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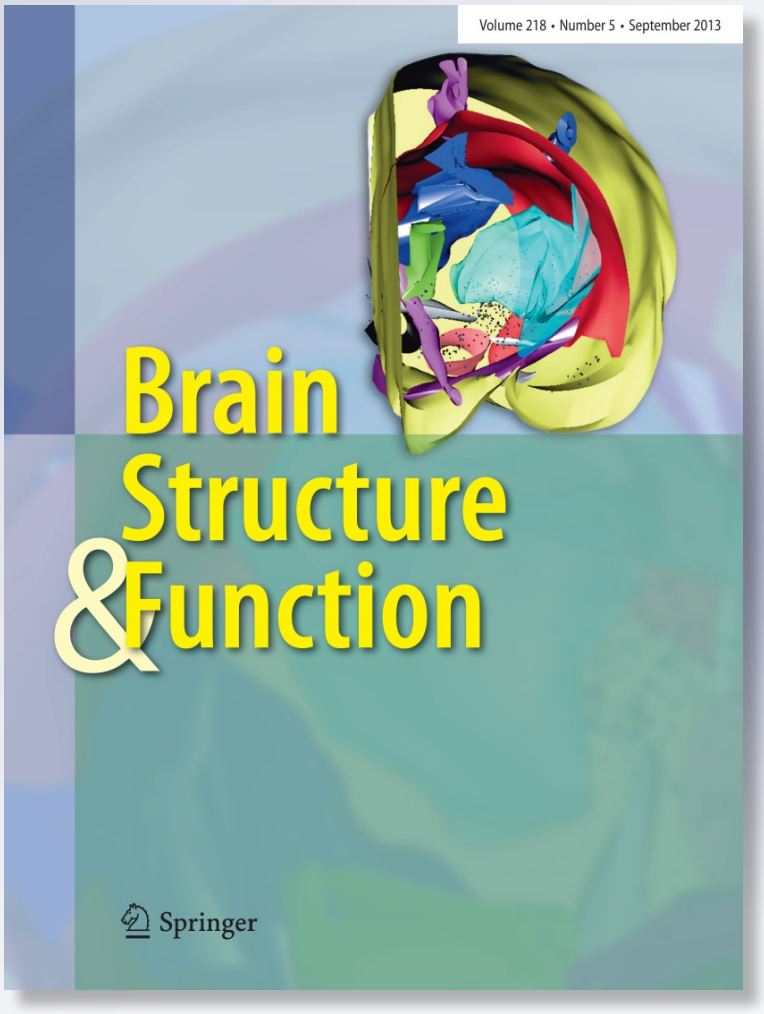

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ORIGINAL ARTICLE

Visual asymmetries and the ascending thalamofugal pathway in pigeons

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Abstract The lateralized visual systems of pigeons and chickens are excellent models to study neural asymmetries at the functional and anatomical level. The aim of the current study was to reveal why these two species closely resemble each other with respect to left–right differences in behavior but not with respect to the pathways involved: While pigeons show an asymmetrically organized tectofugal system, only transient lateralizations of the thalamofugal system have been observed in chickens. Four possible explanations are conceivable. (1) Adult pigeons might also show a hitherto undiscovered thalamofugal asymmetry like chickens. (2) The thalamofugal asymmetry might be transient in both species. (3) Prehatch light stimulation could differentially affect the two visual pathways of chickens and pigeons that mature with different speeds. (4) Tecto- and thalamofugal asymmetries represent species differences, independent of developmental factors. To test these explanations, we injected retrograde tracers into the Wulst of adult pigeons, of hatchlings, and of dark reared pigeons which were monocularly deprived on their left or right eye for one week after hatch. Subsequently we counted labeled cells within the ipsi- and contralateral n. geniculatus lateralis pars dorsalis in search for possible lateralizations of ascending pathways. None of the experimental groups displayed significant differences in the thalamofugal projection pattern. This indicates that visual lateralization in pigeons and chickens depends on tectofugal and thalamofugal asymmetries, respectively. Thus, in different species a highly similar pattern of behavioral asymmetries can be subserved by diverse neural systems.

Keywords Visual system · Tract tracing · Monocular deprivation · Lateral geniculate nucleus · Lateralization

Introduction

Brain asymmetries represent a common principle among vertebrates (Vallortigara and Rogers, 2005). Despite this ubiquity, it is unclear how genetic and epigenetic mechanisms form the emergence of adult asymmetry patterns. Possibly the best model to study this question is the visual system of birds. Pigeons and chickens show a left hemispheric dominance for visual feature analysis (Rogers 2002; Yamazaki et al. 2007; Manns and Güntürkün 2009) and a right sided dominance for relational spatial properties and visually guided emotional responses (Prior et al. 2002; Chiesa et al. 2006; Rosa Salva et al. 2007). In both species, development of left hemispheric visual object discrimination asymmetry is triggered by exposure of the embryo to light. Since avian embryos adopt a turned posture such that the left eye is occluded by the own body and the right eye is close to the egg shell, light traversing the shell induces a right eye stimulation, which then results in an asymmetrical wiring of visual pathways. Dark incubation of pigeon or chicken eggs prevents the establishment of visual lateralization (chicken: Rogers 1982; pigeon: Skiba et al. 2002; Manns and Güntürkün 2003; Freund et al. 2008), while occluding the right eye before hatch in chickens (Rogers and Sink 1988) or directly after hatch in pigeons (Manns and Güntürkün 1999a) reverses behavioral and anatomical asymmetry.

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Thus, in pigeons and chickens visual asymmetry is highly similar with respect to ontogenetic mechanisms and behavior (Güntürkün 1997; Rogers 2008). The neural systems that are asymmetrically organized, however, are different (see Fig. 1). In pigeons, the tectofugal visual pathway (corresponding to the mammalian extrageniculocortical system) shows structural and physiological asymmetries. Here, the numbers of crossing fibers from the tectum to the contralateral thalamic n. rotundus differ between left and right half brains (Güntürkün et al. 1998; Folta et al. 2004; Verhaal et al. 2012). In chickens, however, tectofugal asymmetries are absent (Rogers and Deng 1999). Instead, the thalamofugal pathway (corresponding to the geniculocortical system) is lateralized, with the crossing fibers from the thalamic dorsolateral geniculate (Gld) to the telencephalic visual Wulst being asymmetrically organized (Rogers and Bolden 1991). This thalamofugal asymmetry in chickens is transient and can only be found in hatchlings up to 21 days (Rogers and Sink 1988).

These differences in the anatomical fundaments of avian visual asymmetry are puzzling. Four possible explanations are conceivable. First, adult pigeons might also show a thalamofugal asymmetry like chickens. Then, the two species would only differ with respect to the tectofugal system. Second, the thalamofugal asymmetry could be transient in both species. In this case, we should see a thalamofugal asymmetry in pigeon hatchlings. Third, prehatch light stimulation asymmetry could differentially affect the two visual pathways of precocial chickens and altricial pigeons since these two species mature with different speeds. In chickens and pigeons prehatch biased visual input would then fall into the developmental period of thalamo- and tectofugal systems, respectively (Halpern et al. 2005). If this is true, closing one eye for several days in pigeon hatchlings should prolong asymmetrical visual input into the developmental period of the thalamofugal system, resulting in thalamofugal asymmetries. The fourth possibility is that tecto- and thalamofugal asymmetries represent a species difference that is independent of developmental factors. In this case, pigeons and chickens would realize their highly similar behavioral visual asymmetries with different neural systems. In the following we report a series of experiments to decide between these explanations.

