Impact of Ontogenetic Factors and Commissural Systems onto a Asymmetric Information Processing in the Pigeon (Columba livia)

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CHAPTER 1

General Introduction

1.1 Asymmetries in Humans

Most of living animals are bilaterians, meaning that the left and right side of the body are mirror images of each other. They have a front- and a back-end (anterior-posterior body-axis) as well as a top- and a bottom-side (dorsal-ventral body-axis). Following this body organization, these animals possess a bilateral nervous system, which processes the symmetrical sensory input to a symmetric motor program. In vertebrates, the respective neuronal projections primarily cross to the contralateral side [106], [194]. In contrast to the external symmetry, some internal organs are predominantly asymmetrically organized. There is only one heart, one liver, and one stomach etc. This asymmetry of the visceral organs correlates with an asymmetry of the innervating autonomic nervous system. Also our brain, which at first glance seems to be symmetrical organized, demonstrates anatomical left-right differences. In addition to anatomical left-right differences, functional asymmetries exist, which can be traced back to a lateralized processing of the brain. Specific functions are not symmetrically processed within the two hemispheres of the brain, rather one hemisphere is dominant for a specific task. This was firstly described by the observations of Broca (1861) [21]. Broca discovered that patients with language impairment primary display a lesion of the left hemisphere [21]. Accordingly, he argued that human language is dominantly processed within the left hemisphere and, thus a lateralized function. From this point on, numerous studies were carried out,
which discovered sensory, cognitive, emotional or motoric lateralized functions of the brain.

The left-hemispheric dominance of speech processing and the right-handedness of most of the people, belong to the most famous lateralization in humans. In contrast, a prominent example for a right-hemispheric lateralization is the control for visuospatial attention [135]. Functionally, this dominance is characterized by an attention bias to the left visual field. The so called 'pseudoneglect' was first described by Bowers and Heilman (1980) [19]. Several tests were conducted to analyze this phenomenon, such as the line bisection or cancellation task. In the latter test, the test subject had to cancel a particular letter out of a mixture of different letters [3]. The test subject demonstrates in this task an attention bias to the left hemifield and, accordingly, a dominance of the right hemisphere. For the interhemispheric integration of information from the left and right extracorporate space the corpus callosum is used [59], [79]. With more than 250 million fibers, the corpus callosum is the main projection course between the hemispheres. For a long time the influence of the corpus callosum on interhemispheric communication was not clear. Studies with split-brain patients, people who had a complete or partial transection of the corpus callosum demonstrated the important influence of this commissural structure for interhemispheric communication and exchange [60].

In summary, the lateralization of different brain functions is a fundamental processing principle of the human brain. The appearance of functional laterlization of the brain in some aspects correlates with structural differences in the left and right hemisphere [193]. This aspect of lateralization is introduced in the next section.

1.2 Structure and Function Relations

There is evidence that functional lateralization develops in close relation to structural asymmetries [193]. The interest in structure and function relations started with the discovery that the left planum temporale, which includes parts of Wernickes area, is larger than the right one [62]. This finding correlates with the functional lateralization of the left-hemispheric language processing [34]. In addition to the planum temporale, structural brain asymmetries have also been observed in other parts of the language system, several somatosensory and motor areas, as well as in areas involved in spatial
cognition and the limbic system [148]. Despite this variety of structural asymmetries in the human brain, it is difficult to correlate them with behavioral asymmetries. For example, studies which directly investigate the relationship between the structural left-hemispheric lateralization of the planum temporale and behavioral lateralization have shown contradictory results [46]. Since asymmetries in volume or size of specific regions do not indicate differences in neuronal connectivity with great functional relevance, recent research starts to investigate asymmetries in connectivity pattern and focuses on structural asymmetries in different fiber tracts. Barrick et al. (2007) identified a left-asymmetric pathway connecting the parietal and frontal lobes to the temporal lobe, which may be related to left-hemispheric language dominance [15]. Moreover, they found a right-asymmetric pathway connecting the posterior temporal lobe to the superior parietal lobe. This pathway is may be related to the right-hemispheric dominance of auditory spatial attention.

In summary, the last two sections describe how several functions are lateralized processed within the brain (1.1) and these functional asymmetries correlate to some extent with structural left-right-differences. Thus, the next section introduces mechanisms that affect the development of lateralization.

1.3 Ontogenetic Models of Lateralization

Factors which are involved in the development of structural and functional asymmetries are genetic components [7], cytoplasmatic gradients, hormones [61], environmental factors [51], and sociocultural influences like education. The concerted action of so many different factors raises question concerning developmental patterns.

Early research-models assume that after birth both hemispheres are equipotential; only during early childhood does dominance of one hemisphere develop due to the high plasticity of the brain during this time period [63]. Most current hypotheses, however, postulate a very early invested asymmetry of the brain [105], because structural left-right-differences of the brain, like the size of the planum temporale, are already visible in the early fetus [193]. On behavioral level, newborns show an asymmetry in their posture, thumb-sucking, orientation- and arm-movement. Babies prefer to move their head to the right than to the left side [182]. Accordingly, invariance and parallel-development hypotheses assume the early generation of cerebral asymmetries.
Thereby, the parallel-development hypothesis suggests a critical involvement of interhemispheric interactions. The final lateralization pattern is mediated by a primary inhibitory interaction of the hemispheres through the corpus callosum. This lateralization pattern is presumably affected by environmental factors. Experiments in rats demonstrate that additional experiences with the environment result in a stronger activation of the cortex, which leads to a stronger callosal connection and accordingly to a stronger inhibition of the contralateral hemisphere (for review see [146]).

In contrast, the invariance hypothesis postulates an independent development of both hemispheres, which leads to an asymmetry and the adoption of one hemisphere for one special task. These asymmetries can be genetically fixated [7], induced by different developmental speed [61], [63], different cell division rates, or different activation of one hemisphere [115]. Accordingly, a functional asymmetry is only stabilized, not induced, by interhemispheric interactions. However, the functional and ontogenetic relations of structural and functional asymmetries are far from being clear. Animal models are helpful for deeper insight into the interactions creating a lateralized information processing.

1.4 Functional Lateralization in Animals

For a long time, it was assumed that cerebral lateralization is a human specific feature [127], which developed in correlation with human specific properties like upright walking [152], cultural evolution, language use, or the appearance of consciousness. In the meantime, however, a variety of functional lateralizations were also found in several animal species. Therefore, it was concluded that lateralized information processing is a widespread feature in the animal kingdom. So, left-right-differences in the neuronal system were found in all vertebrate classes and beyond, in invertebrates like octopus [24], fruit flies [150], bees [118], and even nematodes [180]. In addition to functional asymmetries also a variety of structural asymmetries were shown in different animals. There are asymmetries in the brain morphology, connectivity, and transmitter distribution (for review see [148]). Certainly, often the correlation of structural and behavioral asymmetries is not clear. The situation is different in the visual system of birds. Because the efferent fibers from the retina in birds
cross almost completely at the optic chiasm, cognitive functions of one hemisphere can be easily tested by occluding one eye. Such monocular tests revealed that the left and the right hemisphere analyze different aspects of visual stimuli [35], [41]. The left hemisphere is specialized for detailed object analysis and processes local features of visual stimuli [202]. The better discrimination ability of the right eye and therefore, left hemisphere is shown in a grain-grit discrimination task in pigeons [75], chicks [162], quails [184], and zebra finches [4]. A left hemispheric-dominance for fine analysis of visual stimuli is not only visible in food discrimination tasks but also in the discrimination of two dimensional patterns [67]. Further studies discovered a dominance of the left hemisphere for the detection of geometric optic illusions [68], color reversal learning [40], and the categorization of the object category human [202].

In contrast to the left hemisphere, the right hemisphere handles global aspects of a visual scene and, is accordingly dominant in spatial cognition [25], [42]. Comparable to pseudoneglect in humans (see chapter 1.1), birds also show a tendency to attend to the left visual field [25], [42]. When birds like chicks or pigeons were confronted with evenly distributed grains in a restricted area in front of them, they display a tendency to peck more to the left visual field [25], [42]. Apart from spatial processing, the right hemisphere is also specialized for species typical and instinct based behavior as well as social cognition and fight [185], fear and escape behavior, and mating behavior [66].

Functional asymmetries in birds are closely related with anatomical left-right differences in the ascending visual projections of the thalamo- and tectofugal pathways as well as of the interplay of commissural systems [127], [130].

1.5 The Visual System in Birds

1.5.1 Structural Lateralization within Ascending Systems

In birds, as in other amniotes, visual information is processed via two ascending pathways. In birds these two pathways, the thalamo- and tectofugal system, display structural asymmetries depending on the bird species and developmental stage (Fig 1.1 A, B) [127].
The Visual System in Birds

Figure 1.1: Schematic drawing of the ascending visual systems in birds. A: tectofugal pathway: fibers from the retina project to the contralateral optic tectum (TO), from there ascending fibers project bilaterally onto the nucleus rotundus (RT). In the pigeon this projection is asymmetrically organized. From RT unilateral fibers project to the entopallium (E). B: thalamofugal pathway: axons from the retina project to the contralateral nucleus geniculatus lateralis, pars dorsalis (GLD). From there, fibers bilaterally project onto the visual wulst. In chicken this projection is asymmetrical organized. Arrows show the projection direction and dashed lines indicate a decreased projection.

The thalamofugal system corresponds to the geniculocortical system in mammals and transfers retinal information via the contralateral nucleus geniculatus lateralis, pars dorsalis (GLD) of the thalamus bilateral to the telencephalic visual wulst [76], [178]. This pathway demonstrates a transient asymmetry in the number of ascending fibers in chicks (Fig 1.1 B)[108]. Thereby, the left thalamus sends more efferents to the contralateral right telencephalon than vice versa [108], [164]. A comparable asymmetry is not present in the pigeon, neither in juvenile nor in adult animals [178].

The tectofugal system corresponds to the extrageniculocortical system in mammals and projects via the contralateral optic tectum (TO) on mesencephalic level bilateral to the diencephalic nucleus rotundus (RT) and from there to the ipsilateral
entopallium in the telencephalon [127]. While the majority of tectal efferents ascend to the ipsilateral RT, a subpopulation of cells project to the contralateral hemisphere. Thereby, more fibers cross from the right TO to the left RT than vice versa (Fig 1.1 A) [73]. The resulting stronger bilateral innervation of the left RT correlates with enlarged cells on this side [124]. In accordance with the stronger bilateral input, electrophysiological studies demonstrate a majority of neurons in the left RT answering to contra- as well as ipsilateral afferents [56]. Thus, anatomical and electrophysiological studies suggest that the stronger bilateral input to the left RT results in a more complete presentation of the visual scene in the left hemisphere. Accordingly, Güntürkün and Hahmann (1999) showed that lesions of the left RT lead to deficits in acuity of the contralateral right and ipsilateral left eye [72]. In contrast, lesions of the right RT alone did not affect the performance. Further evidence for better left-hemispheric handling of information from the left and right visual hemifield came from studies analyzing hemispheric specific access to transfer information [119], [183]. Pigeons can be tested under monocular conditions in a color discrimination task. In such tasks, each eye learned to discriminate a different color pair. Due to the almost completely crossed optic nerve, each hemisphere had direct experience with only one color pair, resulting in a known and unknown color pair for each hemisphere. Confronting the animals with the known color pairs showed no difference in the performance of the left and right eye. However, confrontation with the unknown color pairs demonstrates a better performance with the right eye in comparison to the left eye. These results suggest that the left hemisphere not only receives more visual afferents but also has a better access to information that was previous learned by the contralateral right hemisphere. This finding can be explained by the tectorotundal projection asymmetry alone, however, behavioral and electrophysiological findings imply that top-down and interhemispheric mechanisms affect the lateralized tectofugal processing pattern [58], [183].

1.5.2 Functional and Structural Lateralization within Descending Systems

The visual system in birds demonstrates not only asymmetries in the ascending pathways as described above, but also in descending pathways. Descending pathways from the telencephalon are described by the tractus occipitomesencephalicus, pars
1.5 The Visual System in Birds

Figure 1.2: Schematic drawing of asymmetrical top-down control of the visual wulst. Descending fibers from the visual wulst project ipsilaterally to the optic tectum (TO) and interact on tectal level with intra- and interhemispheric circuits. The inhibitory commissural system modulates the balance between the hemispheres, in which the right TO inhibits the left TO to a larger extend than vice versa.

hypothalami (HOM), tractus occipitomesencephalicus (OM) and tractus septomesencephalicus (TSM). The HOM connects amygdaloid areas of the telencephalon with limbic structures of the diencephalon [110], [206], while OM connects the telencephalic arcopallium with sensory and motor structures of the diencephalon, the brainstem, and the spinal cord [36], [110], [206]. Neurons which compose the TSM are primary localized within the visual wulst in the telencephalon and descend into the TO [120]. Of these three descending fiber tracts, it is known that the TSM functionally mediates an asymmetric top-down effect [58], [183]. Structural this projection is exclusively ipsilaterally organized and displays no asymmetry in the number of projecting neurons (Fig 1.2)[117], [123]. The connection between the telencephalon and TO allows the wulst to control tectofugal processing within the TO.

The influence of the visual wulst in discrimination tasks is shown by Valencia-Alfonso et al. (2009) [183]. They temporally inhibited the left, right, or both wulst
by injections of tetrodotoxin (TTX) during a color discrimination task as described in section 1.5.1. The inhibition of the wulst leads to a decreased discrimination performance for the known and unknown stimuli pairs. A significant effect was only visible in left side inhibition, not only decreasing the performance of the ipsilateral but also the contralateral eye. It appears that without the impact of the left wulst it is more difficult to retrieve the previously learned information in both hemispheres [58], [183]. In addition to this pharmacological study, electrophysiological studies demonstrate that the wulst generated top-down control has impact on tectofugal processing and is primary located in the left forebrain [56], [183]. At the rotundal level, electrophysiological recordings showed that a subpopulation of cells is modulated by top-down influences, which can be detected by very late response components of these cells [56]. These late responses originate exclusively from the left wulst and demonstrate a bilateral modulation of the rotundus [56]. The wulst itself has no direct connection to the rotundus but are indirectly connected via the TO. As already described in section 1.5.1, the TO projects bilaterally and asymmetrically to the RT. Thus, it is likely that the asymmetric bilateral action of top-down projections does not arise from left- right-differences of the descending system itself, but by its interaction with lateralized tectal systems [127].

1.5.3 Interhemispheric Regulation

The descending projections of the TSM-fibers terminate within the TO, from which point the projections lead to all major midbrain targets [82]. Accordingly, lateralized top-down effects as observed in the RT represent secondary consequences of processes on TO level [127]. In general, the TO is a central relay station for visuomotor behavior, different components of visual processing converge in this structure. The TO receives afferents from the retina, the contralateral TO, and the ipsilateral forebrain. It sends ascending visual projections to the forebrain and descending premotoric projections to the brainstem. This connectivity adjudges the TO a central position for the integration and regulation of lateralized visual information [127]. The endings of the TSM-fibers overlap with inhibitory intratectal systems and in this way affect ipsilateral TO circuits [116]. Additionally to the effects on the ipsilateral TO, the TSM-fibers can modulate inhibitory tectotectal interactions. The left and right TO
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are interconnected by an inhibitory commissural system [116], [159]. This massive fiber bundle consists of the commissura posterior (CP) and commissura tectalis (CT). Anatomical studies demonstrate that the two commissures can be structural separated by the number of projecting fibers, the degree of myelination, and the axon diameter. The CP exhibits 250,000 axons, and 32% of it are myelinated while the CT consist of 911,000 axons where 12% are myelinated [53]. The majority of fibers do not terminate within the contralateral TO but within a variety of brainstem nuclei [192]. Electrophysiological studies demonstrate that this commissural system is primarily composed of inhibitory GABAergic fibers [159]. Additionally, the information shift through the commissures is asymmetrically organized with the left TO inhibiting the right TO to stronger extent than vice versa [104]. The critical impact of these commissures on the lateralized control of visuomotor behavior is demonstrated by the transection of this system. [71] showed a reversed lateralization after transection of the CT and CP. In this process the degree of transection critically influences the strength of the lateralization reversal. This hints at a dynamic control of this system on lateralized behavior.

On telencephalic level, interhemispheric exchange in birds is mediated by the anterior commissure and the small hippocampal commissure. Anatomical studies show that the anterior commissure comprises approximately 89,000 axons with approximately 40% of the total number of fibres myelinated [52]. A study of Zeier and Karten (1973) demonstrated that the AC of pigeons interconnects a wide network of forebrain structures [207]. However, the degenerating tracing technique employed by Zeier and Karten (1973) does not allow for a detailed identification of the topographic organization with regard to cells of origin and partly also with regard to termination areas [207]. On a functional level, the impact of the telencephalic anterior commissure on lateralized information processing is not known yet.

To conclude, it is possible that the asymmetric effect of top-down projection has its origin not in a left-right difference itself, but by the interaction with lateralized tectal systems. As a consequence, the inactivation of the left wulst can change the dynamic of the intertectal inhibition to a larger extent than the right wulst and enables the left hemisphere with a dominant executive control. Furthermore, little is yet known about the structural and functional organization of the telencephalic commissural
systems.

1.5.4 Development of Lateralization

As already mentioned above, birds such as pigeons and chicks display functional as well as anatomical left-right differences in the ascending visual pathways. The development of asymmetries can be ascribed to a lateralized light exposure of the embryo before hatching. Shortly before hatching, the embryo consistently adopts a turned posture such that the right eye is close to the eggshell and the left eye is occluded by its own body [113]. This asymmetrical posture results from an asymmetrical turn of the embryonal axis, which correlates to the asymmetrical arrangement of the internal organs and is controlled by left-/right-specific gene expression cascades [30]. As a consequence of the asymmetrical head position, the right eye can be stimulated through the translucent eggshell and the left eye is naturally deprived (Fig 1.3)[127]. Breeding birds regularly turn the eggs and leave their nests for short time periods [23]. As a consequence, the embryo is repeatedly stimulated by light. These light pulses presumably induce long term effects in cellular processes, which are still active when the light stimulation is interrupted by return of the parents [23]. A variety of light effects are mediated by neurotrophic factors like the brain-derived neurotrophic factor (BDNF)[120]. In this process, light adjusts the expression and release of neurotrophic factors and hence, controls the trophic support of target cells [29]. In turn BDNF

![Figure 1.3: Position of a pigeon embryo within the egg. The left eye is occluded by the body, while the right eye is stimulated by light through the translucent eggshell.](image)
controls sprouting, branching and maintenance of axo-dendritic trees. Accordingly, this neurotrophin could be involved in the activity-dependent development of tectofugal asymmetry in pigeons. In fact, BDNF and its high-affinity receptor TrkB are present in the tectofugal system [181]. In response to the asymmetric embryonic light stimulation the TrkB signaling cascade is also asymmetrically activated [128]. The small G-protein p21Ras is a critical molecular switch for relaying neurotrophic actions into morphological changes [84]. Manns et al. discovered that p21Ras induces in the pigeon’s TO light dependent left-right-differences via altering the morphology of intratectal cell populations [128].

The critical impact of an asymmetrical light stimulation can be confirmed by studies which demonstrate that a bilateral light exposure and incubation in complete darkness prevent the development of a functional lateralization [164], [176]. This plasticity of the visual system ends directly after hatching in precocial chicks [164]. In the pigeon, however, it is possible to alter the visual asymmetries even after hatching [122], [124], [125], [153]. In this altricial species, visual development is prolonged up to 20 days after hatching [122], [124], [125]. Occluding the right eye within this critical time window reverses the visual asymmetry and induces a functional advantage of the left eye in visual discrimination tasks. Conversely, left eye deprivation enhances the dominance of the right eye [122], [124], [125]. The pre- and post-hatch plasticity of the visual system in pigeons is characterized by two critical developmental phases: induction and stabilization.

In conclusion, asymmetries are induced by a lateralized light stimulation before hatching and are stabilized after hatching despite of a symmetrical light input [127]. It is possible that a visual light stimulation before or after hatching affects neuronal processes in different neuronal systems. During the embryonic development asymmetric photic stimulation primarily influences the differentiation of retinotectal projections. After hatching, asymmetric modulation of visual experiences mainly appears to the differentiation of the non-retinorecipient components of the tectofugal system, which mediates the stabilization of induced asymmetries [127].
1.6 Research Questions

This section will introduce the scientific research questions of the present thesis. The present thesis combined neuroanatomical and behavioral approaches to analyze the influence of interhemispheric systems on lateralized information processing in the pigeon brain and its ontogenetic emergence.

For that purpose, first the influence of asymmetrical light stimulation on the asymmetrical projection pattern in the ascending visual system was analyzed. A tracing study then investigated the anatomical foundation for an interhemispheric connection on the telencephalic level by tracing the anterior commissure. In a final behavioral experiment, the impact of the previously anatomically analyzed systems on lateralized information processing were analyzed.

**Study 1:** Light-Dependent Development of Projection Asymmetry

The functional lateralization in the visual system of the pigeon is associated with structural left- right-differences in this system. Cell size differences between the left and right hemisphere are visible and the number of projecting fibers differs. This asymmetrical anatomical pattern develops in response to an asymmetrical light stimulation before hatching. Dark-incubation prevents the development of cell size asymmetries but light-dependent effects onto the quantity of projecting fibers in the ascending visual pathway is still unknown in the pigeon brain. Therefore, we conducted a quantitative tracing study in light- and dark-incubated pigeons to compare the number of projecting fibers between these two experimental groups. This can give deeper insight into the effects of envirotypic factors on the quantity of projecting fibers.

