The canonical circuit of the avian forebrain

Inaugural - Dissertation
zur
Erlangung des Grades eines Doktors der Naturwissenschaften
in der
Fakultät für Psychologie
der
RUHR - UNIVERSITÄT BOCHUM

vorgelegt von

Martin Stacho
aus Bochum

Bochum, Mai 2017
Referent: Prof. Dr. Onur Güntürkün
Korreferent: Prof. Dr. Sen Cheng
# Table of Contents

**Abstract**

**1. Introduction**
- The neocortex
  - Structure and circuitry
  - How does the cortex develop its structure?
  - Function of cortical circuits
- Pallium of birds and Karten’s hypothesis
  - Sensory systems and their circuitry in the avian pallium
    - The auditory system
    - Visual System
      - Visual tectofugal system
      - Visual thalamofugal
    - Somatosensory
      - Trigeminal
      - Thalamofugal somatosensory
  - Functional insights into pallial circuits in birds
  - Developmental aspects of the avian pallium
- The mammalian hippocampus
  - The hippocampal circuitry
  - Topography
  - Function
- The avian hippocampus
  - Circuitry of the avian hippocampus
  - Topography
  - Function
- Aim of the study
- Expectations

**2. Material and Methods**
- Animals
- In-vivo tracer application and surgical procedure
In-vitro experiments ................................................................. 31
Histology and Immunohistochemistry ............................................. 31
  2.4.1 Perfusion and brain slicing .............................................. 31
  2.4.2 DAB staining .................................................................... 32
  2.4.3 Fluorescence staining .......................................................... 33
Microscopy and analysis ................................................................. 33

3. Results ................................................................. ................................. 34
Visual tectofugal system ................................................................. 34
The trigeminal system ......................................................................... 44
The auditory system ........................................................................... 51
The wulst .................................................................................................. 56
Summary of the connectivity within the sensory systems ..................... 66
Hippocampus .......................................................................................... 68
  3.6.1 Summary of the in vivo experiments ........................................ 68
  3.6.2 In vitro injections ..................................................................... 73
    3.6.2.1 DL .......................................................................................... 73
    3.6.2.2 DM .......................................................................................... 76
    3.6.2.3 V-complex .............................................................................. 78

4. Discussion ................................................................. ............................... 80
Sensory systems – comparison to previous studies ................................... 80
  4.1 The visual-tectofugal system .................................................. 80
  4.1.2 The auditory system ............................................................. 83
  4.1.3 The trigeminal system ........................................................... 85
  4.1.4 The Wulst ................................................................................. 85
  4.1.5 Connectivity, cortical principles and the avian forebrain ............. 87
    4.1.5.1 Local circuits, layers and columns ......................................... 87
    4.1.5.2 The physiology and function of local circuits - the interplay between excitation and inhibition .......................................................... 90
Hippocampus .......................................................................................... 93
  4.2.1 The circuits and topography .................................................. 93
  4.2.2 Topography and functional speculations ....................................... 95
  4.2.3 Network properties of the hippocampus in birds and mammals .......... 96
4.2.4 The main hippocampal pathway and its comparison to mammals................. 102
4.2.5 Did birds evolve a complex circuitry different from the mammalian
trisynaptic pathway? ...................................................................................................... 103
4.3 What did we learn about the evolution of the forebrain? ........................................ 106

5. Concluding remarks....................................................................................................110

6. Future studies ...........................................................................................................112

7. References ..................................................................................................................114

8. Appendix ..................................................................................................................148
  8.1 Supplementary figures ................................................................. 148
  8.2 List of figures ...................................................................................... 159
  8.3 List of chemicals ............................................................................. 161
  8.4 Abbreviations .................................................................................. 163

Acknowledgements/Danksagung .............................................................................165

ERKLÄRUNG.........................................................................................................167
Abstract

Last decades of comparative neurobiological research tremendously changed our view on the avian forebrain. Anatomical, electrophysiological and gene expression studies strengthened the conjectures that the avian and mammalian pallia utilize similar organization despite massive macroscopical differences. We performed a comprehensive study of local circuitry in the avian forebrain to elucidate its connectivity principles and hence to better understand its organization and evolution. We used anatomical in vivo and in vitro tracings and focused on the hippocampus, the hyperpallium, as well as the trigeminal, visual and auditory regions of the avian dorsal ventricular ridge. We found that the avian hippocampus comprises a complex recurrent circuitry and identified a putative main forward pathway based on the connectivity strength, which displayed a clear topographical organization. However, many tentative pathways with extensive feedback projections exist within the avian hippocampus. Looking behind the borders of the well-established framework of the mammalian trisynaptic pathway, we emphasize that those are also peculiar features of the mammalian hippocampus. Moreover, our results showed that the avian hyperpallium and the dorsal ventricular ridge contain local circuitry which appears stunningly comparable to the mammalian neocortical circuits. The circuits interconnect different cell populations organized in layers whereby the axons running between the layers form impressive anatomical columns. We conclude that specific connectivity principles probably evolved before the divergence of mammalian and avian lineages and were conserved and further refined in both species. Reptilian cortex suggests that birds and mammals independently segregated genetically defined subtypes of neurons into several layers. The neurons within the superficial layers lost their extratelencephalic projections and acquired intratelencephalic targets providing an anatomical basis for the columnar organization. Despite a controversy about the homologies of specific avian pallial areas to the mammalian forebrain, these data support the original hypothesis of Harvey Karten about the homology of pallial circuits and cell types between birds and mammals. Thus, our study yields an important contribution to the theories in the evolutionary and comparative neurobiology but also furnished an anatomical foundation for further modern studies, which can seek for brain mechanism at the circuitry level.
1. Introduction

The avian brain has puzzled comparative neuroanatomist for centuries. The biggest riddle has been the comparability and homology of the forebrain to the mammalian counterpart. Why? The answer is simple: The avian brain does not have a cortex. Indeed, it looks like the mammalian and avian forebrains have almost nothing in common (Figure 1). If we know that mammals and birds share a common ancestor, who already had a forebrain, then how do we relate such dissimilar structures of modern animals to each other? While the majority of the mammalian telencephalon is covered by a six-layered neocortex divided by a thick band of white matter from the basal ganglia, in birds the situation is different. Here, we see a weakly laminated dorsomedial part called hyperpallium (or wulst), with a ventrally adjacent non-laminated nuclear structure protruding into the lateral ventricle called accordingly the dorsal ventricular ridge (DVR). The DVR can be further subdivided into mesopallium, nidopallium and the more caudoventrally positioned arcopallium (Reiner et al., 2004). Until one and a half decades ago, the majority of the avian forebrain structures were entitled with names, which indicated that these structures are purely of striatal nature. This classical view of the forebrain evolution originated from the opinions of early comparative neuroscientists around the turn of the twentieth century, such as Ludwig Edinger, who combined Darwin’s ideas about the evolution with the Aristotle’s concept of “scala naturae” (Striedter, 1997; Northcutt, 2001; Jarvis et al., 2005). According to this view of evolution as a unilinear and progressive process, the avian brain is only capable of instinctive behavior owing to the prevailing belief at that time that the majority of the avian telencephalon is constituted by basal ganglia. A more sophisticated behavior, as seen in mammals, shall require a unique brain structure only found in mammals, the so called neocortex. Towards the end of the twentieth century, this view started to change (Reiner et al., 2004; Jarvis et al., 2005). It was recognized that bird’s behavior is much more sophisticated than previously thought and that the basal ganglia constitute a much smaller portion of the avian telencephalon, the rest being of pallial nature (Karten, 1969; Reiner, 2004; Shimizu, 2009; Striedter, 2013; Güntürkün and Bugnyar, 2016). However, different opinions have been developed about the homology of the avian and mammalian pallium (Karten, 1969; , 1997; Bruce and Neary, 1995; Striedter, 1997; Puelles et al., 2000; Puelles, 2001; Butler and Molnár, 2002; Puelles
and Medina, 2002; Reiner et al., 2005; Butler et al., 2011; Puelles, 2011; Puelles et al., 2016). The findings that the avian nido- and mesopallium develop from pallial sectors which in mammals give rise to the pallial amygdaloid and claustral nuclei led people recognize that a theory of a direct homology between the neocortex and the avian pallium can not fully account for the data (Fernandez et al., 1998; Striedter et al., 1998; Puelles et al., 2000). Several researchers argued that nido- and mesopallium of birds are homologous to the mammalian claustro-amygdaloid complex based on their connectivity (Bruce and Neary, 1995; Martinez-Garcia et al., 2002; Moreno and Gonzalez, 2007) and gene expression patterns (Puelles et al., 2000; Puelles, 2001). Puelles et al. (2016) also suggested a homology of the mesopallium to the mammalian insula. Others tried to reconcile the discrepancies and proposed that the avian (and reptilian) DVR are homologous as a field to a set of structures in the mammalian brain including the amygdala, claustrum and the lateral cortex without postulating one-to-one homologies (Butler and Molnár, 2002). Similarly, instead of an attempt to find one-to-one homologies, Aboitiz stressed that the mammalian and avian pallia share conserved genetic and developmental mechanisms, which became differently modulated in these species (Aboitiz, 2011; Aboitiz and Zamorano, 2013). The amplification of different sets of regulatory genes led to the formation of the neocortex and rather expanded dorsal pallium in mammals, while in birds changes occurred which favored the expansion of the DVR. In order to figure out similarities and differences between the mammalian and avian forebrain, we briefly outline the structure and function of the neocortex followed by a similar description of the avian pallium. Since the brain development during the embryogenesis can help us to understand the structural organization of different brain areas, and provide us with important insights into the evolution and homology, we also included some developmental aspects in the description. In the end, we turn to the phylogenetically older part of the pallium, the hippocampus, and outline its structural and functional organization in both birds and mammals.
1.1 The neocortex

1.1.1 Structure and circuitry

The neocortex is constituted by six layers composed of different neuron types including spiny pyramidal, spiny stellate as well as a class of aspiny nonpyramidal neurons (DeFelipe and Fariñas, 1992). The spiny pyramidal neurons are the principal neurons of the cortex. With few exceptions, these neurons, which can vary in size, dendritic and axonal processes as well as in spine density and connectivity, are the major projection neurons of the cortex. The high degree of similarity in the cellular arrangement of the cortex across areas and species sparked the idea of an universal canonical excitatory circuit of the neocortex (Douglas et al., 1989; Douglas and Martin, 1991; Thomson and Bannister, 2003; Douglas and Martin, 2004; Bannister, 2005; Douglas and Martin, 2007a, b; Bastos et al., 2012; Harris and Mrsic-Flogel, 2013; Harris and Shepherd, 2015). The circuit begins in the layer 4, where the thalamic input from sensory relay nuclei mainly arrives (Figure 2). Although, this is quantitatively not the main input of this layer (Binzegger et al., 2004), these afferents can nevertheless drive the responses of the neurons in this layer, possibly via synchronized firing.
INTRODUCTION

(Bruno and Sakmann, 2006). The lamina 4 neurons forward the information to all layers but mainly to the superficial layers 2 and 3 (Harris and Mrsic-Flogel, 2013). Neurons in layer 4 receive only little excitatory feedback from other layers but seem to possess area-specific specializations for processing external thalamic input or input from other cortical areas (Harris and Shepherd, 2015). Layer 2/3 neurons integrate the layer 4 input with multiple inputs from different cortical areas and give rise to a major descending intralaminar axon branching extensively especially in the layer 5 (Feldmeyer, 2012; Petersen and Crochet, 2013; Harris and Shepherd, 2015). In addition, they distribute the information to higher cortical areas including the contralateral side (Feldmeyer, 2012). Layers 5 and 6 are the sources of long-range connections to other cortical and subcortical areas including the thalamus and striatum (Guillery and Sherman, 2002; Sherman and Guillery, 2006; Feldmeyer, 2012; Harris and Mrsic-Flogel, 2013). It is thought that these circuits between excitatory neurons across the six layers form a repeating basic pattern which is modified differently in different areas and species (Harris and Shepherd, 2015).

The excitatory circuitry is embedded in a complex inhibitory network which can significantly shape and control the responses of the pyramidal neurons (Gupta et al., 2000; Martin, 2002; Markram et al., 2004; Hestrin and Galarreta, 2005; Petersen and Crochet, 2013; Pi et al., 2013; Hu et al., 2014; Tremblay et al., 2016). The inhibitory interneurons are an at least as diverse class as the excitatory ones. They differ in their morphology, laminar distribution, physiological properties, gene expression as well as their innervation patterns (Markram et al., 2004; Tremblay et al., 2016).

Together principal neurons and interneurons form multiple highly structured subnetworks in each cortical area (Yoshimura et al., 2005; Yoshimura and Callaway, 2005; Hofer et al., 2011;
Harris and Mrsic-Flogel, 2013). For instance, Yoshimura et al. (2005) could show that excitatory connections from the layer 4 to layers 2 and 3 in the visual cortex define subpopulations of selectively interconnected neurons. This means that neurons in layer 2/3 share common input from layer 4 with a higher probability if they are connected to each other. In contrast, excitatory feedback from layer 5 and inhibitory connections from layer 4 target neurons in the layer 2/3 with the same probability irrespective of the subnetworks the layer 2/3 neurons belong to. The same is true for the intralaminar inhibitory projections within the layer 2/3 (Yoshimura et al., 2005). Moreover, the neurons forming one subnetwork within the layer 2/3 share similar responses to external stimuli indicating that these subnetworks constitute distinct functional entities (Ko et al., 2013; Wertz et al., 2015). Interestingly, the establishment of such functionally specific structural interconnections during the ontogeny seems to be driven by the sensory input (Ko et al., 2013).

Furthermore, due to the interlaminar neocortical circuitry, the cortex seems to consist of distinguishable vertical structures termed cortical columns (Mountcastle, 1997). Many definitions of a cortical column exist which differently stress its anatomical or functional aspects (Mountcastle, 1997; da Costa and Martin, 2010; Horton and Adams, 2005; Rockland, 2010). The cortical column can refer to interconnected neurons expanding vertically across cortical layers sharing a certain set of properties (Rockland, 2010). An impressive example of such a columnar organization of the neocortex has been demonstrated in the somatosensory cortex of rodents. Here, discrete, well-defined structures named barrels are located in the layer 4, each barrel representing one whisker of the animal (Petersen, 2007). Axons of the layer 4 neurons projecting to the superficial layers 2 and 3 define anatomically a column with the width corresponding to the horizontal extent of the barrel (Petersen and Sakmann, 2001). Activation of a barrel by whisker deflection (Plomp et al., 2014) or direct electric stimulation (Petersen and Sakmann, 2001) revealed that after only a short delay, the activity from layer 4 is propagated to layer 2/3. The striking finding of such studies was that the activity remained restricted to the vertical column defined anatomically by the layer 4 axons (Petersen and Sakmann, 2001; Petersen, 2007). In accordance, layer 2/3 neurons can be activated mainly by neurons of layer 4 located within the vertical column (Shepherd et al., 2003; Shepherd and Svoboda, 2005).

Historically, the cortical columns date back to the seminal work of Mountcastle (1957) who observed that neurons in cat’s somatosensory cortex recorded along a vertically penetrating
electrode share identical physiological properties. He was inspired by Lorente de Nó’s conviction that radially oriented units are the elementary components of the cortex, which he based on the preponderance of vertically connected neurons observed in his careful studies of Golgi sections (Nieuwenhuys et al., 2008). Therefore, Mountcastle proposed that the somatosensory cortex is constituted by vertically organized interconnected cells extending across the cortical layers, the columns, which are the elementary organizational unit of this cortex (Mountcastle, 1957). Soon after that, Hubel and Wiesel (1962, 1963), who were influenced by Mountcastle’s thinking, described similar columnar organization of orientation selective neurons in the visual cortex of the cat. Although, the cortical column became an influential framework in our research and understanding of the cortex, the columnar concept (Rockland, 2010), the structural and functional overlap of columns (Horton and Adams, 2005) as well as the anatomical existence of columns at all (da Costa and Martin, 2010) have been doubted and criticized and remain a matter of controversy.

1.1.2 How does the cortex develop its structure?

The neuroepithelium of the early telencephalic wall gives rise to radial glia cells which establish the ventricular zone, the main proliferative inner layer of the developing brain (Haubensak et al., 2004). The radial glia also generate intermediate progenitors and outer radial glia, which establish the subventricular zone, a proliferative zone found in mammals, which might have been a major achievement in the evolution of a large, six-layered neocortex (Molyneaux et al., 2007; Cheung et al., 2010). The first structure which occurs during the neocortical development is the so called preplate (Greig et al., 2013). The preplate consists of tangentially migrating Cajal-Retzius cells, which reside in the most superficial cortical layer and express reelin, a glycoprotein which has an necessary function in the development of the neocortical layers (Nomura et al., 2008; Franco et al., 2011; Sekine et al., 2014). The preplate is later divided into a marginal zone (future layer 1) and a subplate. The subplate plays an important role in the guidance and maintenance of incoming thalamocortical axons (López-Bendito and Molnár, 2003; Greig et al., 2013). Between the subplate and the marginal zone, the cortical plate is developing, which gives rise to future layers 2-6. Here, the neurons organize themselves in an inside-out manner, such that early born neurons reside in the deep layers (5 and 6), while new born neurons migrate to progressively more superficial layers (4-2; Molyneaux et al., 2007; Greig et al., 2013). Due to
this inside-out pattern, new born neurons are forced to migrate through early-born neurons, which might facilitate the establishment of connectivity and possibly led to an increase in thickness of the neocortex (Aboitiz, 1999). One model of cortical development assumes that neurons of both superficial and deep layers are produced by a single progenitor lineage and the progenitors become restricted in their competence to produce deep layer neurons later in development (Greig et al., 2013). Alternatively, some evidence also shows that there might be different progenitor lineages for superficial and deep layer neurons (Franco et al., 2012; Greig et al., 2013).