Experiment 1

Materials and methods

In this experiment we test the first possible explanation, which supposes the existence of a thalamofugal asymmetry in adult pigeons. To this end, we analyzed the projection patterns of the left and the right Gld by means of retrograde tracer injections into the visual Wulst. 16 adult pigeons (*Columba livia*) of both sexes from local breeders were used in this first experiment. Pigeons were held in a 12/12 h light/dark cycle and had free access to food and water. In order to examine thalamofugal projections, the retrograde tracer Choleratoxin subunit B (CtB; Sigma, Deisenhofen, Germany) was injected either into the left or the right visual Wulst (left $n = 8$, right $n = 8$). We targeted following substructures: n. interstitialis hyperpallii apicalis, hyperpallium intercalatum and hyperpallium densocellulare. For tracer applications,

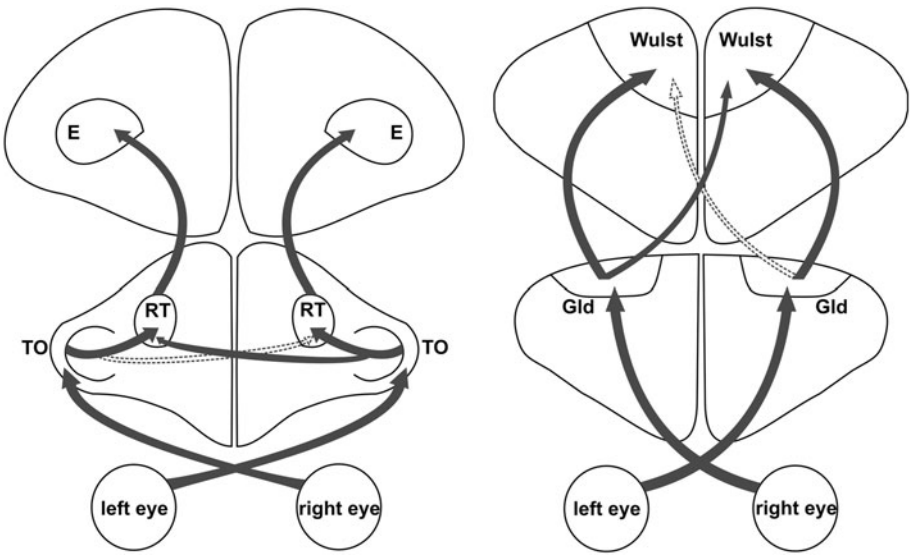

Fig. 1 The tectofugal pathway of pigeons (*left side*) and the thalamofugal pathway of juvenile chickens (*right side*) are asymmetrically organized. In pigeons the projection from the right Tectum opticum (TO) to the left Nucleus rotundus (RT) are stronger than projection from the right TO to the left RT. In chickens the contralateral projections from left Nucleus geniculatus lateralis pars dorsalis (Gld) to the right visual Wulst are stronger than projections from the right Gld to the left visual Wulst. Both of these anatomical asymmetries lead to a lateralized behavior in each species (*E* entopallium)

animals were deeply anesthetized with ketamine/xylazine (0.12 ml/100 g body weight) and fixed in a stereotactic apparatus (Karten and Hodos 1967). After removing feathers from the skull and incising the skin, a small hole was drilled into the skull overlying the Wulst. A glass micropipette (inner tip diameter 12–15 μm) mounted to a nanoliter injector (WPI, USA) was used to inject the tracer into nine distinct sides of the Wulst at positions A 11-13, D 2.7-1.3, L 2.9 according to stereotaxic coordinates of the pigeon brain atlas (Karten and Hodos 1967; Güntürkün et al. 2012). At each injection side 23.4 nl CtB as a 1 % solution (w/v) in distilled water was injected in steps of 2.6 nl over a 15–20 min period. The large scale and the high volume of CtB injections were chosen to equally cover all regions of the Wulst therefore excluding effects of inhomogeneous Gld–Wulst projections. After 48 h survival time, pigeons were transcardially perfused with 4 % paraformaldehyde and brains were removed. After 2 h of postfixation (4 % paraformaldehyde + 30 % sucrose) and cryoprotection (30 % sucrose in phosphate buffer) for 24 h, brains were cut in 40 μm thin frontal slices with a microtome (Leica Microsystems, Wetzlar, Germany). To map CtB, every tenth section was immunohistochemically stained using a goat-anti-CtB antibody (Calbiochem, Merck, Darmstadt, Germany, Cat. no.: 227040) in a dilution of 1:5,000. Antibody visualization was achieved by an ABC-DAB labeling with cobalt–nickel intensification (Manns and Güntürkün 2003). Thalamic sections according to the atlas of Karten and Hodos (1967) were analyzed using a Zeiss Axio Imager M1 Microscope (Carl Zeiss MicroImaging, Göttingen, Germany) with 20x objective (numerical aperture of the lens 0.8). Borders of Gld were defined according to Güntürkün and Karten (1991). The number of ipsi- and contralateral cells in all sections were counted manually by only one person to reduce interpersonal counting differences. For each animal, a lateralization index (LI) was calculated using the following term: LI = (number of ipsilateral neurons − number of contralateral neurons)/(number of ipsilateral neurons + number of contralateral neurons).

Higher values indicate a stronger ipsilateral projection whereas smaller values point towards a bilateral projection.

For direct comparison to the chicken data of Rogers and Bolden (1991), we also used the CI-index. In this Contra/Ipsi Index (CI) the number of labeled cells in contralateral Gld is divided by the number of cells in the ipsilateral Gld. As both measures are independent of the applied tracer amount, no correction procedures were used. Pictures were taken with an AxioCam MRM (Carl Zeiss MicroImaging, Göttingen, Germany) and the software AxioVison 4.8 (Carl Zeiss MicroImaging, Göttingen, Germany). Pictures were adjusted using the software Corel PHOTO-PAINT X4 (Corel Corporation, Unterschleißheim, Germany). Statistical analysis was performed by using the software SPSS (IBM, Ehningen, Germany). All experiments were performed in compliance with the guidelines of the National Institutes of Health for the care and use of laboratory animals and were approved by a national committee (North Rhine-Westphalia, Germany).

Results

All tracer applications into the visual Wulst resulted in labeling of Gld cells (see Fig. 2). On the ipsilateral side, these were mainly located within the Gld subnuclei n. dorsolateralis anterior thalami, pars magnocellularis (DLAmc), n. dorsolateralis anterior thalami, pars lateralis (DLL) and n. suprarotundus (SpRt), with the majority of cells localized within DLL. Only a small subset of cells was labeled within the n. superficialis parvocellularis (SPC). On the contralateral side, labeled neurons were mainly found in the SPC and, in smaller numbers, in the DLL (Fig. 2). In addition, cells in several other diencephalic and mesencephalic areas were found that are outside the scope of this project and will not be reported here. In Table 1 we give the number of ipsi- and contralaterally labeled neurons in DLAmc, DLL, SPC and SpRt. However, we do not include the SPC into our subsequent quantitative analysis since this structure, although sometimes subsumed as a GLd-subcomponent (e.g. Koshiba et al. 2003), is in fact a multimodal structure with projections beyond the visual Wulst (Güntürkün and Karten 1991). Thus, the SPC is not part of the avian GLd and therefore outside the scope of the present study.