**Study 2:** Structural Organization of the Anterior Commissure

As described in the introduction, ontogenetic models suggest the corpus callosum has a crucial impact on the pattern and development of lateralized information processing. In our model organism, the pigeon, numerous functional asymmetries are described despite the absence of a corpus callosum. The largest commissural system on telencephalic level in the pigeon is the anterior commissure, but not much is known
about the connectivity of this system. Therefore, we analyzed the target structures of the anterior commissure and the corresponding cells of origin in a tracing study. The resulting similarities and dissimilarities of the anterior commissure in the pigeon, in comparison to the corpus callosum in mammals, can give deeper insight of the impact of commissural systems onto the ontogeny of a lateralized information processing.

**Study 3: Light-Dependent Lateralization of Visuospatial Attention**

Study 1 and 2 clarified the structural foundations of asymmetrical information transfer. As such a behavioral study followed to analyze the light-dependent pattern of left- and right-hemispheric functional development. Therefore, the visuospatial attention was examined by using a cancellation task. This task was performed with two experimental groups, dark-incubated pigeons and anterior commissure commissurectomized pigeons, in comparison to a control group (light-incubated pigeons).
CHAPTER 2

Study 1: Light-Dependent Development of Projection Asymmetry

As already mentioned in the general introduction, functional asymmetries in birds develop in close relation to an asymmetric embryonic light exposure. However, several functional asymmetries develop independently of asymmetric light stimulation. This leads to the question to which extent structural and functional asymmetries are correlated.

2.1 Introduction

Birds process visual information within two ascending pathways, the tecto- and thalamofugal system [70]. These two systems display species specific structural asymmetries. The thalamofugal pathway transfers retinal information via the contralateral geniculate complex (GLD) bilaterally to the visual wulst [70]. In the chicken, this system is lateralized, showing a transient asymmetry in the number of ascending fibers with more efferents from the left GLD to the right visual wulst than vice versa [108], [166]. Comparable asymmetries are neither present in young nor in adult pigeons [178]. The second ascending visual system is the tectofugal pathway. This system projects via the contralateral mesencephalic optic tectum and the diencephalic nucleus rotundus to the telencephalic entopallium [18], [70]. Different from the thalamofugal pathway, this system is characterized by anatomical left- right-differences in the pigeon [124], [125], but not in chickens [164]. In the tectofugal pathway, the
2.2 Material and Methods

2.2.1 Subjects

20 adult pigeons (Columba livia) of undetermined sex from local breeders as well as 18 adult dark-incubated animals from lab-owned breeding pairs received injections.
of the mainly retrograde transported tracer cholera toxin subunit B (CTB, Sigma, Deisenhofen, Germany). For dark-incubation, fertilized eggs from pairs of breeding pigeons were incubated in two still-air incubators kept in darkness at a constant temperature (38.3°C) and humidity (60-75%) throughout the entire period of incubation. Directly after hatching, the nestlings were banded and swapped with the artificial eggs the breeding birds were sitting on [176].

The tracer was administered into the nucleus rotundus (RT) at the coordinates A 6.00, L 3.00 at two different depth (7.5 mm and 8.0 mm from the brain surface) [102]. In 9 light-incubated (4 left, 5 right) and 14 dark-incubated pigeons (7 left, 7 right) successful RT-injections were performed. These animals were used for quantitative analysis of the tectorotundal projection pattern.

2.2.2 Tracer Application

Following the injection of 0.2 ml dolorex (Intervet, Unterschleißheim, Germany), the pigeons were anesthetized with isoflurane (abbvie, Ludwigshafen, Germany). The tracer CTB (CTB, 1% in deionized water) was injected through a glass micropipette (inner tip-diameter 15-20 μm) with a mechanic pressure device (WPI Nanoliterinjector; World Precision Instruments, Berlin, Germany). The injection volume was 460 nl. Following a survival time of 2 days, the animals were deeply anesthetized with equithesin (0.45 ml per 100 g body weight) and perfused with 0.9% sodium-chloride followed by ice-cold 4% paraformaldehyde (PFA) in 0.12M phosphate-buffered saline (PBS), pH 7.4. Brains were removed and postfixed for 2 h in PFA with a supplement of 30% sucrose. Subsequently, the brains were cryoprotected overnight in a solution of 30% sucrose in PBS. Brains were cut in frontal plane at 30 μm on a freezing microtome (Leica Microsystems; Wetzlar, Germany). The slices were collected in 10 parallel series and stored in PBS containing 0.1% sodium azide.

2.2.3 Immunohistochemical Staining of CTB

The tracer was immunohistochemically visualized by using 3’3-diaminobenzidine (DAB; Sigma, Steinheim, Germany). For an unbiased quantitative analysis one of the 10 parallel series was randomly selected for staining. Slices were pretreated with 0.3% H2O2 for 30 min. After washing in PBS, they were blocked in 10%
normal rabbit serum for 1h, followed by overnight incubation in PBS containing a goat anti-CTB antibody (Calbiochem; Cat no. 227040; 1:10,000) and 0.3% Triton X-100 at 4°C. After being rinsed in PBS, the sections were incubated for 60 min at room temperature in the biotinylated rabbit anti-goat IgG and 0.3% Triton X-100 (Vectastain ABC-Elite kit, Vector, Camon; 1:200). Finally, the sections were incubated in avidin–biotin–peroxidase solution and 0.3% Triton X-100 (Vectastain ABC-Elite kit, Vector, Camon; 1:100) for 60 min at room temperature. After washing, the peroxidase-activity was detected using a heavy-metal intensified DAB-reaction, modified by the use of β-D-glucose/glucose-oxidase [2], [175](Sigma, Steinheim, Germany; 1 mg/ml). Sections were mounted on gelatin-coated slides, dehydrated and coverslipped with DPX.

2.2.4 Histological Analysis
Sections of the optic tectum from A 5.50 to A 1.25 were analyzed using a Zeiss Axio Imager M1 Microscope (Carl Zeiss MicroImaging, Göttingen, Germany) equipped with an AxioCam MRM (Carl Zeiss MicroImaging, Göttingen, Germany) and the software AxioVison 4.8 (Carl Zeiss MicroImaging, Göttingen, Germany). To estimate the injection volume the area within the RT was calculated with the program ImageJ (Image Processing and Analysis in Java; open source) depending on the staining intensity. To control for interindividual differences, we set a threshold for the staining intensity, depending on the background staining of every animal. Only parts of the RT, which were 25% darker stained than the background, were included to the area estimation of the RT. Every area of the RT measured this way was summarized and multiplied by the slice thickness, resulting in the effective injection volume. In every analyzed tectal section, labeled neurons of the complete extent of layer 13 of the left and right tectum were counted by making a mosaic-photo with an x20 objective. The cell counts were corrected by the injection volume.

Nomenclature used in the present study is based on the Avian Brain Nomenclature Forum [156] and the pigeon brain atlas [102].
2.2.5 Statistical Analysis
The statistical analysis was performed with IBM SPSS 20 by running nonparametric Wilcoxon matched pair test to analyze differences in ipsilateral and contralateral projections from the tectum to the rotundus within light- or dark-incubated pigeons. To analyze differences in the projection pattern of light- and dark-incubated pigeons the nonparametric Mann Whitney U-test was conducted. Due to the small sample size, a normal distribution of results was not expected. Therefore, we conducted nonparametric instead of parametric statistical analysis.

2.3 Results
2.3.1 Tectorotundal Projection in Light-Incubated Pigeons
Successful injections into the rotundus labeled high numbers of tectal neurons within the ipsi- and contralateral tectum throughout the complete dorsoventral axis (Fig 2.1 A-D)[80]. Qualitatively, the number of tectal cells projecting ipsilateral to the left and right RT look very similar (Fig 2.1 A, B). Additionally, the contralateral projection from the right TO to the left RT seems to be comparable to the ipsilateral projections (Fig 2.1 D). The fewest cells project from the left TO to the contralateral right RT (Fig 4.1 C).

Quantitative analysis confirmed this pattern (Fig 2.1 E and Tab 2.3). Statistical analysis revealed that the ipsilateral tectorotundal projections do not significantly differ from each other (ipsilateral RT left: 200.33 +/- 55.12 cells/nl, ipsilateral RT right: 97.42 +/- 13.53 cells/nl; Wilcoxon: z = 1.46, p = 0.14). The contralateral projections to the left and right rotundus also reached no significance in the number of projecting cells (contralateral RT left: 148.86 +/- 31.34 cells/nl, contralateral RT right: 66.02 +/- 9.52 cells/nl; Wilcoxon: z = 1.46, p = 0.14). Because the difference in both contralateral projections from the tectum to the rotundus in light-incubated pigeons was one of the key effects in Güntürkün et al. (1998) [73], we further followed up on the non-significant post-hoc test in this group. By using G*Power 3.17 (CIT), we conducted a power-analysis to determine the required sample size for the effect to reach significance in our sample. For calculation, we used an alpha error probability of 0.05, a power of 0.95 and an effect size dz of 2.03, as calculated by the G*Power
2.3 Results

Figure 2.1: Retrograde labeled cells/ injection volume (nl) within the ipsilateral and contralateral TO in light-incubated pigeons. Frontal sections through the pigeon’s TO after right- A, C and left-sided B, D CTB injections into the rotundus. A and B show tectal layer 13 neurons after ipsilateral rotundal injections and C and D after contralateral injections into the rotundus. Bar=100 μm. E represents number of labeled ipsi- and contralateral tectal neurons. Error bars indicate standard error. * = p < 0.05., (*) = p = 0.07.
software from the means and standard deviations in the two conditions. The total sample size required for the effect to reach significance was 6 animals.

Furthermore, the comparison of ipsilateral and contralateral projections revealed that more neurons from the ipsilateral than from the contralateral tectum project to the right rotundus (ipsilateral right RT: 97.42 +/- 13.53 cells/nl; contralateral right RT: 66.02 +/- 9.52 cells/nl; Wilcoxon: $z = 2.02, p < 0.05$). The number of ipsi- and contralateral projecting tectal cells to the left rotundus showed no significant difference but a tendency, with more ipsilaterally projecting cells (ipsilateral left RT: 200.33 +/- 55.12 cells/nl; contralateral left RT: 148.86 +/- 31.34 cells/nl; Wilcoxon: $z = 1.83, p = 0.07$). Also the number of cells projecting from the left tectum to the ipsi- and contralateral rotundus showed a clear but not statistically significant trend (ipsilateral left RT: 200.33 +/- 55.12 cells/nl; contralateral right RT: 66.02 +/- 9.52 cells/nl; Wilcoxon: $z = 1.83, p = 0.07$). The projections from the right tectum to the ipsi- and contralateral rotundus did not quantitatively differ from each other (ipsilateral right RT: 97.42 +/- 13.53 cells/nl; contralateral left RT: 148.86 +/- 31.34 cells/nl; Wilcoxon: $z = 1.10, p = 0.27$).

The results for every single pigeon showed that after injections into the left RT as well as into the right RT, the ipsilateral projection was stronger than the contralateral projection, except for subject P-307 this animal showed a similar ipsi- and contralateral projection (Tab 2.1). The comparison between the individual pigeons demonstrated that after injections into the left RT, every individual animal showed stronger ipsi- and contralateral projections than after right rotundal injections. Only two animals showed the opposite projection pattern. Subject P-72 demonstrated a generally weaker projection after left rotundal injections in comparison to the other animals, and subject P-49 demonstrated a generally stronger projection after injections into the right RT than the other animals (Tab 2.1).

2.3.2 Tectorotundal Projection in Dark-Incubated Pigeons

A qualitative analysis of the tectorotundal projection pattern in dark-incubated pigeons revealed similar to the projection pattern in light-incubated pigeons a high number of tectal neurons within the ipsi- and contralateral tectum throughout the complete dorsoventral axis (Fig 2.2 A-D). Qualitatively, the number of tectal cells
Figure 2.2: Retrograde labeled cells/injection volume (nl) within the ipsilateral and contralateral TO in dark-incubated pigeons. Frontal sections through the pigeon’s TO after right- A, C and left-sided B, D CTB injections into the rotundus. Labeled cells of tectal lamina 13 figure A and B are ipsilateral and figure C and D contralateral to the injection side within the rotundus. Bar = 100 𝜇m. E Number of labeled ipsi- and contralateral tectal neurons. Error bars indicate standard error. * = p < 0.05., (*)& = p = 0.06.
2.3 Results

Table 2.1: Number of tectorotundal projecting cells/injection volume (nl) of the individual animals in light incubated pigeons.

<table>
<thead>
<tr>
<th>Pigeon</th>
<th>Ipsi RT left</th>
<th>Contra RT left</th>
<th>Ipsi RT right</th>
<th>Contra RT right</th>
</tr>
</thead>
<tbody>
<tr>
<td>P38</td>
<td>210.53</td>
<td>177.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P72</td>
<td>102.99</td>
<td>68.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P133</td>
<td>351.32</td>
<td>214.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P307</td>
<td>136.46</td>
<td>134.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P30</td>
<td>99.70</td>
<td>63.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P49</td>
<td>144.52</td>
<td>97.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P69</td>
<td>96.54</td>
<td>70.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P71</td>
<td>84.54</td>
<td>58.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P299</td>
<td>61.80</td>
<td>39.44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

projecting ipsilateral to the left and right RT look very similar (Fig 2.2 A, B). Additionally, also the contralateral projections seemed not to differ from each other (Fig 4.2 C, D). However, generally the contralateral projections seemed to be more weakly pronounced than the ipsilateral projections.

Quantitative analysis confirmed this pattern (Fig 2.2 E and Tab 2.3). The statistical analysis showed no significant difference between the ipsilateral projections (ipsilateral left RT: 126.50 +/- 19.12 cells/nl; ipsilateral right RT: 96.29 +/- 20.13 cells/nl; Wilcoxon: $z = 1.01$, $p < 0.31$). Additionally, also the contralateral projections did not differ from each other (contralateral left RT: 76.61 +/- 12.30 cells/nl; contralateral right RT: 63.27 +/- 13.93 cells/nl; Wilcoxon: $z = 0.51$, $p < 0.61$).

Furthermore, the comparison of ipsi- and contralateral projections revealed that more neurons from the ipsi- than the contralateral tectum projected to the right rotundus (ipsilateral right RT: 96.29 +/- 20.13 cells/nl; contralateral right RT: 63.27 +/- 13.93 cells/nl; Wilcoxon: $z = 2.37$, $p < 0.05$). The number of ipsi-and contralateral projecting tectal cells to the left rotundus also showed a significant difference, with more ipsilaterally projection cells (ipsilateral left RT: 126.50 +/- 19.12 cells/nl; contralateral left RT: 76.61 +/- 12.30 cells/nl; Wilcoxon: $z = 2.37$, $p < 0.05$). The number of cells projecting from the left tectum to the ipsi- and contralateral rotundus showed a clear but not statistically significant trend (ipsilateral left RT: 126.50 +/- 19.12 cells/nl; contralateral right RT: 63.27 +/- 13.93 cells/nl;
Wilcoxon: $z = 1.86, p = 0.06$). The projections from the right tectum to the ipsi- and contralateral rotunds did not significantly differ from each other (ipsilateral right RT: 96.29 +/- 20.13 cells/nl; contralateral left RT: 76.61 +/- 12.30 cells/nl; Wilcoxon: $z = 1.10, p = 0.27$).

The results of the mean data on populational level were also reflected in the individual animal data (Tab 2.2). In contrast to the light-incubated animals, only one of the dark-incubated pigeons showed a divergent projection pattern to the mean data.

### 2.3.3 Comparison of Light- and Dark-Incubated Pigeons

To analyze the tectorotundal projection pattern of ipsi- and contralateral projections between light- and dark-incubated pigeons, we used non-parametric Mann-Whitney U tests. The analysis revealed no significant differences between light- and dark-incubated pigeons (all $p's > 0.07$; for overview see Tab 2.3). The contralateral projection from the right tectum to the left rotundus, however, demonstrated a tendency to have a stronger projection in light-incubated pigeons in comparison to

<table>
<thead>
<tr>
<th>Pigeon</th>
<th>Ipsi RT left</th>
<th>Contra RT left</th>
<th>Ipsi RT right</th>
<th>Contra RT right</th>
</tr>
</thead>
<tbody>
<tr>
<td>P463</td>
<td>182.45</td>
<td>147.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P465</td>
<td>135.00</td>
<td>77.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P498</td>
<td>103.56</td>
<td>59.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P501</td>
<td>100.22</td>
<td>59.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P655</td>
<td>74.66</td>
<td>52.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P764</td>
<td>206.35</td>
<td>74.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P802</td>
<td>83.25</td>
<td>65.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P436</td>
<td>66.73</td>
<td>36.96</td>
<td></td>
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</tr>
<tr>
<td>P462</td>
<td>28.37</td>
<td>21.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P503</td>
<td>165.73</td>
<td>91.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P504</td>
<td>64.62</td>
<td>40.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P760</td>
<td>80.42</td>
<td>58.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P761</td>
<td>98.05</td>
<td>63.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P803</td>
<td>170.15</td>
<td>129.79</td>
<td></td>
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</tr>
</tbody>
</table>
Table 2.3: Mean number of tectorotundal projecting cells/ injection volume (nl) in light- and dark-incubated pigeons.

<table>
<thead>
<tr>
<th></th>
<th>Light-Incubated (cell/nl)</th>
<th>Dark-Incubated (cell/nl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ipsilateral RT left</td>
<td>200.33 +/- 55.12</td>
<td>126.50 +/- 19.12</td>
</tr>
<tr>
<td>ipsilateral RT right</td>
<td>97.42 +/- 13.53</td>
<td>96.29 +/- 20.13</td>
</tr>
<tr>
<td>contralateral RT left</td>
<td>148.86 +/- 31.34</td>
<td>76.61 +/- 12.30</td>
</tr>
<tr>
<td>contralateral RT right</td>
<td>66.02 +/- 9.52</td>
<td>63.27 +/- 13.93</td>
</tr>
</tbody>
</table>

Figure 2.3: A: Comparison of the number of labeled ipsi- and contralateral tectal neurons in light- and dark-incubated pigeons. Error bars indicate standard errors. (*) = p = 0.07. B: Bilaterality index (BI) of left and right nucleus rotundus. A BI of 0 indicates perfectly symmetric tectal afferents from both hemispheres, while a BI of 1 denotes a completely ipsilaterally organized system.

dark-incubated pigeons (contralateral RT left: light-incubated: 148.86 +/- 31.34 cells/nl, dark-incubated: 76.61 +/- 12.30 cells/nl; Mann-Whitney U: z = 1.80, p = 0.07).

In order to show the proportion of bilateral projections in relationship to the ipsilateral projections we calculated a bilaterality index:

\[ \text{BI} = \frac{\text{ipsi} - \text{contra}}{\text{ipsi} + \text{contra}} \]

A BI with a score of minus one indicated a perfectly contralateral projection and a BI of one indicated a completely ipsilateral projection. All BIs had positive
scores, indicating that in all projections, the number of ipsilateral projecting neurons was higher than of contralateral projecting cells. However, the comparison of the BI between left a right rotundal projections and light- and dark-incubated pigeons showed no significant differences (all \( p' s > 0.16 \)).

2.4 Discussion

2.4.1 Summary of the Main Results
Our retrograde tracing data in light-incubated pigeons showed qualitatively the projection pattern observed by Güntürkün et al. (1998) [73]. However, due to low number of successfully injected cases, differences failed to reach significance. This contrasted the results of dark-incubated birds. The results in dark-incubated pigeons revealed that the ipsilateral projections are stronger, in contrast to the contralateral projections. At the same time, the ipsilateral projections to the left and right rotundus do not differ from each other as much as the contralateral projections. The comparison of light- and dark-incubated pigeons demonstrates that an embryonic light-stimulation stabilizes fibers, which are reduced in dark-incubated animals.

2.4.2 Statistical Confirmation of Projection Asymmetry in Light-Incubated Pigeons
Our data displayed a nonsignificant trend confirming previous data which showed significant differences in the contralateral tectorotundal projection pattern. To verify whether the absence of a significant difference in this projection was due to a small sample size and not to a small effect size, we conducted a power analysis. The power analysis confirmed that with only two more correct injections into the left and right rotundus, our results would reach significance. Due to the results of the power analysis and the complementary trend with the study of Güntürkün et al. [73], in the following discussion we act on the assumption that in light-incubated pigeons the projection from the right TO to the left RT is stronger than vice versa.