During the development, different domains of the neocortical primordium secrete diverse morphogens and signaling molecules, which create expression gradients (Alfano and Studer, 2012; Greig et al., 2013). This graded expression initiates the arealization of the cortex and determines the size and position of different cortical areas (Alfano and Studer, 2013; Greig et al., 2013). Furthermore, thalamocortical axons also contribute to the arealization through both, activity-dependent and independent mechanisms, especially in the later stages of development (López-Bendito and Molnár, 2003).

1.1.3 Function of cortical circuits

The mammalian neocortex plays an important role in cognition, sensory processing, learning and memory, sensorimotor transformation and motor control. The circuitry mentioned above is able to perform complex computations allowing the cortex to extract relevant stimuli and stimulus features which can form a representation of the external world and guide the behavior (van Hooser, 2007; Murayama et al., 2009; Nassi and Callaway, 2009; Mao et al., 2011; Harris and Mrsic-Flogel, 2013; Petersen and Crochet, 2013; Glickfeld et al., 2014). The specifically organized inhibitory and excitatory networks allow different cortical neurons to employ different strategies for information encoding (Harris and Mrsic-Flogel, 2013). Two different strategies related to spiking rate and the fraction of neurons that are activated within a neuronal population at a given time are sparse and dense coding. Sparse coding means that only a small subset of neurons within a population encodes the information at any given instant, while in dense code many active neurons encode information by variations in their firing rates. Growing evidence shows that principal neurons in layer 2/3 show high stimulus selectivity supported by sparse firing, while output neurons in layer 5 utilize the dense coding strategy with high spiking frequencies graded to a
broad range of stimuli (Harris and Mrsic-Flogel, 2013; Petersen and Crochet, 2013). Consistent with this notion, it was shown that distinct populations of layer 2/3 neurons represented different contexts in a contextual fear conditioning paradigm (Xie et al., 2014). Such specific coding of layer 2/3 neurons occurred in various cortical areas including visual, somatosensory, motor as well as retrosplenial cortices (Cowansage et al., 2014; Xie et al., 2014). Moreover, several elegant studies demonstrated that remodeling of dendritic spines on layer 2/3 and layer 5 pyramidal neurons are associated with learning and performance of motor tasks (Xu et al., 2009; Hayashi-Takagi et al., 2015) as well as fear conditioning and extinction (Lai et al., 2012). Together, these studies indicate that cortical circuits form functional cell ensembles which possess a high degree of highly selective synaptic plasticity allowing the storage of distinct memories (Wiltgen et al., 2004; Poo et al., 2016).

1.2 Pallium of birds and Karten’s hypothesis

In the early 1960s, Harvey J. Karten and his colleagues started a series of anatomical studies devoted to the understanding of the organization of the avian brain (Karten, 1967; 1968; Karten and Hodos, 1970; Karten et al., 1973; Benowitz and Karten, 1976; Husband and Shimizu, 1999). They investigated the ascending sensory systems and discovered that birds possess unimodal visual, auditory and somatosensory pathways, which terminate in discrete regions of the avian telencephalon. Harvey Karten emphasized the astonishing similarities of the auditory and visual pathways to the same systems known in the mammalian brain (Karten, 1969; Karten and Shimizu, 1989). He developed a theory, which became a very influential framework of comparative studies of the forebrain (Karten, 1969, 1997; Reiner et al., 2005; Butler et al., 2011; Karten, 2015). Karten’s hypothesis states that the avian brain, both the wulst and the DVR contains neuronal cell populations, which are homologous to neurons in certain layers of the neocortex. Specifically, he proposed common origin of the superior part of the neocortex and the wulst on the one hand, and common origin of parts of the temporal cortex and the DVR on the other hand (Reiner et al., 2005). This hypothesis is supported by hodological, morphological and topological data (Butler et al., 2011). Since the DVR and the neocortex develop from different pallial sectors (Karten, 1997; Puelles et al., 2000), Karten proposed a developmental model in which he argues that tectofugal-recipient
neurons in mammals migrate tangentially to reach the pallial mantle and to form the layer 4 of auditory and visual temporal cortices, while the tangential migration of equivalent neurons in birds is limited leading to an enlargement and a protrusion of the DVR into the lateral ventricle (Karten, 1969, 1997; Reiner et al., 2005; Butler et al., 2011). Although tangential migration of neurons within the developing forebrain has been described, these tangentially migrating neurons originate in the subpallial part of the subventricular zone and become GABAergic interneurons within the cortex rather than glutamatergic projection neurons (Marín and Rubenstein, 2001; Ma et al., 2013). Few years ago, tangential migration of a glutamatergic neuron population was indeed discovered, however despite their seemingly important role in the corticogenesis, these neurons form only a negligible fraction of adult neocortical neurons (Teissier et al., 2010). These facts remained the source of major criticism on Karten’s theory (Puelles, 2011). Nevertheless, recent anatomical, physiological and genetic studies yield further important insights into the organization of the avian pallium and the data seem still congruent with this hypothesis (Wang et al., 2010; Dugas-Ford et al., 2012; Pfenning et al., 2014; Ahumada-Galleguillos et al., 2015; Calabrese and Woolley, 2015). Below, we briefly outline the ascending sensory pathways in birds and highlight some interesting comparisons to their mammalian counterparts.

1.2.1 Sensory systems and their circuitry in the avian pallium

1.2.1.1 The auditory system

The auditory input from the hair calls in the cochlea reaches the cochlear nuclei in the medulla (Boord and Rasmussen, 1963; Ryugo and Parks, 2003). The information from the cochlear nuclei is then directly or indirectly (via the superior olive, lemniscal nuclei or nucleus laminaris) conveyed to the nucleus mesencephalicus lateralis, pars dorsalis (MLd), the homologue of the mammalian central nucleus of the inferior colliculus. The MLd projects upon the nucleus ovoidalis (Ov), which in turn gives rise to telencephalopetal projections terminating in the avian auditory region called Field L (Karten, 1967, 1968; Figure 3). This pathway resembles the mammalian ascending auditory projection from the inferior colliculus to the thalamic relay, the medial geniculate nucleus, and thence to the layer 4 of the primary auditory cortex (Jarvis, 2009). The telencephalic auditory region of birds is located in the caudomedial part of the forebrain and includes the caudomedial mesopallium.
covered by the CMM and the caudal nidopallium (NCM) adjacent caudally and laterally to Field L. Further areas involved in the auditory systems are the dorsal nidopallium (Nd) which is part of the NCL (Kröner and Güntürkün, 1999) and the ventromedial portion of the intermediate arcopallium (Aivm). The Field L of birds has been subdivided into 3 layers L1-L3 based on cytoarchitecture and parvalbumin and cytochromoxidase staining (Müller and Scheich, 1985; Wild et al., 1993; Wang et al., 2010). The layer L2 is the main thalamorecipient layer of the Field L, which consists mainly of small granule neurons with extremely thin dendrites forming a relatively small dendritic arbor (Wild et al., 1993; Wang et al., 2010). It has a granular appearance in Nissl staining and is distinguishable by its immunoreactivity for parvalbumin and cytochromoxidase (Wild et al., 1993; Wang et al., 2010). Wang et al. (2010) performed an in vitro tracing study of the auditory system in chickens and confirmed that the organization of this system is clearly laminar with CMM, L1-3 being individual layers, with some of them subdivided into further sublayers. Wang et al. (2010) further explored, whether the layers of this auditory complex form individual functional units or whether they operate in vertical modules across the layers. To this end, they injected a highly sensitive tracer into individual layers in slice preparations of the chick brain. They found that regardless of which layer was injected, the transport of the tracer always occurred in a column perpendicular to the layers crossing all the layers. They found that the thalamorecipient layer L2 projects upon L1 and they both project to CMM. The layer L2 seems to be also interconnected with L3. CMM has

Figure 3: Ascending auditory system in pigeons. The auditory information from the ear reaches the cochlear nuclei (CN) and is then conveyed directly or via further brainstem nuclei to the main midbrain station, nucleus mesencephalicus lateralis, pars dorsalis (Mld). Thence, the information is passed to the Field L in the telencehalon via the thalamic nucleus ovoidalis (Ov). The field L is subdivided into three layers (L1-L3). The L2 is the major recipient of the thalamic input. The L1 and L3 form the output to other regions (not shown). The Field L is also interconnected with the overlying caudomedial mesopallium (CMM). Simplified and modified from Güntürkün et al. (2017). Telencephalic connections are based on Wild et al. (1993) and Wang et al. (2010).
feedback projections onto all these layers. Together the layers L1 and L3 form the major output out of the vertical column to Nd, which than projects to the Aivm (Wang et al., 1993). Wang et al. (2010) also showed that the external (directly adjacent to CMM) part of L1 seems to be the major source of direct output to Aivm. The striking conclusion of this study is a remarkable similarity in the organization of this system in birds with the auditory cortex in mammals. Based on cell morphology, thalamic input and parvalbumin staining, the authors emphasize the correspondence of Field L2 to the lamina 4 of the mammalian auditory cortex. Consequently, the L1 and CMM as the targets of L2, and L1 as major output station, are suggested to be homolog to the superficial layers 1-3 of the auditory neocortex (Wang et al., 2010). Within this framework, the layer L3 together with Nd and Aivm might be crucial components somehow comparable to mammalian deep layers 5-6.

1.2.1.2 Visual System

1.2.1.2.1 Visual tectofugal system

The visual information in the avian visual tectofugal system is propagated from the retina to the optic tectum, from which it is further conveyed to the thalamic nucleus rotundus (Shimizu and Bowers, 1999; see Figure 4). The nucleus rotundus projects topographically upon the entopallium, the primary visual telencephalic station of the avian forebrain (Fredes et al., 2010). The information is distributed form the entopallium to further associative areas including NFL, MVL, TPO and NIL (Husband and Shimizu, 1999; Krützfeldt and Wild, 2005). The entopallium can be divided into a ventral internal part and dorsal external part based on parvalbumin staining and connectivity (Krützfeldt and Wild, 2005; Ahumada-Galleguillos et al., 2015). The internal part seems to be the source of projections to the lateral striatum (LSt). While Krützfeldt and Wild (2005) found that the internal rather than the external part of the entopallium is interconnected with the above located ventrolateral MVL in pigeons, this interconnection was observed for both but predominantly for the external part of the entopallium in chickens (Ahumada-Galleguillos et al., 2015). Similar to the study of the auditory system (Wang et al., 2010), Ahumada-Galleguillos et al. (2015) used in vitro tracing to investigate the local circuitry of the visual system in chickens. They found that this system is organized in three main layers, the thalamorecipient entopallium, the dorsally adjacent NI and the overlying MVL, which can be further subdivided into different sublayers. They found a strong highly topographically organized interconnection between an external layer of MVL
and the entopallium (mainly the external part). A weaker projection also reached the dorsal mesopallium (MD). Together, MD and MVL project back to the entopallium, leaving many collateral terminations in NI on their way down, up to the internal part of the entopallium. Fibers connecting the meso- and entopallium formed discrete bundles and run orthogonally to the layers giving rise to an apparent vertically oriented column. The important conclusion of their study was that a columnar and layered organization of reciprocal interlaminar circuits previously demonstrated in the auditory system also applies to the visual system and thus might be a general characteristic of the avian pallium (Wang et al., 2010, Ahumada-Galleguillos et al., 2015).

Figure 4: Visual pathways in the pigeon.
The retinal projections give rise to a tectofugal (blue) and a thalamofugal (green) pathway. The latter runs via the dorsal part of the lateral geniculate nucleus (GLd) to the visual wulst. The tectofugal pathway reaches the entopallium via the thalamic nucleus rotundus (nRt), which itself receives input from the optic tectum. The intratelencephalic connectivity is based on Shimizu et al. (1995) and Ahumada-Galleguillos (2015).

1.2.1.2.2 Visual thalamofugal
The retinal input within the thalamofugal system arrives in a subset of dorsal thalamic nuclei collectively called the lateral dorsal geniculate nucleus (GLd; Güntürkün and Karten, 1991). The GLd than projects to the visual wulst (Ströckens et al., 2013). The information arrives mainly in the interstitial part of the hyperpallium apicale (IHA; Karten et al., 1973; Streit et al., 1980). The IHA forwards the information mainly to apical part of the hyperpallium (HA), which is the source of projections outside the wulst including extratelencephalic targets (Shimizu et al., 1995). The other hyperpallial layers including the intercalated (HI) and the densocellular (HD) parts of the hyperpallium, which are the source of efferent projections to
the CDL and hippocampus, also project to HA (Shimizu et al., 1995). The thalamofugal pathway in pigeons is concerned with the lateral visual field and with more distant visual objects (Güntürkün and Hahmann, 1999; Budzynski and Bingman, 2004).

1.2.1.3 Somatosensory

1.2.1.3.1 Trigeminal

The trigeminal system differs from the other modalities in two important aspects. First, the nucleus basorostralis pallii (BAS), which is the primary telencephalic target zone of this modality, receives its input directly from the brainstem principal sensory trigeminal nucleus (PrV) via the quintofrontal tract (Wild et al., 1985; Figure 5). Thus, in contrast to all other sensory systems, the ascending trigeminal pathway bypasses the thalamus. Second, the BAS is not unimodal but in addition to its trigeminal input, it also collects auditory and vestibular input from the lateral lemniscal nucleus and the superior vestibular nucleus, respectively (Schall et al., 1986). This kind of input makes the BAS extremely suitable for guiding the pecking behavior (Witkovsky et al., 1973; Wild et al., 1984; Schall, 1987). Detailed anatomical
in vitro studies similar to those performed in the visual and auditory systems have not been carried out yet. However, the evidence gathered from older in vivo tracing studies suggest that the trigeminal system is also constituted by multiple interconnected layers and sublayers, with vertically oriented fibers crossing the layers (Wild et al., 1984, 1985; Dubbeldam and Visser, 1987).

1.2.1.3.2 Thalamofugal somatosensory

While the BAS contains representation of the beak and the oral cavity (Witkovsky et al., 1973), the rest of the body is represented within the thalamofugal system in the rostral wulst (Delius and Bennetto, 1972; Wild, 1987). The somatosensory information from the body is conveyed here from the dorsal column nuclei via the thalamic nucleus dorsalis intermedius ventralis anterior (DIVA; Wild, 1987; Figure 5). However, differences exist between different avian species. For instance, the BAS of barn owl also receives information from the dorsal column nuclei, and the whole body is represented here, while the somatosensory wulst of the barn owl contains only the representation of the claw (Manger et al., 2002; Wild et al., 2008). Interestingly, the claw is represented in a dual and plastic representation map (Manger et al., 2002). Unfortunately, we lack a detailed knowledge of the local circuitry of the somatosensory wulst. We can only reiterate the findings from the in vivo tracing by Shimizu et al., (1995) who showed the above mentioned connectivity involving projections from IHA, HI and HD to HA.

1.2.2 Functional insights into pallial circuits in birds

Although, several studies yielded substantial contribution to the understanding of the functional organization of the avian brain (Scheich, 1983; Delius et al., 1984; Jäger, 1990; Bolhuis and Gahr, 2006; Nagel et al., 2011; Koenen et al., 2016; Stacho et al., 2016), our knowledge of the function of specific circuits is very limited. Recent functional studies of the visual system involving the above mentioned circuitry between the entopallium, MVL and NI indicate that these circuits might be involved in the processing and discrimination of visual pattern, color and motion stimuli (Koenen et al., 2016; Stacho et al., 2016). Furthermore, neurons in these circuits seem to possess an inherent categorical representation of visual
stimuli suggesting that these circuits are crucial for categorization of visual stimuli (Koenen et al., 2016).

The visual wulst of birds seem to possess multiple representations of the visual field, which differ in their physiological properties, including response latencies and direction preferences (Bischof et al., 2016). The subnuclei of GLd contain separate clusters of neurons contributing to these different visual maps (Bischof et al., 2016). Neurons with selectivity for orientation, direction of movement and binocular disparity have been identified in the visual wulst of the barn owl (Pettigrew and Konishi, 1976; Pettigrew, 1979). The neurons have various receptive field properties with different degrees of complexity (Pettigrew, 1979). The majority of neurons, especially in the binocular overlap zone of the wulst, are binocular with a small fraction of neurons without orientation selectivity being monocular. The visual wulst of barn owl was shown to contain orientation selectivity map organized in pinwheel-like manner (Liu and Pettigrew, 2003). All these characteristics strikingly resemble the properties of the mammalian visual cortex (Hubel and Wiesel, 1962; Pettigrew, 1979). The wulst has been shown to be involved in spatial information processing, motion detection, fine-detail, pattern and color discrimination and reversal of the reward contingencies (MacPhail, 1976; Powers et al., 1982; Martinoya et al., 1983; Hodos et al., 1984; Watanabe, 2003; Budzynski and Bingman, 2004; Watanabe et al., 2011). However, functional analysis of local connectivity is missing.

Much more details are known about the auditory processing provided mainly by the research on songbirds. Neurons in different layers of the auditory system possess different temporal tuning for auditory sounds, while spectral tuning varies along the mediolateral axis perpendicular to the layers (Kim and Doupe, 2011). Moreover, Calabrese and Woolley, (2015) could show that the avian auditory circuit follows a hierarchical processing strategy where information is first processed within the thalamorecipient layer and then forwarded to adjacent superficial and deep layers. Furthermore, they could identify two main types of neurons within these circuits including the putative excitatory principal neurons and putative inhibitory neurons. A striking finding of their study was that the property of these neurons and their differences across layers pretty much matched those observed in the neocortex. In both neuron types, the response sparseness, the stimulus selectivity and the complexity of the receptive field increased in successive processing stages. Based on spontaneous and evoked pairwise activity correlation, they further provided physiological evidence that
putative inhibitory neurons are stronger interconnected than putative excitatory neurons. In addition, the interconnection among neurons was stronger and less dependent on anatomical distance in deeper than in superficial layers for both neuron types.