The DLAmc, an ovoid shaped nucleus reaching from A7.25 to A6.75, contained the lowest amount of labeled cells, with labeling only present on the side ipsilateral to Wulst injections (see Fig. 3a, b). Labeled DLAmc-cells were mostly located in the dorsolateral part, had mainly large somata and were clearly distinguishable from cells in the surrounding nuclei. The DLL reaches from A7.25 to A6.00 and exhibited a heterogeneous distribution of labeled cells. Ipsilaterally, most labeled neurons were located in ventral DLL, where cells were packed densely and showed strong labeling of somata and fibers. On the contrary, labeled neurons in the dorsal subdivision showed a lower density as well as weaker staining. For both, the ventral and the dorsal DLL, density of labeled neurons was highest in the central portion while density decreased laterally. On the contralateral side only few labeled cells were visible and these were located in the dorsal DLL (Fig. 3a–d). This is in accordance with older studies, which found a dorso-ventral segregation of DLL with the ventral part

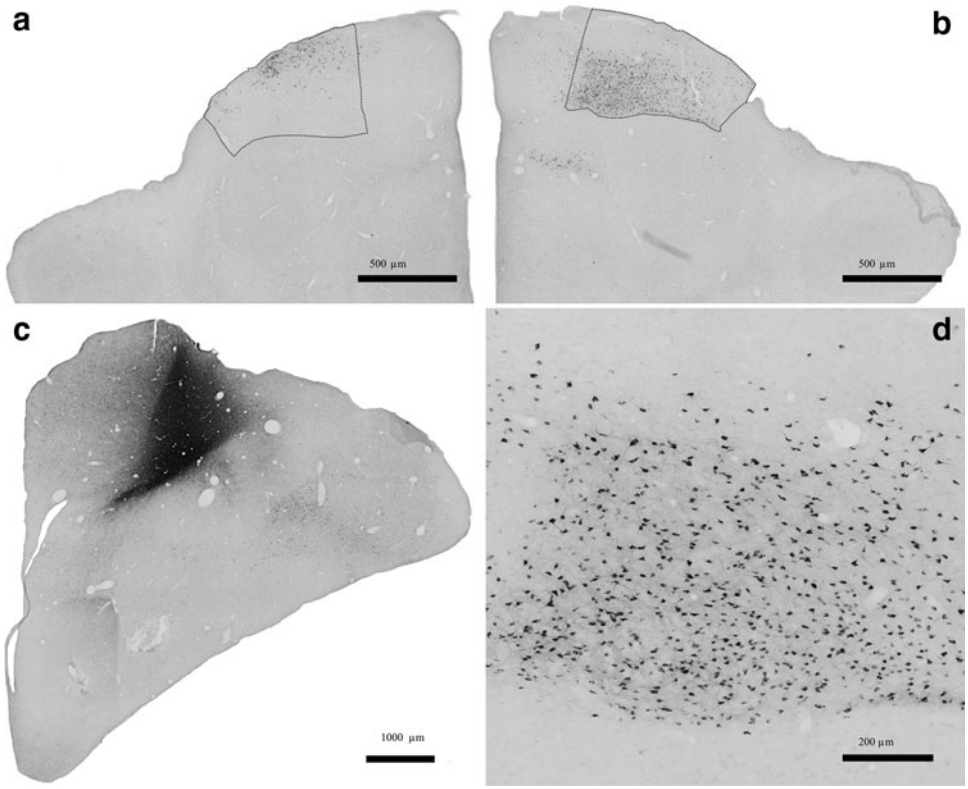

Fig. 2 Immunohistochemical CtB labelling after injections of CtB into the right visual Wulst. **a**, **b** Labelled cells within left and right Gld at A 6.50 (*dotted black line*). On the *right side* cells are mainly labelled within the Gld subnuclei n. dorsolateralis anterior thalami, pars lateralis (DLL) and n. suprarotundus (SpRt). On the *left side* labelled cells are mainly located in the n. superficialis parvocellularis (SPC). Note the higher cell number on the *right side* due to stronger ipsilateral projections of the Gld to the Wulst. **c** Injection side in visual Wulst at A 11.00. **d** Higher magnification of cells shown in (**b**)

projecting ipsilaterally, while the dorsal part has mostly contralateral projections (Miceli et al. 1975; Bagnoli and Burkhalter 1983). The SpRt, lying from A6.50 to A6.00 in a thin band above the n. rotundus, contained densely packed labeled neurons that only projected ipsilaterally (Fig. 3c, d). The SPC contained few ipsilaterally labeled neurons that were mainly located at its ventral and dorso-medial border. On the contralateral side however, labeled cells were densely packed throughout the whole SPC with the exception of the dorso-lateral corner. Here, cells were more scattered probably due to the fibers of the tractus septomesencephalicus, which pass through the SPC (Güntürkün and Karten 1991). The SPC therefore represents the strongest contralateral thalamic projection to the Wulst.

On average the GLd subnuclei contained 2,955 (±341 SEM) labeled cells with 15.9 % of them projecting contralaterally. A detailed summary of cell numbers within all subnuclei are provided in Table 1. To compare the ascending projection patterns of left and right Wulst injected animals, LI values were analyzed. LI values for left sided injections ranged from 0.56 to 0.77 ($\bar{x} = 0.66$, $\sigma = 0.08$) and those for right sided injections from 0.57 to 0.88 ($\bar{x} = 0.70$, $\sigma = 0.09$). No significant asymmetry could be revealed (two-tailed independent samples t test ($t(14) = -0.85$; $p = 0.41$), see Fig. 4). All variables were normally distributed (Kolmogorov–Smirnov tests, $p = 1.00$). To keep our analyses comparable with the data from chickens, we then also calculated CI values. Comparison of CI values for left and right Wulst injections (left = 0.2, $\sigma = 0.07$, right = 0.18, $\sigma = 0.06$) were also normally distributed (Kolmogorov–Smirnov tests, $p = 1.00$) and showed no significant effect (two-tailed independent samples t test (t (14) = −0.86; $p = 0.41$). We then analyzed if these non-significant effects eventually resulted from limitations of sample size. To this end, we calculated a power analysis for CI values using G*Power 3.1.2 (http://www.psycho.uni-duesseldorf.de/aap/projects/gpower; Buchner et al. 1997) to determine the sample size that would be needed for the effect to reach significance. Cohen's d was 0.46 and the power analysis revealed that the effect would not reach significance until an overall n of 288 animals. Compared to chickens, were the effect reached significance for an n of 7 (Rogers and Bolden 1991), these results make it likely that, different from chickens, Gld projections in adult pigeons are not asymmetrically organized.

Discussion of experiment 1

Several authors had previously analyzed the GLd-projection onto the Wulst in pigeons in descriptive terms. The

Table 1 Average number of labeled neurons in the thalamic nuclei DLAmc, DLL, SPC and SpRt after left or right sided injections into the Wulst of adult animals (experiment 1), hatchlings (experiment 2) and dark incubated animals with either a left, a right or no eye cap for one week after hatch (experiment 3)

