of precursor cells and axon collaterals (reviewed Snider 1994).

Thus, BDNF and NT-3 as well as their corresponding receptors seem to have differential pre- and posthatch effects. Before hatch they probably regulate tectal mechanisms that are not related to retinal input. Therefore, prehatching induction of visual asymmetry has to be mediated via mechanisms that do not depend on any of these neurotrophins. After hatch, however, BDNF as well as NT-3 seem to be massively involved in the shaping of retino-tectal circuits. The posthatch horizontal proliferation of tectal asymmetry and the consolidation of visual lateralization might therefore be intimately related to these neurotrophins.

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References

Adams JC (1981) Heavy metal intensification of DAB-based HRP reaction product. J Histochem Cytochem 29:775

Bagnoli P, Porciatti V, Lanfranchi A, Bedini, C (1985) Developing pigeon retina: light-evoked responses and ultrastructure of outer segments and synapses. J Comp Neurol 235:384–394

Bagnoli P, Porciatti V, Fontanesi G, Sebastiani L (1987) Morphological and functional changes in the retino-tectal system of the pigeon during the early posthatching period. J Comp Neurol 256:400–411

Bartheld CS von (1998) Neurotrophins in the developing and regenerating visual system. Histol Histopathol 13:437–459

Bartheld CS von, Byers MR, Williams R, Bothwell M (1996a) Anterograde transport of neurotrophins and axodendritic transfer in the developing visual system. Nature 379:830–833

Bartheld CS von, Williams R, Lefcort F., Clary DO, Reichardt LF, Bothwell M. (1996b) Retrograde transport of neurotrophins from the eye to the brain in chick embryos: roles of the p27NTR and trkB receptors. J Neurosci 16:2995–3008

Benowitz LI, Karten HJ. (1976) Organization of tectofugal visual pathway in pigeon: retrograde transport study. J Comp Neurol 167:503–520

Biffo S, Dechant G, Okazawa H, Barde YA. (1994) Molecular control of neuronal survival in the chick embryo EXS 71:39–48

Bovolenta P, Frade JM, Martí E, Rodríguez-Peña MA, Barde YA, Rodríguez-Tébar A (1996) Neurotropin-3 antibodies disrupt the normal development of the chick retina. J Neurosci 16: 4402–4410

Catsicas M, P´equignot Y, Clarke PG (1992) Rapid onset of neuronal death induced by blockade of either axoplasmic transport or action potentials in afferent fibers during brain development. J Neurosci 12:4642–4650

Cellerino A, Maffei L (1996) The action of neurotrophins in the development and plasticity of the visual cortex. Prog Neurobiol 49:53–71

Cohen-Cory S, Fraser SE. (1994) BDNF in the development of the visual system of *Xenopus*. Neuron 12:747–761

Cohen-Cory S, Fraser SE (1995) Effects of brain-derived neurotrophic factor on optic axon branching and remodelling in vivo. Nature 378:192–196

Crossland WJ, Cowan WM, Rogers LA (1975) Studies on the development of the chick optic tectum. IV. An autoradiographic study of the development of retino-tectal connections. Brain Res 91:1–23

Escandon E, Soppet D, Rosenthal A, Mendoza-Ramirez JL, Szonyi E, Burton LE, Henderson CE, Parada LF, Nikolics K (1994) Regulation of neurotrophin receptor expression during embryonic and postnatal development. J Neurosci 14:2054–2068

Fawcett JP, Bamji SX, Causing, CG, Aloyz R, Ase AR, Reader TA, McLean JH, Miller FD (1998) Functional evidence that BDNF is an anterograde neuronal trophic factor in the CNS. J Neurosci 18:2808–2821

Fournier AE, Beer J, Arregui CO, Essagian C, Aguayo AJ, McKerracher L (1997) Brain-derived neurotrophic factor modulates GAP-43 but not T alpha1 expression in injured retinal ganglion cells of adult rats. J Neurosci Res 47:561–572