1.2.3 Developmental aspects of the avian pallium

The avian wulst and the mammalian neocortex both develop from the dorsal pallial sector (Puelles et al., 2000). Therefore, these two areas are homologous from a developmental point of view. Although the avian wulst is a laminated structure, these layers are not considered as true layers and are therefore sometimes referred to as pseudolayers (Medina and Reiner, 2000). The layers in the avian wulst differ in many aspects from the mammalian neocortical layers (Medina and Reiner, 2000). In contrast to neocortical layers, the layers of wulst are organized parallel to the radial glial fibers, indicating that each layer originates in a different sector of the neuroepithelium (Medina and Reiner, 2000; Montiel et al., 2016). Furthermore, the neurons in the wulst do not seem to branch their dendrites across different layers (Medina and Reiner, 2000).

As mentioned above, the mammalian neocortex develops in an inside-out fashion, with deep layer neurons produced earlier than neurons designed for superficial layers. Although, the ability of new cells to migrate through older cells might not be exclusive to mammalian cortical development (Striedter and Keefer, 2000), such a well-organized inside-out pattern is only evident in mammals (Montiel et al., 2016). Due to differences in pallial organization, it was thought that this temporal sequence in the genesis of different neuron subtypes is a mammalian innovation. However, Suzuki et al. (2012) discovered that neurons expressing superficial and deep layer markers are generated in the same temporally ordered manner in the chicken pallium. The difference to mammalian cortical development was that the superficial and deep neurons originated in spatially separated domains. While the deep layer neurons originated from the medial sector, the lateral sector gave rise to superficial layer neurons. Interestingly, in vitro culture of neuronal progenitor cell from the medial and lateral domain revealed that both progenitor types are able to generate deep and superficial layer neurons. This study indicates that birds and mammals share an evolutionary conserved neurogenetic program, whose extrinsic regulation has been modified in mammals allowing
the homogenous allocation of layer-specific neurons across the neocortex (Suzuki et al., 2012; Suzuki and Hirata, 2012, 2013).

1.3 The mammalian hippocampus

The hippocampus forms a phylogenetically old part of the mammalian cortex referred to as the archicortex. This part of the cortex can be subdivided into dentate gyrus, Ammon’s horn and subiculum. The dentate gyrus and Ammon’s horn are both trilaminar structures with a cellular layer flanked by plexiform layers, which can be further subdivided into different sublayers (Nieuwenhuys et al., 2008). The cellular layers of the dentate gyrus and the Ammon’s horn are continuous during early development but became separated later on forming the characteristic interlocking of these two layers by the convolution of the dentate gyrus (Nieuwenhuys et al., 2008; Hevner, 2016). The cellular layer of the Ammon’s horn consists of pyramidal neurons and is located between the stratum oriens above and stratum radiatum underneath. Adjacent to stratum radiatum is the stratum lacunosum and stratum moleculare sometimes called collectively stratum lacunosum-moleculare (Förster et al., 2006; Nieuwenhuys et al., 2008). The dentate gyrus consists of an outer molecular layer, a central granule cell layer and deep polymorph layer also called hilus. The Ammon’s horn can be further subdivided into three subdivisions CA1 to CA3 based on size and density of the pyramidal neurons.

Adjacent to and continuous with the subiculum the entorhinal cortex is located. The entorhinal cortex is a six-layered structure clearly distinguishable from the rest of the neocortex by the presence of a cell-poor fiber layer called lamina dissecans instead of layer 4 (Canto et al., 2008). The entorhinal cortex provides the main entrance to the hippocampus and is a nodal point between hippocampus and the rest of the neocortex (Kerr et al., 2007).

1.3.1 The hippocampal circuitry

The main hippocampal loop begins with the projection from the superficial layers of the entorhinal cortex to dentate gyrus via the perforant path (Tamamaki and Nojyo, 1993). The inputs to the dentate gyrus originate mainly in the large stellate neurons of layer 2 of the entorhinal cortex (Witter and Amaral, 1991; Varga et al., 2010) which form excitatory
synapses on the dendrites of dentate gyrus granule neurons. This perforant pathway axons synapsing on the granular neurons of the dentate gyrus were one of the first where the long-term potentiation was discovered (Bliss and Lømo, 1973). The granule cells of the dentate gyrus send axons to CA3 which end as mossy fibers on the dendrites of pyramidal neurons in the stratum radiatum in which they form a separate sublayer termed stratum lucidum (Acsády et al., 1998; Nieuwenhuys et al., 2008). Axons of the pyramidal neurons in the CA3 region give rise to collaterals known as Schaffer collaterals which contact the basal and apical dendrites of pyramidal neurons in CA1 in stratum oriens and lacunosum, respectively. CA1 pyramidal neurons project to subiculum which then closes the loop with its projection to the entorhinal cortex. The subicular axons target the deep layers of the entorhinal cortex, which then project back to the superficial layers (Nieuwenhuys et al., 2008). The canonical pathway from the entorhinal cortex to the CA1 via the dentate gyrus and the CA3 region is known as the trisynaptic pathway which received the major attention in the hippocampus circuitry research. However, a reduction of the hippocampal processing to this pathway is an oversimplification and other intrinsic hippocampal connectivities have to be taken into account (Amaral, 1993; Kitamura et al., 2015). For instance, the information from the entorhinal cortex reaches the regions of the Ammon’s horn and the subiculum not only via the dentate gyrus but also directly (Witter and Amaral, 1991; Tamamaki and Nojyo, 1993; Amaral, 1993; Witter et al., 2000; Kitamura et al., 2015). The layer 2 neurons project to the CA2 and CA3, while the direct entorhinal inputs to CA1 pyramidal neurons and to the subiculum originate in neurons located in the layer 3. Furthermore, the intrinsic excitatory circuitry is embedded into a complex system of inhibitory interneurons which exerts an important modulation of the excitatory signals (Freund and Buzsáki, 1996; Maccasferri and Lacaille, 2003; Galván et al., 2011). For example, the direct excitatory input from the entorhinal layer 3 neurons to dendrites of CA1 pyramidal neurons is controlled by inhibitory neurons in stratum lacunosum activated by axons originating in layer 2 of the entorhinal cortex (Kitamura et al., 2015). Such feedforward inhibition can also be found along the trisynaptic pathway. Both, mossy fibers as well as the Schaffer collaterals contact inhibitory interneurons in addition to their terminations on the principal neurons (Acsády et al., 1998; Pouille and Scanziani, 2001). This is reflected in a sequence of excitatory and inhibitory postsynaptic potentials (EPSP and IPSP) in CA1 pyramidal neurons evoked by the stimulation of Schaffer collaterals. The feedforward inhibition exerted by the Schaffer collaterals seems
to be restricted to the soma of the pyramidal neurons, thus shortening the time window for EPSP summation specifically in the soma, while leaving the dendrites a longer time window to sum incoming activity (Pouille and Scanziani, 2001). In addition, the pyramidal neurons are also inhibited by inhibitory recurrent feedback loops. In an excellent study, Pouille and Scanziani (2004) could show that stimulation of axons of CA1 pyramidal neurons induces inhibition of these neurons. Interestingly, at least two different types of interneurons are recruited by these axons. The two types of the interneurons differ in their target location on the pyramidal neurons as well as their EPSP kinetics and membrane time constant. These different properties of the interneurons result in an early-onset transient somatic inhibition and a late-onset persistent dendritic inhibition of CA1 pyramidal neurons (Pouille and Scanziani, 2004).

1.3.2 Topography

The trisynaptic hippocampal pathway as well as the direct pathways follow a complex topography along the septo-temporal (longitudinal) and proximal-distal (transverse) axis (Amaral, 1993; Witter et al., 2000). The axons of neurons in layer 2 of the lateral (LEC) and medial part (MEC) of the entorhinal cortex terminate in different parts of the molecular layer of the dentate gyrus (outer and middle parts, respectively). In addition, the entorhinal cortex can be subdivided into three different bands along the dorsolateral to ventromedial axis spanning both the LEC and MEC (Dolorfo and Amaral, 1998; Kerr et al., 2007). These bands project to the dentate gyrus in a topographical manner along its longitudinal axis with the most dorsolateral band projecting to the septal (dorsal) part, and the ventromedial bands projecting progressively to more temporal (ventral) parts of the dentate gyrus. The mossy fiber projections of the dentate gyrus are organized along the longitudinal axis without an obvious topography along the transverse axis. The Schaffer collaterals of the CA3 neurons, however, display a specific arrangement along the transverse axis, such that the proximal (i.e. proximal to dentate gyrus) part of CA3 projects to the distal part of CA1, while the projections of the distal part of CA3 reach the proximal CA1 (Figure 6). In turn, proximal CA1 projects to distal subiculum, whereas the distal CA1 project to the proximal subiculum. This might appear confusing at the first glance but if one knows that CA3, CA1 and subiculum are subsequently adjacent to each other, then the proximal-distal topography just describes the fact that the projections connect the juxtaposed and distant parts of these regions,
I

respectively. In addition, the direct projections originating in layer 3 of LEC terminate in
distal CA1 and proximal subiculum, while those from MEC reach the proximal CA1 and distal
subiculum (Witter et al., 2000).

The separation into topographically defined pathways does not originate in the entorhinal
cortex. Rather the LEC and MEC already receive separated input from adjacent perirhinal
and postrihinal cortex, respectively (Burwell, 2000; Witter et al., 2000; Kerr et al., 2007).
Since LEC and MEC as well as post- and perirhinal cortices themselves are interconnected to
different areas, it is thinkable that the different pathways conveys slightly different functions (Burwell, 2000; Kerr et al., 2007; Furtak et al., 2007).

![Figure 6: Topographical organizations of the trisynaptic pathway along the transversal axis.](image)

In contrast to the projections of the dentate gyrus (DG), the
projections, within the Ammon’s horn follow a certain proximal-distal
topography along the transversal axis of the hippocampus. The
proximal part of the CA3 (nearer to the DG) projects to the distal part
of CA1. The distal part of CA3 projects to the proximal part of CA1. The proximal and distal parts of CA1 project to distal and proximal
parts of the subiculum (Sub), respectively.

1.3.3 Function

A number of functions have been associated with the hippocamal complex including spatial
navigation, context representation, as well as encoding, (temporal) storage and
consolidation of episodic memories (Aggleton et al., 2010; Geva-Sagiv et al., 2015; Moser et
al., 2015; Tonegawa et al., 2015a, b). The diverse regions and pathways within the
hippocampus subserve different aspects of these functions (Nakazawa et al., 2004; Strange
et al., 2014; Kitamura et al., 2015). For example, based on available evidence, Kitamura et al., (2015) suggested that the trisynaptic pathway might be mainly important for spatial contextual memory, while the direct entorhinal pathways to CA1 appear crucial for temporal properties of episodic memory.

The hippocampus has been proposed to perform two different operations – the pattern separation and pattern completion (Nakazawa et al., 2004; Fenton, 2007; Treves et al., 2008; Kheirbek et al., 2012). During pattern separation, similar neuronal activity patterns are converted into distinct de-correlated representations. The pattern completion mechanisms lumps distinct representations if differences are small. Thus, pattern completion supports to the ability to reconstruct complete memories when only incomplete set of cues is available, while pattern separation allows distinction of two similar but distinct memories.

The dentate gyrus was suggested as a suitable candidate for pattern separation with various type of evidence supporting this notion (for reviews see Treves et al., 2008; Schmidt et al., 2012). The dentate gyrus might contribute to this process by the sparse coding in the granule cell population and by recruitment of different populations of place cells in the CA3 region via its mossy fiber projections (Treves et al., 2008). This is supported by the fact that minor changes in the context or in the sensory input lead to remapping of the place cell receptive fields in CA3 region (Markus et al., 1995; Treves et al., 2008; Moser et al., 2015). Thus, similar events activate independent firing patterns of CA3 pyramidal neurons via the dentate gyrus, which de-correlates similar inputs and maximizes the capacity of the network (Treves et al., 2008; Schmidt et al., 2012). In accordance, specific lesions of the dentate gyrus impair discrimination performance between similar contexts or object locations with deficits positively correlated with the degree of similarity between correct and incorrect choice (Gilbert et al., 2001; McHugh et al., 2007).

The axons of pyramidal neurons in CA3 have been shown to give rise to extensive recurrent collaterals innervating other neurons in CA3 (Ishizuka et al., 1990; Wittner et al., 2007). This recurrent collateral input is the strongest input of CA3. Due to this property CA3 has been suggested to serve as an associative memory network perfectly suited to provide pattern completion. Nakazawa et al., (2002) indeed demonstrated that intact N-methyl-D-asparate (NMDA) receptors at glutamatergic synapses on the CA3 pyramidal neurons activated by these collaterals are necessary for memory retrieval under limited cue conditions. However, a recent theory of hippocampus function suggests that pattern completion is computed by
the network between the entorhinal cortex and CA1 (Cheng, 2013). In this theory, the recurrent CA3 network produces intrinsic sequences of activation patterns which can be associated with episodic memories. A specific sequence in CA3 can be activated by context reset from the dentate gyrus to initiate recall of a sequence or to enable storage of a new one. The pattern completion by CA1-entorhinal cortex network compensates the distortions caused by noise during CA3 sequence retrieval.

Modern research of the past decades significantly contributed to a better understanding of the storage of individual memories in the brain (Tonogawa et al., 2015a, b; Poo et al., 2016). Memory content seems to be stored in a functional connectivity pattern of ensemble neurons involving the dentate gyrus and the Ammon’s horn. Interestingly, the synaptic changes in these functional networks do not appear to require de-novo protein synthesis, while the plastic changes at the synapses formed by axons originating in the entorhinal cortex and contacting the dentate gyrus engram neurons are protein synthesis dependent (Ryan et al., 2015). These protein-dependent plastic changes are necessary for a correct recall of the memory content (Ryan et al., 2015; Roy et al., 2016). Besides unravelling the fascinating mechanisms of neurobiology of memory, these and other studies point to an intriguing conclusion that a forgotten memory may still be stored in the brain despite the inability to recall it (Fischer et al., 2007; Roy et al., 2016).

The engram ensembles are, of course, not restricted to the hippocampus but involve further regions including amygdala as well as different cortical regions (Cowansage et al., 2014; Kim et al., 2014; Xie et al., 2014). The different areas process different aspects of the memory content (Zelikowsky et al., 2014) and memory information in some cortical circuits might even represent the consolidated long-term memory traces (Xie et al., 2014).

1.4 The avian hippocampus

The hippocampus is a phylogenetically old structure and might have specialized for spatial memory very early in the evolution (Rodríguez et al., 2002; Striedter, 2016). It probably possessed a trilaminar organization already in the amniote ancestor and since then the hippocampus increased in complexity and underwent several changes (Striedter, 2016). However, the mammalian and avian hippocampi, which endured 300 million years of
independent evolution, evolved to strikingly dissimilar structures (Rattenborg and Martinez-Gonzalez, 2011; Herold et al., 2015; Striedter, 2016). In both species, the hippocampus develops from the medial pallium (Puelles et al., 2000) but in contrast to mammals, the avian hippocampus seems to have lost a clear trilaminar organization and lacks a prominent granule or pyramidal cell layer (Striedter, 2016). The lack of a distinct granular layer and mossy fibers opened the question whether birds have something like a dentate gyrus at all or whether it is a mammalian innovation (Kempermann, 2012; Herold et al., 2015; Striedter, 2016).

Similar to mammals, the avian hippocampus contains pyramidal-like neurons which form distant collaterals in multiple directions (Tömböl et al., 2000a, b; Striedter, 2016), it’s a site of adult neurogenesis (Powers, 2013), and can be subdivided along its transverse axis into several continuous regions which differ in their cyto- and chemorachitecture, and connectivity (Erichsen et al., 1991; Kahn et al., 2003; Atoji and Wild, 2004; 2006; Herold et al., 2014; Atoji et al., 2016). Various numbers of subregions have been proposed and different authors came to diverse conclusions about the correspondence of avian and mammalian hippocampal subdivisions (Erichsen et al., 1991; Siegel et al., 2002; Kahn et al., 2003; Atoji and Wild, 2006; Gupta et al., 2012; Herold et al., 2014; Atoji et al., 2016). The avian hippocampus can be subdivided into dorsolateral (DL), dorsomedial (DM) and ventral regions (Atoji and Wild, 2004; Herold et al., 2014; Figure 7). The border between DL and DM is not clearly visible in Nissl staining but it is easily determined by a shallow sulcus running rostro-caudally on the ventricular site of the hippocampus in this region (Atoji and Wild, 2004). Medially, the DL is differentiated from the adjacent area corticoidea dorsolateralis (CDL) by its decreasing thickness towards the CDL. Both DL and DM can be further subdivided into dorsal (DLd and DMd) and ventral components (DLv and DMv; Herold et al., 2014). Atoji and Wild (2004) further distinguished three small regions along the dorsomedial corner which are mainly subsumed by DMd based on Herold et al. (2014). The ventral part of the avian hippocampus is constituted by a triangular region (Tr) flanked by a thin V-shaped layer consisting of more densely packed neurons as compared to the rest of the hippocampus (Atoji and Wild, 2004). The V-shaped layer can be differentiated into a lateral (VL) and a medial (VM) component. Both VL and VM are covered by white, cell poor fiber layers sometimes referred to as paraventricular and medial fiber zone, respectively (pfz, mfz;
Atoji and Wild, 2004, 2006). However, the V-shaped layer is clearly differentiated only in some birds e.g. including pigeons but not chickens (Puelles et al., 2007; Striedter, 2016).