	DLAmc			DLL			SPC			SPpRt			Sum		
	Ipsi	Contra	Total	Ipsi	Contra	Total	Ipsi	Contra	Total	Ipsi	Contra	Total	Ipsi	Contra	Total
Adult															
Left injection	2 (±7)	0	2 (±7)	2,048 (±436)	460 (±127)	2,508 (±472)	115 (±112)	384 (±127)	498 (±224)	226 (±65)	0	226 (±65)	2,391 (±504)	843 (±183)	3,234 (±600)
Right injection	22 (±26)	0	22 (±26)	2,312 (±1,334)	485 (±259)	2,797 (±1,548)	125 (±95)	367 (±165)	493 (±258)	356 (±198)	0	356 (±198)	2,814 (±1,498)	853 (±414)	3,667 (±1,874)
Hatchlings[a]															
Left injection	–	–	–	–	–	–	–	–	–	–	–	–	600 (±406)	154 (±117)	753 (±475)
Right injection	–	–	–	–	–	–	–	–	–	–	–	–	953 (±1,502)	223 (±281)	1,176 (±1,764)
Left cap CtB															
Left injection	168 (±221)	1 (±2)	169 (±220)	3,382 (±112)	674 (±193)	4,056 (±240)	330 (±93)	838 (±313)	1,004 (±156)	178 (±44)	0	178 (±44)	4,057 (±336)	1,422 (±163)	5,479 (±340)
Right injection	116 (±118)	7 (±11)	123 (±126)	2,781 (±658)	511 (±128)	3,292 (±713)	264 (±172)	535 (±269)	775 (±213)	164 (±140)	0	164 (±140)	3,325 (±854)	1,053 (±297)	4,378 (±1,093)
Left cap FG															
Left injection	46 (±66)	1 (±1)	47 (±67)	764 (±516)	80 (±65)	844 (±579)	27 (±19)	100 (±73)	107 (±79)	11 (±12)	0	11 (±12)	847 (±524)	181 (±134)	1,028 (±650)
Right injection	78 (±126)	5 (±8)	83 (±133)	1,299 (±507)	242 (±101)	1,541 (±592)	69 (±34)	234 (±141)	312 (±120)	39 (±21)	0	39 (±21)	1,485 (±657)	482 (±230)	1,967 (±879)
Right cap CtB															
Left injection	47 (±84)	1 (±1)	48 (±84)	2,253 (±988)	365 (±176)	2,617 (±1152)	218 (±115)	505 (±218)	583 (±265)	98 (±50)	0	98 (±50)	2,616 (±1,146)	871 (±365)	3,487 (±1,489)
Right injection	10 (±11)	2 (±4)	12 (±9)	2555 (±718)	590 (±306)	3145 (±959)	192 (±74)	544 (±142)	782 (±307)	148 (±56)	0	148 (±56)	2,904 (±735)	1,136 (±389)	4,041 (±1,054)
Right cap FG															
Left injection	33 (±60)	0 (±1)	34 (±61)	1,073 (±941)	152 (±62)	1,225 (±999)	64 (±36)	223 (±116)	216 (±89)	30 (±20)	0	30 (±20)	1,200 (±1,036)	376 (±171)	1,576 (±1,203)
Right injection	30 (±47)	2 (±3)	32 (±50)	1,119 (±807)	167 (±82)	1,286 (±880)	82 (±67)	165 (±55)	250 (±144)	48 (±45)	0	48 (±45)	1,279 (±920)	335 (±132)	1,614 (±1,047)
Dark incubation CtB															
Left injection	38 (±72)	4 (±7)	41 (±79)	2,243 (±798)	333 (±229)	2,576 (±956)	138 (±71)	398 (±128)	470 (±241)	98 (±62)	0	98 (±62)	2,516 (±851)	734 (±202)	3,250 (±1,018)
Right injection	0	0	0	1,935 (±990)	310 (±225)	2,245 (±1,211)	156 (±99)	312 (±47)	466 (±186)	85 (±45)	0	85 (±45)	2,177 (±992)	622 (±260)	2,798 (±1,247)
Dark incubation FG															
Left injection	129 (±148)	16 (±11)	145 (±159)	1,982 (±631)	283 (±211)	2,265 (±842)	137 (±95)	282 (±265)	420 (±306)	46 (±12)	0	46 (±12)	2,293 (±862)	581 (±487)	2,873 (±1,349)
Right injection	36 (±47)	1 (±1)	37 (±46)	1,117 (±499)	239 (±134)	1,356 (±633)	84 (±27)	224 (±48)	323 (±107)	68 (±74)	0	68 (±74)	1,304 (±592)	464 (±86)	1,768 (±677)

Ipsilateral, contralateral and total numbers are given for each nucleus. The last column depicts the sum for left or right sided injections in each condition. There were no significant differences in the sum of ipsi- or contralaterally labeled cells after left and right injections (all $p > 0.05$) and no significant differences in the amount of ipsi- and contralateral labeled cells within all groups (all $p > 0.05$). [a] Due to immaturity of the Gld in pigeon hatchlings, differentiation between subnuclei was not possible. Therefore, cell counts are only given for the whole Gld

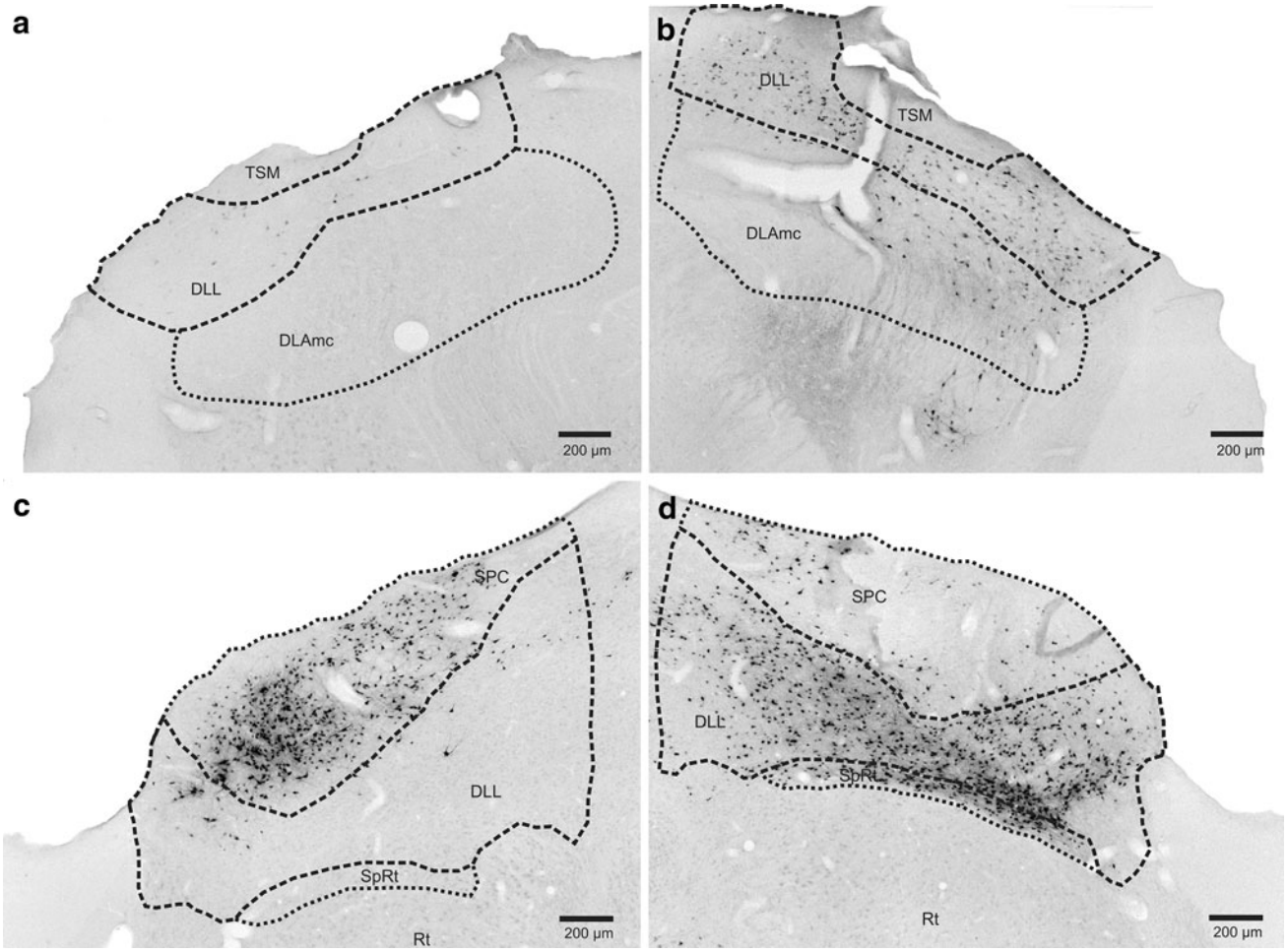

Fig. 3 Borders of the Gld nuclei n. dorsolateralis anterior thalami, pars magnocellularis (DLAmc), n. dorsolateralis anterior thalami, pars lateralis (DLL), n. suprarotundus (SpRt) and the thalamic n. superficialis parvocellularis (SPC) after unilateral injection of CtB into the Wulst. The thalamus contralateral (**a**) and ipsilateral (**b**) to injection side at A7.00 as well as at A6.50 (**c** contralateral, **d** ipsilateral) is depicted