2.4.3 Behavioral and Structural Relations
As already mentioned in the introduction, functional asymmetries in birds are closely related to structural left- right- differences in the ascending visual pathways. In juvenile chicks, structural asymmetries within the thalamofugal system demonstrate
more fibers projecting from the left GLD to the right visual wulst than vice versa [165]. This projection pattern creates a more right hemispheric view of the visual scene in chickens and can explain on functional level right-hemispheric dominance for visuospatial attention [25], [130]. In contrast, in pigeons there is a structural asymmetry in the tectofugal pathway with more fibers projecting from the right TO to the left RT than vice versa, which creates a more complete presentation of the left and right visual scene in the left hemisphere in pigeons [73]. Qualitatively, the results of the present study also supports this view. On functional level the structural asymmetry in pigeons coincides with left-hemispheric dominance for visual feature detection [75], or visual object analysis [176]. As already mentioned in the introduction, functional and structural lateralization in birds depends on asymmetric light-stimulation during embryonic development. Consequently, incubation of embryos in complete darkness prevents the formation of behavioral asymmetries in pigeons and chickens [25], [176]. In chickens, this result comes along with the absence of structural asymmetries in the thalamofugal system [163]. In pigeons, the likewise absence of morphological left-right differences appears in soma-size asymmetries at tectal level [125], [176], rotundal level [124], and tectal side pathways [57]. The present study indicates the influence of asymmetric light experiences on the development of the tectorotundal projection as well. The tectorotundal tracing in dark-incubated pigeons demonstrates, in contrast to light-incubated pigeons, no asymmetry within the contralateral projections. The results show that neither ipsilateral projections nor contralateral projections differ between the hemispheres. Thereby, ipsilateral projections are stronger than contralateral projections. Accordingly, the left and the right hemisphere receive a symmetrical presentation of the left and right visual field in dark-incubated pigeons. However, due to the stronger projection from the TO to the ipsilateral RT than to the contralateral RT the left and right hemisphere know more about the contralateral than the ipsilateral visual hemifield.

2.4.4 Development of the Tectorotundal Projection

Light-dependent modulation in the strength of ipsi- and contralateral projecting fibers raises questions concerning developmental pattern of the tectofugal pathway in pigeons. The neurons from tectal layer 13, which generate the tectorotundal
projection, belong to a multipolar, efferent cell type [45], [80], [81], [100], [121]. Accordingly, these cells constitute tecto-tectal and descending connections [82]. The descending tectobulbar and tectospinal connections are one of the first developing fiber tracts within the embryonic avian brain, directly followed by interhemispheric tecto-tectal connections [45], [109], [173]. The tectorotundal projection demonstrates a decelerated development in comparison to the previously described connections [109], [173]. Additionally, previous studies in the altricial zebra finch and pigeon demonstrate that the development of the tectorotundal projection is not finished after hatching [124], [125], [137]. In zebra finches, the neuropil within the RT matures during the first 20 days after hatching. During this time, the number of synapses and presynaptic terminal continuously increases [137]. A similar maturation of the tectofugal system is suggested for the pigeon. In pigeons, the occlusion of the left or right eye after hatching indicates post-hatch plasticity of the projection pattern [124], [125]. Accordingly, the tectorotundal projection matures post-hatch. Possibly, prior to hatching the tectorotundal projection develops bilaterally and post-hatch the regression of redundant fibers creates the final projection pattern. Such a mechanism is also known for the differentiation of fiber tracts in the mammalian brain, for example for callosal fibers [91]. In light-incubated pigeons post-hatch processes generate a lateralized projection pattern in response to an embryonic asymmetric light trigger with more cells projecting from the right TO to the left RT than vice versa. Accordingly, in dark-incubated birds light deprivation before hatching leads to a symmetrical tectorotundal projection. Thereby, the ipsilateral projection is stronger than the contralateral projection. These results imply for the development of tectorotundal projection pattern that a difference in ipsi- and contralateral projections develops independent of a light-stimulation before hatching. Light increases the innervation of the left rotundus suggesting a light-dependent stabilization of tectorotundal fibers. However, this stabilization cannot be mediated by light-dependent stronger activation of tectal neurons, because this would strengthen the projection from the left TO to the right RT. More likely is a retrograde trophic effect, which is mediated by the left RT.
2.4 Discussion

2.4.5 Neurotrophic Activated Effects

In pigeons it is known that the TO is the first station of the ascending processing stream, where morphological asymmetries are visible. Previous studies demonstrate that the majority of retinorecipient neurons in the left tectum are larger than in the right tectum [69]. As the soma-size of a neuron is an indicator for the extent of the axo-/dendritic arborization pattern, tectal soma-size asymmetries indicate differences in the complexity of left and right tectal circuits. The maturation of the retinotectal pathway is known to be regulated by light activity [153], and consequently changes in retinal activity quickly affect synaptogenetic processes [29], [167]. In this manner, retinal activity differences constitute the first step in the initiation of asymmetric anatomic development.

Many light effects are mediated by neurotrophic factors, and in particular the brain-derived neurotrophic factor (BDNF) is a key player in activity-dependent development [120]. Light adjusts the expression and/or release of neurotrophic factors, and hence regulates trophic support of target cells [29], [190]. In turn, BDNF controls sprouting, branching and maintenance of axo-dendritic trees. Since BDNF and its high-affinity receptor TrkB are present in the developing retinotectal system of pigeons [181], it is possible that retinal activity-differences are mediated by asymmetric BDNF supply. Manns et al. (2005) showed that indeed the tectal TrkB signalling cascade is asymmetrically activated in response to embryonic light stimulation [128]. The small G protein p21Ras is one critical molecular switch for relaying neurotrophic actions into morphological changes [84], [122]. The activation of p21Ras within the TO of pigeons depends on light-stimulation. During development, p21Ras produces left-right differences via altering the morphology of chemically specified cellular populations within the tectum [128]. While the cells of the retinorecipient layers, which are primarily of GABAergic nature [126], are enlarged in the left TO, the efferent cells in the deeper lamina are larger in the right TO [69]. This soma-size asymmetry pattern develops within the first week after hatching in response to reduced TrkB/Ras signaling in the right tectum [128]. The light-dependent stronger activation of GABAergic cells within the left TO can exert an enhanced inhibitory control onto the efferent cells in the left TO in comparison to the right [128]. These results
nicely explain morphological alterations on the tectal level before hatching. Only following these pre-hatch tectal mechanisms it was expected, for the development of the tectorotundal projection pattern, a regression of outgrowing axons from the left tectum, due to a stronger inhibition of rotundal projecting neurons in the left tectum than in the right. Accordingly, the comparison of light- and dark-incubated pigeons should demonstrate a difference in the projection from the left TO to the ipsi- and contralateral RT. However, as already mentioned above, the present study shows an increase in the contralateral projection from the right to the left RT. This result hints at further mechanisms affecting the tectorotundal projection pattern, which could be mediated by a retrograde trophic effect of the left RT. Morphological analysis revealed light-dependent soma-size asymmetries also on rotundal level, with larger somata in the left RT than the right RT [124]. This hints at a light-dependent different activation of the left and right RT. Because GABAergic immunoreactivity in the RT evolves during post-hatch development [14], an inhibitory modulation of the left and right RT can only be mediated by the influence of components, which gain functional significance after hatching [124]. This influence can be mediated by pretectal nuclei like nucleus subpretectalis and nucleus interstitio-pretecto-subpretectalis, which form side pathways of the tectorotundal projections and only mature after hatching [38], [134]. The possible details of the excitatory and inhibitory interactions on rotundal level are far from being clear. However, it is likely that the final size of rotundal cells is determined by the interaction of different systems to different developmental stages: the pre-hatch natural deprivation, which is transmitted by excitatory tectorotundal fibers; the post-hatch stimulation pattern, which represents a mixture of excitatory tectorotundal and inhibitory pretectal inputs [124]. Accordingly, the left RT seems to be stronger activated than the right RT. In response to this activation, the left RT could deliver a higher amount of trophic factors and promote the outgrowth of axons to the left RT.

2.4.6 Interhemispheric Stabilization After Hatching

As already mentioned above, the development of the tectofugal pathway is not finished with hatching. In addition to the positive retrograde trophic effect of the left rotundus, which primarily acts after hatching, asymmetric tectotectal interactions
can strengthen the tectorotundal projection pattern in presence of an symmetrical light-stimulation. Because this intertectal projection is asymmetrically organized with a stronger inhibition of the right through the left TO [104]. Accordingly, neurons from the left TO can inhibit neurons from the right TO including the GABAergic neurons, which project to the layer 13 neurons. This asymmetric tectotectal interaction can stabilize the contralateral projection from the right TO to the left RT due to a stronger inhibition of GABAergic cells in the right TO.

2.4.7 Conclusion
In summary, the asymmetry in the tectorotundal projection in pigeons can be explained due to a stepwise development of the visual system. First, retinal fibers innervate the optic tectum followed by descending tectobulbar, tectospinal and interhemispheric tectotectal fiber tracts. The tectorotundal projections develop more slowly in comparison to the other projections and prolong into the time after hatching. Prior to hatching, due to the asymmetrical light stimulation, inhibitory tectal circuits can be asymmetrically activated and thus induce the asymmetrical innervation of the left and right RT. After hatching, the contralateral projection from the right TO to the left RT can be strengthened in presence of a symmetrical light input by an asymmetrical tectotectal interaction and a positive retrograde trophic effect of the left RT. Thereby, the projection asymmetry is not created by a regression in the contralateral projection from the left TO to the right RT due to inhibitory circuits, but by a progression of the projection from the right TO to the left RT.

2.4.8 Outlook
Several open questions derive from the discussion above:

- The data displayed only a trend, although previous data show significant differences in the contralateral tectorotundal projection pattern. To confirm the previous shown projection pattern, we have to increase the number of successful rotundal injections and analyze the resulting projection pattern.
- The present study cannot answer the question of whether light-stimulation directly affects the number of tectal projecting cells or the number of branching axon-collaterals. To clarify this question, bilateral rotundus injections of a
2.4 Discussion

retrograde tracer can be performed, followed by quantification of bilaterally projecting tectal cells. Quantitative changes in the left and right tectum of bilateral projecting cells could hint at an alteration in the number of axon-collaterals.
CHAPTER 3

Study 2: Structural Organization of the Anterior Commissure

As already mentioned in the general introduction, ontogenetic models suggest the corpus callosum with a crucial impact for the pattern and development of lateralized information processing. The largest commissural system on telencephalic level in the pigeon is the anterior commissure, but about the connectivity of this system not much is known. Therefore, in this study the structural basis of an interhemispheric information processing should be analyzed.

3.1 Introduction

Integration of information from the left and the right side of the body is key for survival. This is accomplished by various commissural systems that interconnect the two halves of the nervous systems in all animals [8], [171]. In the brains of all vertebrates, the three main commissural systems at telencephalic level are the anterior commissure (AC), the hippocampal commissure (HC) and the corpus callosum (CC). The CC is the evolutionarily most recent interhemispheric pathway, and probably developed in conjunction with the cortical expansion in eutherian mammals. In metatherian mammals, like wallabies and opossums, the AC and HC are relatively large, possibly to compensate for the absence of the CC. But, the AC of metatheria is not only large, but also displays a connectivity pattern that is equivalent to a combination of the eutherian AC and CC [64], [131]. Next to mammals, birds are the second major class of vertebrates that are able to process and coordinate complex behavioral and cognitive operations. Like metatherian mammals, birds do not possess
a corpus callosum. Instead, interhemispheric exchange occurs at telencephalic level via the anterior commissure and the small hippocampal commissure. Despite these limitations of commissural pathways, behavioral studies in pigeons demonstrated that not only color discriminations [119], [177], but also cognitive inference information can be transferred between the hemispheres [129]. However, pigeons are virtually unable to transmit interhemispherically conjoint spatial and visual information [65], [144], [195]. Thus, it is possible that the absence of a CC sets limits to what kind of information can be exchanged between hemispheres. Along these lines, the colloquial statement that birds are 'natural split brains' could partly be true. Contrasting these assumptions, the classic study of Zeier and Karten (1973) demonstrated that the AC of pigeons interconnects a wide network of forebrain structures and differs in part substantially from the metatherian organization [207]. Unfortunately, the degenerating tracing technique employed by Zeier and Karten (1973) does not allow for a detailed identification of the topographic organization with regard to cells of origin and partly also with regard to termination areas [207]. However, such knowledge is necessary to compare the AC of birds with those of other vertebrates and especially with the organization in methateria and eutheria. Therefore, more than 40 years after the publication of Zeier and Karten (1973) we decided to re-examine the fiber connections and the chemoarchitecture of the commissura anterior of pigeons with modern tract tracing methods [207].

3.2 Material and Methods

3.2.1 Subjects
Overall, 35 pigeons (Columba livia) of unknown sex were used for the study. In 5 of them the anterior commissure was transected before tracer application, while in further 30 animals the anterior commissure was left intact. The animals received unilateral injections of either the predominantly anterogradely transported tracer biotinylated dextran amine (BDA, 10,000 molecular weight, Molecular Probes, Leiden, Netherlands) or the tracer cholera toxin subunit B (CTB, Sigma, Deisenhofen, Germany) into subunits of the left or right arcopallium to analyze the termination areas of the anterior commissure. Because CTB is transported both in anterograde
as well as retrograde direction, this tracer was also injected into the termination areas to analyze the cells of origin of the anterior commissure. All experiments were carried out according to the specifications of the German law for the prevention of cruelty to animals and hence, the European Communities Council Directive of November 24, 1986 (86/609/EEC). All efforts were made to minimize the number of animals used and their suffering.

3.2.2 Transection of the Anterior Commissure and Tracer Application

Pigeons were anesthetized with a 7:3 mixture of Ketamin (100 mg/ml) and Xylaxin (20 mg/ml). The skull was opened and the sinus sagittalis along the midline of the brain was gently pulled sideward. A surgical microknife was inserted under stereotaxic guidance between the forebrain hemispheres at coordinates A 7.75, L 0 according to the pigeon brain atlas by Karten and Hodos (1967) [102]. The blade was slowly lowered for 9.0 mm dissecting the anterior commissure in the process. After one week recovery the tracer application was performed. Pigeons were anesthetized as described above. For arcopallial tracer injections, a modified device was used, which allowed lateral rotation of the head along the longitudinal axis over 100° to the left and right [80]. Both tracers (BDA, 10% in 3% DMSO and CTB, 1% in deionized water) were injected through a glass micropipette (inner tip-diameter 15-20 μm) with a mechanic pressure device (WPI Nanoliterinjector; World Precision Instruments)

Table 3.1: List of injected brain areas and the respective coordinates.

<table>
<thead>
<tr>
<th>Injected Area</th>
<th>Anteroposterior Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior arcopallium (AA)</td>
<td>A 7.50</td>
</tr>
<tr>
<td>Intermediate arcopallium (AI)</td>
<td>A 7.50; A 6.50; A 6.25</td>
</tr>
<tr>
<td>Medial arcopallium (AM)</td>
<td>A 6.50</td>
</tr>
<tr>
<td>Dorsal arcopallium (AD)</td>
<td>A 6.50</td>
</tr>
<tr>
<td>Posterior pallial amygdala (PoA)</td>
<td>A 6.25; A 5.00</td>
</tr>
<tr>
<td>Caudolateral nidopallium (NCL)</td>
<td>A 6.50</td>
</tr>
<tr>
<td>Caudocentral nidopallium (NCC)</td>
<td>A 6.50</td>
</tr>
<tr>
<td>Medial striatum (MST)</td>
<td>A 11.00</td>
</tr>
<tr>
<td>Apicale hyperpallium (HA)</td>
<td>A 12.00</td>
</tr>
<tr>
<td>Intercalate hyperpallium (HI)</td>
<td>A 12.50; A 11.00; A 10.00</td>
</tr>
</tbody>
</table>
Instruments, Berlin, Germany). CTB was also injected into the nidopallium (A 6.50), the intercalate hyperpallium (A 12.00), and the medial striatum (A 11.00). For every area the injection-volume was between 200 to 400 nl (for overview of injection sites see Tab 3.1). Following a survival time of 2 days (for CTB) and 7 days (for BDA), the animals were deeply anesthetized with equithesin (0.45 ml per 100 g body weight) and perfused with 0.9% sodium-chloride followed by ice-cold 4% paraformaldehyde (PFA) in 0.12M phosphate-buffered saline (PBS), pH 7.4. Brains were removed and postfixated for 2 h in PFA with a supplement of 30% sucrose. Subsequently, the brains were cryoprotected overnight in a solution of 30% sucrose in PBS. Brains were cut in frontal plane at 30 μm on a freezing microtome (Leica Microsystems; Wetzlar, Germany), and the slices were collected in PBS containing 0.1% sodium azide.

3.2.3 Immunohistochemical Staining of CTB and BDA
Both tracers were immunohistochemically visualized by using 3’3-diaminobenzidine (DAB; Sigma, Steinheim, Germany). Slices were pretreated with 0.3% H2O2 for 30 min. After washing in PBS, they were blocked in 10% normal rabbit serum for 1h, followed by overnight incubation in PBS containing a goat anti-CTB antibody (Calbiochem; Cat no. 227040; 1:10,000) and 0.3% Triton X-100 at 4°C. After being rinsed in PBS, the sections were incubated for 60 min at room temperature in the biotinylated rabbit anti-goat IgG (Vectastain ABC-Elite kit, Vector, Camon; 1:200). Finally, the sections for CTB and BDA visualization were incubated in avidin–biotin–peroxidase solution (Vectastain ABC-Elite kit, Vector, Camon; 1:100) for 60 min at room temperature. After washing, the peroxidase-activity was detected using a heavy metal intensified DAB-reaction, modified by the use of b-D-glucose/glucose-oxidase (Sigma, Steinheim, Germany; 1 mg/ml). Sections were mounted on gelatin-coated slides, dehydrated and coverslipped with DPX.

3.2.4 Fluorescence Double Staining CTB-GABA and CTB-TH
For CTB-TH double staining, the slices were incubated for 30 min in 10% normal goat serum. After being rinsed, the slices were incubated for 3 days at 4°C in PBS containing a rabbit anti-CTB antibody (Sigma-Aldrich, Cat no. C3062, Munich, Germany; 1:1,000), a mouse anti-TH antibody (Millipore; Cat no. MAB5280,
1:2,000) and 0.3% Triton X-100, followed by incubation in goat anti-rabbit Alexa-488 IgG (Invitrogen, Darmstadt, Germany; 1:200) and goat anti-mouse Alexa-594 IgG (Invitrogen, Darmstadt, Germany; 1:200). Sections were then mounted on glass slides and coverslipped with fluoromount (SouthernBiotech, Eching, Germany).

For CTB-GABA double staining, a sequential staining was performed, starting with CTB labeling. Slices were incubated for 30 min in 10% normal horse serum, followed by incubation in PBS containing a goat anti-CTB antibody (1:1,000) and 0.3% Triton X-100 for 3 days at 4°C. After rinsing in PBS, the sections were incubated in PBS containing donkey anti-goat Alexa-594 IgG (Invitrogen, Darmstadt, Germany; 1:200) and 0.3% Triton X-100. For the GABA staining the slices were incubated for 3 days at 4°C in PBS containing rabbit anti-GABA antibody (Sigma-Aldrich; Cat no. A2052; 1:1,000) and 0.3% Triton X-100. The GABA-antibody was diluted in an incubation solution (IS; consisting of 2% NaCl, 0.3% Triton, 4% BSA, 5% normal horse serum in 0.05M tris-buffered saline, pH 7.4). The slices were washed in PBS and incubated in PBS containing donkey anti-rabbit Alexa-488 IgG (Invitrogen, Darmstadt, Germany; 1:500 diluted in IS) for 1 h at room temperature. After rinsing in PBS, the incubation in primary antibody solution was repeated for 1 day at 4°C, followed by the incubation in secondary antibody solution for 1h at room temperature. The sections were mounted on glass slides and coverslipped with fluoromount with DAPI (SouthernBiotech, Eching, Germany).

3.2.5 Histological Analysis

DAB stained sections were analyzed using a Zeiss Axio Imager M1 Microscope (Carl Zeiss MicroImaging, Göttingen, Germany), equipped with an AxioCam MRM (Carl Zeiss MicroImaging, Germany) and the software AxioVison 4.8 (Carl Zeiss MicroImaging, Göttingen, Germany). The fluorescence staining was analyzed using a Zeiss LSM 510 Meta Confocal Microscope (Carl Zeiss MicroImaging, Göttingen, Germany) and the software AxioVison 4.8.