1.4.1 Circuitry of the avian hippocampus

Despite several attempts (Hough et al., 2002; Kahn et al., 2003; Atoji and Wild, 2004), we still lack a detailed understanding of the intrinsic connectivity and the functional organization of the avian hippocampus. An overview of the precise intrinsic connectivity pattern is also partly hindered due to variations in boundaries between hippocampal subregions across studies (Hough et al., 2002; Kahn et al., 2003; Atoji and Wild, 2004; Herold et al., 2014). Nevertheless, there are already several insights into the intrahippocampal organization (Figure 7).

The extrahippocampal sensory information seems to both enter and leave the hippocampus mainly via the DL and the DM (Atoji and Wild, 2004, 2005, 2006). These two areas are also connected together since the ventral DL projects to DMv, possibly preferentially to its dorsal part (Kahn et al., 2003). The DMv projects back to DL, however, it targets its dorsal portion, the DLd (Atoji and Wild, 2004). Since DLv and DLd are interconnected, the connections between DLv, DLd and DM might form a short closed loop (Atoji and Wild, 2004). The DM conveys the information further to the V-shaped layers, which in turn project to Tr and back to DM (Kahn et al., 2003; Atoji and Wild, 2004; 2006). DM also seems to project directly to Tr (Atoji and Wild, 2004). In addition, a projection from the Vm to VI has also been described (Hough et al., 2002; Kahn et al., 2003). The Tr projects to DM, DLd and CDL (Atoji et al., 2002; Atoji and Wild, 2004, 2005, 2006). The information from DL is distributed also directly to Tr, to a part of DMd, and to the V-shaped layers (Atoji and Wild, 2004). Thus, the avian hippocampal circuit seems to be constituted by the projection from DL to DM, which then projects to Vm, which in turn projects to VI (Kahn et al., 2003). The VI projects back to DM, which then projects out of the hippocampus. This circuit contains many reciprocal connections, as well as alternative routes, including the route via Tr (Atoji and Wild, 2006).

Moreover, the sensory input reaches the Tr, V-shape and DM also via input from CDL (Atoji and Wild, 2005). CDL also projects to DL, especially to its lateral part (Atoji and Wild, 2004). Unfortunately, there is only very limited knowledge about the physiology of these pathways, yet (Wieraszko and Ball, 1991, 1993; Margrie et al., 1998). Margrie et al. (1998) investigated
the synaptic transmission within the avian hippocampus. They found that, similar to mammals, stimulation of afferent fibers evokes a sequence of an EPSP followed by an IPSP. Furthermore, tetanic stimulation of these fibers elicited an immediate and long-lasting potentiation of the EPSP. This potentiation was specific to stimulated synapse, was NMDA-receptor independent and required presynaptic calcium influx. This kind of LTP resembles the potentiation observed on the mossy fiber synapses, however, in contrast to mammals, the activation of adenylyl cyclase was not necessary for the induction of the LTP in birds (Margrie et al., 1998).

1.4.2 Topography

As described above, the mammalian hippocampal pathways possess a complex and precise topographical organization. However, only few details are known about the topography within the avian hippocampal circuits. The projection from DL to DM appears to be very divergent and hence non-topographic along the anterior-posterior axis (Kahn et al., 2003). The connections between DM and the V-shaped layers seem to follow a certain kind of topographical organization. Kahn et al. (2003) observed that while the Vm was interconnected with the dorsal DM, the projections of VI appeared to target rather the ventral DM. It should be noted that the dorsal DM in Kahn et al. (2003) corresponds to DMd and the dorsal part of DMv as defined by Herold et al. (2014), while the ventral DM corresponds to the ventral part of DMv. Atoji and Wild (2004, 2005) divided DM into lateral...
and medial part and observed that the VI projects to lateral portions of DM, whereas Vm projects to its medial portion (Atoji and Wild, 2004). The lateral and medial DM also seem to differ in sensory input they receive, with lateral and medial DM receiving stronger visual and olfactory input, respectively (Kahn et al., 2003). The DM of Atoji and Wild roughly corresponds to DMv of Herold et al. (2014). In addition, a clear topographical organization along the anterior-posterior axis was observed in the commissural interhippocampal projections (Atoji et al., 2002).

1.4.3 Function

As mentioned above spatial memory might be a conserved feature of the hippocampus (Rodríguez et al., 2002; Striedter, 2016). The interest to the avian hippocampus has been attracted mainly by the remarkable navigational abilities, homing behavior and spatial memory in birds (Bingman et al., 2003; Herold et al., 2015; Mouritsen et al., 2016). The hippocampus of pigeons, especially the left hemisphere, seems to be necessary for the acquisition of a navigational map in young birds (Bingman et al., 2005). This navigational map helps the pigeon to determine the distance and direction of displacement, even from unfamiliar places. However, the map is most probably not stored in the hippocampus, since hippocampus lesions in adult pigeons do not affect the homeward orientation after displacement (Bingman et al., 2005). An interesting study by Gagliardo et al. (1999) could show that the pigeon hippocampus is important for learning a landmark map of the environment, which can be used for navigation over familiar space (Gagliardo et al., 1999, 2009; for a nice review see Herold et al., 2015). Moreover, the hippocampus is probably necessary for the perception of significant environmental landmarks, since birds with a lesioned hippocampus seem to be insensitive to such landmarks both in familiar and unfamiliar environments (Gagliardo et al., 2009, 2014). Consistent with its role in spatial representation, the avian hippocampus was also shown to be important for representation of goal locations based on geometrical cues (Nardi and Bingman, 2007). In accordance, neurons responding to biologically salient locations have been found in the avian hippocampus (Siegel et al., 2005). Another type of neurons, so called path cells, which display increased activity along corridors and are also direction selective may support the computation of navigational trajectory (Hough and Bingman, 2004; Bingman et al., 2006).
Furthermore, the avian hippocampus has been implicated in spatial memory, especially in food-storing birds (Balda and Kamil, 1992; Pravosudov and Smulders, 2010; Herold et al., 2015). The role of the avian hippocampus in spatial memory is supported by a larger hippocampal volume in food-storing species (Krebs et al., 1989) and their impairments after hippocampal lesions (Sherry and Vaccarino, 1989), as well as by increased hippocampal activity of birds relaying on spatial cues in a spatial-feature learning paradigm (Mayer and Bischof, 2012).

As outlined before, the mammalian hippocampus uses pattern separation to store information in distinct neuronal ensembles (Treves et al., 2008; Schmidt et al., 2012; Tonegawa et al., 2015a, b). However, similar studies have not been performed in birds, yet. The role of the avian hippocampus in pattern separation has been inferred from behavioral lesion studies (White et al., 2002; Kahn and Bingman, 2009; Herold et al., 2015). In the study of White et al. (2002), pigeons had to learn the association between a spatial-relational configuration and a correct food bowl. There were two spatial-relational conditions, each associated with one food bowl. A third bowl never contained food. Although, pigeons with a hippocampus lesion learned which two bowls contain food, they were unable to choose the correct one in a given spatial-relational condition. Kahn and Bingman (2009) used five bowls, with two bowls containing a highly desirable or less desirable food item, respectively. Again, hippocampus lesioned pigeons were able to distinguish bowls which contain food from non-food bowls. However, they were unable to learn which one contains the more desirable food item. From these and similar studies, it was concluded that the avian hippocampus is necessary for formation of distinct memory representations, preferentially based on spatial properties of the environment (Herold et al., 2015). However, there is still electrophysiological or immediate early gene data needed to support this idea. Furthermore, a variation in similarity between the different spatial conditions could be implemented in such experiments. Impairments in birds with hippocampus lesions positively correlated to the degree of similarity of the different spatial configurations would yield a more convincing evidence for a pattern separation mechanism in birds (Gilbert et al., 2001; McHugh et al., 2007).
1.5  Aim of the study

The aim of our study is to uncover the local circuitry of different areas of the avian forebrain. We are inspired by two main objectives. First, motivated by the desire of neuroscientists to explain behavior with discrete brain mechanisms, we want to establish the basement for future functional studies of circuits in birds. Second, our study shall help to acquire a comprehensive picture about the degree of similarities between forebrains of birds and mammals. Seeking for similarities and differences in the neuroarchitectures of different species can yield significant contributions to our view of the avian brain in terms of homology and homoplasy. We assume that functionally equivalent structures of different species display a high degree of overlap in their local circuitry despite profound differences in their histological appearance.

Common features of the mammalian and avian hippocampi evoke the question, whether these two structures employ similar organization of their circuits to support spatial memory and navigation (Rodríguez et al., 2002; Herold et al., 2015). Many remarkable similarities have also been described between neocortex and the avian wulst in the visual domain (Pettigrew and Konishi, 1976; Pettigrew, 1979; Liu and Pettigrew, 2003). Furthermore, recent studies point to the remark that cell types, circuitry and computations utilized by the sensory circuits of DVR resemble the mammalian neocortical circuits to a certain degree (Wang et al., 2010; Dugas-Ford et al., 2012; Atoji and Karim, 2012, Jarvis et al., 2013; Chen et al., 2013; Atoji and Karim, 2014; Pfenning et al., 2014; Ahumada-Galleguillos et al., 2015; Calabrese and Woolley, 2015; Harris, 2015). All these studies provided important new insights into the organization of the forebrain circuitry but they also sparked new open questions. First, is the laminar and columnar organization of the auditory and visual system of chickens a characteristic feature of the avian DVR which hence can be extrapolated to other modalities and perhaps to other bird species? Second, is the connectivity pattern similar across the modalities? Third, how does the wulst, the derivate of the dorsal pallium, fit into the picture? Does it share the properties with the rest of the forebrain or are there substantial differences in its connectivity pattern and organization?

To answer these questions, we performed in vivo and in vitro tracing experiments to study the circuitry employed by the trigeminal, visual and auditory systems in the avian DVR, the avian wulst and the hippocampus. We chose two different species, the pigeon and the barn
owl. The barn owl possesses an enlarged, well elaborated wulst making this animal a perfect model for studying the organization of this structure. In addition, data from different avian species could facilitate possible extrapolation of the findings to the whole avian taxon.

1.6 Expectations

We expect that the auditory and visual modalities in DVR in pigeons will display connectivity patterns comparable to those identified in chickens. Furthermore, we hypothesize that a very similar pattern of connectivity as well as laminar and columnar organization can also be found in the avian trigeminal system. We further expect that the avian wulst and the hippocampus will show some typical features of their mammalian counterparts. There are two possibilities for the organization of the avian wulst. It might differ from the rest of the DVR since it is different structure from a developmental point of view. On the other hand, it might in principle resemble the organization of the other systems in DVR. Last but not least, we hypothesize that the avian hippocampus utilizes a main pathway which can be bypassed by several others and these pathways might display a precise topography.
2. Material and Methods

2.1 Animals

This study was performed at the Ruhr-University Bochum at the Department of Biopsychology. In total 77 homing pigeons (Columba livia) and 5 barn owls of both sexes were used for the experiments. The pigeons were obtained from local breeding stocks, while the owls were delivered from the Institute of Zoology in RWT Aachen University. 20 pigeons were used for in vivo hippocampal injections and 18 for those performed in vitro. The studies of sensory circuits involved 27 pigeons for in vitro and 10 for combined in vitro and in vivo experiments. Two additional pigeons were used for in vivo injections into the wulst only.

All procedures were in compliance with the national institutes for the care and the use of laboratory animals and were approved by the National Committee of North Rhine-Westphalia, Germany.

2.2 In-vivo tracer application and surgical procedure

The animals were anaesthetized with either a mixture (7:3; 0.15 ml/100g body weight) of ketamine (100 mg/ml; Zoetis GmbH) and xylazine (20 mg/ml; Bayer Vital GmbH) or with isoflurane (0.5%-5%; AbbVie Deutschland GmbH & Co. KG) combined with the same ketamine/xylazine mixture (0.075 ml/100g). The anaesthetized animals were positioned in a stereotaxic apparatus and their heads were fixed in either a lateral or frontal head-holder. Feathers on the head were cut and the scalp was incised to expose the skull. The surrounding muscles were carefully pulled aside when needed and the cranial bone was opened above the injection site using an electric drill. The meninges were opened and a glass micropipette (15-20 μm inner diameter) filled with the Choleratoxin subunit B (CTB, Sigma, Germany) was lowered according to the stereotactic coordinates of Karten and Hodos (1967) into the brain tissue. Between 200-400 nl of the tracer was applied using a mechanic pressure device (Nanoliterinjector 2000; WPI). For animals assigned to the
combined in vivo and in vitro experiments or the in vivo wulst tracing, the injections were performed in both hemispheres while hippocampal injections were unilateral. After 2 days of survival, the animals were subjected to in-vitro experiments or directly anesthetized for perfusion (see section 2.4).

2.3 In-vitro experiments

For in-vitro tracing, the animals were either directly decapitated or first anesthetized with equithesin (0.45 ml/ 100g) and perfused with an ice-cooled sucrose-substituted Krebs solution (210 mM sucrose, 3 mM KCl, 3 mM MgCl₂·6H₂O₂, 23 mM NaHCO₃, 1.2 mM NaH₂PO₄·H₂O, 11 mM β-D-glucose). The brains were quickly dissected and then submerged in this solution for 2 min. The brains were then cut into 800 µm thick coronal slices with a vibratome (LEICA VT 1000S). The slices were collected in an artificial cerebro-spinal fluid (ACSF; 120 mM NaCl, 3 mM KCl, 1 mM MgCl₂·6H₂O₂, 23 mM NaHCO₃, 1.2 mM NaH₂PO₄·H₂O, 2 mM CaCl₂·2H₂O, 11 mM β-D-glucose) continuously oxygenized with carbogen (95% O₂, 5% CO₂) at room temperature. For injections, the sections were placed in a chamber containing ACSF. Biocytin crystals (Santa Cruz Biotechnology) were deposited on the desired regions using a glass micropipette (20 µm inner diameter). The slices were kept in the continuously carbogenized ACSF for 4-6 hours at room temperature and were thereafter immersed in 4% paraformaldehyde in 0.12M PB (pH = 7.4) for 12 hours. Subsequently, they were cryoprotected in 30% sucrose solution (in PBS; pH = 7.4) until they sunk and re-sectioned on a freezing microtome (LEICA) into 35 µm thick slices. The slices were further processed according to the DAB (3,3 diaminobenzidine tetrahydrochloride) or fluorescence protocols described below. All hippocampal in vitro injections and the histological work with these slices was performed by Noemi Rook.

2.4 Histology and Immunohistochemistry

2.4.1 Perfusion and brain slicing

Animals subjected to in vivo injections without in vitro experiments were deeply anesthetized with equithesin (0.45 ml/ 100g body weight) and perfused transcardially with
0.9% NaCl followed by cooled (4°C) 4% paraformaldehyde in 0.12M PB (pH = 7.4). The brains were dissected and post-fixated in 4% paraformaldehyde solution containing 30% sucrose (4°C for 2 hours). Subsequently, the brains were cryoprotected in a sucrose solution (30% in PBS; pH = 7.4) until they sunk to the bottom (usually 24 hours). To facilitate slicing, brains were embedded in a gelatin block (15% gelatin, 30% sucrose) which was also fixated in paraformaldehyde (4% in PBS for 24h). The brains were cut in coronal plane into 30-40 µm thick slices using a freezing microtome (LEICA) and stored at 4 °C in 0.1% sodium azide until further processing. Every fifth slice was used for staining and the analysis.

2.4.2 DAB staining

Standard DAB staining procedure intensified with nickel and cobalt was used to visualize biocytin (Shu et al., 1988; Hellmann and Güntürkün, 2001). To combine biocytin with CTB staining, one of the tracer was stained with and the other without the nickel and cobalt intensification. In this way, we obtained black-brown biocytin/CTB double staining. Briefly, the slices were shortly rinsed in PBS (1 x 10 min) and incubated in 0.3% hydrogen peroxide (H$_2$O$_2$) in distilled water for 30 min to block endogenous peroxidases. For biocytin staining, slices were washed and (3 x 10 min in PBS) directly incubated in the avidin-biotin-peroxidase complex (ABC; see below). After rinsing, CTB slices were incubated in 10% normal rabbit or horse serum (Vector Laboratories – Vectastain Elite ABC kit) in PBS with 0.3% Triton-X-100 (PBST) to block unspecific binding sites. After the serum incubation, the slices were incubated in a polyclonal goat-anti-CTB antibody (1:10 000 in PBST; Millipore) at 4 °C for 1-3 days. After rinsing in PBS (3 x 10 min), slices were transferred into secondary biotinylated rabbit anti-goat antibody (1:200 in PBST; Vector Laboratories – Vectastain Elite ABC kit) for 60 min at room temperature. After further washing in PBS (3 x 10 min), slices were incubated in ABC (Vector Laboratories – Vectastain Elite ABC kit; 1:100 in PBST) for 60 min at room temperature. Slices were rinsed in 3 x 10 min in PBS, and when nickel and cobalt intensification were used they were additionally washed 1 x 5 min in 0.1 M sodium acetate buffer (pH 6.0) before transferring into a DAB solution. Since the DAB solution contained β-D-glucose, the staining reaction could be induced by adding glucose-oxidase (80-100 µl in 50 ml DAB solution). While the DAB solution was prepared in 0.1 M sodium acetate buffer (pH 6.0) for black staining, the DAB was dissolved in PBS for brown staining. The reaction lasted 30 min (staining intensity was visually controlled) and the reaction solution was changed.
every 10 min. Rinsing the slices in 0.1 M sodium acetate buffer (pH 6.0; 3 x 5 min) or PBS stopped the reaction. After further washing in PBS (3 x 10 min), slices were mounted on gelatin-coated slides and either counterstained with Nissl or directly dehydrated in ethanol and coverslipped with DPX (Fluka).