Garner AS, Menegay HJ, Boeshore KL, Xie XY, Voci JM, Johnson JE, Large TH (1996) Expression of TrkB receptor isoforms in the developing avian visual system. J Neurosci 16:1740–1752

Goldberg S (1974) Studies on the mechanics of development of the visual pathways in the chick embryo. Dev Biol 36:24–43

Güntürkün O (1993) The ontogeny of visual lateralization in pigeons. German Journal of Psychology: 17:276–287

Güntürkün O (1997a) Avian visual lateralization: a review. Neuroreport 8:iii-xi

Güntürkün O (1997b) Morphological asymmetries of the tectum opticum in the pigeon. Exp Brain Res 116:561–566

Güntürkün O, Hellmann B, Melsbach G, Prior H (1999) Asymmetries of representation in the visual system of pigeons. Neuroreport 9:4127–4130

Hallböök F, Backstrom A, Kullander K, Ebendal T, Carri NG (1996) Expression of neurotrophins and trk-receptors in the avian retina. J Comp Neurol 364:664–676

Hannestad J, Germaná A, Catania S, Laurá R, Ciriaco E, Vega JA (1998) Neurotrophins and their receptors in the pigeon caecal tonsil. An immunohistochemical study. Vet Immunol Immunopathol 61:359–367

Hardy O, Leresche N, Jassik-Gerschenfeld D (1985) Morphology and laminar dirstribution of electrophysiologically identified cells in the pigeon's optic tectum: an intracellular study. J Comp Neurol 233:390–404

Hayes BP, Webster KE (1985) Cytoarchitectural fields and retinal termination: an axonal transport study of laminar organization in the avian optic tectum. Neuroscience 16:641–657

Hellmann B, Güntürkün O (1999) Visual field heterogeneity within the tectorotundal projection of the pigeon. Eur J Neurosci 11:2635–2650

Herzog KH, Bartheld CS von (1998) Contributions of the optic tectum and the retina as sources of brain-derived neurotrophic factor for retinal ganglion cells in the chick embryo. J Neurosci 18:2891–2906

Herzog KH, Bailey K, Barde YA (1994) Expression of the BDNF gene in the developing visual system of the chick. Development 120:1643–1649

Huang LH, Li JL, Wang SR (1998) Glutamatergic neurotransmission from the optic tectum to the contralateral nucleus rotundus in pigeons. Brain Behav Evol 52:55–60

Hughes WF, McLoon SC (1979) Ganglion cell death during normal retinal development in the chick: comparisons with cell death induced by early target field destruction. Exp Neurol 66:587–601

Hunt SP, Künzle H (1976) Observations on the projections and intrinsic organization of the pigeon optic tectum: an autoradiographic study based on anterograde and retrograde, axonal and dendritic flow. J Comp Neurol 170: 153–172

Inoue A, Sanes JR (1997) Lamina-specific connectivity in the brain: Regulation by *N*-cadherin, neurotrophins, and glycoconjugates. Science 276:1428–1431

Ip NY, Ibanez CF, Nye SH, McClain J, Jones PF, Gies DR, Belluscio L, Le Beau MM, Espinosa R 3rd, Squinto SP (1992) Mammalian neurotrophin-4: structure, chromosomal localization, tissue distribution, and receptor specificity. Proc Natl Acad Sci USA 89:3060–3064

Karten HJ, Hodos W (1970) Telencephalic projections of the nucleus rotundus in the pigeon (*Columba livia*). J Comp Neurol 140:35–52

Karten HJ, Revzin AM (1966) The afferent connections of the nucleus rotundus in the pigeon. Brain Res 2:368–377

Keysers C, Diekamp B, Güntürkün O (2000) Evidence for asymmetres in the phasic intertectal interactions in the pigeon (*Columba livia*) and their potential role in brain lateralisation. Brain Res 852:406–413

Klein R, Jing SQ, Nanduri V,O'Rourke E, Barbacid M (1991) The trk proto-oncogene encodes a receptor for nerve growth factor. Cell 65:189–197

Lamballe F, Klein R; Barbacid M (1991) Trk C, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. Cell 66:967–979