Nomenclature used in the present study is based on the Avian Brain Nomenclature Forum [156] and the pigeon brain atlas [102].
3.3 Results

3.3.1 Injections into Subareas of the Arcopallium and Posterior Pallial Amygdala

3.3.1.1 CTB retrograde labeling

The anterior commissure in the pigeon brain is localized within the telencephalon and extends in the anteroposterior direction from A 7.50 to A 8.00 (Fig 3.1 A, B). To visualize the neurons of origin of the anterior commissure, a CTB injection was placed at A 7.50 below the pia mater into the rostral portion of the left arcopallium (case P-798) (Fig 3.2 A). Diffusion was restricted to the rostral portion of the arcopallium, including AA and AI and partly innervated PoA. Numerous CTB-labeled neurons were found around the injection site, and ipsilateral arcopallial cell labeling extended between A 7.25 and A 7.75. Contralaterally, a moderate number of labeled neurons were seen in the dorsal AI at A 7.50 and more rostral in AA at A 7.75 close to the border of AD but never crossing this border (Fig 3.2 B, C, J). From A 7.25 to A 7.00 this cluster of CTB-labeled neurons shifted to the ventral part of AI (Fig 3.2 J). Furthermore, caudal to the injection site, at A 6.75 and within the contralateral telencephalon a few CTB-labeled neurons were scattered within PoA (Fig 3.2 J). A further CTB injection was placed into the left and right AM (case P-771, P-656) at A 6.25, with a minute tracer diffusion to the adjacent AI. Contralaterally, a small number of CTB-labeled neurons were observed in the ventromedial AI (AIvm) close to the border of AM, a few of these retrogradly labeled cells were seen in AM (Fig 3.2 E). In the anteroposterior direction, the CTB-labeled neurons extended from A 6.25 to A 7.00. In a more dorsal part of AIvm the retrogradly labeled neurons overlapped with anterogradly labeled fiber endings (Fig 3.2 E, G). In two pigeons similar CTB injections were placed at A 6.50 into the caudal portion of the left arcopallium (case P-04, P-05). The diffusion area of the tracer in these two cases extended from A 6.25 to A 7.25, within AD and lateral AI. Within the contralateral telencephalon a small number of retrogradly labeled cells were found within the ventral AI (A 6.00 to A 6.50 (Fig 3.2 H, I). These CTB-labeled neurons extended from AIvm to the ventrolateral edge of AI (Fig 3.2 H, I, J). More ventrally and within the lateral edge of PoA, a few further neurons were seen (Fig 3.2 H, I). In contrast to case P-771, with tracer injections into the medial AI, the retrogradly labeled cells in case P-04
3.3 Results

Figure 3.1: Location of the AC in the pigeon brain. A: Frontal section at A 7.75 of a Gallyas fiberstaining. Arrow indicates the anterior commissure. B: Sagital view of a schematic drawing at L 0. The blue dot marks the location of the AC.

and P-05 extended to the lateral AI. In contrast to the first described case P-798, the position of the injection site in the last four cases (P-771, P656, P-04, P05) was placed more caudally within the arcopallium. This had an impact on the position of the contralaterally retrogradely CTB-labeled cells. The injection site in the rostral part of the arcopallium resulted in rostrally located CTB-labeled cells, while a more caudal position correlated with caudally labeled cells. Small amounts of CTB were also placed into the left PoA (case P-551) at A 6.25. Diffusion of the tracer was restricted to PoA from A 6.00 to A 6.75 and to the ventral AI. Contralaterally, a few CTB-labeled neurons were seen within the medial PoA at A 6.50. In case P-585, small amounts of CTB were placed into the caudal part of right PoA at A 5.00. The tracer diffused to AD and nidopallial areas immediately surrounding PoA. Within the contralateral telencephalon no CTB-labeled neurons were found. In sum, injections into the arcopallium and PoA revealed retrogradely labeled cells within
Figure 3.2: Cells of origin and termination areas within the arcopallium. **A:** Injection site into AA at A 7.75 (left) with labeled fibers within the AC leading towards the neurons of origin in the contralateral side (right). **B:** CTB labeling within the contralateral AI at A 7.50. **C:** Enlargement of the Box in B. Within a network of fibers retrogradly labeled neurons are found. Arrow indicates a bundle of fibers crossing through AI. **D:** Enlargement of the Box in B. Axosomatic-like fibers coil around perikarya within the ventral AI. **E:** CTB labeling within the contralateral AI at A 6.75. **F:** Enlargement of the Box in E showing many CTB-labeled cells within AI. **G:** Enlargement of the Box in E displaying the fine network of terminating fibers. **H:** CTB labeling within the contralateral AI and PoA at A 6.00. **I:** Enlargement of the Box in H. Few retrogradly labeled cells within the ventral AI and PoA. **J:** Schematic drawing of the cells of origin and termination areas within the arcopallium after CTB injections into the contralateral arcopallium. For abbreviations, see list. Scale bars = 1 mm in A; 500 µm in B, H; 50 µm in C, F, G, I; 20 µm in D; 200 µm in E.
3.3 Results

the contralateral telencephalon that were restricted to areas within AA, AI and PoA (Fig 3.2 J). This cluster of cells shifted from a ventral position caudally to a laterodorsal position rostrally (Fig 3.2 J).

3.3.1.2 BDA/CTB anterograde labeling

For a first overview of the projections of the anterior commissure, massive injections of BDA or CTB were placed into the rostral portion of either the left or right arcopallium. One of the main projection areas in the contralateral telencephalon was the arcopallium, but the fibers also crossed the borders of the arcopallium and terminated in further pallial and subpallial areas.

Termination areas within contralateral arcopallium. In two birds similar BDA-injections were placed at A 7.50 into the left (P-08) or right (P-01) arcopallium. Tracer spread was observed in all subareas of the arcopallium (AA, AM, AI, AD) and the posterior pallial amygdala. Additionally, the tracer diffused to the adjacent NCC, the ventral part of NCL, the lateral striatum and the subpallial amygdala. The injection of BDA into the left (P-08) or right (P-01) arcopallium revealed similar termination areas as described in the following. Within the contralateral arcopallium terminating fibers were visible from A 6.50 to A 7.75. The innervated subareas of the arcopallium in this anterior/ posterior extension were mainly AI. A dense network of axon terminals was also observed at A 6.50 within AI, AIvm and the ventral part of AIdm. This projection pattern was also visible in the already described case P-771 and P-656, demonstrating a network of thin and varicose fibers within the dorsomedial AI (A 7.00 to A 7.25), overlapping within AIvm with retrogradly CtB-labeled neurons (Fig 3.2 E, G, J). More rostrally, a mixture of thin and thick fibers terminated in a dorsolateral portion of AI (A 7.50). Terminating fibers were also found within the contralateral AA and PoA (Fig 3.2 J). The ventral parts of AI and PoA were not as densely innervated as the dorsal part, but the innervating fibers were much thicker (Fig 3.2 A, B). In case P-798 a precise CTB-injection could be placed into AA and the rostral parts of AI and PoA. Due to the rostral position of the injection site, a large number of fibers passed contralaterally through AA and rostral AI (Fig 3.2 B, C). En route, various fibers also terminated within these areas. In a more caudal part of the arcopallium (A 7.00 to A 6.50) a network of
Figure 3.3: Termination areas within the telencephalon after contralateral arcopallial BDA or CTB injections. A: BDA labeled fibers within the contralateral hemisphere at A 6.50 at low magnification. B: Enlargement of the box in A. Thick BDA-labeled Fibers within PoA. C: Enlargement of the box in A. BDA-labeled fiber bundles run dorsal to the LSt. D: Enlargement of the box in A. BDA-labeled fibers within the NCL. E: Enlargement of the box in A. Fine network of BDA-labeled fibers is in in the NCC with crossing fibers with a large number of varicosities. F: BDA labeled fibers within the contralateral hemisphere at A 11.00 at low magnification. G: Enlargement of the box in F. BDA-labeled fibers running from MID into the deeper layer of the mesopallium. H: Enlargement of the box in F. Fine network of thin fibers within NI. I: Enlargement of the box in F. Fine network of terminating fibers within the medial MSt. For abbreviations, see list. Scale bars = 1 mm in A, F; 100 μm in B, C, D, E, G-I.
Figure 3.4: Schematic illustration of the rostrocaudal extent of the termination areas within the telencephalon after arcopallial BDA or CTB injections. Short lines represent anterogradely labeled terminals.
thin fibers innervated nearly the whole arcopallium with a higher concentration of terminating fibers in the dorsal part of AI (Fig 3.2 B). At A 7.50 these fibers coiled around unlabeled perikarya, building basket-like arrangements within AI (Fig 3.2 D). Within the contralateral AD and AM no axon terminals were observed.

_Termination areas within further contralateral pallial areas._ After BDA or CTB injections into the arcopallium (case P-08, P-01, P-798), many fibers passed through the arcopallium within the contralateral telencephalon and continued into neighboring structures (Fig 3.3 A). Following the trajectory of fibers crossing beyond the LAD and running dorsal to the LSt (Fig 3.3 C), a large number of thin BDA-labeled fibers constituted a network within NCL (A 6.00 to A 7.75) (Fig 3.3 D, 3.4 A, B) and NCC (A 6.00 to A 7.00) (Fig 3.3 E, 3.4A). A few varicose fibers ran through the NCL and terminated more rostrolaterally within TPO, NIL and NFL (Fig 3.4 C, D, E). This rostrolaterally oriented trajectory turned dorsomedially at A 10.50 into MID (Fig 3.3 F, G) and terminated within MFD and MFV (A 12.25 - 13.50) (Fig 3.4 E, F). At 11.50 this network of terminating fibers within NIL expanded rostromedially into NFT (A 12.50 to A 13.50) (Fig 3.4 E, F). A distinct network of thin fibers within NI was visible dorsal to the medial striatum and medial to the entopallium at A 11.00 to A 12.50 (Fig 3.3 H, 3.4 D). A medially oriented fiber stream terminated within the medial striatum and built a network of very thin fibers (A 9.00 to A 11.00) (Fig 3.3 I, 3.4 D).

3.3.2 Injections into the Nidopallium

3.3.2.1 CTB retrograde labeling

A CTB injection into the right NCL (case P-959) was placed at A 6.50, and the tracer diffused to the immediately surrounding NCC (A 7.00 to A 5.75) (Fig 3.5 A). Contralaterally, a moderate number of CTB-labeled neurons were seen in the rostral part of AI, extending medially into AM and ventrally into PoA (Fig 3.5 B, C). A few retrogradely labeled neurons were contralaterally scattered within AA. Beyond the borders of the arcopallium and the posterior pallial amygdala no CTB-labeled neurons were found in the contralateral telencephalon.
Figure 3.5: CTB labeling after injections into the contralateral nidopallium and MSt. **A:** Injection site of CTB into the nidopallium at A 6.50. **B:** CTB-labeled neurons within the ventral AI after injections into the contralateral nidopallium. **C:** Enlargement of the box in B. There are many retrogradly labeled cells within AI. **D:** Injection site of CTB within MSt at A 11.00. **E:** Retrogradly labeled cells within the ventral AI and PoA after CTB injections into the contralateral MSt. For abbreviations, see list. Scale bars = 1 mm in A, D; 200 μm in B, E; 50 μm in C.
3.3 Results

3.3.3 Injection into the Medial Striatum

3.3.3.1 CTB retrograde labeling

The injection of CtB was placed at A 11.00 into the right medial striatum (case P-669). Diffusion of the tracer was restricted to MSt (A 11.25 to A 10.75) (Fig 3.5 D). Within the contralateral telencephalon a small number of CtB-labeled neurons were scattered within the ventrolateral AI and the lateral PoA at A 6.50 (Fig 3.5 E). Beyond the borders of the arcopallium and the posterior pallial amygdala, no CTB-labeled neurons were found in the contralateral telencephalon.

3.3.4 Injection into the Intercalate Hyperpallium

3.3.4.1 CTB retrograde labeling

Our massive BDA injections into the arcopallium revealed no anterograde fiber staining within the contralateral hyperpallium. However, previous studies had revealed a participation of the hyperpallium to interhemispheric projections [13], [207]. We, therefore, performed CTB injections into subareas of the hyperpallium. Injections of CTB were placed at A 12.00 into the left or right hyperpallium apicale (HA) (case P-62, P-63). The tracer injection was restricted to HA, with no tracer spread into the adjacent intercalate hyperpallium (HI) (Fig 3.6 A). Within the contralateral arcopallium no retrogradly labeled neurons were found (Fig 3.6 B). Further CTB injections were performed into the left or right HI at different anteroposterior positions (case P-520, P-449, P-382; P461). In case P-520 tracer was injected at the dorsal border of HI at A 12.50. Diffusion of the tracer slightly crossed the border to HA but never touched the ventral border to HD or mesopallium (Fig 3.6 C). Contralaterally, a small number of CTB-labeled neurons were found in the ventral part of AI and PoA (A 6.50 to A 6.75) (Fig 3.6 D). Beyond the borders of the arcopallium and the posterior pallial amygdala, no CTB-labeled neurons were found in the contralateral telencephalon. More caudal tracer injections at A 11.00 to A 10.00 (P-382; P461) revealed no CTB-labeled cells within the contralateral arcopallium or the remaining contralateral telencephalon.
Figure 3.6: CTB labeling after injections into the contralateral hyperpallium. A: Injection site into HA at A 12.00. B: No CTB labeling within AI and PoA after CTB injections into the contralateral HA. C: Injection site of CTB into HI at A 12.50. D: Retrogradely labeled cells within the ventral AI and PoA after CTB injections into the contralateral HI. For abbreviations, see list. Scale bars = 1 mm in A, C; 100 μm in B, D.
3.3 Results

Figure 3.7: Contralateral projections after AC commissurectomy. **A:** Transection of the AC at A 7.75. Arrows indicate the remaining fibers of the AC in the left and right hemisphere. **B:** Injection site of CTB into the arcopallium at A 7.50. **C:** No CTB labeling within AI and PoA after CTB injections into the contralateral arcopallium of commissurectomized pigeons. For abbreviations, see list. Scale bars = 1 mm in A, B; 200 μm in C.

3.3.5 Injections of CtB or BDA after AC Commissurectomy

In four of five pigeons the transection of the anterior commissure was successful (Fig 3.7 A). Injections of CTB were placed into right arcopallium (case P-526) at A 7.50, and the tracer diffused within the ipsilateral arcopallium to AI and AA (Fig 3.7 B). In another case (P-477) the CTB injection was placed at A 7.00 into the ventral part of the AI with diffusion observed into PoA. Within the contralateral telencephalon no projections were found after the transection of the anterior commissure in any of these cases (Fig 3.7 C). Further injections of CTB were placed into the nidopallium (case
3.3 Results

P-629) at A 6.50 and also showed no labeling within the contralateral telencephalon.

3.3.6 Cytochemistry of AC Projecting Cells

_GABA-CTB double labeling._ In a first overview on low magnification, different brain areas could easily be distinguished based on the density of GABA-like fibers and neurons. It was, thereby, already visible that the large fiber tract of the anterior commissure at A 7.75 was unstained (Fig 3.8 A), and the arcopallium appeared to be labeled less than the remaining telencephalon (Fig 3.8 A). Within the arcopallium and PoA the GABA-like staining demonstrated a moderate and regular distribution of perikarya that was similar throughout different arcopallial subareas (AA, AI, AM, AD) and PoA. At a higher magnification, the GABA-positive neurons could be subdivided in two different cell types (Fig 3.8 B, C). A very small population had large somata with a diameter around 15 μm (Fig 3.8 B). The majority of GABA-positive neurons within the arcopallium was small with cell body diameters around 10 μm (Fig 3.8 C). Contrary to the GABA-positive cells, the commissural CTB-positive neurons showed a rather uniform perikarya size with a diameter of around 15 μm (Fig 3.8 D). With fluorescent double staining, the arcopallial, nidopallial and medial striatal CTB-tracing was counterstained with GABA. With confocal microscopy it was shown that the CTB-labeled commissural cells were not co-localized with GABA (Fig 3.8 E, F, G). Thus, commissural neurons of the AC are very likely not GABAergic.

_TH-CTB double staining._ A fluorescent double staining against TH and CTB in P-798 (the commissural projection pattern was described above) revealed a very high density of TH-positive fibers within AD and a high density within AM (for detailed description see [49], [201]). Within AI and PoA, the density of TH-positive fibers was low. However, the double staining with CTB demonstrates a close connection between TH-positive fibers and commissural projecting CTB-positive neurons within AI (A 7.50) (Fig 3.9 A; B). This suggests the possibility that the cells, originating within the arcopallium, receive catecholaminergic and possibly dopaminergic input.
Figure 3.8: GABA-CTB double staining. 

A: Overview of a GABA-staining (DAB staining) at A 7.75. Arrows indicate the fibers of the AC which are not stained.

B: GABA-positive (green) large neuron within the arcopallium counterstained with DAPI (blue). Arrows indicate the dendrites of the neuron.

C: GABA-positive (green) small cell within the arcopallium counterstained with DAPI (blue)

D: CTB-positive (red) commissural neuron within the arcopallium counterstained with DAPI (blue)

E: CTB-staining (red) within the arcopallium counterstained with DAPI (blue)

F: GABA-staining (green) within the arcopallium counterstained with DAPI (blue)

G: Overlay of photo E and F demonstrating no co-localization of commissural CTB and GABA. Scale bars = 1 mm in A; 20 μm in B, C, D, E, F, G.
3.4 Discussion

3.4.1 Summary of the Main Results

The results of the present study show that the anterior commissure of pigeons originates in the arcopallium/amygdala-complex and has reciprocal and homotopic connections to this complex in the contralateral hemisphere. In addition, the arcopallium/amygdala-complex projects via the anterior commissure in a widespread, unidirectional and heterotopic manner to predominantly secondary sensory, multimodal and limbic structures of the contralateral telencephalon. Furthermore, the arcopallial cells that give rise to the commissural system are very likely not GABAergic and receive a catecholaminergic input of presumably dopaminergic nature. Overall, different from an often repeated statement, the bird cerebrum is not a natural split brain but has prominent interhemispheric connections that reach major portions of the contralateral telencephalon. However, these widespread projections originate from a small cluster of somatomotor and limbic neurons in the caudal telencephalon.

3.4.2 Homotopic Projections of the Anterior Commissure in Pigeons and Other Avian Species

3.4.2.1 Amygdaloid Projections

The avian amygdala consists of the posterior pallial amygdala (PoA), the subpallial amygdala, and the nucleus taeniae of the amygdala [156]. The present study shows
that only the PoA has interhemispheric projections via the anterior commissure. The PoA can be subdivided into two parts, a more dorsal part (PoAc) that projects predominantly to the lateral part of the bed nucleus of the stria terminalis, with some fibers reaching the medial hypothalamus, and a more ventral part (PoAb) that extends more rostrally along the base of the telencephalon and projects heavily onto the hypothalamus [9]. According to the present study, it is only the PoAb that exclusively participates in interhemispheric projections and forms homotopic connections with the contralateral PoAb.

3.4.2.2 Arcopallial Projections

The arcopallium consists of four subareas the anterior (AA), dorsal (AD), intermediate (AI), and medial (AM) arcopallium. While the connections of the first three components clearly show a somatomotor instead of an amygdaloid character, the somatic or limbic identity of AM is less clear [156]. AA and AI are somatomotor structures that innervate subpallial areas down to medullary levels [47], [206]. At the same time AA and AI are associative structures that receive input from the caudolateral nidopallium (NCL), auditory [199], trigeminal [198], somatosensory [110], and visual structures [13], [89]. According to Shanahan et al. (2013), the AI represents one of the most central hubs of the pigeons’ connectome [172]. The present study demonstrates that the reciprocal and homotopic interhemispheric projections via the anterior commissure are primarily mediated by the somatomotor structures AA and ventral AI. The AIvm is the auditory component of AI and also projects to the contralateral arcopallium [199]. The present findings accord with some previous studies in pigeons [207], chicken [36], and mallards [48]. All of these studies had demonstrated commissural afferents to AA and projections from AA towards the contralateral arcopallium. Projections towards the contralateral AI were only reported for pigeons [207] and mallards [48].
3.4.3 Heterotopic Projections of the Anterior Commissure in Pigeons and Other Avian Species

3.4.3.1 Projections to Higher Sensory Areas

**Trigeminal system.** Somatosensory input from the oral region in birds is conveyed to the nucleus sensorius principalis nervi trigemini in the brainstem and is then sent via the tractus quintofrontalis without thalamic relay to the nucleus basorostralis pallii (Bas) in the forebrain [169], [198]. Bas gives rise to efferents towards the frontal-trigeminal nidopallium (NFT) which then sends connections to AI and the trigeminal component of the NCL [110], [198]. The present study shows that the arcopallium projects via the anterior commissure to the contralateral NFT. This is similar to mallards which, in addition, also have connections to the contralateral dorsal (MD) and ventral (MV) mesopallium [48]. In pigeons, there is a second trigeminal pathway that runs through the frontoventral mesopallium (MFV) that is reciprocally connected to Bas and NFT [11]. The present study shows that both NFT and MFV are interhemispherically connected via arcopallial projections. Thus, interhemispheric trigeminal information is integrated both at primary (Bas) and at secondary (NFT, MFV) levels.

**Visual system.** Visual information is conveyed from the retina to the telencephalon via two main visual pathways, the tectofugal and the thalamofugal pathway. Within the tectofugal pathway visual information is transferred from the thalamic nucleus rotundus to the entopallial core, which then projects to a surrounding shell, the perientopallium. The perientopallium receives a relatively sparse direct projection from nucleus rotundus and is itself a major source of projections to wider regions of the hemisphere, including NFL, intermediolateral nidopallium (NIL), NCL, the area temporo-parieto-occipitalis (TPO) and arcopallium [89], [111]. The present study reveals that the arcopallium projects via the anterior commissure to the contralateral NFL, NIL and TPO. This result is in line with previous studies in pigeons [207], and mallards [48]. Furthermore, these results indicate that the arcopallium modulates visual tectofugal information in a widespread telencephalic network through the anterior commissure.
In the thalamofugal pathway of pigeons, visual information is transferred from the thalamic lateral geniculate nucleus, pars dorsalis to the interstitial part of the hyperpallium apicale/ hyperpallium densocellulare, pars lateralis of the visual wulst [74]. The visual information is then primarily sent to the supragranular layer in the visual wulst (HA), which, in turn, projects to NFL, NCL, intermediale nidopallium (NI), AI, lateral hyperpallium (HL), and LSt/MSt [110], [174]. NFL receives a projection from the perientopallium and constitutes reciprocal connections with HA. Thus, NFL is an area of integration between the thalamofugal and the tectofugal pathway [174]. In contrast to pigeons, Davies et al (1997) did not report interhemispheric commissural projections from the arcopallium to NFL or NIL in chicken [36]. Instead, the chicken AA seems to have strong interhemispheric connections with all layers of the wulst, including HA. Thus, both in chicken and in pigeons the visual system is interhemispherically connected via the AC. But while the AC of chicken only interconnects the thalamofugal system, the AC of pigeons integrates information from tertiary visual areas of both visual pathways. Additionally, the present study revealed an interhemispheric connection between AI and PoA and the contralateral HI. This projection was already described by Zeier and Karten (1973) [207] and Bagnoli et al. (1983) [13] in pigeons. A similar commissural projection was also discovered by Dubbeldam et al. (1997) in the mallard [48]. Additionally, the present study demonstrated that primarily the anterior part of HI receives contralateral input from the arcopallium/amygdala-complex. This part of HI receives afferents from the thalamic nucleus dorsolateralis and nucleus superficialis, parvocellularis, which both are thought to belong to the somatosensory, spinothalamic tract [103]. Accordingly, the anterior part of HI primary processes somatosensory instead of visual information, suggesting that somatosensory information is interhemispheric controlled by the contralateral arcopallium/amygdala complex on a primary processing level.