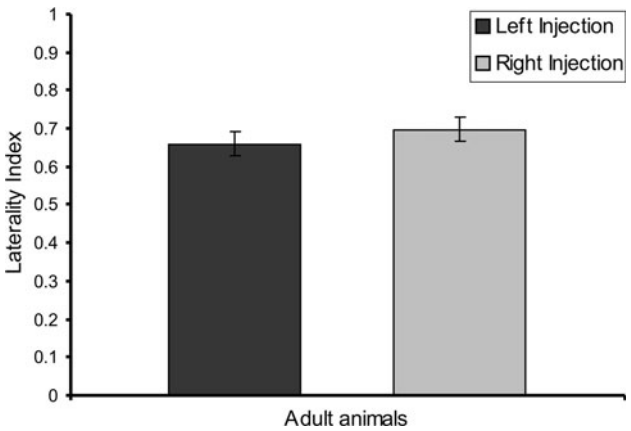

Fig. 4 Laterality indices of Gld projections after left- and right-sided CtB-injections into the visual Wulst of adult pigeons. There are no significant differences in LI values. *Error bars* depict standard errors

observed anatomical distribution of labelings of the present study is well in line with the literature. Combining our results with previous reports (Miceli et al. 1975, 1990; Bagnoli and Burkhalter 1983; Güntürkün and Karten 1991) we can safely say that ipsilateral Gld to Wulst projections mainly arise from DLL, DLAmc and SpRt, whereas contralateral projections originate primarily in SPC and, to a smaller extent, in DLL.

At the quantitative level, we could not reveal significant asymmetries in the relation of ipsi- and contralateral thalamofugal projections in adult pigeons. It is always difficult to discuss negative data. But our power analysis shows that, even if an asymmetry in the thalamofugal system would exist, it is extremely subtle and definitely constitutes a considerably smaller effect than in chickens. Thus, it is safe to conclude that adult pigeons develop a permanent

asymmetry in the ascending projections of the tectofugal system (Güntürkün et al. 1998; Folta et al. 2004; Verhaal et al. 2012) but very likely not of the thalamofugal one. Therefore, the first explanation assuming a thalamo- and a tectofugal lateralization in adult pigeons can be rejected. However, since thalamofugal asymmetries in chickens are only transient, there might also be a transient lateralization in the pigeon's thalamofugal system with only the tectofugal system being permanently lateralized. To test this second scenario, we went on to analyze the projection patterns of the left and the right Gld in pigeon hatchlings.

Experiment 2

Materials and methods

Pigeon hatchlings of posthatch day 4 from lab own breeding pairs were used for our second experiment. Animals received CtB tracer injections in either left or right visual Wulst as described in experiment one. However, injections were made under visual control since no atlas of pigeon hatchling brains exists. After 24 h survival time, the animals were sacrificed. Immunohistochemical staining was performed as stated above. The in comparison to adult animals' shorter survival time was chosen, since hatchling brains at this age are relatively small (1.2 cm). Therefore, 24 h are more than enough for a fast transported tracer like CtB with an average transportation speed of 2 cm/day (Köbbert et al. 2000) to cover such distance. Hatchlings in which the injections missed the Wulst were discarded from analysis. In total six animals with right Wulst and six animals with left Wulst injections were used for analysis. LI and CI Values were obtained as described before.

Results

Tracer applications into the visual Wulst resulted in bilateral labeling of Gld cells. Due to the immaturity of the dorsal thalamus (Manns et al. 2008), it was not possible to draw with certainty clear borders between the different Gld subnuclei and the SPC. Therefore, cell counts in hatchlings include SPC, resulting in a higher number of contralaterally labeled neurons. In the ipsilateral dorsal thalamus (Gld + SPC, for simplicity called Gld in this section) large sized labeled cells were mainly localized in more lateral portions, while smaller occupied medial areas. Only very few cells were labeled at the dorsal and ventral Gld borders (Fig. 5a). In the posterior GLd, labeled cells shifted laterally, while medial and dorso-medial parts contained almost no cells (Fig. 5c). On the contralateral side, significantly fewer neurons were labeled. These were mainly localized in the dorsolateral Gld (Fig. 5b, d).

In hatchlings, significantly fewer neurons were counted compared to adult pigeons, averaging 965 (±361 SEM) cells with 19.5 % of them projecting contralaterally. Localization of cells within Gld was comparable to adult birds. LI values for left and right Wulst injections in hatchlings ranged from 0.24 to 0.93 ($\bar{x} = 0.60$, $\sigma = 0.28$) in left injected animals and from 0.02 to 0.80 ($\bar{x} = 0.55$, $\sigma = 0.30$) in right injected hatchlings (see Fig. 6). No significant side difference could be evinced (two-tailed independent samples t test ($t(10) = -0.34$; $p = 0.74$). All variables were normally distributed (Kolmogorov–Smirnov tests, $p > 0.63$). To keep our analysis comparable with the results from the lab of Lesley Rogers, we again also computed CI values (left = 0.35, $\sigma = 0.32$, right = 0.28, $\sigma = 0.24$). CI values were normally distributed (Kolmogorov–Smirnov tests, $p > 0.46$) and showed no significant effect (two-tailed independent samples t test ($t(10) = -0.37$; $p = 0.72$). Cohen's d for CI values was 0.21 and the power analyses revealed that the effect would not reach significance until an overall n of 1,118 animals.

Discussion of experiment 2

Our second experiment in pigeon hatchlings also showed no differences in projection strengths between the left and the right Gld. The general pattern of distribution of labeled cells within the Gld was comparable to adult birds. However, the amount of contralaterally labeled cells was slightly higher in comparison to adult birds (15.9 % of total cells on the contralateral side in adult animals versus 19.5 % in hatchlings). It is likely that such a difference was caused by an incorporation of the SPC cells into our analysis. In the first experiment we could show that the SPC has a strong contralateral projection to the Wulst (for direct comparison see Table 1). Since this effect was present in left as well as in right side injected animals, CI and LI values should not be affected. In total, we counted considerably lower absolute cell numbers, suggesting an ongoing development of thalamofugal projections. This was expected since pigeon brains including the thalamic relay systems are relatively immature after hatch (Manns et al. 2008). This immaturity makes it likely that our tracing analysis covered the ongoing sensitive phase of the thalamofugal system. Since our data did not show a lateralization of the thalamofugal system in pigeon hatchlings, the second explanation suggesting a transient thalamofugal lateralization in pigeons has also very likely to be rejected. However, we can not completely exclude a transient asymmetry during later stages of thalamofugal development.