2.4.3 Fluorescence staining

In addition to DAB double staining, fluorescence staining was also used for visualization of biocytin and CTB injected sections. Slices were washed in PBS (3 x 10 min) before incubation in 10% normal horse serum (30 min, Vector Laboratories – Vectastain Elite ABC kit; in PBST). After that, they were incubated in a polyclonal goat anti-CTB antibody (Sigma-Aldrich; 1:5000 in PBST) for 72 hours. The next day, the slices were washed in PBS (3 x 10 min) and incubated in a mixture of the secondary antibody Alexa-488 donkey anti-goat (Invitrogen; 1:1000 in PBST) and Alexa-594 streptavidin (Invitrogen; 1:1000 in PBST) for 60 min. Streptavidin, like avidin binds strongly to biotin and therefore can be used to visualize biocytin, if conjugated to a fluorophore. Finally, the slices were washed in PBS (3 x 10 min), mounted on polarized glass slides and coverslipped with DAPI-Fluoromount (SouthernBiotech).

2.5 Microscopy and analysis

The slices were analyzed with a ZEISS Imager.M1 AXIO microscope equipped with an AxioCam MRm ZEISS 60N-C 2/3” 0.63x camera. The ZEISS filter sets 45 (excitation: BP 560/40, beam splitter: FT 585, emission: BP 630/75) and 38 (excitation: BP 470/40, beam splitter: FT 495, emission: BP 525/50) were used for the analysis of the fluorescence stainings. Pictures of the relevant slices were taken via the computer software AxioVision (AxioVision, Version: 4.8.1.0). Color, contrast and brightness adjustments were accomplished via the AxioVision software as well as the CorelDRAW X5 (Version 15.2.0.686; Corel Corporation). Confocal analysis was performed with the aid of a confocal laser scanning microscope (LSM 510, Zeiss) in combination with Zeiss 40x (Plan-Neofluar, NA 1.3) oil immersion lenses. All figures were prepared in CorelDRAW X5.
3. Results

3.1 Visual tectofugal system

The main telencephalic parts of the tectofugal visual system, which we focused on, are the entopallium, the overlying mesopallium and the intermediate nidopallium intercalated in-between these two structures (Figure 8). In Nissl staining, entopallium displayed a sort of concentric organization of mainly polygonal and triangularly shaped medium sized neurons. This arrangement of neurons rapidly changed at the dorsal border of the entopallium. Here, different types of neurons in NI, slightly varying in shape and size, formed thin vertically oriented rows separated by neuropil and fibers interconnecting the entopallium with the mesopallium (see below). The mesopallium was separated from the ventrally adjacent NI by a clearly visible lamina called the lamina mesopallialis (LM). The vertical arrangement of neurons in NI very often continued dorsally behind the LM up to the ventral MVL. The transition between the NI and MVL was also noticeable by a preponderance of rather round neurons in MVL with a tendency to build chunks consisting of up to 7-8 neurons. These chunks were restricted mainly to the most ventral part of the MVL possibly demarcating the internal sublayer of

Figure 8: Transversal section of the avian telencephalon. Nissl stained transversal section of the telencephalon of a pigeon. The visual tectofugal system includes the entopallium (E), intermediate nidopallium (NI) and the overlaying ventral and dorsal part of the lateral mesopallium (MVL, MD). The MVL can be further subdivided into the external and the internal part (MVL-in, MVL-ex).
RESULTS

MVL (MVL-in) identified previously in chickens (Ahumada-Galleguillos et al., 2015). Neurons in the external part of the MVL formed less and smaller chunks, which also clearly distinguished this part of the MVL from the dorsally contiguous dorsal mesopallium (MD). We performed biocytin crystal depositions into all three structures to investigate the connectivity of the visual tectofugal system.

Figure 9: Entopallium connectivity.
The entopallium is reciprocally connected with the MVL and NI. A, D: Anterogradelly labelled fibers after deposition of a biocytin crystal into the entopallium. Fibers form bundles and run vertically towards the mesopallium, where they terminate. The square in (A) shows the position of the magnified region from a consecutive slice shown in (D). B - C: Injections into E also resulted in retrograde labelling within the MVL. The fibers in fiber bundles leave collaterals in the NI. E: Retrogradelly labelled neurons in NI after injections into entopallium. F: Magnification of the collaterals demerging from the fibers interconnecting the entopallium with the MVL. The square in (C) shows the approximate region where the picture in was taken in another animal. Scale bar in F applies to all photograps. The section in (B) is counterstained with Nissl. A = 200µ; B, C, E = 100µm; D, F = 20µm.
RESULTS

Due to successful both retrograde and anterograde transport of biocytin, the injections into entopallium already uncovered plenty of aspects of the connectivity within this system (Figure 9). First of all, the entopallium was heavily interconnected with the MVL. Fibers leaving the entopallium formed thick bundles (15-50 µm) running dorsally towards the mesopallium, while also leaving few collaterals in the inter-bundle space (Figure 9C, F). Shortly after they crossed the LM, the majority of the fibers terminated within the MVL (Figure 9D). A minority of fibers continued further dorsally to reach the MD. The termination field within the MVL largely overlapped with the location of retrogradelly labelled neurons which gave rise to a strong feedback projection to the entopallium (Figure 9B, C).

![Figure 10: Different types of neurons in NI.](image)

Retrogradelly labelled neurons in NI after entopallium injections had triangular (A), polygonal (B), oval (C), fusiform and round shape (D). Neurons were embedded in-between the fiber bundles interconnecting the meso- and entopallium. A: Neuron with a triangular cell body and dendrites bifurcating distantly from the soma. The neurons often possessed a “main” dendrite (white arrow) which was thicker than the other primary dendrites. B: Polygonally shaped neuron with one thick bifurcating dendrite (white arrow) and three other dendrites demerging from the soma in different directions. C: Oval neuron with stellate-like dendritic arrangement with very sparsely branched dendrites. D: Left - two round neurons with dendrites leaving the body in different directions. Right – a fusiform neuron with two thick dendrites arising from one site of the cell body and two thinner dendrites in the opposite direction. Scale bar = 20µm, applies to A-D.
The retrogradelly labelled neurons in MVL were mainly round to polygonal with dendrites leaving the cell bodies in all directions with rather sparse arborizations. Some neurons also had triangular shape and had distantly bifurcating processes which gave rise to broader dendritic arbors. The entopallium was previously divided into internal (ventral) and the external (dorsal) part (Husband and Shimizu, 1999; Krützfeldt and Wild, 2005). Krützfeldt and Wild (2005) showed that it is mainly the ventral part which is interconnected with the MVL. In our experiments, both injections into the dorsal and ventral part gave rise to the mesopallial connections (Figure 12).

![Figure 11: Spiny neurons in NI.](image)

**Figure 11: Spiny neurons in NI.**

A - B: Two examples of a spiny neuron type in NI labelled retrogradelly after entopallium injection. The somas of these neuron have a triangular shape, they give rise to four primary dendrites which than split into two to three further branching dendritic shafts. The dendrites of these neurons contain dendritic spines, and extend in different directions across several fiber bundles, which run between ento- and mesopallium. The sections are counterstained with Nissl. Scale bar = 10 μm.

Furthermore, these injections also revealed that the NI projects back upon the entopallium (Figure 9E). The occasional Golgi-like staining of biocytin labelled neurons enabled us to inspect the morphology of the NI neurons. We observed neurons with various soma shapes including polygonal, triangular, fusiform and oval somas (Figure 10). These neurons were located in-between the above described fiber bundles stretching their dendrites across several bundles in all directions. For many polygonal, oval and fusiform neurons, we observed only sparse dendritic arbor, while the triangular neurons usually displayed widely ramifying spiny dendrites (Figure 11). The dendrites of these neurons usually first bifurcated distantly (up to 8 μm) from the soma into two or three branches. In some neurons, one dendrite was usually thicker than the rest. This was also true for some sparsely ramifying neurons (Figure 10A, B).
Figure 12: Dorsal and ventral aspects of the entopallium project to MVL.
A: Example of an injection into the ventral part of the entopallium. B: Magnification of the fibers projecting to MVL after the injection shown in (A). C: Even small injections into the most dorsal aspect of the entopallium produced dense anterograde labelling of fibers projecting to MVL. D: Magnification of fibers labelled by the injection shown in C. Scale bars = 500 μm in A and C; 100 μm in B and D.
Crystal depositions into NI labelled the dorsal aspects of the fiber bundles in MVL. We observed anterogradely labelled fibers and retrogradely labelled neurons in MVL (Figure 13). Few fibers left the NI also ventrally running towards the entopallium but we were not able to see terminations there. We found few retrogradely labelled neurons restricted to the most dorsal aspect of the entopallium (Figure 13B). These data indicate that in addition to its interconnection with entopallium, NI seems to be reciprocally connected also to MVL. In addition, we observed many short cut fibers apparently leaving the injection site medioventrally, however, they immediately changed their direction rendering it impossible to follow them further in this transversal section (Figure 13C).

Figure 13: NI is interconnected with MVL.
A: Nissl stained section showing the location of the crystal deposition in NI. B: Magnification of the selected region in (A). Labelled neurons and fibers were found in MVL. The arrows point to location of retrogradely labelled neurons in MVL and entopallium. The inserts in top right and left low corners are higher magnifications of labelled neurons in MVL and entopallium, respectively. C: Fibers also left the injection site medioventrally. These fibers seem to have been cut during the in vitro procedure, indicating that they changed they direction. Black arrows point to examples of such fibers. Scale bar in C applies to all photos. A= 400 µm; B and C = 50 µm; 30 µm in the inserts in B.
Although the injections into the MVL were less successful to produce retrograde staining they nevertheless confirmed the above described connectivity revealed by NI and entopallium injections. After MVL injections, many fibers, arranged in bundles, left the MVL ventrally towards the entopallium (Figure 14A). We could follow these fibers up to the middle part of the entopallium with few fibers continuing to the most ventral aspect of the entopallium. The fibers in the dorsal two-thirds of the entopallium gave rise to many thin collaterals, which appeared to form terminal-like structures. These fibers also contained plenty of varicosity-like structures making synaptic contacts very likely, in both dorsal and ventral parts of the entopallium (Figure 14C, Figure 16). Sporadically, retrogradelly labelled neurons were located in the entopallium, with the majority found in the most ventral aspect closely adjacent to the pallio-subpallial boundary (insert in Figure 14C). Several retrogradelly labelled neurons were also found in NI scattered between the LM and the entopallium (Figure 14A). In addition, the fibers running to the entopallium displayed varicosities and

Figure 14: MVL injections confirmed the connectivity between NI and entopallium and MVL.
A: Injections into MVL produced dense anterograde fiber labelling as well as occasional retrograde labelling of neurons in NI (black arrows) and entopallium (insert in C, black arrows). B: Varicose fibers in NI. Arrows point to a long horizontal collateral. C: Varicose branching fibers in the dorsal entopallium. Scale bars: 100 µm in A; 20 µm in B and C; 50 µm in the insert in C.
RESULTS

branched off many short and few long collaterals in NI (Figure 14B). Thus, these findings confirm the reciprocal connections between the MVL and NI and the entopallium. In addition, consistent with the anterograde labelling of fibers in MD after entopallium injections, MD injections labelled neurons in NI and entopallium as well as fibers descending towards NI and entopallium (Figure S1). Moreover MVL and MD seem to be interconnected too (Figure S2).

![CTB, Biocytin](image)

**Figure 15: NCL projecting neurons in NI located in-between the bundles.**

CTB-labelled neurons (green) after injection into the NCL (Figure S3) and biocytin labelled fibers after injections into the entopallium. The neurons are located in-between the bundles but seem to expand their dendrites across them (white arrow).
**RESULTS**

Figure 16: Mesopallium projects to dorsal and ventral entopallium.

A: Injection into the MVL labelled many fibers organized into fiber bundles running towards the entopallium. The fibers reach both the dorsal and ventral part of the entopallium. B: Magnification of labelled fibers in entopallium from a neighboring slice. In this particular case it seems that the labelling in Ed is stronger than in Ev. Scale bars 50 µm in A; 100 µm in B.

Figure 17: Neurons in NI forward the visual information to the NCL.

A-B: Two examples of labelled neurons in NI after CTB (green) injections into NCL. The neurons received input from bypassing fibers labelled with biocytin (red) injected into the entopallium. White arrows point to colocalization of green and red signal. The pictures were taken with a confocal microscope. The sections are counterstained with DAPI (blue). Scale bar = 5 µm.
So far, the results showed that the visual areas entopallium, NI and MVL form a tightly interconnected network. To complement the understanding of this circuitry, we further investigated how the information leaves these circuits. To this end, we combined in vitro tracing with in vivo injections of CTB into the caudolateral nidopallium (NCL), an associative area, analogous to the mammalian prefrontal cortex, and two motor output structures, the arcopallium (Arco) and the apical part of the hyperpallium (HA). While HA and Arco CTB-injections resulted in retrograde labelling of neurons in the lateral part of the medial intermediate nidopallium (NIMI), a structure located medially to the entopallium, injections into the NCL labelled neurons in NI. With confocal microscopy, we could show that the fibers labelled by biocytin depositions into entopallium most likely contacted these neurons on their way between ento- and mesopallium (Figure 17). The neurons were located predominantly in-between the fiber bundles but possibly extended their dendrites across the bundles (Figure 15).
3.2 The trigeminal system

Within the trigeminal system, we investigated the BAS, the overlying NFT and the mesopallium (Figure 18). Like in the NI, neurons in NFT formed vertical rows in Nissl stained sections. The BAS was clearly distinguishable by the lack of such rows and a higher density of neurons. The cytoarchitecture of the overlying mesopallium was similar to the one in the visual domain, with noticeable differences between ventral (MFV) and dorsal (MFD) part, including a thin layer in the most ventral part (MFV-in).

![Figure 18: Sagittal section of the avian telencephalon.](image)

Nissl stained sagittal section of the telencephalon of a pigeon. The trigeminal system consists of the nucleus basorostralis pallii (BAS), the frontotrigeminal nidopallium (NFT), and the ventral and dorsal part of the overlying mesopallium (MFV and MFD).

Similar to the visual system, crystal depositions of biocytin into the BAS uncovered a strong projection to the overlying mesopallium with axons forming a vertically oriented column. The axons displayed a slight tendency to be grouped into bundles but in contrast to the visual system, the bundles here were far less distinct. The fibers crossed the LM and the majority terminated there, while a small fraction continued to the dorsal mesopallium (Figure 20A, Figure 19). Although, we did not observe retrogradelly labelled neurons in MFV,
we found plenty of neurons in NFT (Figure 20B, C). Again, in some cases the occasional Golgi-like staining revealed some morphological features of these neurons (Figure 20C). Similar to NI, we found neurons with round, oval, polygonal as well as triangular soma shapes with wide dendritic ramifications. The dendrites left the soma in many directions and branched into 2-3 further branches distantly from the soma. In several neurons, one dendrite appeared thicker than the rest. In order to dissociate ventral and dorsal BAS projections, we deposited crystals into ventral and dorsal BAS in two consecutive sagittal vibratome slices (800 µm), respectively. In this particular case, we noticed that the ventral BAS injection labelled profoundly less fibers running towards the MFV (Figure 21). This data indicate that the dorsal BAS might be stronger connected to MFV than the ventral part.

Figure 19: BAS targets both the ventral and dorsal mesopallium. 
A: BAS projects to both ventral and dorsal mesopallium. B: Whereas the majority of axons terminate in the ventral mesopallium (white arrow), a minor branch (black arrow) continues towards the dorsal mesopallium. C: Example of a fiber terminating in the MD. Scale bars = 500 µm in A; 20 µm in B-C.
Figure 20: BAS connectivity.
A: Vertically oriented fibers emerging from BAS projecting to overlying mesopallium. B: Injections into BAS also labelled neurons in NFT. C: Example of a neuron from NFT with wide dendritic ramifications in all directions. Dendrites ramify distantly from the soma into two-three father branches. One dendrite appeared to be thicker than the others (white arrow). Scale bar = 100 µm in A; 50 µm in B; 20 µm in C.
Figure 21: Dorsal BAS projections.
A: Small biocytin crystal placed into the dorsal portion of the BAS yield massive labelling of fibers running towards the mesopallium. B: The trigeminal column under higher magnification. C: On the other hand, ventral portion of the BAS was much less connected to the NFT and MFV. D: Magnification of the BAS and NFT in C. Scale bar = 500 µm in A and C; 100 µm in B and D.
After MFV injections, we could follow some fibers up to the BAS and found also few retrogradely labelled neurons in BAS (Figure 22B). In addition, several retrograde labelled neurons as well as anterograde labelled fibers could be detected in NFT (Figure 22C-D). Injections into NFT further labelled neurons in BAS, however, we could see virtually no fibers or cells in the MFV (Figure 23). These data show that the BAS is strongly interconnected with overlaying NFT and projects also densely to the MFV. The back projections from MFV were seen only weakly in our cases. In addition, NFT and MFV are also reciprocally connected. This connection also appeared weaker in our slices.