*Multisensory system.* NCL is reciprocally connected with the secondary sensory areas of all modalities [110]. Due to this multimodal input and the broad overlap of terminations, the NCL is a true associative forebrain structure and the largest hub in the pigeons’ forebrain connectome [172]. The present study revealed that AI sends a massive projection to the contralateral NCL that mostly terminate in the
representational areas of the trigeminal, tectofugal visual and somatosensory systems ([110]). This observation is in line with the results from Zeier and Karten (1973) in the pigeon [207].

3.4.3.2 Limbic System

The frontomedial nidopallium (NFM) is reciprocally connected to the PoA [9] and also projects to the medial MSt [110], [189]. Another limbic pathway is processed via the frontodorsal mesopallium (MFD), which connects reciprocally with NFM, receives thalamic input from the subrotundus, and projects to AD, AI, and medial MSt [11]. In addition, MFD has descending fibers to the hypothalamus and connects reciprocally with limbic ventral arcopallium, PoA, and the area corticoidea dorsolateralis [9], [206]. The present study demonstrates a contralateral connection from the arcopallium to entire MFD and MID. The origin of this projection within the arcopallium seems to be the AI [11]. MID is connected to the limbic caudocentral nidopallium (NCC) and appears to be associated with the limbic system [10]. As shown in the present study, especially the limbic NCC receives strong input from the contralateral arcopallial AI [10].

3.4.3.3 Motoric System

The striatum of birds are constituted by a lateral and a medial component. The present study reveals an exclusive projection from limbic PoA and ventral AI to the contralateral MSt via the anterior commissure. The MSt can be further subdivided into a lateral and a medial part. The more lateral MSt receives pallial input from somatic regions, such as those involved in somatosensory, visual, auditory and motor functions [20], [101], [143], [189], [197], [199]. By contrast, medial MSt appears to be more viscerolimbic, since its pallial input arises from such regions as hippocampus, piriform cortex and the limbic subareas of NCL [110], [112], [188], [189]. In the present study, the anterogradely labeled fibers in the contralateral MSt were primarily found in the lateral part of MSt suggesting an interhemispheric influence onto striatal information within the more somatic lateral MSt.
3.4 Discussion

3.4.4 Comparative Aspects of the Anterior Commissure in Other Vertebrates

3.4.4.1 The AC in Fish, Amphibians and Reptiles

Both the hippocampal (pallial) and the anterior commissure also exist in fish, amphibians and reptiles. Unfortunately, in none of this vertebrate species we were able to find a publication, which exclusively analyzes the pattern of the anterior commissure. Tracing studies in fish primary focus on the connectivity of the olfactory bulb, and in this context also some anterior commissural connections were described. In agnathans, cartilaginous fish and teleosts the anterior commissure interconnects the olfactory bulbs (agnatha: [136]; cartilaginous fish: [203]; teleost: [55]; [140]). In birds, the olfactory bulb is not interconnected via the anterior but via the habenular commissure [36], [151], [207]. Interestingly, secondary olfactory axons of cartilaginous and bony fish decussate not only through the anterior commissure, but also through the habenular and the postoptic commissure [141], [179], [203]. Studies of commissural systems in amphibians and reptiles report interhemispheric projections that could be transmitted via either the hippocampal and/or the anterior commissure (amphibians: [107], [142]; reptiles: [22], [87], [139]). Overall, these studies show that most of the extent of the pallium of amphibians and reptiles is interhemispherically connected through commissural systems without clearly separating which commissural system contributes to which pathway.

3.4.4.2 The AC in Eutherian and Metatherian Mammals

Projections of the paleocortex. In all mammals, the paleocortex, including the olfactory bulbs (OB), their associated nuclei and the amygdala (human: [44]; higher eutherians: [95], [96], [97], [149]; lower eutherians: [98]) of both hemispheres, are interconnected through the anterior commissure (Fig 3.10 A, B). In contrast, the present study could not reveal olfactory interconnections through the anterior commissure in pigeons (Fig. 3.10 C). In pigeons, olfaction plays an important role for navigation. Olfactory information is bilaterally transferred from the OB to several telencephalic areas, including the olfactory tuberculum, prepiriform cortex, nucleus taeniae of the amygdala, OB and piriform cortex [12], [151]. Rieke et al. (1978) described that the contralateral efferents of the OB run through the AC [158]. Also Zeki (1973) described a number of projections through the AC to olfactory
Figure 3.10: Some principles of the structural organization of interhemispheric connections. A: Callosal connections are largely homotopic-reciprocal but heterotopic-unidirectional projections also exist as shown in the dorsal view of the hemispheres. B: AC connections in mammals show nearly the same amount of fibers for homotopic-reciprocal as well as heterotopic-unidirectional connections. C: AC connections in aves are largely heterotopic-unidirectional. Only a small amount of homotopic, reciprocal projecting fibers exist.
Figure 3.11: Schematic diagram showing the main interhemispheric connections through the CC and the AC in A: eutherian mammals B: metatherian mammals C: Aves. Line widths between the blocks are about proportional to the magnitude of the connections.
3.4 Discussion

nuclei like olfactory tuberculum and piriform cortex in pigeons [208]. Davies et al. (1997) described efferents from AA to the contralateral OB, piriform cortex and olfactory tuberculum through the AC in chickens [36]. In contrast, Reiner and Karten (1985) showed that a bundle of fibers from the OB enters the diencephalon via the stria medullaris, crosses in the habenular commissure and ascends to the contralateral telencephalon [155]. The projection to the contralateral hemisphere via the habenular commissure has also been reported in reptiles and amphibians but not in mammals [138], [139], [155], [168]. Thus, there is strong evidence that interhemispheric olfactory information in birds is not transferred via the AC, but the habenular commissure. This is in accordance with the present results that could not evoke an olfactory projection via the AC in pigeons (Fig 3.10 C). In sum, while the transfer of interhemispheric olfactory information in mammals is achieved via the anterior commissure, this is not the case for birds, reptiles, and amphibians. Besides olfactory areas, the anterior commissure in mammals also interconnects amygdaloid areas. This is also true for pigeons as demonstrated in the present study (Fig. 3.10 C).

**Projections of the neocortex.** In most mammals, the anterior commissure also contains fibers originating from the neocortex. In eutherians, it is primarily the temporal pole that is interconnected through the anterior commissure [44], while the remaining neocortical structures are interconnected by the corpus callosum (Fig. 3.10 A) (man: [28]; rhesus monkey: [93], [114]; cat: [50], [93]; rat: [93]). Metatherian mammals like wallaby and opossum, do not possess a corpus callosum. In these animals, the whole neocortical mantle projects through the anterior commissure (Fig 3.10 B)[64], [131]. So, the corpus callosum in eutherians overtook most of the interhemispheric connectivity that is subserved by the anterior commissure in metatherians [64], [131]. Although, the pigeons’ AC resembles the commissural systems of eutherian and metatherian mammals in some aspects, there are some important differences: One of them is that the AC of pigeons does not interconnect any of the primary sensory forebrain areas (Fig. 3.10 C). This is different to eutherians with respect to the corpus callosum [28], [93], [114] and to metatherians with respect to the AC (Fig. 3.10 A, B)[64], [131]. In contrast, secondary sensory areas are connected via the AC in pigeons and metatheria [50], [64] and the corpus callosum
in eutheria [28]. Multimodal and ‘prefrontal’ areas are interconnected via the AC in pigeons (present study) and via the corpus callosum in eutheria (Fig. 3.10 A, C)[114].

Differences in anatomical organization between birds and mammals. In contrast to the AC of birds, the connections of the mammalian corpus callosum are largely homotopic and reciprocal (Fig. 3.11 A)[92]. Also the mammalian AC has a large amount of homotopic and reciprocal connections, but in comparison to the corpus callosum the proportion of homotopic reciprocal to heterotopic unidirectional connections is different to the corpus callosum (Fig. 3.11 B)[115]. In eutherians, an example for a nonreciprocal AC connection is the heterotopic connection between the olfactory bulb and the piriform cortex (Fig. 3.11 B)[78]. The AC of metatherians is much larger than that of eutherians and has both strong homotopic and heterotopic projections (Fig. 3.11 B). The present study in the pigeon shows that a homotopic reciprocal fiber organization is only true for arcopallial and amygdaloid projections. These constitute only a small fraction of the connectivity that runs through the AC. The majority of AC-connections in birds are heterotopic and nonreciprocal (Fig 3.11 C). In addition, the avian AC is constituted by a rather small population of arcopallial and amygdaloid fibers. Although these neurons reach a vast contralateral territory with heterotopic projections, their constituent cell population is restricted to small part of the bird pallium.

3.4.5 Conclusion

In summary, the present study demonstrates that the AC of pigeons interconnects a wide network of forebrain structures. The telencephalic commissures in mammals and birds overlap in some aspects of organization but also evince several important differences. In contrast to the mammalian AC, the avian AC interconnects no olfactory areas. Furthermore in birds, interhemispheric information is predominantly integrated at a later processing stage due to the absence of interconnections between primary sensory areas (Fig 3.12). The only exception is somatosensory information, which is already interhemispherically integrated at a primary processing level.

But the main differences in the interhemispheric connectivity, between birds and mammals, are found at two levels of structural organization. First, the AC in
birds differs from the corpus callosum and the AC of mammals in its proportion of homotopic reciprocal to heterotopic unidirectional projections. In contrast to the situation in mammals, in birds only a small amount of cells interconnect the two hemispheres in a homotopic and reciprocal fashion. Instead, most of the cells project heterotopically and in unidirectional manner. Second, in birds the absolute majority of pallial areas do not participate by themselves in interhemispheric exchange. Instead, a rather small arcopallial and amygdaloid cluster is key for commissural interactions. According to Ehrlich and Mills (1985), only 89,000 crossing fibers constitute the AC in chicken [52]. Thus, the colloquial statement of birds as ‘natural split-brains’ is wrong, when the pallial areas are considered that interhemispherically interact via the AC. It is true, however, when taking into account how small the proportion of pallial neurons is that constitute interhemispheric exchange.
CHAPTER 4

Study 3: Light-Dependent Lateralization of Visuospatial Attention

The following described project was conducted in cooperation with Emre Ünver. Therefore, parts of this project are also described in the thesis of Emre Ünver. Data analysis and interpretation were prepared under each person’s own authority; only data acquisition of 20 light-incubated pigeons was a shared responsibility. Four pigeons with transection of the anterior commissure were tested by Emre Ünver.

In this chapter, several aspects of the development of functional asymmetries and the impact of structures that affect a lateralized information processing are analyzed:

1. In pigeons, several left-hemispheric functions develop in response to an asymmetric light-stimulation. However, not much is known about the ontogenetic mechanisms leading to right-hemispheric lateralization. To analyze a possible light-dependent development of right-hemispheric lateralization, the well-known asymmetry for visuospatial attention control was examined. This cerebral asymmetry is lateralized in the right hemisphere in birds as well as humans.

2. Furthermore, an open question in the development of functional lateralization is to what extent left- and right-hemispheric asymmetries develop dependently or independently from each other. Therefore, the impact of the light-dependent
left-hemispheric lateralization on right-hemispheric attentional control is analyzed.

3. As already mentioned in the general introduction, interhemispheric systems have a critical impact on lateralized information processing. In the pigeon it is known that on mesencephalic level the tectotectal commissural system strongly affects a lateralized behavioral output. However, less is known about the control of functional lateralization on telencephalic level.

4.1 Introduction

In humans, lateralized attention control is shown in a visuospatial bias to the left visual field in tasks like line-bisection [94] and cancellation tasks [191]. This phenomenon is named 'pseudoneglect' and was described the first time by Bowers and Heilman (1980) [19]. This asymmetry is usually explained by neuronal circuits in the right hemisphere, which are capable of attending to and representing both sides of space, while the left hemisphere is concerned only with the contralateral right side [16], [106], [133]. Recently, two species of birds, the domestic chick (*Gallus gallus*) and the pigeon (*Columba livia*), were tested in a task investigating the visuospatial attention of these animals [25], [42]. The test used closely matched the cancellation task which is employed in humans. In this task human-subjects are required to delete a target stimulus located between other similar diverting items [3]. In the avian-version, chicks and pigeons were confronted with grains centered in front of them and had free choice to orient and peck at the grains [42]. Similar to human pseudoneglect phenomena, a selective allocation of attention to the left hemifield is seen in the cancellation task in birds [25], [42]. In chicks the bias to the left visual hemifield vanishes after incubation of the eggs in complete darkness [25], indicating that the emergence of right-hemispheric specialization depend on asymmetric light-stimulation as well. This is comparable to left-hemispheric dominance in object discrimination [176]. In the same study, Chiandetti (2011) analyzed the effect of left-hemispheric dominance for object discrimination onto the right-hemispheric dominance for visuospatial attention [25]. The combination of these two functional asymmetries leads in light-incubated chicks to no visible attention bias, indicating that left- and right-hemispheric dominance affect each other. Because chicks and pigeons
display similar light-dependent development, it is conceivable that right-hemispheric dominance for visuospatial attention also develops in response to asymmetric visual stimulation in pigeons. Accordingly, also the critical interaction of left- and right-hemispheric asymmetries could be similar to that in chicks. On the other hand, structural asymmetries differ between chicks and pigeons, indicating differences in the mechanisms of the development of left-right-asymmetries and its reciprocal interactions [130].

In this study, we tested light- and dark-incubated pigeons in the cancellation task to analyze light-effects, which are mediated through ascending visual systems on right-hemispheric asymmetries. To test the critical interaction of left- and right-hemispheric asymmetries, we combined the cancellation task with a discrimination task and tested light- and dark-incubated pigeons in this test. To analyze the impact of telencephalic interhemispheric transfer onto a lateralized information processing, we transected the anterior commissure in pigeons, and tested pre- and post-surgery the behavioral performance in the cancellation task.

4.2 Material and methods

4.2.1 Subjects

Behavioral testing was performed with three experimental groups of adult pigeons (*Columba livia*) of undetermined sex: 30 light-incubated pigeons, 18 dark-incubated pigeons, and 4 pigeons with the anterior commissure transected (transection procedure see chapter 3.2.2). The light incubated pigeons came from local breeders. The dark-incubated animals stem from lab-own breeding pairs (see chapter 2.2.1).

The birds were housed individually and were placed on a 12/12 h light/dark cycle. Animals were maintained on 85-90% of their free feeding weight throughout the experiments. Food was provided during the experiment and after experimental sessions.

4.2.2 Apparatus

The apparatus consisted of a gray plastic box (30 cm wide x 30 cm high x 30 cm deep) with a 7 cm wide hole in the middle of the front panel (Fig 1A). In front of the box a square board was placed (25 cm x 25 cm) with 13 x 10 round cavities. In
4.2 Material and methods

9 x 9 of these cavities a single pea was placed (Fig 4.1 A). In a second test condition, all of the cavities were filled with grit. In 9 x 9 of these grit filled cavities a single Dari-grain was placed (Fig 4.1 B)[75]. The shape, size and color of the Dari-grain were very similar to the grit to increase the discrimination difficulty of the test. The behavior of the animals was recorded with a video-camera (Sony DCR-SR210) positioned in front of the experimental apparatus.

4.2.3 Procedure

The animals of the different experimental groups were first positioned inside the box and habituated to the experimental setup to protrude their head through the window in the front panel to directly start pecking grains. Pigeons did this quiet spontaneously as the box was dark and the experimental room was illuminated. Habituation procedure was repeated three times for 10 min. During testing-phase, the pigeons were placed inside the box and observed for a total of 5 min while they were free to peck the food (peas or grit/Dari-grain), in the 9 x 9 array as described above. This testing-phase was repeated three times with each pigeon under three vision conditions (binocular, left eye uncovered, right eye uncovered). For monocular testing, one of the pigeon’s eyes was temporally covered by an eye cap. In order to cover one eye, a velcro-ring was adhered to the feathers around the eye with
non-toxic glue. A cap could then be gently velcroed over either eye.

4.2.4 Analysis

The recorded videos were analyzed on a computer using the program windows media player. For analysis, the surface was divided into nine vertical columns: the central midline (CTR), four left (L1, L2, L3, L4) and four right (R1, R2, R3, R4) columns. The video recordings were analyzed for of the number of pecks in each column. Due to high peck accuracy, pigeons were able to make only a few pecks, and after each successful peck, their attention shifted to the areas where grains remained. As such, the pecking order is an accurate way of assessing pigeons' attention. To include this factor in our analysis, the spatial position of the first 25 pecks was scored based on the order in which they occurred, with the first peck given the highest score of 25.

Every peck was counted independently whether it was successful or not, however, repeated pecking in one cavity was counted as one peck. Not every pigeon performed 25 pecks for each trial, in particular for the grain-grit cancellation task under monocular vision. Every session with less than 20 pecks was excluded from the analysis, and the weighted pecks for every column were normalized with performed pecks.

The statistical analysis was performed with IBM SPSS 20 by running analysis of variance (ANOVAs). For Post-hoc comparisons Bonferroni corrected t-test were conducted. For the test-group with a transection of the anterior commissure a bootstrap analysis was calculated due to the small sample size (4 pigeons).

4.3 Results

4.3.1 Comparison of Light- and Dark-Incubated Pigeons

4.3.1.1 Pea-Cancellation Task under Binocular Condition

The results of the pea-cancellation task were analyzed using a $2 \times 2 \times 4$ mixed analysis of variance with the between-subjects factor Group (dark-incubated animals, light-incubated animals) and the two within-subject factors Side (left, right) and Column (1, 2, 3, 4) (Fig 4.2 A, B).

The analysis revealed a main effect of Side ($F_{(1,46)} = 8.00; p < 0.01; \text{partial } \eta^2 = 0.15$). Overall, animal groups showed a significant bias for pecking on the right
Figure 4.2: Visuospatial attention to the left and right hemifield in the peacock-cancellation task. Outlined is the average score of the order in which pigeons pecked in every column. Highlighted is the difference between the order of left and right pecks and the central midline (CTR). Error bars indicate standard errors. A: Results of binocular testing in light-incubated pigeons. B: Results of binocular testing in dark-incubated pigeons.
4.3 Results

side (11.68 +/- 0.60) compared to the left side (8.43 +/- 0.56). Importantly, this asymmetry was modulated by the factor Group as indicated by a significant interaction Side × Group ($F_{(1,46)} = 7.21; p < 0.01; \text{partial } \eta^2 = 0.14$). To further analyze this effect, we conducted Bonferroni-corrected post-hoc tests. This analysis revealed that dark-incubated animals showed a pronounced left-right-pecking asymmetry (right side: 13.04 +/- 0.95; left side: 6.70 +/- 0.89; post-hoc test: $p < 0.001$). In contrast, no left-right-difference was observed in the light-incubated group (right side: 10.31 +/- 0.74; left side: 10.15 +/- 0.69; post-hoc test: $p = 0.91$). Thus, the significant main effect was driven by the dark-incubated animals. This pecking pattern is in contrast to the observation made by Diekamp et al. (2005) [42]. Therefore, we tested, whether a divergent pattern resulted from sample size. By using G*Power 3.17 (CIT) we conducted a power-analysis to determine the sample size required to reach significance in our sample. For calculation, we used an alpha error probability of 0.05, a power of 0.95 and an effect size dz of 0.0237, as calculated by the G*Power software from the means and standard deviations in the two conditions. The total sample size required for the effect to reach significance was 23,102 animals.