Leibrock J, Lottspeich F, Hohn A, Hofer M, Hengerer B, Masiakowski P, Thoenen H, Barde YA (1989) Molecular cloning and expression of brain-derived neurotrophic factor. Nature 341:149–152

Levi-Montalcini R (1987) The nerve growth factor 35 years later. Science 237:1154–1162

Lindholm D, Castrén E, Berzaghi M, Blöchl A, Thoenen H (1994) Activity–dependent and hormonal regulation of neurotrophin mRNA levels in the brain – implications for neuronal plasticity. J Neurobiol 25:1362–1372

Liu ZZ, Zhu LQ, Eide FF (1997) Critical role of TrkB and brain-derived neurotrophic factor in the differentiation and survival of retinal pigment epithelium. J Neurosci 17:8749–8755

Luksch H, Cox K, Karten HJ (1998) Bottlebrush dendritic endings and large dendritic fields: motion-detecting neurons in the tectofugal pathway. J Comp Neurol 396:399–414

Manns M (1998) Die Ontogenese visueller Lateralisation bei der Taube (*Columba livia*): Entwicklung und Plastizität. Thesis, Ruhr-Universität Bochum, Germany

Manns M, Güntürkün O (1997) Development of the retino-tectal system in the pigeon: a cytoarchitectonic and tracing study with cholera toxin. Anat Embryol 195:539–555

Manns M, Güntürkün O (1999a) Monocular deprivation alters the direction of functional and morphological asymmetries in the pigeon's (*Columba livia*) visual system. Behav Neurosci 113: 1257–1266

Manns M, Güntürkün O (1999b) 'Natural' and artificial monocular deprivation effects on thalamic soma sizes in pigeons. Neuroreport 10:3223–3228

Okazawa H, Kamei M, Imafuku I, Kanazawa I (1994) Gene regulation of trkB and trkC in the chick retina by light/darkness exposure. Oncogene 9:1813–1818

Puelles L, Bendala MC (1978) Differentiation of neuroblasts in the chick optic tectum up to eight days of incubation: a Golgi study. Neuroscience 3:307–325

Remy M, Güntürkün O (1991) Retinal afferents to the tectum opticum and the n. opticus principalis thalami in the pigeon. J Comp Neurol 305:57–70

Rosa RJ de la, Arribas A, Frade JM, Rodriguez-Tebar A (1994) Role of neurotrophins in the control of neural development: neurotrophin-3 promotes both neuron differentiation and survival of cultured chick retinal cells. Neuroscience 58:347–352

Schecterson LC, Bothwell M (1992) Novel roles for neurotrophins are suggested by BDNF and NT-3 mRNA expression in developing neurons. Neuron 9:449–463

Shu S, Ju G, Lingzhi F (1988) The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system. Neurosci Lett 85:169–171

Snider WD (1994) Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. Cell 77:627–638

Theiss C, Helmann B, Güntürkün O (1998) The differential distribution of AMPA-receptor subunits in the tectofugal system of the pigeon. Brain Res 785:114–128

Thoenen H (1995) Neurotrophins and neuronal plasticity. Science 270:593–598

Vanselow J, Dütting D, Thanos S (1990) Target dependence of chick retinal ganglion cells during embryogenesis: cell survival and dendritic development. J Comp Neurol 295:235–247

Vázquez E, Water TR van de, Valle M del, Vega JA, Staecker H, Giraldez F, Represa J (1994) Pattern of trkB protein-like immunoreactivity in vivo and the in vitro effects of brain-derived neurotrophic factor (BDNF) on developing cochlear and vestibular neurons. Anat Embryol 189:157–167

Ventimiglia R, Mather PE, Jones BE, Lindsay RM (1995)The neurotrophins BDNF, NT-3 and NT-4/5 promote survival and morphological and biochemical differentiation of striatal neurons in vitro. Eur J Neurosci 7:213–222

Zhou XF, Rush RA (1996) Endogenous brain-derived neurotrophic factor is anterogradely transported in primary sensory neurons. Neuroscience 74:945–953