Figure 22: MFV is interconnected with NFT and BAS.
A: Overview of a Nissl stained section showing an example of MFV injections. B: Fibers from the injection site placed in MFV can be followed up to the BAS. C: Retrograde labelled neurons and anterogradely labelled fibers (black arrows) were found in NFT, indicating a reciprocal connection between NFT and mesopallium. D: Additional example of anterograde and retrograde labelling in NFT from another case. Insert in B shows retrogradely labelled neurons in BAS after mesopallium injections. Scale bar = 200 µm in A; 20 µm in B; 100 µm in B-insert; 20 µm in C.
As in the previous section, we were interested how the information exits the trigeminal sensory circuit. Previous work showed that NFT projects to the arcopallium as well as to the NCL (Wild et al., 1985; Kröner and Güntürkün, 1999). In accordance, we observed retrogradely labelled neurons in NFT after CTB injections into both arcopallium (Figure 24A-B; Figure S5B) and NCL (Figure 24; Figure S5A). Biocytin labelled fibers running from the BAS to the MFV seem to contact these neurons. We observed varicosities in the proximity of cell bodies and dendrites indicating possible synaptic contacts. In addition, in one case we observed anterogradely labelled varicosity-rich fiber on a neuron retrogradely labelled after BAS injection (Figure 24D). The latter finding indicates that a portion of neurons in NFT receives input from BAS and directly feeds back to it.

Figure 23: NFT injections labelled BAS neurons.
A: Nissl stained section showing biocytin injection placed into NFT. B: This injection retrogradely labelled neurons BAS. Scale bar in A = 500 µm; 20 µm.
Figure 24: NFT forwards the information to NCL and arcopallium.

A: CTB labelled neurons (green) in NFT projecting to the arcopallium. The neurons are contacted by varicosity-rich fibers labelled with biocytin (red) which was injected into the BAS. B: Enlargement of the region marked with a rectangle in (A). Varicosities on the biocytin labelled fibers were found in a close proximity to the cell somas and proximal part of the dendrites (white arrows). C: Neurons labelled with CTB (green) injected into NCL. Fibers coming from the BAS labelled with biocytin (red). D: Magnification of NFT in a consecutive slice. Similar to the arcopallium case, we observed varicosity-rich fibers apparently contacting the CTB labelled neurons projecting to NCL (white arrows). The arrowhead points to an example of varicosity rich fiber found on a neuron retrogradelly labelled with biocytin. Scale bar = 20 µm in A-B and D; 100 µm in C.
3.3 The auditory system

The main parts of the auditory system include the Field L, the CMM and the NCM (Figure 25). The field L is recognizable by densely packed mainly round neurons. It can be subdivided into L1-L3, with L1 lying adjacent to the CMM, L2 and L3 are juxtaposed further ventrally. As in the other sensory systems, the auditory mesopallium was clearly separated by the LM from the Field L and distinguishable by neurons grouped into chunks. In contrast to NI and NFT, we did not observe vertically oriented rows in neither of the auditory regions.

Figure 25: The auditory system of the pigeon. Transversal Nissl stained section of the forebrain of the pigeon showing the parts of the auditory system including the Field L with its three subdivisions (L1-L3), caudomedial mesopallium (CMM). The NCM extends also further caudally behind the Field L. Further components of the auditory system include the dorsal nidopallium (Nd, part of the NCL) and the ventrointermediate acropallium. Both structures are not depicted in the figure as they are located caudally to the slice shown.
Figure 26: Connectivity within the auditory system.
A: Injection site into the L2 of the Field L. B: Injections into the L2 labelled vertically oriented fibers running towards the CMM. Retrogradely labelled neurons in L1 were also observed. C: Example of a neuron in L1 labelled by injection into the L2. D: Injection into L1 labelled neurons and fibers in CMM. Scale bar = 500 µm in A; 50 µm in B; 20 µm in C; 100 µm.
Injections into area L2 of the Field L produced labelling of fibers running towards the CMM oriented orthogonally to the LM (Figure 26 A-B). Similar to previous description of the visual and trigeminal systems, fibers contained varicosities and some of them gave rise to collaterals oriented perpendicularly to the dorsally running fibers (Figure 28). The dorsally running fibers crossed the LM and reached the CMM. Several of these fibers could be followed far dorsally up to the dorsal aspects of the CMM. Few neurons were occasionally labelled in CMM and L1 (Figure 26 B-C). In addition, fibers also left the injections site into other directions, including ventrally and caudally. Furthermore, L1 was also interconnected with CMM (Figure 26D). Considerable amount of fibers run also ventrally from the L1 injections possibly reaching the L3. Few fibers towards L3 could also be followed from L2.

In vitro investigations of the auditory system combined with in vivo injections into the associative and motor output areas uncovered a very interesting organization of this system. First, the arcopallium injections showed that the output of the auditory circuitry might

---

**Figure 28:** Fibers running to the CMM and their collaterals. The picture shows fibers originating in the L2 running towards the CMM (white arrows). The fibers contained varicosities and some branched collaterals (black arrows) running perpendicular to the rest of the fibers. Scale bar = 20 µm.

**Figure 27:** Output of the auditory system to the arcopallium. CTB labelled neurons projecting to the arcopallium (green) are located in the CMM. These neurons are reached by the fibers coming from the L2. Scale bar = 20 µm
directly reach the arcopallium via the CMM, where neurons projecting to arcopallium and anterograde labelled fibers coming from the L2 were found (Figure 27). Second, similar to the NFT and NI in the trigeminal and visual systems, the L1 contained neurons projecting to the NCL (Figure 29). These neurons are embedded in a dense plexus of fibers originating in L2. Third, the L1 also contained neurons which project to the HA and might be contacted by fibers originating from the L2 as well as from the CMM (Figure 31, Figure 30). CMM injections also labelled few neurons in L1, which were intermingled with the HA projecting neurons. Thus the information from the auditory column constituted by the Field L and CMM can be further propagated to both the NCL and the HA via the L1 and to the arcopallium via the CMM. In addition, the HA injections also uncovered a further cell population arranged concentrically around the very most caudal pole of the medial pallium. These cells are contacted by fibers leaving the L2 caudally.

Figure 29: Output of the auditory system to the NCL.
A: CTB labelled neurons projecting to the NCL (green) are located in the L1. The neurons are embedded in fibers coming from the L2. B: Confocal image of NCL projecting neurons in L1 contacted by the bypassing fibers connecting the L2 with the CMM. Scale bars = 500 µm in A; 10 µm in B-C.
**Figure 31: Output of the auditory system to the HA.**
A: CTB labelled neurons in the caudalmost part of the nidopallium projecting to HA. B: Fibers from L2 reaching this location. C: Overlay of A and B. D: Low power picture of the caudomedial pallium in a sagittal section showing the auditory Field L, with biocytin injected into the L2 and CTB labelled neurons projecting to HA located in L1 and the caudal portion of the brain. E: Schematic drawing of a sagittal section demarcating the region showed in A-E. The black line in the picture of the brain depicts the approximate position of the slice. Scale bar = 50 µm in A-C; 500 µm in D.

**Figure 30: HA projecting neurons contacted by fibers from CMM.**
A: CTB labelled neurons in L1 projecting to HA. B: Fibers coming from CMM labelled by biocytin. C: Overlay of (A) and (B). White arrow points to an example of a CTB labelled neuron contacted by a biocytin labelled fiber coming from CMM. Scale bar = 50 µm.
3.4 The wulst

As outlined above (1.5), an important aspect in the understanding of the forebrain circuitry is to resolve the circuitry utilized by the avian wulst. For this purpose, we chose the barn owl as a model, as this animal possesses a large and well elaborated wulst. Thus, we describe the results of our in vitro tracings of the owl first and then switch to the pigeon.

**Figure 32: Forebrain of the barn owl.**
Transversal Nissl stained forebrain section of the barn owl depicting the different layers recognizable in Nissl staining. The wulst is constituted by the apical (HA), the interstitial (IHA), the intercalated (HI) and the densocellular (HD) part. The HA is usually subdivided into pars externa (ex-IHA) and pars interna (in-IHA).
The hyperpallium of the barn owl consists of several layers including the HA, IHA, HI and HD (Figure 32). The HA is a thick layer consisting of large neurons interspersed with smaller loosely packed granulate neurons. The dorsal part of the HA (dHA) appeared to contain more large neurons and less granulate cells than the ventral part (vHA). Contiguous with the vHA, a prominent, thick and easily recognizable granular layer is located. The layer is arched alongside the whole extend of the HA. This layer contains exclusively densely packed small

Figure 33: Interlaminar connectivity of the wulst in the barn owl.
A: Terminations in ex-IHA and vHA observed after biocytin crystal depositions into HI. B: Injections into the ex-IHA with spread into the vHA but no spread into in-IHA labelled many neurons in in-IHA and in the dorsal aspect of HI. C: As revealed by injections into HI, HD projected heavily to this layer. D: HD showed a strong intralaminar connectivity. Fibers run within the HD along its long axis. E: Higher magnification of the fibers within the HD. Scale bars = 20 µm in A and E; 50 µm in B; 100 µm in C; 200 µm in D.
RESULTS

granulate neurons differently arranged along the dorsoventral width of the layer. While the neurons in the most dorsal and the most ventral aspects of this layer were randomly distributed, the middle portion, which constituted the largest part of the layer, appeared to be arranged into vertical rows. We considered the dorsal part of the IHA to be the external part (ex-IHA), while the middle and ventral part was assigned as the internal part of IHA (in-IHA). Ventral to IHA, the density of granular neurons progressively decreased and they were intermixed with bigger-sized neurons within the HI.

Figure 34: In-vitro injections into the HA.
Left, two Nissl stained sections from a case in which biocytin was injected into the HA. Labelled fibers could be followed from the injection towards the other hyperpallial layers. The fibers crossed the IHA and could be found also in HI and HD. The fibers usually did not terminate there, but often continued to the mesopallium and in some cases reached the NFL. A: Higher magnification of varicosity-rich fibers and terminals found in the IHA at the position marked by a red rectangle in the left upper slice. B: Higher magnification of a region in HI marked by the red rectangle in the lower slice left. C: Higher magnification of a region in HD marked by the red rectangle in the lower slice left. Black arrows point to examples of varicosities and axon terminals. Scale bar = 20 µm.

Further ventrally the HD, a thinner layer of densely packed bigger neurons was located. This layer was virtually devoid of granular neurons and was also clearly distinguishable from the ventrally adjacent mesopallium (Figure S7B). The mesopallium possessed a fascinating arrangement of cells reminding of a flourishing meadow (Figure S7A). Small granular neurons surrounded a bigger cell or cell cluster. Such cell groups were also found in other
layers such as the HI and HA but were by far most densely disseminated within the mesopallium.

An exceedingly pronounced connection was observed between the in-IHA and ex-IHA and between the dorsal portion of HI and ex-IHA and vHA (Figure 33B). Biocytin crystals deposited into the ex-IHA with spread into the vHA but no spread into in-IHA labelled many neurons in in-IHA and in the dorsal aspect of HI. The axons of these neurons formed a very dense fiber column with fibers running in-between the thin vertical rows of the in-IHA. Anterogradely labelled fiber reached also the HI. The labelled neurons in in-IHA were small granulate with sparse dendritic ramifications, while the neurons in HI were bigger and had also triangular or oval soma shape with larger dendritic ramifications. In accordance, injections into the HI labelled fibers terminating within both, ex-IHA and vHA (Figure 33A). Furthermore, fibers also left the injection ventrally to innervate the HD. Except of a shorter bunch of fibers, which immediately entered the HD, another loose bundle of fibers run lateroventrally to enter the HD in a more distant location from the injection site and then continued running along the HD. Only few fibers could be found in the mesopallium. In addition, HI injections also labelled many neurons in HA and HD (Figure 33C). The neurons in HA had various shapes and many of them had a stellate-like appearance. They were quite

**Figure 35: In-vitro injections into the IHA.**
Left, a Nissl stained section showing the injection into the IHA. Labelled fibers with varicosities and terminal like structures were found in HA (A) and HI (B). A: Region marked with a red rectangle in HA in the slice left. B: Region marked with a red rectangle in HI in the slice left. Black arrows point to examples of varicosities and axon terminals. Scale bar= 20 µm.
broadly distributed within the HA. The projection of HA to HI was also confirmed by injections placed into HA. Fibers running towards the HI appeared to contact neurons in the IHA. Moreover, injections placed into the most dorsal aspect of HA retrogradelly labelled neurons in vHA indicating an interconnection of the two HA subdivisions. The retrogradelly labelled neurons in HD were mostly concentrated underneath the injection site in HI with several neurons also found distributed further along the medio-lateral axis (Figure 33C). This projection was also confirmed anterogradelly by injections into HD. HD also projected to the mesopallium. However, the most impressive fiber labelling after HD injection was found within the HD itself. Here, many fibers run along the layer, only very rarely crossing the boundary of the HD (Figure 33E). As these fibers run along the layer in contrast to the interlaminar fibers which cross the layers, we refer to this connectivity pattern with the term horizontal connectivity.

The barn owl, as a model with a remarkably elaborated wulst, helped us to identify the hyperpallial circuitry. For a comparative interpretation of these data, it is important to explore to which degree this circuitry is present in birds with less elaborated wulst. To this end, we investigated the hyperpallial circuitry in the pigeon. The wulst of pigeons is much
smaller than that of an owl but is constituted by the same layers including the thalamo-
recipient IHA, the laterally adjacent HI and HD, and the output layer HA located medially.

![Figure 37: Projections of the border layer between HD and MD.](image)

**A:** Injections into the arcopallium labelled plenty of neurons in the border layer between HD and MD. Depicted is a medial sagittal section. **B:** Magnification of the region marked by the rectangle in (A). Scale bars = 500 µm in A; 100 µm.

The IHA in pigeons is tremendously thinner than the one in the owls and cannot be
differentiated into further sublayers. It is recognizable in the Nissl staining by its slightly
higher density of neurons and the presence of granular neurons (though much less than in
the owl) at the lateral aspect of HA. Lateral to IHA, there is the HI and HD. In contrast to the
owl, the cytoarchitecture of HI and HD is very similar making it more difficult to find the
border between the two, especially in the rostral wulst, where the lamina frontalis suprema
(LFM) is not clearly visible. Nevertheless, the neurons in HD are little more densely packed
and might have higher tendency to form chunks making it possible to distinguish these two
layers. In the rostral slices, this border is usually aligned with the vallecula. The HD is
separated from the ventrolaterally adjacent MD by the lamina frontalis superior (LFS).
However close examination of Nissl stained sections revealed that another layer, which is
often neglected, is interposed between HD and MD. This layer, however has clearly different
architecture resembling neither HD nor MD and possibly also possesses an idiosyncratic
connectivity pattern (see below; Figure 37). Therefore, this border layer may be considered
as an individual distinct layer possibly belonging to the wulst or the mesopallium. The
density of the neurons in this layer appeared lower and the neurons, which are rather small
and round, are arranged into many short elongated chunks oriented alongside the layer.
RESULTS

In vitro injection of biocytin into the HA labelled fibers which left the injection site towards the other hyperpallial layers (Figure 34). The fibers crossed the IHA and could be found also in HI and HD. However, they usually did not terminate there, but often continued to the mesopallium and in some cases reached the NFL. We observed both thin fibers with varicosities as well as much thicker fibers without such structures. We supposed that the thick, varicosity-free fibers possibly represent some long-distance connections, while the varicose fibers found in IHA, HI as well as HD indicate that HA projects to all the other hyperpallial layers. In addition, in several cases, we observed fibers leaving the injection site ventrally, possibly running towards the medial striatum (Shimizu et al., 1995).

![Image](image_url)

**Figure 38: In-vitro injections into the HD.**

Left, a Nissl stained section showing the injection into the HD. Labelled fibers with varicosities and terminal like structures were found in HI and IHA. **A:** Region marked with a red rectangle at the border between IHA and HI. **B:** Region marked with a red rectangle in HI in the slice left. Black arrows point to examples of varicosities and axon terminals. Scale bar= 20 µm.

After injections into the IHA, we observed many fibers with varicosities and occasionally with terminal like structure in HA and fewer fibers terminating in HI (Figure 35). HI injections revealed a projection from HI to IHA and HA (Figure 36). In some cases, few fibers crossed the border towards the HD. Injections into HD labelled fibers containing varicosities and axon terminals within the HI (Figure 38). Several fibers continued further medially to reach the IHA and in some cases also the HA. Given the dense fiber labelling within the HD described above in owls, we wondered whether such a prominent intralaminar connectivity is also present in HD of pigeons. Interestingly, we did not observe such a strong horizontal connectivity in HD but it was present at the border between HD and MD, in the border layer mentioned above (Figure 39). To see whether the mesopallium is involved in the wulst
circuitry, we placed biocytin crystals into this structure (Figure 40). Such injections labelled fibers running towards the HA across all the hyperpallial layers. However, these fibers contained much less varicosities and hence the projections of the mesopallium to the wulst might be only minor (see also below).

![Figure 39: Intralaminar connectivity in the wulst of pigeon.](image)

A: Injection was placed into the border layer between HD and MD. Fibers were found running within this layer. B: Higher magnification of fibers in the rectangle in A. Scale bars = 500 µm in A; 20 µm in B.