The main effect of Group failed to reach significance ($F_{(1,46)} = 3.36; p = 0.73; \text{partial } \eta^2 = 0.07$), indicating that there were no systematic biases in performance between the two groups that might have affected the asymmetry effects. As expected, we also observed a significant main effect of Position ($F_{(3,138)} = 332.06; p < 0.001; \text{partial } \eta^2 = 0.88$) indicating that in general, animals showed a tendency to peck closer to the midline (column 1: 18.48 +/- 0.40; column 2: 13.06 +/- 0.33; column 3: 7.20 +/- 0.47; column 4: 1.47 +/- 0.21). This effect was modulated by Column and Group as indicated by significant interactions Column × Group ($F_{(3,138)} = 4.97; p < 0.01; \text{partial } \eta^2 = 0.10$), Side × Column ($F_{(3,138)} = 7.81; p < 0.001; \text{partial } \eta^2 = 0.15$) and Side × Column × Group ($F_{(3,138)} = 4.35; p < 0.01; \text{partial } \eta^2 = 0.09$). In light-incubated animals, the difference between left and right pecks was small, independent of column (column 1: right: 17.85 +/- 1.04; left: 16.80 +/- 1.02; column 2: right: 14.39 +/- 1.13; left: 13.35 +/- 1.04; column 3: right: 7.33 +/- 1.20; left: 8.26 +/- 0.97; column 4: right: 1.68 +/- 0.37; left: 2.19 +/- 0.41). In contrast, dark-incubated animals showed much larger asymmetries, which were modulated by column, with columns closer to the midline eliciting larger absolute left-right-
4.3 Results

differences than those farther away (column 1: right: 24.52 +/- 1.34; left: 14.74 +/- 1.31; column 2: right: 16.17 +/- 1.45; left: 8.34 +/- 1.31; column 3: right: 10.21 +/- 1.55; left: 2.10 +/- 1.25; column 4: right: 1.28 +/- 0.47; left: 0.73 +/- 0.53).

4.3.1.2 Grain-Grit Cancellation Task under Binocular Condition

The results of the grain-grit cancellation task were analyzed using a $2 \times 2 \times 4$ mixed analysis of variance with the between subjects factor Group (dark-incubated animals, light-incubated animals) and the two within-subject factors, Side (left, right) and Column (1, 2, 3, 4) (Fig 4.3 A, B).

The only main effect that reached significance was Column ($F_{(3,126)} = 145.12; p < 0.001; \text{partial } \eta^2 = 0.78$), indicating that the animals pecked less the farther away a pecking column was from the midline (column 1: 17.08 +/- 0.54; column 2: 14.12 +/- 0.41; column 3: 7.74 +/- 0.54; column 4: 2.16 +/- 0.46). This effect was modulated by the factor Group as indicated by a significant interaction Column $\times$ Group ($F_{(3,126)} = 3.35; p < 0.05; \text{partial } \eta^2 = 0.07$). All other main effects and interactions failed to reach significance (for all, $p > 0.40$). Thus neither light-nor dark-incubated pigeons showed a pecking asymmetry.

4.3.1.3 Relationship Between the two Tasks under Binocular Condition

In order to test, whether the test results of the two tasks were independent or not, we determined an overall LQ for each task by summing up the scores for all four columns for each side and calculating the LQ using the following formula:

$$LQ = \left[\frac{(R-L)}{(R-L)}\right] \cdot 100$$

$R =$ score for the right side, $L =$ score for the left side

We then compared the LQ, as well as the scores for the left and the right side between the two tasks, using dependent-samples t-tests (Tab 4.1, Fig 4.4 A, B).

All scores for the left and right side did not differ except the score for the left side in the dark-incubated animals. Here, the dark-incubated animals showed a higher score in the grain-grit cancellation task (36.09 +/- 4.51) than in the pea-cancellation task (26.81 +/- 3.49).
Figure 4.3: Visuospatial attention to the left and right hemifield in the grain-grit cancellation task. Outlined is the average score of the order in with pigeons pecked in every column. Highlighted is the difference between the order of left and right pecks and the central midline (CTR). Error bars indicate standard errors. A: Results of binocular testing in light-incubated pigeons. B: Results of binocular testing in dark-incubated pigeons.
4.3 Results

**Figure 4.4:** Comparison of the pecking order in light- and dark-incubated pigeons between pea-cancellation task and grain-grit cancellation task. **A:** Score of the pecking order to the left and right side. **B:** Lateralization quotient (LQ). A LQ of 0 indicates a perfectly symmetric pecking distribution, while a LQ of +100 denotes exclusive pecking to the right and -100 to the left. Error bars indicate standard errors. * = p < 0.05.

**Table 4.1:** Comparison of the scores from the left and right side and LQ between pea-cancellation and grain-grit cancellation task.

<table>
<thead>
<tr>
<th></th>
<th>Light-Incubated Pea</th>
<th>Light-Incubated Grain-Grit</th>
<th>Dark-Incubated Pea</th>
<th>Dark-Incubated Grain-Grit</th>
</tr>
</thead>
<tbody>
<tr>
<td>score left</td>
<td>40.60 +/- 2.79</td>
<td>41.89 +/- 3.38</td>
<td>26.81 +/- 3.49</td>
<td>36.90 +/- 4.51</td>
</tr>
<tr>
<td>score right</td>
<td>41.25 +/- 2.77</td>
<td>41.37 +/- 3.14</td>
<td>52.17 +/- 4.20</td>
<td>44.24 +/- 5.06</td>
</tr>
<tr>
<td>LQ</td>
<td>0.65 +/- 5.50</td>
<td>-0.52 +/- 6.46</td>
<td>25.36 +/- 7.56</td>
<td>7.34 +/- 9.49</td>
</tr>
</tbody>
</table>

4.3.1.4 Pea-Cancellation Task under Monocular Conditions

The results of the pea-cancellation task under monocular conditions were analyzed using a $2 \times 2 \times 2 \times 4$ mixed analysis of variance with the between subjects factor Group (dark-incubated animals, light-incubated animals) and the three within-subject factors seeing Eye (left, right), Side (left, right) and Column (1, 2, 3, 4) (Fig 4.5 A, B, C, D).

The analysis revealed a significant main effect of column ($F_{(3,135)} = 85.54; p < 0.001$;
4.3 Results

Figure 4.5: Visuospatial attention to the left and right hemifield in the peacock cancellation task under monocular conditions. Outlined is the average score of the order in which pigeons pecked in every column. Highlighted is the difference between the order of left and right pecks and the central midline (CTR). Error bars indicate standard errors. 

A: Results of left eye vision in light-incubated pigeons. 
B: Results of right eye vision in light-incubated pigeons. 
C: Results of left eye vision in dark-incubated pigeons. 
D: Results of right eye vision in dark-incubated pigeons.
partial $\eta^2 = 0.66$), indicating that the animals pecked less, the farther away a pecking position was from the midline (column 1: 14.60 +/- 0.46; column 2: 13.06 +/- 0.27; column 3: 10.17 +/- 0.41; column 4: 4.78 +/- 0.49). This effect was modulated by Group, as indicated by significant interaction Column $\times$ Group ($F_{(3,135)} = 10.72; p < 0.001; \text{partial } \eta^2 = 0.19$). This interaction showed that light-incubated animals pecked less, the farther away a pecking position was from the midline (column 1: 16.36 +/- 0.77; column 2: 12.59 +/- 0.33; column 3: 8.44 +/- 0.50; column 4: 3.63 +/- 0.61; post-hoc test: $p < 0.001$ for all columns). In contrast, dark-incubated pigeons showed continuous pecks for the first three columns, and pecks decreased for column 4 (column 1: 12.83 +/- 0.72; column 2: 13.52 +/- 0.42; column 3: 11.90 +/- 0.64; column 4: 5.93 +/- 0.77; post-hoc test: $p < 0.001$ only for column 4).

As expected, we observed a significant interaction Eye $\times$ Side ($F_{(1,45)} = 879.82; p < 0.001; \text{partial } \eta^2 = 0.95$), indicating that the pigeon pecked more to the side with the seeing eye (left eye vision: right side: 2.58 +/- 0.28; left side: 18.55 +/- 0.42; post-hoc test: $p < 0.001$; right eye vision: right side: 18.73 +/- 0.47; left side: 2.75 +/- 0.33; post-hoc test: $p < 0.001$). This effect was also modulated by Group and Column as indicated by significant interaction Eye $\times$ Side $\times$ Group ($F_{(1,45)} = 35.05; p < 0.001; \text{partial } \eta^2 = 0.44$) and Eye $\times$ Side $\times$ Column ($F_{(3,135)} = 43.55; p < 0.001; \text{partial } \eta^2 = 0.49$). These interactions demonstrate that light-incubated pigeons pecked more
often to the side contralateral to the seeing eye compared with dark-incubated pigeons (left eye vision: right side: light-incubated: 3.66 +/- 0.34; dark-incubated: 1.49 +/- 0.44; post-hoc test: p < 0.001; right eye vision: left side: light-incubated: 4.05 +/- 0.41; dark-incubated: 1.44 +/- 0.52; post-hoc test: p < 0.001). The main effect of Group reached significance (F(1,35) = 20.61; p < 0.001; partial η² = 0.31), indicating that there were systematic biases in pecks between the two groups. This difference in the mean pecking order between the two groups could be the result of different pecks to the midline. We therefore compared the pecks to the midline between light- and dark-incubated pigeons, using independent-samples t-tests (Fig 4.6). The analysis revealed that light-incubated pigeons, in comparison to dark-incubated, showed a higher tendency to peck to the midline under both the left eye vision (light-incubated: 18.51 +/- 0.99; dark-incubated: 12.47 +/- 1.57; F(45) = 1.54; p < 0.01) and right eye vision (light-incubated: 17.26 +/- 0.96; dark-incubated: 10.79 +/- 1.37; F(46) = 0.03; p < 0.001). Accordingly, despite monocular vision light-incubated pigeons show a tendency to peck to the midline.

4.3.1.5 Grain-Grit Cancellation Task under Monocular Conditions

The results of the grain-grit cancellation task were analyzed using a 2 x 2 x 2 x 4 mixed analysis of variance with the between subjects factor Group (dark-incubated animals, light-incubated animals) and the three within-subject factors seeing Eye (left, right), Side (left, right) and Column (1, 2, 3, 4) (Fig 4.7 A, B, C, D).

The analysis revealed a significant main effect of Column (F(3,108) = 21.28; p < 0.001; partial η² = 0.37). In contrast to the pea-cancellation test, the column effect was not only reflecting the distance from the midline. In the grain-grit cancellation test, the animals did not peck less the farther away a pecking position was from the midline, but the animals showed equivalent low pecks for column 1 and 4 (column 1: 8.70 +/- 0.51; column 4: 9.09 +/- 0.73), which differed significantly from column 2 and 3 with equivalent high pecks (column 2: 13.42 +/- 0.43; column 3: 15.07 +/- 0.68). We also observed as expected, a significant interaction of Eye x Side (F(1,36) = 1522.01; p < 0.001; partial η² = 0.98), indicating that the pigeons pecked more to the side with the seeing eye (left eye vision: right side: 0.97 +/- 0.37; left side: 22.38 +/- 0.49; right eye vision: right side: 21.90 +/- 0.42; left side: 1.04 +/-
4.3 Results

Grain Grit Cancellation Task

Figure 4.7: Visuospatial attention to the left and right hemifield in the grain-grit cancellation task under monocular conditions. Outlined is the average score of the order in which pigeons pecked in every column. Highlighted is the difference between the order of left and right pecks and the central midline (CTR). Error bars indicate standard errors. A: Results of left eye vision in light-incubated pigeons. B: Results of right eye vision in light-incubated pigeons. C: Results of left eye vision in dark-incubated pigeons. D: Results of right eye vision in dark-incubated pigeons.
4.3 Results

This interaction was modulated by the factors Group and Column as indicated by the significant interactions Eye × Side × Group ($F_{(1,36)} = 7.06; p < 0.05; \text{partial } \eta^2 = 0.16$) and Eye × Side × Column ($F_{(3,108)} = 37.94; p < 0.001; \text{partial } \eta^2 = 0.51$).

The Bonferroni-corrected post-hoc tests revealed that dark-incubated pigeons pecked more to the left side under left eye vision in comparison to light-incubated pigeons (left eye vision: left side: dark-incubated: 23.48 +/- 0.80; light-incubated: 21.28 +/- 0.57; post-hoc test: $p < 0.05$). In contrast, with right eye vision no difference between dark- and light-incubated animals was visible (right eye vision: right side: dark-incubated: 22.73 +/- 0.68; light-incubated: 21.07 +/- 0.50; post-hoc test: $p > 0.05$).

The main effect of Group reached significance ($F_{(1,36)} = 7.22; p < 0.05; \text{partial } \eta^2 = 0.17$), indicating that there were systematic biases in performance between the two groups. This difference in pecking order could be a result of different pecks to the midline. We therefore compared the pecks to the midline between light- and dark-incubated pigeons, using independent-samples t-tests (Fig 4.8). The analysis revealed, that light-incubated pigeons, in comparison with dark-incubated, showed a higher tendency to peck to the midline under left eye vision, as it was also shown for the pea-cancellation task (light-incubated: 9.42 +/- 1.18; dark-incubated: 3.80 +/- 1.22; $F_{(36)} = 1.89; p < 0.01$). In contrast to the pea-cancellation task, in the
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grain-grit cancellation task light- and dark-incubated pigeons showed no significant
difference in pecks to the midline under right eye vision ($p > 0.80$).

4.3.1.6 Relationship Between the two Tasks under Monocular Conditions

In order to test whether the pecking pattern differed between the two task version
under monocular condition, we analyzed the LQ using a $2 \times 2 \times 2$ mixed analysis
of variance with the between subjects factor Group (dark-incubated animals, light-
incubated animals) and the two within-subject factors seeing Eye (left, right), and
Test (pea-cancellation task, grain-grit cancellation task) (Tab 4.2, Tab 4.3, 4.9).

The analysis revealed a main effect of Test ($F_{(1,36)} = 50.74; p < 0.001$; partial $\eta^2 = 0.59$). Overall, animals showed higher pecking scores in the grain-grit cancellation
 task than in the pea-cancellation task (grain-grit cancellation LQ: 84.54 +/- 2.17;
pea-cancellation LQ: 63.68 +/- 2.47; post-hoc test: $p < 0.001$). This effect was
modulated by the factor Group as indicated by the significant interaction Test x Group
($F_{(1,36)} = 7.03; p < 0.05$; partial $\eta^2 = 0.16$). To further analyze this effect, we
conducted Bonferroni-corrected post-hoc tests. This analysis revealed that dark-
incubated animals showed in comparison to light-incubated animals higher peck

| Table 4.2: Comparison of the scores from the left and right side and LQ between pea-cancellation and grain-grit cancellation task under left eye vision. |
|-----------------|-----------------|-----------------|-----------------|
|                  | Light-Incubated | Light-Incubated | Dark-Incubated  |
|                  | Pea             | Grain-Grit      | Pea             |
|                  |                 |                 | Grain-Grit      |
| score left       | 64.60 +/- 3.05  | 81.83 +/- 4.23  | 81.59 +/- 2.65  |
| score right      | 14.18 +/- 1.56  | 5.26 +/- 1.95   | 5.94 +/- 1.47   |
| LQ               | 52.16 +/- 3.49  | 79.63 +/- 4.53  | 75.65 +/- 3.99  |

| Table 4.3: Comparison of the scores from the left and right side and LQ between pea-cancellation and grain-grit cancellation task under right eye vision. |
|-----------------|-----------------|-----------------|-----------------|
|                  | Light-Incubated | Light-Incubated | Dark-Incubated  |
|                  | Pea             | Grain-Grit      | Pea             |
|                  |                 |                 | Grain-Grit      |
| score left       | 15.79 +/- 1.82  | 6.10 +/- 1.68   | 5.77 +/- 1.57   |
| score right      | 66.95 +/- 2.46  | 81.04 +/- 3.95  | 83.44 +/- 2.57  |
| LQ               | 51.16 +/- 4.22  | 77.93 +/- 3.74  | 77.67 +/- 4.03  |


scores in both pea-cancellation and grain-grit cancellation task (pea-cancellation task: dark-incubated: 77.20 +/- 4.01; light-incubated: 50.16 +/- 2.90; post-hoc test: p < 0.001; grain-grit cancellation task: dark-incubated: 90.29 +/- 3.52; light-incubated: 78.78 +/- 2.54; post-hoc test: p < 0.05). All other main effects and interactions failed to reach significance (for all, p > 0.60).

Table 4.4: Comparison of the scores from the left and right side and LQ pre- and post-transection of the anterior commissure.

<table>
<thead>
<tr>
<th></th>
<th>Pre-Surgery</th>
<th>Post-Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>score left</td>
<td>28.41 +/- 4.63</td>
<td>58.27 +/- 12.18</td>
</tr>
<tr>
<td>score right</td>
<td>49.49 +/- 3.31</td>
<td>23.58 +/- 7.94</td>
</tr>
<tr>
<td>LQ</td>
<td>26.04 +/- 11.17</td>
<td>-39.34 +/- 20.40</td>
</tr>
</tbody>
</table>
4.3 Results

Average pecking order for each lateral position

Position to the left (L) and right (R) from the central midline (CTR)

Figure 4.10: Comparison of visuospatial attention to the left and right hemifield in the pea-cancellation task of pigeons with an intact and a transected anterior commissure (AC). Highlighted is the difference between the order of left and right pecks and the central midline (CTR). Error bars indicate standard errors. **A**: Results of binocular testing in light-incubated pigeons before transection of the AC. **B**: Results of binocular testing in light-incubated pigeons after transection of the AC.
4.3 Results

Figure 4.11: Comparison of the pecking order in pigeons with and without transection of the anterior commissure (AC) in the pea-cancellation task. A: Score of the pecking order to the left and right side. B: Lateralization quotient (LQ). A LQ of 0 indicates a perfectly symmetric pecking distribution, while a LQ of +100 denotes exclusive pecking to the right and -100 to the left. Error bars indicate standard errors.

4.3.2 Anterior Commissure Transected Animals Pre-/Post-Surgery)

In order to test whether the results of the pea-cancellation task are modulated by the AC, we tested the animals before and after transection of the AC. Due to the small sample size of four animals, we analyzed the pecking performance for every column (L1, L2, L3, L4, R1, R2, R3, R4, central midline) and the LQ pre- and post-surgery using a dependent-samples t-tests (Tab 4.4, Fig 4.10 A, B).

Only the comparison of pecks to column R1 reached significance ($t(3) = 4.74; p < 0.05$). Here, the animals showed before transection of the AC a higher pecking score (27.44 +/- 2.18) than after surgery (9.54 +/- 4.78). All other effects failed to reach significance (for all, $p > 0.10$).

Also due to the small sample size of four animals, we repeated the analysis by conducting a bootstrap analysis with 1000 sample-repetition. The results of this analysis showed, as expected, a significant change in pecking score for R1 ($p < 0.01$; based on 892 samples). Additionally, the results for R4 ($p < 0.01$; based on 682 samples) and LQ ($p < 0.01$; based on 892 samples) reached significance.
4.4 Discussion

4.4.1 Summary of the Main Results

The results of the present study demonstrate that light-incubated pigeons show no visuospatial attention shift to the left or right visual hemifield. In contrast, dark-incubated pigeons display a strong attentional bias to the right visual hemifield. Additionally, transection of the anterior commissure results in a change of the lateralization quotient according to a bootstrap analysis, suggesting a possible impact in shaping visuospatial attention.

As expected, under monocular conditions the visuospatial attention in light- and dark-incubated pigeons is primarily centered to the hemifield ipsilateral to the seeing eye. In contrast to dark-incubated pigeons, light-incubated pigeons shift their attention more often to the hemifield contralateral to the seeing eye.

In a modulated version of the cancellation task, the grain-grit cancellation task, an additional discrimination process was induced. Light-incubated pigeons showed no visuospatial attention bias under this task condition. Additionally, the strong attentional bias, which dark-incubated pigeons show in the pea-cancellation task, is decreased in the grain-grit cancellation task. Accordingly, the lateralization quotient in dark- and light-incubated pigeons does not differ between the different task conditions. As expected, light- and dark-incubated pigeons center their attention to the hemifield ipsilateral to the seeing eye under monocular conditions. However, in contrast to the binocular condition, light- and dark-incubated pigeons show a stronger bias to the visual field ipsilateral to the seeing eye within the grain-grit cancellation task in comparison to the pea-cancellation task.

4.4.2 The Cancellation Task in Birds: an Indicator of Lateralized Visuospatial Processing

In humans, the cancellation task is a test in which subjects are asked to cancel visual targets on a sheet of paper centrally placed in front of them. This is one of the tests used to diagnose visuospatial attention deficits in human patients. In this test, normal subjects show right lateralized inattention [191]. In previous studies, two bird species, chicks (Gallus gallus) and pigeons (Columba livia), were tested in an adapted version of this task. Here, chicks and pigeons showed a significant bias for the left
visual field [25], [42]. The present study could not replicate this pattern. Here, normal pigeons allocated attention symmetrically to the left and right hemispace. We checked these contradictory results in our study in comparison to Diekamp et al. (2005) by conducting a power analysis [42]. This analysis demonstrated that on basis of our effect size we had to test 23,102 animals to reach a significant effect. It is more likely then, that the finding from Diekamp et al. (2005) resulted from a random left hemifield bias distribution in the sample population [42].