It is possible that the difference in thalamofugal asymmetry between pigeons and chickens result from the

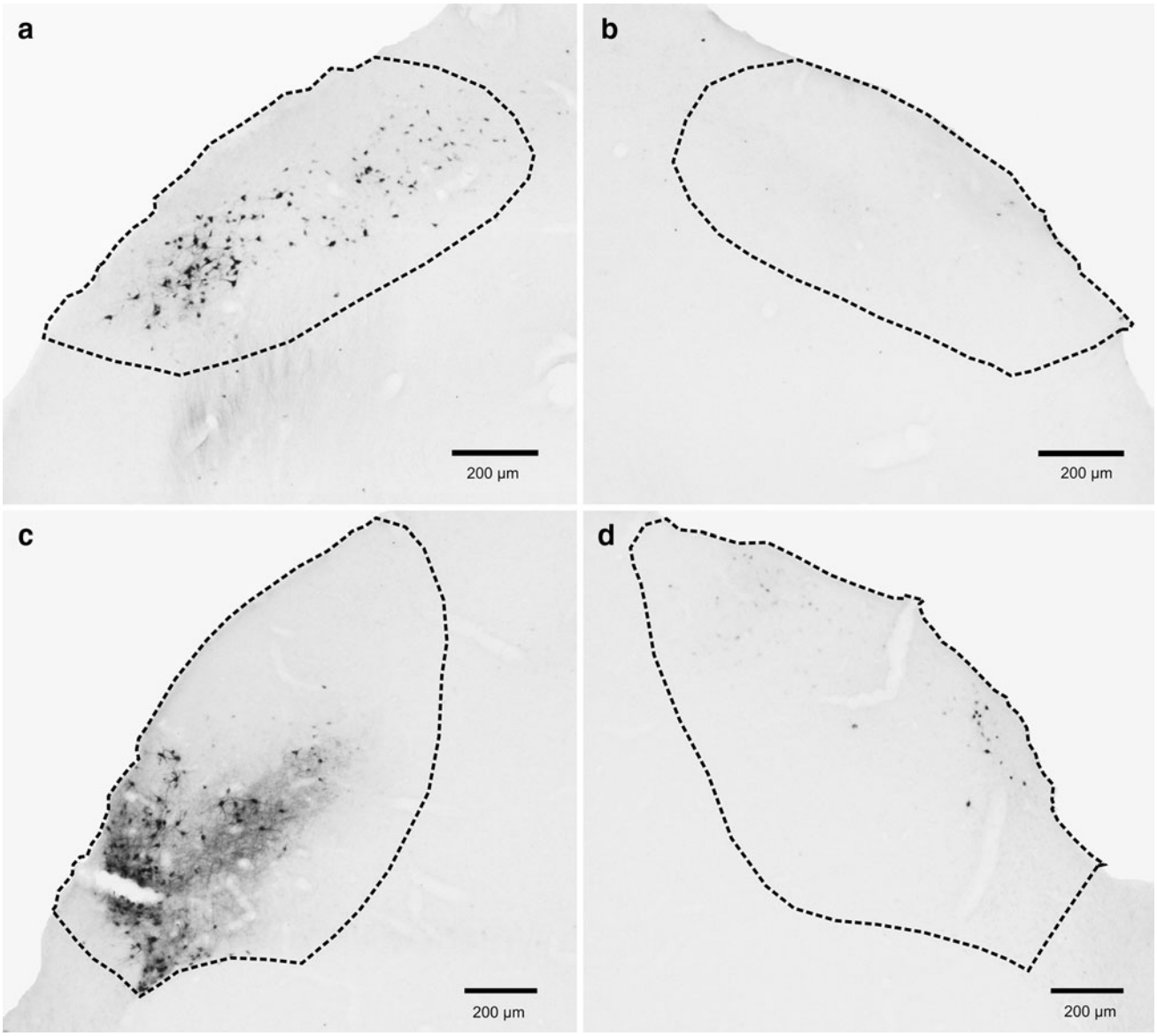

Fig. 5 Outline of the GLd ipsilateral (*left*) and contralateral (*right*) at anterior (**a**, **b**) and medial (**c**, **d**) sections of the dorsal thalamus of posthatch day 4 hatchlings after unilateral Wulst injections. Note that a classification of subnuclei comparable to experiment one was not possible since borders could not been drawn with certainty at this immature state

different developmental speed of these two species. As depicted in Fig. 7, chickens are precocial birds that are relatively mature when they hatch after 21 days. Pigeons, however, are altricial when they hatch after 17 days and need a lengthy rearing period before being able to feed on their own. In birds, the tectofugal systems matures earlier than the thalamofugal one (Rogers and Bell 1989; Deng and Rogers 2002). It is therefore likely, that in pigeons the tectofugal system is sensitive to biased environmental input before hatch (Manns and Güntürkün 1997), while the thalamofugal pathway just starts to develop after hatch (Manns et al. 2008). In the later hatching chicken, however, the thalamofugal system would be in the midst of its sensitive phase when the embryo is undergoing asymmetrical light input (Deng and Rogers 2002). As a result, it is conceivable that the different developmental speeds of pigeons and chickens result in anatomical asymmetries of tecto- and thalamofugal pathways, respectively (Halpern et al. 2005).

We designed our third experiment to test this explanation. For this, we raised dark incubated pigeon hatchlings and placed an eye cap for 1 week directly after hatch. In

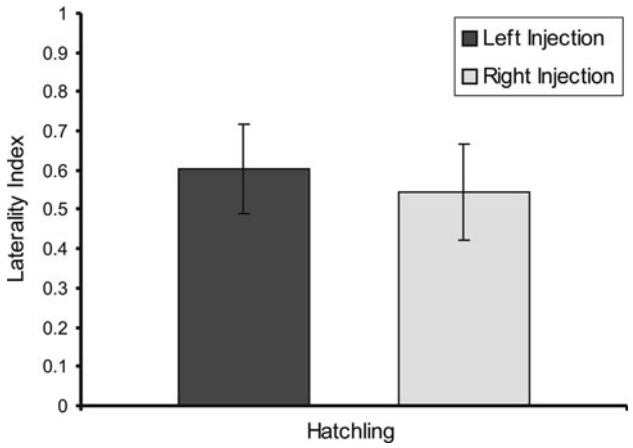

Fig. 6 Laterality indices of Gld projections after left- and right-sided CtB-injections into the visual Wulst of posthatch day 4 pigeon hatchlings. There are no significant differences in LI values. *Error bars* depict standard errors

these animals no embryonic light input could affect the development of any visual pathway. After hatch and thus during the developmental phase of the thalamofugal system, the unilateral eye cap should produce a lateralized visual stimulation. If the thalamofugal pathway of pigeons is affected by this left–right difference of photic stimulation, we should observe an anatomical thalamofugal asymmetry during adulthood (see Fig. 7).

Experiment 3

Materials and methods

37 pigeon eggs were taken away from the nests directly after laying and were exchanged by plaster replica. Eggs were placed into a dark incubator at 38.5 °C and 65 % air humidity until day 14, followed by 80 % air humidity until hatch (Skiba et al. 2002). After 17–18 days, hatching occurred and hatchlings were monocularly deprived by placing opaque caps either over the left (left cap) or the right eye (right cap) for 1 week (Manns and Güntürkün 1999a, b; see Fig. 8). Caps were attached directly to the skin surrounding the eye with nontoxic, water-soluble glue and were carefully removed after 1 week.