Since our in vitro injections in the pigeon wulst were less successful in retrograde transport of the tracer, we performed in vivo injections of CTB to further investigate and confirm the inputs and outputs of different layers of the wulst. CTB injections into the HA labelled many fibers running towards the HI and the HD as seen in the in vitro cases. Many neurons were labelled within HA including IHA around the injection site (Figure 41). From the hyperpallial layers HD provided by far the strongest input to the HA, since plenty of retrogradelly labelled neurons were observed there. In addition, HI also significantly contributed to the HA afferents, however this projection was weaker than from HD. While in the most rostral slices, many neurons were found in the lateral aspect of the HI, near the border to HD, in slightly caudal slices, the retrogradelly labelled neurons were virtually restricted to the HD. The medial part of the HI had only minor projections to HA.
In addition, the bunch of fibers running across the hyperpallial layers continued through the mesopallium to innervate the NFL. The NFL also contained labelled neurons showing that HA is reciprocally connected with NFL. Both neurons and fibers in the NFL were predominantly restricted to its most ventral aspect. Many neurons were also found in the mediolateral aspect of the NFL, closely adjacent to the LM. This population of neurons was distributed further up to the NFT but remained ventrally juxtaposed to the LM. Only very few neurons were scattered throughout the mesopallium. Furthermore, as indicated by the in vitro experiments, fibers from the HA travelled also ventrally to reach the lateral aspect of the medial striatum. In a case, in which the injection was place mainly into the dorsal aspect of the HI, with a small spread into HA and HD, we observed retrogradelly labelled neurons within HI, HA and HD. Further neurons were also found in NFL and few in MD. Neurons in HD and MD were mostly restricted to the dorsal portions of the regions aligned to the dorsal location of the injection site.

Figure 40: In-vitro injections into the MD.
Left, a Nissl stained section showing the injection into the MD. Labelled fibers were observed in all hyperpallial layers. Only few varicosities could be observed. A: Region marked with a red in HA. B: Region marked with a red rectangle in IHA in the slice left. C: Region marked with a red rectangle at the border between IHA and HI. Black arrows point to examples of varicosities and axon terminals. Scale bar= 20 µm.
Figure 41: In vivo injection into the HA in the pigeon.

A: Many neurons were labelled within the HA around the injection site. Fibers travelling across the layers were observed as noticed also in the in vitro tracings. Plenty of neurons were retrogradelly labelled within the HD. Less but still a lot of neurons were found in the lateral HI and only few neurons were found in the medial aspect of HI. B: Low power photograph of a section showing the injection site in HA. Scale bars = 100 µm in A; 1000 µm in B.
3.5 Summary of the connectivity within the sensory systems

Before we turn to the hippocampus data, we shortly summarize the sensory connectivity outlined above. The Figure 42 gives a schematic overview over the connectivity uncovered by our tracing studies. As can be seen, the primary sensory areas within the visual-tectofugal, trigeminal and auditory system were all interconnected with the overlaying mesopallium, mainly with its ventral part but a smaller fraction of fibers always continued further dorsally. The intercalated parts of the nidopallium (NI, NFT, L1) were interconnected with both the mesopallium and the primary sensory areas and gave rise to projections to further associative and output areas. Although the general connectivity pattern appeared comparable across these modalities, differences existed between them mainly in terms of the strength of the projections. While the reciprocal connection between the entopallium and MVL was relatively (and absolutely) strong, the NI was only weakly interconnected with the MVL and projected sparsely to the entopallium. In contrast, the BAS received weak input from the mesopallium, but was much stronger interconnected with the NFT. The auditory system is different in that it contains a third layer, the L3, located ventrally to the primary station. The injections into L2 seemed to label fibers more diffusely in comparison to the visual-tectofugal and the trigeminal system. Many fibers often left the injection site in different directions, including a caudal stream towards the NCM and its most caudal portion. In all systems, the intermediate regions (NI, NFT, L1) projected to the NCL. The NFT additionally also projected to the arcopallium, whereas the L1 also heavily projected to the HA. Furthermore, within the auditory system, the arcopallium could be reached by the CMM, which contained retrogradelly labelled neurons after arcopallium injections. In addition, the primary sensory regions in the DVR seem to have direct striatal projections (Figure S8).

Although the wulst interacted with several nidopallial areas (NIMI, NFL and NCL) and to some extent also with the mesopallium, the local intrahyperpallial connectivity was rather separated from the meso-and nidopallial areas. Despite a much more differentiated wulst in the barn owl, we found a comparable connectivity pattern in both animal species. The IHA projected to the HA and HI, the HI was interconnected with HA, HD and IHA, and HA seemed to project to all layers of the wulst, except of the HD in the barn owl. However, there were two main differences between the barn owl and the pigeon. First we did not observe a
projection from HD to HA in the barn owl, while in pigeons HD massively projected to the HA. Second, while the HD in the barn owl contained many intralaminar fibers running along the layer (termed horizontal connectivity above), we found such fiber labelling in a distinct layer separating the HD and MD. This layer also heavily projected to the arcopallium (Figure 37) and NCL and possibly received input from HA (not shown).

**Figure 42:** Summary of the connectivity within the sensory systems.
The primary sensory areas in the nidopallium were interconnected with the overlying mesopallium and the intercalated nidopallium. The intercalated nidopallial areas were also reciprocally connected to the mesopallium. The relative strength of the connections varies between the modalities. These circuitries also forwarded the information to further associative and motor output areas. The main source of such long-range horizontal projections were mainly the intercalated nidopallial reigons (NI, NFT, L1).
3.6 Hippocampus

The data on hippocampus were acquired together with Noemi Rook. She performed all in vitro injections and the histological work on cases discussed in the section 3.6.2. The in vivo injections summarized in the section 3.6.1 were performed by myself.

As described above (1.4), the avian hippocampus consists of different subregions. In pigeons, the VI and Vm are clearly visible in the Nissl section, distinguished by a higher density of neurons. In-between these two layers, the Tr is located which dorsally borders on the DMv. DMv is delimited dorsomedially by the DMd and laterally by the hippocampal sulcus. The DL is located between the CDL and the sulcus and can be subdivided into its dorsal and ventral half.

3.6.1 Summary of the in vivo experiments

We clustered our in vivo injections into three main groups, depending on spread of the injection into the different regions (Figure S10). First, we had injections that only involved the DMv. The second group contains cases where injections involved the DMd, Tr and in some cases included the most medial part of the DMv. The injections in the third group were mainly restricted to the Vm and VI.

The Figure 43 gives a summary of retrograde labelling within the hippocampus across all cases in all three groups. The analysis revealed an interesting pattern. While the DMv injections labelled neurons in VI, only few were labelled there in the second group. On the other hand, the second group contained neurons within the Vm, although these were rather scarce. These data indicate that VI and Vm have different projection targets. The VI seems to innervate the DMv whereas the Vm may targets the dorsomedial aspects of the hippocampus. The latter projection might be reciprocated by the DMd (Figure 45E-F; Figure 47). The different injection groups also revealed a dissociation of DLd and DLv projections. While DLv was shown to project to the DMv, the DLd contained labelled neurons only after injections including Vm or DMd (Figure 44). CTB injections into DMv covering both the ventral (DMvv) and dorsal (DMvd) aspect of DMv also labelled fibers leaving the DMvd targeting the DLd with few fibers sometimes crossing to the DLv (Figure 44C and F). Some fibers also left the DMvv and reached the DLv. In addition, long-
range fibers left both DMvd and DMvv and run along the dorsal and ventral aspect of the CDL, respectively.

Figure 43: Retrograde labelling within the hippocampus and CDL.
**RESULTS**

Furthermore, the input to the hippocampus from CDL seemed to arrive mainly in its ventral part. Plenty of neurons were labelled within the CDL, mainly its central part, after injections in the second and third group, while injections placed into DMv labelled only few if any neurons within the CDL (Figure 43; Figure 46).

**Figure 43 continued:**

The in vivo hippocampal injections were clustered into three groups. **A:** The first group contained cases where the injections were placed within the DMv. **B:** In the second group, the injections involved the DMd, the Tr and in some cases the medial part of DMv. **C:** The third group of injections were mostly restricted to the Vi and/or Vm. **D:** Summary of retrograde labelling in the different injection groups. The circles represent labelled neurons and the color corresponds to one of the three groups. Scale bars = 500 μm in A-C.

**Figure 44:** Dissociation of the dorsal and ventral DL projections. **A:** Injection into DMd and medial DMv. **B:** Injection into ventral hippocampus including the V-shape, Tr and part of the DMd. **C:** Injection restricted to the DMv. **D:** Magnification of the DL in case (A). Both DLv and DLd contain retrogradelly labelled neurons. **E:** Magnification of the DL of the case shown in (B). Again, both DLd and DLv contain CTB labelled neurons. **F:** Magnification of DL in case (C). DMv injections labelled neurons only in the DLv. Thus the DLd might target mainly the DMd but neither DMvv nor DMvd. On the other hand, DLv seem to target the DMvv (see also in vitro data). Scale bars = 500 μm in A – C; 100 μm D - F.
Figure 45: DMd projections.
A: In vivo injections into DMd labelled neurons within the DMvd. B: Magnification of CTB-labelled neurons within DMvd. C: Injections into DMv showed that the connection between DMvd and DMd might be reciprocal (see also Figure 47).
Taken together these data indicate that the hippocampus may be topographically organized into a dorsomedial and ventrolateral part, each consisting of several interconnected subregions. This idea is further supported by the finding that injections into the dorsomedial
aspect including DMd and Vm labelled neurons within the DMv but only in its dorsal portion (Figure 45A-B). Thus, the DMv might possibly also be subdivided into a dorsal (DMvd) and ventral (DMvv) parts based on the connectivity. Interestingly, injections placed predominantly into the DMvv also labelled descending fibers which run across the lateral half of the Tr. On the other hand, when the injections involved predominantly the DMvd, descending fibers were located in the medial half of the Tr (fig. A 21). The DMvd but not DMvv injections also labelled neurons within the DMd (fig. A 21). To further investigate the topographical organization and intrinsic connectivity of the hippocampus, we switched to in vitro tracing which enables us to deposit tracer into different subregions with a high precision.

3.6.2 In vitro injections

3.6.2.1 DL

The in vivo data suggested that DLd and DLv project to different hippocampal targets (Figure 44). To further confirm this dissociation, we deposited biocytin crystals into DLd and DLv in vitro (Figure 48). After DLd injections, bunches of fibers run through the DMvd and DMd continued further along both the medial fiber zone as well as across the hippocampus crossing the medial DMv and Tr. Some of these fibers contained varicosities and occasionally gave rise to medial and lateral collateral branches in Tr (Figure 48B-C). The fibers were mostly restricted to the medial half of the Tr with only few collaterals extending further.

Figure 47: DMd projects to DMvd.
A: Injection located mainly in DMvd with spread to DMvv. B: Injection located in DMvv with spread into DMvd. C: Retrogradelly labelled neurons within the DMd were found in case (A). D: However, no labelled neurons in DMd were found in the case shown in (B). These data suggest that DMd targets predominantly the DMvd rather than DMvv. Scale bars = 500 µm in A-B; 50 µm in C-D.
RESULTS

A large amount of fibers was usually situated along the dorsomedial pial surface of the sections. These fibers were rather thick and sometimes contained varicosities. On their way down to the septum they branched of some collaterals in DMD and few if any in VM and TR. Furthermore, we found many retrogradely labelled neurons within the DMD and occasionally very few neurons within the DMV.

After crystal depositions into the DLV labelled thin varicose fibers were observed within the ventral part of the DMV (DMV; Figure 48G). Few fibers containing varicosities also reached the VI (Figure 48F). In addition, biocytin labelled neurons within the DLV built a dense plexus of dendritic and axonal processes. Many of these processes were oriented perpendicular to the dorsal pial surface and reach up to the most dorsal aspects of the DLd.

Figure 48: Different projections of the dorsal and ventral DL.

A: A section counterstained with Nissl showing a case in which biocytin crystal was placed into the DLd. B: Fibers run along the dorsomedial hippocampus via the DMD, and medial TR. These fibers occasionally gave rise to collateral branches running medially (black arrows) C: Similar collaterals also branched laterally (black arrows). The fibers often contained varicosities, which were especially large in some fibers (arrow heads). D: Furthermore, DLd injections often retrogradely labelled neurons within the DMD (white arrows). E: A section counterstained with Nissl showing a case in which biocytin crystal was placed into the DLv. F: In contrast to DLd injections, DLv injections labelled fibers in the ventrolateral aspect of the hippocampus, with some fibers reaching the VI. Arrowheads point to example of varicosities within VI, indicating en passant synaptic contacts. G: A large amount of fibers was observed within the DMVv. Scale bars = 500 µm in A und E; 50 µm in B and D; 20 µm in C and F-G.
Figure 49: Dissociation of DMvd, DMvv and DMD projections. 
A: Injection placed into the DMvd. B: Labelled fibers and neurons in DMD in the case shown in (A). C: Case with an injection placed into the DMvv. D: In contrast to DMvd, DMvv injections labelled fibers in VI and the lateral aspect of Tr. E: Injection placed into DMD. F: Labelled fibers and neurons were found within the medial half of Tr. Fibers also run through Vm and many fibers were observed running along the mfz. Black arrows point to examples of putative synaptic contacts, while white arrows point to examples of retrogradely labelled neurons. Scale bars = 500 µm in A, C and E; 50 µm in B, D and F.
RESULTS

3.6.2.2 DM

Consistent with the in vivo data, injections into DM revealed a clear topographical pattern (Figure 49). The DMv could be dissociated into a dorsal and ventral part (DMvd and DMvv, respectively) based on their projections. While the dorsal injections labelled fibers running dorsally via the DMd as well within the fiber zone along the dorsomedial aspect of the hippocampus (Figure 49A-B), the ventral injections labelled fibers running ventrally via the lateral part of the Tr and the Vl (Figure 49C-D). In the ventral apex of the hippocampus the ventral fibers entered the paraventricular fiber zone and left the hippocampus. These fibers showed clear varicosity-like structures and appeared to contact neurons in both the lateral Tr and Vl (Figure 49D). On the other hand, the fibers taking the dorsal route displayed some varicosities within the dorsomedial hippocampus near the injection site and within the DMd (Figure 49B). However, few if any varicosities have been found in the most medial aspect of the DMd. While the fibers running within the medial fiber zone could be followed further ventrally up to the V-shape, the fibers crossing the DMd apparently run in a different plane and hence could not be followed besides the DMd. Thus, the main intrahippocampal target of the DMvd might be the DMd, whereas DMvv projects to lateral Tr and Vl. In addition, the DMvv and DMvd are heavily interconnected. Both subregions appeared to project to each other. Interestingly, the neurons within DMvd and DMvv possess long dendrites extending down to the ventricular or up to the pial surface of the DMv, respectively (Figure S9C-D). Furthermore, when injections were placed into the DMd, fibers run across the central and medial part of the Tr (Figure 49E). They displayed many varicosities and appeared to contact neurons within the Tr and Vm (Figure 49F, Figure S9A-B). In many cases only fractions of the fibers were seen, indicating that they run in a plane different from our cutting angle. In sum, these data suggest that the projections of the DM are organized parallel to the dorsolateral-to-ventromedial curvature of the hippocampus (further referred to as the transversal axis). Moreover, the connections of the DM were often reciprocal. For instance, we observed retrogradelly labelled neurons in DMd after DMvd injection (Figure 49B), as well as retrogradelly labelled neurons located mainly in the central and medial Tr after DMd injections (Figure 49F).
**Figure 50: Intrinsic connectivity of the V-complex.**

**A:** Nissl stained section showing the injection of biocytin into the Vm. **B:** Labelled fibers and terminals (black arrows) were observed within the DMd. **C:** Another example of terminals labelled within DMd after Vm injection. Vm also projected to the VI (F). **D:** Slice showing an example injection into the VI. In such case, many fibers were observed within the Tr. **E:** Example of a fiber, which possibly contacts neurons in Tr (black arrows) labelled after injection into the Vm. **F:** Magnification of the rectangle F in (A) showing projections to VI and Tr from Vm (black arrows). **G:** Injection example with the injection placed into the Tr. **H:** Many labelled fibers were observed within the DMvv. **I:** Neurons in Tr extend their dendrites medially up to the mfz. The dendrites are organized parallel to each other and orthogonal to the fibers running via the mfz. Scale bars = 500 µm in A, D, G; 20 µm in B, C, E, F, H, I.
3.6.2.3  V-complex

Three main projections were revealed by injections into the Vm (Figure 50A-C). First, fibers left the injection dorsally and innervated the DMd. Some fibers were also labelled within the mfz, dorsally to the Vm injection. Second, Vm injections labelled axons running medially across the Tr. Many of them reached the opposite Vl, with some fibers extending up to the pfz. On their way to Vl, they also seem to contact neurons within Tr, as these fibers showed many varicosities. In addition, many fibers were observed within the Tr itself. Some neurons were located close to the Vm within the medial Tr labelled possibly due to the spread of the tracer or due to extension of their dendrites into Vm. We were able to follow the axon in one of these neurons and saw that it turned laterally and run via the Tr towards the lateral site of Tr. Although, we could not determinate the final termination target of this axon, these data indicates that Tr could contain some intrinsic circuits interconnecting its lateral and medial part. Similar to the Vm injections, Vl main targets was the Tr, where many fibers were observed (Figure 50D-F). Few fibers also left the injection dorsolaterally towards the DMv. A prominent fiber bundle was labelled within the pfz ventrally leaving the hippocampus. Furthermore, injections into the Tr revealed a dense projection to the DMv but not DMd (Figure 50G-I). A very interesting organization of Tr neurons was uncovered by these injections. They displayed long dendritic trees which expanded across the Vm and extended up to the mfz where they crossed with orthogonally running fibers of this

Figure 51: Summary of the intrahippocampal connectivity.
The figure summarizes the connectivity as revealed by the in vivo and in vitro tracings. Projections of all subregions are shown. The thickness of the arrows indicates the strength of the connection. The dashed line represents the transversal axis of the hippocampus. The projections within the hippocampus seem to be topographically organized parallel to this axis. Our data suggest that the hippocampus can be subdivided into a dorsomedial and ventrolateral part lying above und underneath this axis, respectively. These two subdivisions contain separate pathways, which interact with each other at different levels. The main projections of DLd are to the DMd and medial Tr (Trm), while DLv targets mainly the DMvv. The DMvd is reciprocally connected to the DMd, which further projects to the Vm and Trm and also back to DLd. The V-shape layers are connected to Tr and Vm projects also to Vl, while VI projects to DMvv. The Trl projects back to DMvv whereas mTr projects back to DMd.
fiber zone. In addition, many labelled fibers left the injection ventrally and left the hippocampus.