4.4.3 The Ascending Visual Pathways: Tectofugal and Thalamofugal System

Behavioral output of visual tasks in birds is strongly influenced by the structural organization of the ascending visual pathways (for review see [130]). In chicks the thalamofugal pathway is asymmetrically organized, resulting in a stronger bilateral presentation in the right hemisphere [163], [164]. Studies to investigate the asymmetries in visuospatial attention processing show that in chicks have an attentional bias to the left visual hemispace [25]. Behavioral and physiological studies demonstrate that the visual wulst in birds, which is the target structure of the thalamofugal system, is involved in attentional processes [17], [196], suggesting this system with a prior position in attention control. Thus, the attentional bias for the left hemifield can be explained by a stronger bilateral presentation of the visual scene in the right hemisphere. This is in contrast to pigeons, which do not show left-right differences in thalamofugal projections [178]. Given that structural asymmetries are related to the emergence of lateralized spatial attention, the absence of attentional bias can be explained by symmetrical visual systems. Indeed, the results of the present study in pigeons show no bias for the left or right visual hemifield. When we take the thalamofugal system as functional basis for the processing of attentional processes, the absence of an attentional bias in the pigeon can be explained by the absence of a structural asymmetry in the thalamofugal system. In contrast to the tectofugal system, the thalamofugal system in pigeons is not lateralized neither in hatchlings nor in adult animals [178]. These results support that lateralized bottom-up processing modulates lateralized functional processes [130].
4.4 Discussion

Figure 4.12: Representation of the left and right visual field within the tectofugal system in light- A and dark-incubated B pigeons. Circles represent the left field and rectangles the right field. Visual information is processed via the eyes to the optic tectum (TO), to the rotundus (RT), to the Entopallium (E). Dashed-lines represent weaker projections in comparison to the others. Red edging indicates the dominant hemisphere. Gray circles or rectangles illustrate a weaker representation within the respective structure resulting from weaker projections.

4.4.4 Light-Dependent Modulation of Attentional Processes

The structural organization in the ascending visual pathways of pigeons and chicks are shaped by ontogenetic light conditions [39], [57], [124], [125], [126], [164], [176]. Structural asymmetries in these systems develop in response to an asymmetric embryonic light-stimulation. As already mentioned, in chicks, this causes an asymmetry in the projection of the thalamofugal system [108], [163], [164], while in pigeons the tectofugal pathway is affected [73]. Dark-incubation of eggs prevents establishment of several asymmetries [57], [126], [165], [176]. As expected, in dark-incubated chicks
the bias to the left hemifield in the cancellation task vanishes [25], which can be explained by the absence of structural lateralization in the thalamofugal system [163]. Since dark-incubated pigeons likewise do not display projection asymmetries [178], no attentional bias could be expected. Surprisingly, the results in dark-incubated pigeons reveal an attentional bias to the right visual hemifield, suggesting a dominance of the left hemisphere. This asymmetry, thus, is not explained by a structural lateralization in the ascending visual pathways. This attentional bias is another example of a light-independent asymmetry in pigeons [119], which is not related to projection asymmetries. Accordingly, this asymmetry in dark-incubated pigeons can no longer be explained alone by the sensory input of the ascending thalamofugal system. The tectofugal system, in addition to the thalamofugal system, is the second ascending pathway processing visual information in birds. In pigeons, the tectofugal pathway is lateralized with stronger projections from the right tectum opticum to the contralateral left nucleus rotundus than vice versa [73]. This structural asymmetry, however, is only present in light-incubated pigeons and vanishes after dark-incubation (detailed discussion see Study 1, chapter 2). Accordingly, the attentional bias to the right hemifield in dark-incubated pigeons cannot be the result of an asymmetric presentation of visual information within the left and right hemisphere due to a structural asymmetry within the ascending visual pathways. Therefore, it is likely that the attentional bias to the right visual field in dark-incubated pigeons is the result of an endogenously determined left-hemispheric asymmetry.

However, this pattern is modulated by embryonic light experiences as indicated by light-incubated pigeons, which show no attentional bias. It is conceivable that the light-induced projection asymmetry levels lateralized performance, due to the hemispheric-specific representation of the two visual half fields. As we cannot find comparable asymmetry in light-incubated pigeons, it is possible that the structural asymmetry within the tectofugal system creates a complete scene of the left and right visual hemifield in the executing left hemisphere. This better bilateral representation in the left hemisphere results in a symmetrical attention to the left and right side (Fig 4.12 A). In contrast, in dark-incubated pigeons, due to the absence of the structural asymmetry (detailed discussion see Study 1, chapter 2), the dominant left hemisphere is able to process more information from the contralateral right hemifield than the
ispilateral left hemifield and accordingly, the attention is shifted to the contralateral visual field (Fig 4.12 B).

4.4.5 Light-Dependent Modulation of the Visual-Field Extension

In birds, which have an almost completely crossing optic nerve, the information from the left eye is primarily processed within the contralateral right hemisphere and the information from the right eye in the left hemisphere. Thus, the specialization of one hemisphere can be easily tested by occluding one eye. In our cancellation task, we can retrace how the information from only one hemifield is processed within the left and right hemisphere.

The monocular testing in the cancellation task shows that the visuospatial attention is primarily centered on the hemifield ipsilateral to the seeing eye with no attentional differences between the left and right eye conditions. In line with the discussion above about the binocular results, we would not expect a difference in the attentional focus between the left and right eye in light-incubated pigeons. The attention controlling left hemisphere can be activated nearly to the same extent in both contralateral right and ipsilateral left eye conditions, due to the stronger projection from the right tectum to the left hemisphere (Fig 4.13 A, B). In dark-incubated pigeons, we would expect that the ipsilateral left eye cannot activate the dominant left hemisphere to the extent of the contralateral right eye, due to the absence of projection asymmetry in the tectorotundal projection (Fig 4.14 A, B). However, we would not expect this differential activation of the dominant left hemisphere to affect the attention to the left or right hemifield under the monocular condition, because independent of the vision condition, the left hemisphere has no information about the visual field of the occluded eye (Fig 4.14 A, B). There is no difference either in light- or in dark-incubated pigeons, in the attentional focus between the left and right eye, however, the attentional shift between the groups differs significantly. The light-incubated pigeons cross the midline more often to the visual hemifield contralateral to the seeing eye than dark-incubated pigeons do. This result could hint at a wider frontal visual field in light- than in dark-incubated pigeons (Fig. 4.13 and Fig 4.14). As already mentioned, in birds, visual information is processed via two ascending pathways the tectofugal and thalamofugal pathway. The thalamofugal system primarily processes
4.4 Discussion

Figure 4.13: Representation of the left and right visual field within the tectofugal system under left eye vision A and right eye vision B condition in light-incubated pigeons. Circles represent the left field and rectangles the right field. Dotted-lines represent the frontal visual field of one eye. Visual information is processed via the eyes to the optic tectum (TO), to the rotundus (RT), to the Entopallium (E). Dashed-lines represent weaker projections in comparison to the others. Red edging indicates the dominant hemisphere. Gray circles or rectangles illustrate a weaker representation within the respective structure resulting from weaker projections.

Visual cues from the lateral monocular visual field and the tectofugal system from the frontal binocular field [157]. The origin of these projections is also visible in a different morphological organization of the retina. So, the TO of the tectofugal system primarily receives afferents from the red field [157]. In contrast, the retinal ganglion cells outside the red field send efferents to the thalamofugal GLD [157], which render the thalamofugal pathway largely frontally blind [70]. The red field can be morphological separated from the surrounding area due to a higher cell density.
and synaptic complexity [70], [204]. Several studies confirm that light-dependent cell activity affect the development of cell morphology [176], sprouting and branching of axon collaterals [120], and number of projecting cells [154], [165]. Thus, it is possible that also the organization of the red field in the pigeon retina is strongly influenced by light, possibly resulting in lesser efferents from the red field into the tectofugal system in dark-incubated pigeons and accordingly to a smaller visual field than in light-incubated pigeons. This could explain the difference between light-
dark-incubated pigeons in the visuospatial attention under monocular conditions, and may indicate that peripheral processing affects attention.

4.4.6 Task-Dependent Changes

In a modulated version of the cancellation task, we combined the testing for visuospatial attention [42] with the grain-grit task for testing discrimination abilities [75]. Based on the assumption that attentional processes controlled by the thalamofugal system [17], [196] (see discussion above section) and the ability to discriminate differences in pattern, color, or intensity is processed in the tectofugal system [85], we would expect a necessary functional interaction of this two ascending visual systems for the grain-grit cancellation task. The combination of these two tasks in chicks shows a disappearance of the previous visible bias to the left visual field. This result is explained by the activation of the left hemisphere, which is dominant for fine discrimination and stimulus categorization [5], and counteracts the right-hemispheric dominance for attentional processes [25]. In light-incubated pigeons we find no difference in visuospatial attention comparing the two test conditions. This result can be explained by the fact that in pigeons, as opposed to chicks, visuospatial attention is primarily processed by the left and not the right hemisphere (see discussion above), hence both attentional and discrimination processes should be processed in the same hemisphere. Therefore, we expect no conflict between the hemispheres resulting in a different attentional bias between the pea-cancellation and grain-grit cancellation task. Additionally, this result could be a hint that different tasks lateralized in the same hemisphere do not interfere with each other. Interestingly, in dark-incubated pigeons, the strong attentional bias to the right hemifield in the pea-cancellation task is decreased resulting in no significant bias to the left or right hemifield. Due to the fact that left-hemispheric dominance for discrimination vanishes after dark-incubation [176], this result could indicate a task-dependent modulation of the attentional focus, which is mediated by an interaction of lateralization processed in the same hemisphere. Possibly, the addition of a discrimination component could lead to a stronger attention to the lateral visual field, or rather a more symmetrical view of the visual scene. This view is supported by the results under monocular vision in the grain-grit cancellation task. In the grain-grit cancellation task both light- and
dark-incubated pigeons show stronger inattention to the frontal binocular field than in the pea-cancellation task. Additionally, because within light- and dark-incubated pigeons, the width of the frontal binocular field changes depending on the task, this cannot be explained by possible light-dependent structural changes of the red field within the retina (see discussion above in section 4.4.5). Interhemispheric interactions could potentially control the attention extension. The transection of the telencephalic anterior commissure demonstrates a variation in the lateralization quotient in comparison to pigeons with an intact anterior commissure. This result could imply that the anterior commissure influence the transfer of information between the hemispheres and dynamically modulates the direction of attention depending on the task condition.

4.4.7 Conclusion

In summary, the data indicate that in pigeons, attentional processes are controlled by an endogenously determined left-hemispheric dominance. The light-dependent structural changes within the ascending visual pathways provide the foundation for how much information from the left and right visual hemifield arrives the dominant left hemisphere. Additionally, the direction of attention is modulated by interhemispheric interactions of the anterior commissure. This commissural system can modulate the attentional focus in a dynamic way depending on the task that is performed.

4.4.8 Outlook

Several open questions derive from the discussion above:

The monocular data demonstrate a general difference in the attention focus between light- and dark-incubated pigeons. It remains a question, whether this difference is a result due to light dependent changes on a basal sensory level or if this difference is mediated on higher processing. This question can be answered by analysis of the retinal morphology in light- and dark-incubated pigeons. On higher processing level, commissural systems could mediate a dynamic modulation of attention to the left or right hemispace. Due to the small sample size of commissurotomized pigeons in this study, a clear impact of the AC onto attention cannot be deviated. Therefore, the cancellation task should be repeated with a higher number of commissurotomized
pigeons.
CHAPTER 5

General Discussion

5.1 Summary of the Main Results

- **Light-Dependent Development of Tectorotundal Projection - Emergence of Bottom-Up Asymmetries:**

  This study address the question of to what extent projection asymmetries in pigeons depend on asymmetric light-stimulation as a morphological substrate of lateralized visual processing.

  Only light-incubated pigeons show left-right differences in the tectorotundal projection. In dark-incubated pigeons this asymmetry is absent, indicating a light-dependent development of structural bottom-up asymmetries. This leads to a higher amount of fibers stabilized within the stronger activated left hemisphere. However, the stabilization of fibers acts by an indirect effect, since especially contralateral projecting cells from the right tectum as well as ipsilateral cells from the left tectum are affected. The development of this projection is prolonged beyond hatching, indirect mechanisms might be mediated by inhibitory circuits and retrograde trophic effects leading to enhanced stabilization of fibers innervating the left rotundus.

  This pattern must be explained by a step-wise development of the visual system. First retinal fibers innervate the tectum followed by the emergence of descending tectobulbar, tectospinal and interhemispheric tectotectal fiber tracts. The tectorotundal projections develop slowly in comparison to the other projections,
continuing beyond hatching [124], [125]. Prior to hatching, due to asymmetrical light-stimulation, inhibitory tectal circuits can be asymmetrically activated and thus induce the asymmetrical innervation of the left and right RT. After hatching the contralateral projection from the right TO to the left RT can be in presence of a symmetrical light input strengthen by an asymmetrical tectotectal interaction and a positive retrograde trophic effect of the left RT. As a result, the projection asymmetry is not created by a regression in the contralateral projection from the left TO to the right RT due to inhibitory circuits, but by a progression in the projection from the right TO to the left RT.

- **Structural Organization of the Anterior Commissure - The Role of Commissural Systems:**

  This neuroanatomical tract tracing study unravels the connectivity pattern of the anterior commissure as the major commissural system in the avian telencephalon. This knowledge is a necessary prerequisite for an understanding of the functional organization of lateralized processing.

  In summary, the study demonstrates that the anterior commissure of pigeons interconnects a wide network of telencephalic structures. The telencephalic commissures in mammals and birds overlap in some aspects of organization, but also evince several important differences. In contrast to the mammalian AC, the avian AC interconnects no olfactory areas. Furthermore, in birds, interhemispheric information is integrated at a later processing stage due to the absence of interconnections between primary sensory areas. But the main differences in the interhemispheric connectivity between birds and mammals are found at two levels of structural organization. First, the AC in birds differs from the CC and the AC of mammals in its proportion of homotopic reciprocal to heterotopic unidirectional projections. Unlike mammals, in birds, only a small amount of cells interconnect the two hemispheres in a homotopic and reciprocal fashion. Instead, most of the cells project heterotopically and in unidirectional manner. Second, in birds, the absolute majority of pallial areas do not participate by themselves in interhemispheric exchange. Instead, a rather
small arcopallial and amygdaloid cluster is the key mediating commissural interactions.

- **Lateralized Visuospatial Attention - The Role of Embryonic Light-Stimulation:**

This behavioral study deals with the question of to what extent embryonic light-stimulation affects the balance of left-right-hemispheric dominance as indicated by visuospatial attention.

In contrast to previous studies, the present data do not demonstrate an attentional bias to the left in light-incubated pigeons, as indicated by symmetrical pecking pattern in the visual hemifields. Instead, dark-incubated birds display an attentional bias to the right visual hemifield.

In summary, our data indicate that in pigeons attentional processes are controlled by an endogenously determined left-hemispheric dominance. This hemisphere produces by default an attentional bias to the right visual hemifield. This pattern is modulated by embryonic light-stimulation and might be related to the emergence of structural asymmetries in the ascending visual pathways. The resulting more complete representation of both hemifields enables the left hemisphere to allocate attention to both hemifields. Thus, light does not change the dominance of one hemisphere, but rather access to visual information resulting in more complete control over visuomotor behavior. However, this pattern is modulated by task-complexity. An additional discrimination component modifies the pattern. This effect points to critical role of interhemispheric systems. In the pigeon, this interhemispheric integration is probably mediated by the anterior commissure.

In summary, the data of the different studies in the present thesis indicate complex interactions of endogenous and environmental (light) factors, which determine the lateralized functional organization of the pigeon’s visual system. As such, ascending (tectorotundal projection, Study 1, chapter 2) and interhemispheric (anterior commissure, Study 2, chapter 3) projections affect the direction and degree of lateralization at the functional level (visuospatial attention, Study 3, chapter 4).
5.2 Development of Structural Asymmetries

The origin of most visual lateralization in birds is an asymmetric light stimulation before hatching. This asymmetric sensory experience can be traced back to a postural asymmetry of the body, which presumably is encoded in the asymmetric activation of early embryonic gene cascades [128], [161], but may also affect asymmetrical expression of neuronal genes and may be related to the generation of endogenous neuronal asymmetries (for review see [31]). In several vertebrates, an asymmetrical distribution of gene products determine for an asymmetrical bending of the embryonic body, which results in an invariant posture asymmetry of the embryo [30], [205] and thus an asymmetrical exposure of environmental sensory input. In birds, this asymmetrical body posture brings the right eye close to the translucent eggshell, while the left eye is occluded by the body and therefore, naturally deprived of light-stimulation [113]. This asymmetric light-stimulation correlates with the emergence of structural and functional asymmetries in the visual system of birds [73], [127], [130]. Alteration in the light conditions affect the development of these lateralization, so the incubation in complete darkness prevents the occurrence of hemispheric-asymmetries in visual object discrimination [39], [176] and structural left-right differences in the ascending visual system (see Study 1, chapter 2) [124], [125], [163], [176]. These results suggest a strong correlation of functional and structural asymmetries in bottom-up systems. Because the visual systems of precocial chickens and altricial pigeons mature with different developmental speeds and hence, with presumably differential sensitive phases for light experiences, an asymmetrical light trigger shortly before hatching exerts differential effects in both species. While it is the thalamofugal system that is sensitive to photic-stimulation before hatching [163], it is the tectofugal one in pigeons, which is still plastic after hatching [124], [125]. It is thought that species differences are determined by the stage of development of each visual system at the time of light exposure [39]. Ströckens et al. (2013) demonstrated that no asymmetry has been found in the thalamofugal system of pigeons regardless of light-exposure, dark-rearing or exposure of either the left or right eye [178]. Thus, the asymmetrical development of different visual systems is a clear species-dependent difference. These results show that in birds visual experiences with the environment
shape the structural maturation of the visual system. Thereby, species-dependent
differences in pathways of the visual system are affected, suggesting that species-
dependent different systems to different time points are sensitive to environmental
factors.

This raises questions concerning the interrelations between structural and functional
asymmetries, which will be addressed in the following section.

5.3 Emergence of Functional Lateralization

5.3.1 Structure and Function Correlations

The functional lateralization pattern, which is visible in the behavioral output, is not
only a reflection of bottom-up projections. Rather, it is a combination of interacting
bottom-up and top-down mechanisms. As discussed above, the ascending sensory
systems in birds demonstrate structural asymmetries. These structural asymmetries
are traced back to an asymmetric sensory input (see Study 1, chapter 2), and highly
correlate with functional asymmetries. However, no structural asymmetries within
the telencephalon or top-down projections are known in birds [123].

Several visual asymmetries which bear on higher cognitive processing mechanisms
are induced by light. Light-incubated chicks use their left hemisphere to distinguish
grain from pebbles and their right hemisphere to respond to predators, attack other
chicks and copulate (for review see [161]). Chicks incubated in complete darkness
lack these asymmetries. Chicks that have been manipulated so that the left eye of the
embryo is exposed to light while the right eye is occluded have reversed asymmetries
in that they use their right and not their left hemisphere to discriminate grain
from pebbles [160]. Similar effects of light on the development of functional visual
lateralization have also been shown in the pigeon. Light exposure of the embryo
induces specialization of the left hemisphere for distinguishing grain from grit [75],
categorizing stimuli, and decreasing in the right hemisphere visuomotor speed [176].
These specific visual functions are examples of lateralization, which correlates with
structurally lateralized bottom-up systems. Accordingly, a structural asymmetry
seems to critically affect the development of higher cognitive asymmetries.

However, in chicks and pigeons several functional asymmetries develop indepen-
Emergence of Functional Lateralization

Light- as well as dark-incubated chicks show a right hemispheric dominance for novelty detection [160], [163], visual choice in approaching a social partner [6], detouring around an obstacle [26], or preferential monocular sleep [132]. Additionally, the present thesis shows a light-independent lateralization for visuospatial attention in pigeons (see Study 3, chapter 4). These asymmetries clearly develop independent of structural asymmetries (see Study 1, chapter 2). The mismatch in structure-function correlation in light-independent visual asymmetries of higher cognitive processes suggest more complex mechanisms creating a functionally lateralized brain.

Evidence for lateralization of higher telencephalic functions in pigeons provide the functional asymmetry of top-down effects. A lateralized top-down effect is mediated by the visual wulst. The wulst is the primary visual projection area of the ascending thalamofugal pathway and, additionally, the source of the largest pallial descending fiber system, the TSM. The TSM-neurons, which are primarily localized within the visual wulst descend within the optic tectum [120]. As already mentioned in the general introduction (section 1.5.3) the optic tectum is a central relay station for visuomotor behavior, different components of visual processing converge in this structure. The optic tectum receives afferents from the retina, the contralateral TO, and the ipsilateral forebrain. It sends ascending visual projections to the forebrain and descending premotoric projections to the brainstem. Accordingly, telencephalic top-down effects allow the wulst to control tectal processing circuits [127]. The temporal inhibition of the left or right wulst demonstrates that the left wulst has a significantly higher impact on tectofugal processing than the right wulst [58], [183]. Since the number of telencephalotectal neurons within TSM demonstrates no difference between left and right [123], the differences observed in functional impact of the wulst do not derive from quantitative differences in the number of descending axons. This example shows that higher cognitive asymmetries are not necessary in order to exhibit structural asymmetries and that light is the primary factor affecting the structural maturation of bottom-up systems. Possibly, asymmetries of top-down processing may not necessarily be pre-wired but are coded in terms of synaptic strength [58]. Thus, the functional lateralization in dark-incubated birds without structural lateralization could be reflecting top-down mechanisms without
structural lateralization. However, this assumes that a lateralized top-down control is endogenously determined and does not develop in response to an asymmetrical light-stimulation. Certainly, the effect of sensory input onto lateralized top-down mechanisms is not yet known.