A subgroup of nine hatchlings did not receive a cap and served as control (dark incubation only). After 6 months of maturation, pigeons received injections of retrograde tracers CtB in the right and FluoroGold (Fluorochrome, LLC, Denver, USA) in the left visual Wulst or vice versa, with the sides of CtB and FluoroGold injections being balanced. Double injections allowed minimizing the number of animals needed. Injection protocols were identical to those in experiment one. Subsequent to perfusion and brain cutting, sections were stained against CtB or FluoroGold using a rabbit-anti-FluoroGold antibody (Fluorochrome, LLC, Denver, USA) in a concentration of 1:5,000. Visualization

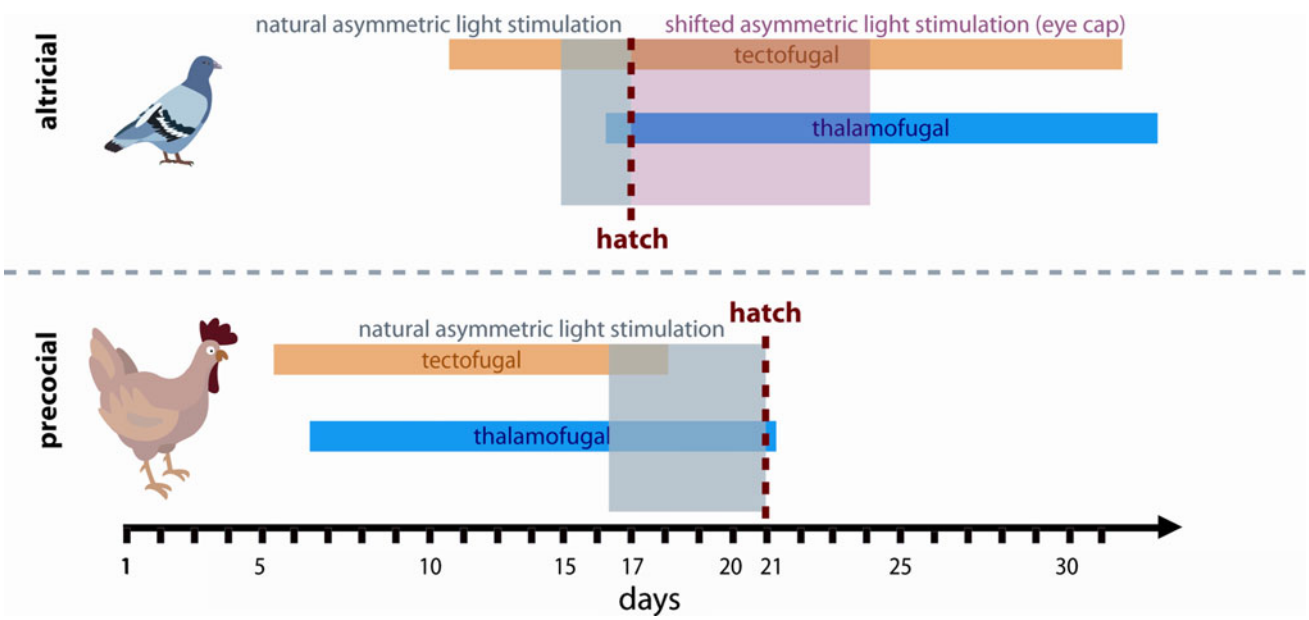

Fig. 7 Development of the tectofugal and the thalamofugal system in pigeons and chickens. Due to ontogenetic differences in developmental speed of altricial pigeons and precocial chickens, asymmetrical light stimulation (*rectangle* before *dashed line*) occurs during different developmental stages. Biased lateralized visual stimulation during the last days before hatch (*dashed line*) could therefore mainly affect the tectofugal or thalamofugal systems in pigeons and chickens, respectively. In our experiment, we shifted asymmetric light stimulation during thalamofugal development in pigeons into the posthatch period of thalamofugal development by placing an eye cap over the left or the right eye (*rectangle* after *dashed line*) in dark-incubated animals. Figure adapted from Deng and Rogers (2002)

Fig. 8 Two-day-old hatchling with covered left eye which was used in experiment 3

of antibodies, analyses of sections and statistical analyses also followed the same protocol as stated in experiment one. To cope for variability in tracing sensitivity while using different tracers (Güntürkün et al. 1993), a correction factor C of 0.985 was introduced. This factor was multiplied by all LI values of FluoroGold, to align CtB and FluoroGold results. The correction factor C was calculated using the term:

$$\frac{1}{3}\sum_{i=1}^{3} x_i$$

with

$$x_i = \left(\frac{\frac{\bar{x}_{\text{CtBleftcap}}}{\bar{x}_{\text{FGleftcap}}} + \frac{\bar{x}_{\text{CtBrightcap}}}{\bar{x}_{\text{FGrightcap}}} + \frac{\bar{x}_{\text{CtBdark}}}{\bar{x}_{\text{FGdark}}}}{3} \right).$$

For CI values, a correction factor of 1.157 was used. Due to animal losses and misplaced or unsuccessful tracer injections/labeling the final amount of successful injections (either CtB or FluoroGold) in each group was reduced to: left cap $n = 20$ (left injection: 9, right injection: 11), right cap: $n = 22$ (left injection: 13, right injection: 9), dark incubation only $n = 11$ (left injection: 6, right injection: 5). Again, labeled cells in SPC were excluded from analysis.

Results

CtB Injections resulted in labeled Gld neurons with a similar distribution as observed in experiment one. Also the distribution of cells within the different Gld subnuclei resembled the first experiment. On average 3,236 (±195 SEM) labeled Gld cells were counted and 14.6 % of them projected contralaterally. As expected, significantly less neurons were labeled with FluoroGold: 1,429 (±188 SEM) with 12.8 % of them projecting contralaterally. As mentioned above, the correction factor C was used to cope for these differences. For the left cap group, LI values for left sided injections ranged from 0.61 to 0.89 ($\bar{x} = 0.75$, $\sigma = 0.09$) whereas LI values for right sided injections ranged from 0.59 to 0.82 ($\bar{x} = 0.71$, $\sigma = 0.08$). For the right cap group, LI values for left sided injections ranged from 0.55 to 0.80 ($\bar{x} = 0.72$, $\sigma = 0.09$) whereas LI values for right sided injections ranged from 0.56 to 0.80 ($\bar{x} = 0.67$, $\sigma = 0.10$). In dark incubated animals LI values for left sided injections ranged from 0.62 to 0.87 ($\bar{x} = 0.75$, $\sigma = 0.10$) whereas LI values for right sided injections ranged from 0.65 to 0.84 ($\bar{x} = 0.72$, $\sigma = 0.07$, see Fig. 9). All variables were normally distributed (Kolmogorov–Smirnov tests, all $p > 0.80$) and were analyzed using a univariate analysis of variance (ANOVA) with the between subject factors injection side (left, right) and group (left cap, right cap, dark-incubation only). Both the main effects of injection side ($F(1,47) = 2.43$; $p = 0.13$) and group ($F(2,47) = 1.20$; $p = 0.31$) were not significant, as was the interaction injection side × group ($F(2,47) = 0.01$; $p = 0.99$). As in experiment one, we aimed to be comparable in our analyses to the published data from Lesley Rogers' lab and therefore also calculated CI values for each group (left cap: left = 0.15, $\sigma = 0.06$, right = 0.18, $\sigma = 0.06$, right cap: left = 0.18, $\sigma = 0.07$, right = 0.21, $\sigma = 0.08$, dark-incubation only: left = 0.15, $\sigma = 0.07$, right = 0.18, $\sigma = 0.06$). These CI values were normally distributed (Kolmogorov–Smirnov tests, $p > 0.69$) and were analyzed using a univariate analysis of variance (ANOVA) with the between subject factors injection side (left, right) and group (left cap, right cap, dark-