5.3.2 Development of Functional Asymmetries

As already mentioned, birds develop several functional visual asymmetries in response to an early embryonic light-stimulation. For the development of lateralization could this mean that asymmetric light-stimulation induces asymmetries on telencephalic level, which manifests functional asymmetries in the adult animal. It is possible that changes in gene-transcription could be involved in this process, as they induce not just transient but permanent functional left-right differences [54]. It is feasible that an increased neuronal activity, mediated by the asymmetric embryonic light-stimulation, affects the expression pattern of different genes by epigenetic factors in the more strongly stimulated hemisphere and thus long lasting changes are induced. In contrast, light-independent asymmetries cannot be explained by an epigenetically mediated asymmetric expression of different genes. Such functional asymmetries must have their origin in endogenously determined asymmetries. However, light-independent asymmetries are not completely unaffected by light-dependent processes. Light- and dark-incubated pigeons display a reversed pattern in accessing transfer information [119]. This example indicates that endogenously determined lateralization can be modified by light. This is also demonstrated by the present thesis (see Study 3, chapter 4). The present study demonstrates that the left-hemispheric dominance for visuospatial attention in pigeons develops independently of light. This pattern is modulated by embryonic light-stimulation, however not in that way that light shapes the emergence of this functional lateralization by the activation of epigenetic factors. Rather, the light-dependent structural asymmetries in the ascending visual pathways create a more complete representation of both hemifields within the left hemisphere, which enables the left hemisphere to allocate attention to both hemifields. Thus, light does not change the dominance of one hemisphere, but access to visual information resulting in more complete control over visuomotor behavior.

In summary, different mechanisms affect the development of functional asymmetries,
5.3 Emergence of Functional Lateralization

and as such the interaction of endogenous and epigenetic factors are critically involved in this process.

5.3.3 Interhemispheric Interactions

5.3.3.1 Functional Implications in the Pigeon Brain

Apart from examining bottom-up processes, several authors point out that the interhemispheric interactions critically influence the development and stabilization of cerebral asymmetries. In humans and other mammals these interhemispheric interactions are primarily mediated by the corpus callosum.

The corpus callosum is the largest fiber tract on telencephalic level interconnecting the two hemispheres and appears to play a role in numerous aspects of brain function. Its most important and unquestionable role is the integration of information from the left and right hemispace to create a complete view of the world via the visual, tactile and auditory modalities (for review see [146]). For the visual modality this means that since each hemisphere contains a representation of the contralateral visual hemifield, callosal fibers connect these two hemi-representations to a whole visual field. In the pigeon, due to the completely crossing optic nerve and crossing fibers on tectorotundal level, both hemispheres receive a complete presentation of the left and right visual hemifield (see also Study 3, chapter 4). Thus, an interhemispheric integration of information solely to create a representation of the whole visual field in both hemispheres is not necessarily needed. This also strongly suggests that interhemispheric connections are not solely used for an exchange of information. Possibly, commissural systems have an active role in mediating lateralized information processing. In birds, which do not possess a corpus callosum, it is known that the tectotectal commissural system on mesencephalic level mediates an asymmetric interaction [104]. The critical impact of this system onto the lateralized control of visuomotor behavior is demonstrated by the transection of this system. Güntürkün and Böhringer (1987) showed a reversed lateralization after transection of the tectotectal commissures [71]. In this process, the degree of transection critically influences the strength of lateralization reversal. This hints to a dynamic control of this system onto lateralized behavior. On the telencephalic level, the two hemispheres in birds are interconnected by the anterior commissure (see study 2, chapter 3)[207].
The functional impact of this system on lateralization is suggested in Study 3, chapter 4. The transection of the anterior commissure in pigeons suggests a modification of the attention pattern.

5.3.3.2 Structure and Function Interactions

The interhemispheric impact of the corpus callosum onto a lateralized information processing is controversy discussed, confronting both excitatory and inhibitory models. Excitatory models assume that lateralized information processing is not exclusively dependent on one hemisphere, rather cerebral functions often rely on a dynamic cooperation between the two hemispheres, which needs interhemispheric transfer [88]. Therefore, the basic excitatory model of callosal functions postulates a cooperative sharing of information between the hemispheres mediated by the corpus callosum [60]. Since the corpus callosum primarily connects homotopic areas, the activation of cell groups in one hemisphere stimulates the activation of the mirror image within the contralateral hemisphere. Thus, a system such as the corpus callosum is likely to increase the probability of common activation [170].

However, interhemispheric communication does not necessarily have to be an exclusively cooperative process. Other models suggest an inhibitory effect of callosal communication [37]. The corpus callosum may inhibit the activity of one hemisphere, thus allowing the other to take over the information processing. The role of the corpus callosum in this model is to maintain independent processing in the two hemispheres [146]. Accordingly, a high interhemispheric connectivity through the corpus callosum leads to an enhancement of lateralized information processing [146].

Based on the homotopic organization of the corpus callosum, the inhibitory processes acts not on the general hemisphere, but rather more locally. Cook postulates that the activation in one hemisphere generates an inhibition within the contralateral homologous region [32], [33]. In accord with this model, homotopic areas of the two hemispheres are involved in processing complementary aspects of information, and the negative relationship of the mirror image is established by selective inhibition mediated by the interhemispheric influence of the corpus callosum [146].

In a further theory, the excitatory and inhibitory influence of the corpus callosum is combined [27]. Because the interconnection of different functional areas is
characterized by different morphologies of the projecting fibers, of different functional modules of interhemispheric communication are derived [1]. Within restricted functional regions, differences in the fiber organization exist. Primary sensory and motoric areas tend to be interconnected by thicker fibers, and associative areas are interconnected by thinner fibers [1], [114]. The pattern of specific fiber types, which mediate specific interhemispheric processes, hint at the presence of different functional modules of interhemispheric communication [1]. The different modules possess different functional properties and conduction times [90], [145]. Accordingly, every functional module can locally modulate a specific functional lateralization and not centrally one hemisphere.

The theories for interhemispheric influence on functional lateralization have one thing in common; they base on the homotopic organization the corpus callosum. Thus, interhemispheric connections by the corpus callosum locally mediate an excitation or inhibition of the homogenous mirror region followed by more parallel or lateralized information processing, respectively. Due to the structural organization of the anterior commissure in pigeons, which is characterized by mostly heterotopic connections (see Study 2, chapter 3), no direct influence on homologous areas can be mediated. This kind of organization presumably enables generalized effects on the contralateral hemisphere [146]. Accordingly, when interhemispheric systems affect asymmetric information processing, this is not only traced back to a direct influence on homotopic areas.

5.4 Nature and Nuture Interaction

Several aspects of human typical hemispheric-specialization are also present in other vertebrate species [31], [147], [187]. In this way, the left hemisphere tends a categorical, serial, or analytic processing of local stimuli, and the right hemisphere works more in parallel or configural and tends to process global stimuli [43]. These correspondences hint at a common phylogenetic ancestor for cerebral asymmetry patterns and thus presumably genetically determined development [31], [34], [83], [186]. This assumption is also supported by the emergence of light-independent visual lateralization in birds. Genetic foundations are supported by inheritance models [7], [31], [83], [148], asymmetric expression of genes during embryogenesis,
and the early occurrence of anatomical and behavioral asymmetries [31], [83], [148]. Genetic factors presumably mediate asymmetric differentiation processes, which induce fundamental differences in hemispheric processing. The light-independent left-hemispheric lateralization for visuospatial attention in pigeons support the influence of genetic factors for the establishment of functional lateralization (see Study 3, chapter 4). However, it does not support the theory that a genetic determination increases the probability of species comprehensive similar directed lateralization. Our testing of visuospatial attention in pigeons revealed a left-hemispheric dominance, although in chicks and humans a right-hemispheric dominance is suggested [25], [133]. These species-specific differences in the direction of lateralization could be explained by two theories. On the one hand, this could raise the possibility that for efficient processing in the brain, the direction of lateralization is of minor importance and variability in species-specific lateralization pattern is already established on genetic level. On the other hand, the differences in lateralization pattern could be a result of species-specific divergent processing strategies. In the context of visuospatial attention, this implies that humans and chicks prefer to analyze different stimuli in a more global context resulting in a right-hemispheric lateralization, while pigeons analyze the stimuli in a more detailed way, resulting in left-hemispheric lateralization [130].

Further variability of lateralization pattern can be seen in interactions with environmental factors. In humans, studies with monozygotic twins show a high level of plasticity in the development of lateralization, which is in particular suggested by a divergent structural and functional lateralization pattern in these infants [51], [77], [83]. The animal model discussed here, especially the comparison of light- and dark-incubated birds, provides deeper insights into nature and nurture interactions. In particular, this highlights the influence of an asymmetrical environmental visual input. Additionally, recent studies demonstrate that not only an asymmetric sensory stimulation affects the development of lateralization, but also the cross-reaction of different modalities can change or enhance lateralization. The exposure of cuttlefish (Sepia officinalis) eggs to the odor of seabass, which is a predator of the species [99], enhances the left side turning bias in the cuttlefish hatchlings. This lateralized behavior is normally triggered by visual input, as indicated by a correlation of the
asymmetry of optic lobe size and leftward turning bias [99]. Accordingly, there must be a cross modality effect of visual and olfactory experiences during embryonic development. Furthermore, in humans the tactile stimulation of the left side of the body can enhance auditory detection on the left side, suggesting an interaction between input in these two modalities within the right hemisphere [86].

In summary, it is shown that the development of functional lateralization is affected by a critical interaction of genetic and environmental factors in which the interaction of sensory input from different modalities can shape the lateralization pattern.

5.5 Outlook
The present thesis answers some questions regarding the ontogenesis of structural asymmetries and its correlation to functional lateralization. Furthermore, the impact of interhemispheric systems on a lateralized information processing was analyzed. It is also now clear that this is only the beginning of understanding the complex interactions of genetic, environmental and structural factors controlling behaviorally relevant hemispheric asymmetries. Therefore, several interesting questions for future research can be asked based on the present results.

- As pointed out in the general discussion, the functional lateralization results from an interaction of lateralized bottom-up and top-down mechanisms. In contrast to the top-down system, the bottom-up pathway demonstrated obvious light-dependent structural asymmetries, which clearly reflects the environmental impact on the sensory pathway. However, whether environmental factors also shape top-down mechanisms is unclear. To answer this question, the emergence of lateralized top-down effects should be analyzed in dark-incubated pigeons.

- As pointed out in the general discussion, it is highly likely that forms of lateralization are determined by partly independent endogenous mechanisms that interact with environmental factors. Thereby, it is not clear how bottom-up asymmetries are translated into higher processing lateralization. As suggested in the discussion, it is plausible that asymmetrical environmental influences mediate activity differences on telencephalic level, resulting in an altered
expression pattern of different genes in the left and right hemisphere. To substantiate this epigenetic influence, changes in the methylation-pattern of visual structures on the telencephalic level should be analyzed in light- and dark-incubated pigeons.

- Furthermore, the interhemispheric impact on lateralized information processing is still a riddle. The present study can not clearly define the impact of telencephalic commissures on lateralized information processing and especially not on the development of lateralization. Further lesion studies in light-incubated adult pigeons, where the anterior commissure is transected, can help to define the functional impact on lateralized behavior. Transection of the anterior commissure in hatchlings, while the brain is still plastic, can give deeper insight into the developmental impact of telencephalic commissures on functional lateralization.

5.6 Conclusion

The aim of the present thesis was to investigate the influence of ontogenetic and interhemispheric factors on lateralized information processing on an anatomical and behavioral level. The anatomical analysis of the visual system in pigeons reveals that an asymmetrical light stimulation before hatching induces quantitative changes in the ascending visual pathway, implying that sensory input in a first step affects the structural maturation of sensory systems. As such, structural asymmetries within ascending systems can mediate different functions. On the one hand, they can asymmetrically activate epigenetic signaling cascades on a telencephalic level and thus directly affect the establishment of asymmetries of higher functions. On the other hand, functional lateralization can be genetically determined and develop independently of an asymmetric sensory stimulation and the resulting structural alterations. However, the structural organization of sensory systems controls the sensory information to the dominant hemisphere. The impact of commissural systems on lateralized information processing are less clear. Possibly, interhemispheric interactions can dynamically control the direction of asymmetries. The comparison of the anterior commissure in birds and the corpus callosum in mammals suggests evolutionary development from a generalized interhemispheric effect to a more and
more structural and functionally specific interaction between the two hemispheres. In summary, the emergence of a functional lateralized brain is the result of complex interactions of genetic, environmental and interhemispheric components.
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## APPENDIX A

### Abbreviations

#### A.1 Abbreviations of Brain Structures

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<tbody>
<tr>
<td>AA</td>
<td>arcopallium anterius</td>
</tr>
<tr>
<td>AC</td>
<td>commissura anterior</td>
</tr>
<tr>
<td>AD</td>
<td>arcopallium dorsale</td>
</tr>
<tr>
<td>AI</td>
<td>arcopallium intermedium</td>
</tr>
<tr>
<td>AIdm</td>
<td>arcopallium dorsomediale</td>
</tr>
<tr>
<td>AIvm</td>
<td>arcopallium ventromediale</td>
</tr>
<tr>
<td>AM</td>
<td>arcopallium mediale</td>
</tr>
<tr>
<td>AV</td>
<td>arcopallium ventrale</td>
</tr>
<tr>
<td>Bas</td>
<td>nucleus basorostralis pallii</td>
</tr>
<tr>
<td>CC</td>
<td>corpus callosum</td>
</tr>
<tr>
<td>CP</td>
<td>commissura posterior</td>
</tr>
<tr>
<td>CT</td>
<td>commissura tectalis</td>
</tr>
<tr>
<td>E</td>
<td>entopallium</td>
</tr>
<tr>
<td>GLD</td>
<td>nucleus geniulatus lateralis, pars dorsalis</td>
</tr>
<tr>
<td>HA</td>
<td>hyperpallium apicale</td>
</tr>
<tr>
<td>HD</td>
<td>hyperpallium densoceullare</td>
</tr>
<tr>
<td>HI</td>
<td>hyperpallium intercalatum</td>
</tr>
<tr>
<td>HL</td>
<td>hyperpallium laterale</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>HOM</td>
<td>tractus occipitomesencephalicus, pars hypothalami</td>
</tr>
<tr>
<td>LSt</td>
<td>striatum laterale</td>
</tr>
<tr>
<td>MD</td>
<td>mesopallium dorsale</td>
</tr>
<tr>
<td>MFD</td>
<td>mesopallium frontodorsale</td>
</tr>
<tr>
<td>MFV</td>
<td>mesopallium frontoventrale</td>
</tr>
<tr>
<td>MID</td>
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</tr>
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<td>striatum mediale</td>
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<tr>
<td>MV</td>
<td>mesopallium ventrale</td>
</tr>
<tr>
<td>NCC</td>
<td>nidopallium caudocentrale</td>
</tr>
<tr>
<td>NCL</td>
<td>nidopallium caudolaterale</td>
</tr>
<tr>
<td>NFL</td>
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<tr>
<td>NFM</td>
<td>nidopallium frontomedial</td>
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<td>nidopallium intermedium</td>
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<tr>
<td>NIL</td>
<td>nidopallium intermedium lateralis</td>
</tr>
<tr>
<td>OB</td>
<td>bulbus olfactorius</td>
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<tr>
<td>OM</td>
<td>tractus occipitomesencephalicus</td>
</tr>
<tr>
<td>PoA</td>
<td>posterior pallial amygdala</td>
</tr>
<tr>
<td>RT</td>
<td>nucleus rotundus</td>
</tr>
<tr>
<td>ThA</td>
<td>nucleus taeniae of the pallial amygdala</td>
</tr>
<tr>
<td>TSM</td>
<td>tractus septomesencephalicus</td>
</tr>
<tr>
<td>TO</td>
<td>optic tectum</td>
</tr>
<tr>
<td>TPO</td>
<td>area temporo-parieto-occipitalis</td>
</tr>
</tbody>
</table>

**A.2 Further Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
</tr>
<tr>
<td>BI</td>
<td>bilaterality index</td>
</tr>
<tr>
<td>CTB</td>
<td>cholera toxin subunit B</td>
</tr>
<tr>
<td>DAB</td>
<td>3′3′-diaminobenzidine</td>
</tr>
<tr>
<td>g</td>
<td>gramm</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma-aminobutyric-acid</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>hydrogen peroxide</td>
</tr>
<tr>
<td>IgG</td>
<td>immunoglobulin G</td>
</tr>
<tr>
<td>LQ</td>
<td>lateralization quotient</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>ml</td>
<td>milliliter</td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>nl</td>
<td>nanoliter</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate-buffered saline</td>
</tr>
<tr>
<td>PFA</td>
<td>paraformaldehyde</td>
</tr>
<tr>
<td>pH</td>
<td>pondus hydrogenii</td>
</tr>
<tr>
<td>TTX</td>
<td>tetrodotoxin</td>
</tr>
<tr>
<td>μm</td>
<td>micrometer</td>
</tr>
</tbody>
</table>
APPENDIX B

Chemicals and Equipment

B.1 Solutions

Acetate-buffer \((0.2 \text{ M}, \text{pH 5.8})\)

\[
16.4 \text{ g sodium-acetate dissolve in 1 l A. dest and pH adjust to 5.8 with acetic acid.}
\]

Acetate-buffer \((0.1 \text{ M}, \text{pH 6.0})\)

\[
0.2 \text{ M acetate-buffer 1/1 dilute with A. dest and pH adjust to 6.0 with acetic acid.}
\]

DAB-Solutions

25 ml solution A, 20 ml solution B, 5 ml Solution C:

- Solution A: 5 g ammonium-nickel(II)-sulfate and 800 mg \(\beta\)-D-glucose dissolve in 100 ml acetate-buffer (0.2 M).
- Solution B: 20 mg DAB dissolve in 80 ml A. dest (20 mg = 2 pellets).
- Solution C: 80 mg ammonium-chlorid and 80 mg cobalt(II)-chlorid(x 6 \(\text{H}_2\text{O}\)) dissolve in 20 ml A.dest.

Paraformaldehyde-Solution 4% (PFA)

\[
40 \text{ g paraformaldehyde dissolve in 800 ml A. dest at 60^\circ\text{C}. Add 6 drops NaOH and wait till the solution is clear. Add 200 ml 0.6 M PBS, filtrate the solution and cool it down to 4^\circ\text{C}.}
\]
**Phosphate-Buffered Saline** *(PBS; 0.12 M, pH 7.4)*

Dilute PBS-stock-solution (0.6 M) 1/5 with 0.9 % sodium-chloride. For 1 l 0.6 M stock-solution dissolve 87.09 g K₂HPO₄ and 13.80 g NaH₂PO₄·H₂O in 1 l A. dest; adjust pH with hydrochloric acid to 7.4.

**Storage-Buffer**

Dilute 0.1 % sodium azide with 0.12 M PBS.
B.2 Chemicals

acetic acid Merck, Darmstadt, Germany
ammonium-chlorid Sigma, Steinheim, Germany
ammonium-nickel(II)-sulfate Sigma, Steinheim, Germany
cobalt(II)-chlorid-hexahydrate Merck, Darmstadt, Germany
3’3-diaminobenzidine Sigma, Steinheim, Germany
dolorex Intervet, Unterschleißheim, Germany
ethanol Merck, Darmstadt, Germany
fluoromount SouthernBiotech, Eching, Germany
β-D-glucose Sigma, Steinheim, Germany
β-D-glucose-oxidase Sigma, Steinheim, Germany
heparin Ratiopharm, Ulm, Germany
hydrogen-peroxide J.T. Baker, Deventer, Netherlands
isoflurane abvie, Ludwigshafen, Germany
isopropanol Merck, Darmstadt, Germany
paraformaldehyde Prolabo, Leuven, Belgium
sodium-acetate J.T. Baker, Deventer, Netherlands
sodium-chloride J.T. Baker, Deventer, Netherlands
sodium-hydroxide Merck, Darmstadt, Germany
sodium-phosphate J.T. Baker, Deventer, Netherlands
succrose J.T. Baker, Deventer, Netherlands
triton-X-100 Sigma, Steinheim, Germany
xylene J.T. Baker, Deventer, Netherlands
xylocain Bayer, Leverkusen, Germany
B.3 Equipment

- anesthesia-machine MDI, Victoria, Australia
- AxioCam MRM Zeiss MicroImaging, Göttingen, Germany
- AxioImager M1 Microscope Zeiss MicroImaging, Göttingen, Germany
- AxioVison 4.8 Zeiss MicroImaging, Göttingen, Germany
- IBM SPSS 20 IBM software, Germany
- ImageJ Image Analysis in Java, open source
- microtome RM2136 Leica Microsystems, Wetzlar, Germany
- video-camera Sony DCR-SR210
- WPI Nanoliterinjector World Precision Instruments, Berlin, Germany
- LSM 510 Meta Confocal Microscope Zeiss MicroImaging, Göttingen, Germany
Publications

Submitted Manuscripts


Submissions to international conferences


Submissions to national conferences

- **Manns M., Patzke N., Verhaal J., Letzner S.** (2012). Ontogenetic light experience induces lateralized information transfer in pigeons by affecting the mesencephalic commissural system. *FENS Forum of Neuroscience*, Barcelona,

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Danksagung

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