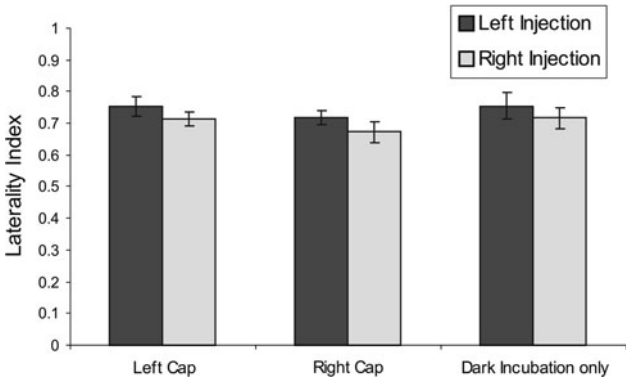

Fig. 9 Laterality indices of Gld projections after left- and right-sided CtB-injections into the visual Wulst of adult pigeons with a right, a left or no eye cap for 1 week after hatch. There were no significant differences in LI values neither between cap conditions nor groups. *Error bars* depict standard errors

incubation only). Both the main effects of injection side ($F(1,47) = 2.86$; $p = 0.10$) and group ($F(2,47) = 1.10$; $p = 0.34$) were not significant, as was the interaction injection side × group ($F(2,47) = 0.02$; $p = 0.98$). For the between subject factor injection side, Cohen's d was 0.25 and the power analyses revealed that the effect would not reach significance until an overall n of 217 animals. For the between subject factor group, Cohen's d was 0.22 and the power analyses revealed that the effect would not reach significance until an overall n of 331 animals.

Discussion of experiment 3

Also our third experiment did not reveal any significant asymmetries of Gld projections to the visual Wulst. Neither after left nor after right monocular deprivation for one posthatch week could we reveal any thalamofugal lateralization. Furthermore, we did not find differences of the LI- or CI-values between monocularly deprived and dark incubated animals. The power analyses revealed that, if there was an effect at all, it would only reach significance after testing hundreds of birds. Thus, we assume that it is safe to say that an asymmetrical visual stimulation that falls within the developmental period of the thalamofugal system in pigeons does not result in sizable asymmetries in the numbers of cells that project from the GLd to the visual Wulst. However, it should be noted that our monocular deprivation might have induced a transient lateralization, which was not detected by our experiment.

It is very likely that our monocular deprivation was indeed conducted during the period of thalamofugal maturation. Manns et al. (2008) compared the developmental pattern of the Gld in posthatch day 2 and posthatch day 4 hatchlings and found vast differences in the distribution of calbindin-, parvalbumin- and $GABA_A$ receptor expressing cells between the posthatch developmental states as compared to the adult state. In contrast, retino-thalamic projections were already mature at posthatch day 2. Thus, the authors therefore suggested an immaturity of the Gld after hatching which therefore still is possibly sensitive to modulations of visual experience.

Overall discussion

We conducted a series of three experiments to search for a thalamofugal asymmetry in pigeons that resembles the chicken pattern. In the first and the second study we did not find a thalamofugal asymmetry in adult and in newly hatched pigeons. In the third study, we tested if asymmetrical visual stimulation during the developmental period of the thalamofugal system induces asymmetrical thalamofugal projection patterns in pigeons. Again, we found neither in left- or right eye-deprived nor in dark incubated pigeons a thalamofugal asymmetry in the adult condition. However, we can neither exclude the presence of a transient lateralization of thalamofugal projections in pigeons before posthatch day 4 nor a transient lateralization induced by artificial asymmetrical light stimulation after hatch in the thalamofugal system of pigeon hatchlings. Keeping this limitation in mind, we can nevertheless state that the thalamofugal system in pigeons is not lateralized with respect to its bilateral projection pattern. Since pigeons and chickens are not in the same family of the avian taxon (Hackett et al. 2008), it is conceivable that the ontogenetic susceptibility of the tectofugal (pigeons) or thalamofugal system (chickens) represents a true genetic species difference. When drawing this conclusion, we have to take into account that it is never possible to exclude a false negative result. Yet, our power analysis revealed that an effect would not reach significance until an overall n of 228 animals for experiment one, an overall n of 1,118 for experiment two and an overall n of 331 for experiment three. Since previous experiments used a rather small number of chicks for each injection side ($n = 4$–8 per group, Rogers and Sink (1988); $n = 6$–8 per group, Rogers and Bolden (1991); $n = 8$ per group, Rogers and Rajendra 1993) and found significant effects, it is unlikely that our negative results are only due to sample size effects.

If similar environmental factors affect different neural systems of two bird species, the analysis of closely related species could reveal the evolutionary pattern of visual asymmetries at the anatomical level. For chickens, a comparison to ducks or geese of the Anseriformes phylum would be warranted (Hackett et al. 2008). For pigeons, a comparison to the Podicipediformes (grebes) might be helpful (Hackett et al. 2008).

If tecto- and thalamofugal asymmetries of pigeons and chickens, respectively, represent a species and not a system-dependent difference, both species must realize their behavioral visual asymmetries with different neural systems. These behavioral left–right differences are highly similar at least for the right eye/left hemisphere. Here, both species show a higher performance in food searching tasks (pigeon: Güntürkün and Kesch 1987; Skiba et al. 2002, chicken: Mench and Andrew 1986; Rogers 1990), a better visual pattern memory recall (pigeon: von Fersen and Güntürkün 1990, chicken: Gaston and Gaston 1984) and a general dominance for the analysis and categorization of object specific cues (pigeon: Güntürkün 1985; Prior et al. 2002; Yamazaki et al. 2007, chicken: Vallortigara et al. 1996; Tommasi and Vallortigara 2001, 2004). Similarities of the left eye/right hemispheric system between the two species are less clear, mainly due to a smaller number of studies for this system in pigeons. In chickens, there is plenty of literature reporting a left eye/right hemispheric

dominance for visual spatial tasks (Vallortigara et al. 1996) and usage of global and geometrical cues for orientation (Vallortigara et al. 2004; Tommasi and Vallortigara 2001, 2004). Also pigeons show a right hemispheric superiority in the control of visuospatial attentional resources (Diekamp et al. 2005), the analysis of visuospatial configurations (Yamazaki et al. 2007) and navigation inside a hierarchical organized flock (Nagy et al. 2010). However, in contrast to chickens, pigeons display no asymmetry in the usage of global cues to orient in space (Prior and Güntürkün 2001; Prior et al. 2002; Wilzeck et al. 2009). There is even good evidence that pigeons rely strongly on left hemispheric resources when navigating during flight (Prior et al. 2004; Gagliardo et al. 2005; Siegel et al. 2006; Nardi and Bingman 2007). Thus, at least for one right hemispheric functional domain it is conceivable that visual asymmetries of pigeon and chicken differ. However, it should be noted that at least in chicken not all forms of visual lateralization are triggered by light and can be found even in dark incubated animals, including imprinting, social recognition and discrimination of certain position-specific and object-specific cues (Vallortigara et al. 2001; Andrew et al. 2004; Chiandetti et al. 2005).

This short overview reveals that most, but not all, behavioral patterns of visual asymmetry in pigeons and chickens are highly similar. Also the ontogenetic factors that induce visual lateralization at an early developmental period are identical (Rogers 1982; Skiba et al. 2002). Yet the anatomical systems subserving these behavioral left–right differences are different. This result makes it likely that the ascending tecto- and thalamofugal pathways have the principle potential to produce highly similar behavioral lateralization patterns. In more general terms, our data reveal that the functional specification of the two parallel ascending visual pathways shows high degrees of freedom over phylogenetic time.

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