Taken together, the projections within the hippocampus seem to be topographically organized parallel to its transversal axis (Figure 51). The in vivo and in vitro tracing data suggest that the hippocampus can be subdivided into a dorsomedial and a ventrolateral subdivision. These two hippocampal partitions contain separate pathways, which interact with each other via the interconnections of DLv and DLD, DMvv and DMvd as well as the V-shape projections to both medial and lateral Tr (Trm, Trl). In brief, the main projections of DLD reach the DMD and the medial Tr (Trm). The DMD conveys the information to the Vm and Trm and also back to DLD and is also reciprocally connected with DMvd. The Vm in turn projects to the Tr and Vl. On the other hand, the DLv targets mainly the DMvv, which further projects to Trl and Vl. The Vl projects back to the DMvv as well as to the Tr. The medial and lateral aspects of Tr projects to DMD and DMvv, respectively.
4. Discussion

We performed an anatomical in vivo and in vitro study of local circuits in the avian forebrain. To our knowledge, this is the first study applying in vitro tracing procedure to adult pigeons and barn owls and one of few in vitro tracing studies of the avian forebrain in total. We have several important findings. First, the avian hippocampus possesses reciprocal circuitry organized topographically parallel to its transversal axis. Second, the sensory circuitry within the forebrain is comparable across modalities and is composed of reciprocally connected layers. Third, the connections between the layers also define anatomical columns oriented perpendicular to the layers. Within the DVR, the primary sensory areas are reciprocally connected with the overlying mesopallium. The intercalated nidopallial areas are reciprocally connected to both primary and mesopallial areas. Nidopallial and mesopallial layers are the sources of projections to other associative and motor output areas. Our data add an important contribution to the understanding of the forebrain circuitry and have implications for evolutionary conjectures. We begin with the discussion of the connectivity of the sensory systems. We first compare our findings to existing anatomical knowledge and discuss then the parallels to the mammalian circuitry pattern. After that, we elaborate more on the physiology of neocortical circuits to sparkle some speculations about the circuitry properties of the avian forebrain. Then we turn our discussion to the hippocampal circuitry. Here, we highlight the topography and main pathways as well as the comparison to the mammalian counterpart. Last but not least, we draw some evolutionary conclusions followed by notes for future studies.

4.1 Sensory systems – comparison to previous studies

4.1.1 The visual-tectofugal system

Our results on the connectivity within the entopallial system are consistent with previous tracing studies (Husband and Shimizu, 1999; Krützfeldt and Wild, 2005). A strong topographically organized interconnection between the entopallium and MVL has been reported in different species including pigeons (Husband and Shimizu, 1999; Krützfeldt and
Wild, 2005), chickens (Ahumada-Galleguillos et al., 2015) and zebra finches (Krützfeldt and Wild, 2004). Thus, this connection appears to be an important component of the visual system in birds (Figure 52). Interestingly, the subdivisions of the entopallium which are interconnected with the MVL seem to be the main origin the entopallial striatopetal efferents (Krützfeldt and Wild, 2004, 2005). This circuitry could therefore be an important component of the network controlling the visuomotor behavior.

However, we noticed slight discrepancies within this connection between our data and the previous observations in pigeons. Krützfeldt and Wild (2005) found that the internal (ventral) rather than the dorsal part of the entopallium is the source of the projections to the MVL. In addition the ventral part also received the majority of the MVL input. In contrast, both the ventral and the dorsal parts appeared to be interconnected with MVL in our study. However, due to the sparseness of retrogradely labelled neurons after mesopallium injections, we can not completely exclude the possibility that at least a fraction of the anterograde projections to the MVL labelled after injections into the dorsal entopallium could result from labelling of bypassing fibers originating in the ventral part of the entopallium. We nevertheless observed that the mesopallial input targets both parts of the entopallium. Our data are therefore rather consistent with the projection pattern observed by Ahumada-Galleguillos et al. (2015) in chickens. Similarly, these authors found that both parts of the entopallium were interconnected with the MVL. However, they reported much stronger interconnection with the dorsal part. Although the exact topography within this connection might vary across different bird species, the differences between our findings and those from Krützfeldt and Wild (2005) are unlikely to result from different tracer sensitivities (biocytin vs. CTB) or methods (in vitro vs. in vivo) and hence remain unclear.

Furthermore, the NI was interconnected with both the MVL and the entopallium (Figure 52). This is in line with previous studies where input to the NI from both MVL and entopallium and its projection to the mesopallium has been described (Husband and Shimizu, 1999; Krützfeldt and Wild, 2005; Atoji and Wild, 2012). However, the back projection to the entopallium has not been reported before. We often observed at least few retrogradely labelled neurons within the NI after entopallium injections. We noticed that these neurons were of different types and many of them showed extensive dendritic ramifications spanning several thick fiber bundles interconnecting the entopallium with the MVL. Due to these characteristics, the neurons appear perfectly suited to sample input from different
parts of the visual field or possibly combine different features of the visual scene (Wang et al., 1993; Hellmann et al., 2004; Nguyen et al., 2004; Cook et al., 2013). With their direct feedback to the entopallium they could affect the information gating or the tuning properties of the neurons. Although we know nothing about the physiology and neurochemistry of these neurons, this is nevertheless a valid speculation. For instance, in the mammalian visual cortex both excitatory and inhibitory recurrent intracortical feedback connections appear to be important for orientation selectivity tuning of the neurons in the visual cortex (Somers et al., 1995; Shapley et al., 2007; Chariker et al., 2016; but see also Priebe and Ferster, 2008).

Previous studies showed that NI projects to further visual areas as well as to the NCL and arcopallium (Husband and Shimizu, 1999; Kröner and Güntürkün, 1999; Krützfeldt and Wild, 2005). Thus the NI is the major output region of the entopallial circuitry distributing the sensory information to further associative and motor areas (Figure 52). We showed that neurons in NI projecting to the NCL are directly contacted by the collaterals of the entopalliofugal fibers reaching the MVL. Although, we consistently observed massive projections to the arcopallium from the border layer between HD and MD, the projections from the mesopallium were restricted to its caudal pole belonging to the auditory system. However, previous studies imply that in addition to NI, the MVL and the surrounding mesopallium could also forward the information to other associative areas (Husband and Shimizu, 1999; Kröner and Güntürkün, 1999; Krützfeldt and Wild, 2005; Atoji and Wild, 2012).

Our data indicate that similar entopallial connectivity is also present in the barn owl (Figure S4). Interestingly, the interconnection between the entopallium and the MVL appeared much weaker than in the pigeon. While an unfortunate cutting angle is a plausible reason to explain the difference, it is intriguing to speculate that the strength of this connection may co-vary with the degree to which an animal relies on this system (see also sections 4.1.2). Pigeons have a large entopallium and possess high visual acuity in their tectofugal visual system (Shimizu and Bowers, 1999; Güntürkün, 2000; Nguyen et al., 2004; Stacho et al., 2016). On the other hand, the barn owls have relatively small entopallium but tremendously enlarged hyperpallium which is well differentiated and possesses visual characteristics akin to the primate visual cortex (Pettigrew and Konishi, 1976; Pettigrew, 1979; Liu and Pettigrew, 2003; Harmening and Wagner, 2011). It is therefore thinkable that
the observed connectivity strength differences might correspond to divergent utilization of the two visual systems in the different species.

**Figure 52: Summary of the intratelencephalic sensory connectivity.**
The figure summarizes the main aspects of the connectivity discussed in the text. All sensory systems within the DVR including the trigeminal (blue), visual (dark green), auditory (red) as well as the wulst (light green) contain different “layers” which are reciprocally interconnected. The primary sensory areas within DVR are interconnected with both the overlying nido- and mesopallial fields. These regions are the source of projections to further associative and motor areas including the NCL and the arcopallium. Similarly, the HI and HD layers of the wulst are the source of projections to other regions e.g. to the hippocampus and to the HA, the motor output structure. The dotted lines represent connections that were not demonstrated in our experiments but are known from previous studies.

### 4.1.2 The auditory system

Previous studies have yielded a quite comprehensive picture of the connectivity within the auditory system (Wild et al., 1993; Vates et al., 1996; Wang et al., 2010). Our results are in line with these previous findings (Figure 52). Both L2 and L1 have been shown to be interconnected with the overlying mesopallium (Wild et al., 1993; Wang et al., 2010). In our study, the L2 projected to the CMM quite strongly but received only weak back projections. As in vivo studies convincingly demonstrated rather massive terminations within L2 in
pigeons (Wild et al., 1993; Atoji and Wild, 2012), our lack of retrogradelly labelled neurons in CMM after L2 injections can be most probably explained by wrong cutting plane or unsuccessful retrograde transport of biocytin. Curiously however, the projection from L2 to the CMM was shown in chickens (Wang et al., 2010) and zebra finches (Vates et al., 1996) but appeared to be missing in pigeons (Wild et al., 1993; Atoji and Wild, 2012). Since we found this projection in both pigeons and barn owls, it is thinkable that the discrepancies arise from different methodologies rather than different species. In addition, L3 seems to be interconnected with CMM too, although we did not demonstrate it in our study (Vates et al., 1996; Wang et al., 2010; Atoji and Wild, 2012).

The auditory system is connected to other telencephalic areas especially via the L1 and CMM (Figure 52). We showed that neurons in L1 project to the NCL and HA, while neurons in CMM convey the information to the arcopallium. Consistent with these findings, reciprocal connection between L1 and HA and a projection to the NCL were demonstrated previously (Wild et al., 1993; Kröner and Güntürkün, 1999). The connection from CMM to the arcopallium was observed in some (Kröner and Güntürkün, 1999) but not all studies (Atoji and Wild, 2012). In addition, L1 was also shown to project to the arcopallium (Kröner and Güntürkün, 1999; Wang et al., 2010). Interestingly, we found that the periventricular margin of the caudomedial nidopallium contained a thin layer of neurons projecting to the HA. These neurons appeared to be contacted by caudal projections of the L2. This projection may correspond to fibers leaving caudally the Field L described by Wild et al. (1993). Thus, the wulst appear to be an important exit station of the auditory system. The presence of auditory projection to the wulst might explain auditory responses within this structure (Adamo and King, 1967).

Interestingly, we found that the interconnection between the CMM and Field L2 was very pronounced in the barn owl (Figure S6). Given the excellent auditory capabilities of the owl (Peña and DeBello, 2010), this finding may further pursue the above mentioned idea that the strength of the interconnection between the primary sensory area and the overlying mesopallium to some extent varies with the degree of elaboration and utilization of the given sensory modality.
4.1.3 The trigeminal system

The original description of the connectivity of the pigeon trigeminal system emphasized a feedforward pathway from the BAS to the NFT, which in turn gives rise to the fronto-arcopallial tract targeting the arcopallium and the NCL (Wild et al., 1985; Figure 52). In addition to its projection to the NFT, the BAS was shown to project to the mesopallium in zebra finch (Wild and Farabaugh, 1996) and this connection was reciprocal in mallard (Dubbeldam and Visser, 1987). Later studies revealed that BAS in pigeons is indeed also reciprocally connected to the overlying mesopallium (Atoji and Wild, 2012), indicating that this connection is a hallmark of the avian trigeminal system. This is consistent with our observation of orthogonally running fibers from the BAS crossing the NFT and terminating in the MFV. The back projection from the MFV to the BAS appeared weaker in our study. Although methodological problems might have contributed to the sparseness to some degree, the BAS received strong feedback from the NFT rather than MFV. We observed that NFT was also interconnected with the MFV congruent with previous studies (Kröner and Güntürkün, 1999; Atoji and Wild, 2012). The reciprocal connectivity between the different subdivisions as well as the output to the arcopallium and NCL resembles the connectivity pattern found in the visual and auditory modalities. Similar to the auditory and the visual system, we also observed evidence for a projection to the striatum after BAS injections. However, this projection has not been reported before (Wild et al., 1984, 1985; Wild and Farabaugh, 1996; Dubbeldam and Visser, 1987). In mallards and zebra finches, projection to the striatum arrived from the NFT (Dubbeldam and Visser, 1987; Wild and Farabaugh, 1996) and a similar projection might also exist in pigeons (Veenman et al., 1995). Thus, the projection of BAS to the striatum might be very scarce if present at all and the observed projection in our study could in principle have originated from labelling of other striatopetal axons running through the BAS, for instance those originating in the NFT.

4.1.4 The Wulst

A handful of studies investigated the anatomical organization of the circuitry of the avian wulst (Karten et al., 1973; Streit et al., 1980; Reiner and Karten, 1983; Shimizu et al., 1995; Deng and Rogers, 2000). These studies identified the input and output layers of the wulst and its connections to other telencephalic areas. However, the intrinsic connectivity of the wulst remained rather enigmatic. We described the connectivity in an animal model with a
large and elaborated wulst, the barn owl as well as in the pigeon, a bird with a tremendously smaller wulst in comparison to the owl. We found a comparable connectivity pattern in both species (Figure 52). In brief, the main thalamorecipient input layer IHA projected directly to the output layer HA as well as to its other neighboring layer, the HI. Both projections were reciprocal. HI was also interconnected with HA and HD. The HD received input from HA in pigeons but not in the owl. We do not know whether this is a genuine species difference. It is likely that we did not observe this connection in the owl because the distance between the two layers is much bigger in this bird than in pigeons. Thus with in vitro tracing, there is a chance that we missed this connection based on the orientation of the cutting plane or the survival of the long-distance axons. In sum, putting this difference aside, the connectivity picture reveals that each layer of the wulst is reciprocally connected with the output layer HA as well as with its adjacent layers. Interestingly, this situation fits to the pattern found in the rest of the sensory forebrain (Figure 34).

Moreover, the wulst and the sensory circuits apart from the wulst interact with each other via associative areas including NCL, NFL and NIMI as well as via the arcopallium (Kröner and Güntürkün, 1999; our data). The interaction via the mesopallium, which lies directly juxtaposed to the wulst is surprisingly weak. Furthermore, a direct connection to the wulst from the local sensory circuitry also exists, at least in the auditory system (Figure 52). However, the organization of the wulst and the rest of the sensory forebrain also differ in few aspects. First, the layer which provides the extratelencephalic output is located directly adjacent to the other layers in the wulst but distantly in the rest of the forebrain. Second, possibly owing to the proximity of the output layer HA, the main thalamorecipient layer IHA projects directly to the HA and omits projections to one of the remaining layers (the HD). In contrast, the thalamorecipient layers in the rest of the forebrain (including BAS whose afferents bypass the thalamus) projects to both their directly attached nidopallial area as well as to the further dorsally located mesopallium. However, they do not project directly to the output layers (arcopallium or HA). It should be noted that at least in the barn owl, the thalamic input also targets the HD (Karten et al., 1973), which perhaps renders the projection of IHA to HD unnecessary. Third, while the wulst can reach the hippocampus directly via the HD, the information from the other modalities investigated in our study reach the hippocampus only via longer polysynaptic pathways (Atoji and Wild, 2004, 2005, our data).
4.1.5 Connectivity, cortical principles and the avian forebrain

Decades of anatomical research yielded an overwhelming bulk of information on the connectivity of the avian telencephalon. Analysis of these intrapallial connections with graph theory provided astonishing new insights (Shanahan et al., 2013). It revealed that the organizational principles of the avian and mammalian (including human) connectomes are very similar. In both cases the forebrains networks display small-world properties and can be subdivided into several modules. Regions belonging to one module are interconnected stronger with each other than with regions of other modules. Furthermore, the networks contain several nodes identified as hubs – nodes which are extremely suited for mediating the flow of information within the network. Among other nodes, prefrontal-like and hippocampal areas were identified as hubs in mammals and birds. Thus, despite macroscopically different organizations and the presence of non-homologous structures, the overall connectivity pattern and properties of the networks share remarkable similarities between birds and mammals (Kröner and Güntürkün, 1999; Reiner et al., 2004; Shanahan et al., 2013; Güntürkün et al., 2017).

The similarities in the large-scale network organization spark the question, whether the forebrains of birds and mammals perform similar kind of computations during sensory processing. In other words, can we also find similarities within the local forebrain circuitry? Given the structural differences between the neocortex and the avian pallium, it would not be surprising if the local computational units differ between birds and mammals. However, recent anatomical, genetic and functional studies sparked the possibility that hidden forms of cortical layers and columns can be identified within the avian forebrain (Wang et al., 2010; Dugas-Ford et al., 2012; Jarvis et al., 2013; Pfenning et al., 2014; Ahumada-Galleguillos et al., 2015; Calabrese and Woolley, 2015; Güntürkün and Bugnyar, 2016). It is therefore possible that local neocortical and avian pallial circuitry may share similar organization to some extent.

4.1.5.1 Local circuits, layers and columns

Based on anatomical and electrophysiological evidence Douglas and his colleagues (Douglas et al., 1989; Douglas and Martin, 1991) developed a local canonical circuit of the neocortex, which successfully modeled cortical responses evoked by stimulation of thalamocortical afferents. This circuit incorporates excitatory pyramidal and stellate neurons of layers 2-4,
which are interconnected with each other as well as reciprocally connected to the pyramidal neurons of deep layers 5-6. The excitatory neurons contact inhibitory neurons which provide inhibition to both superficial and deep layers. The thalamic input contacts both inhibitory and excitatory neurons and arrives stronger in the superficial than in deep layers. Douglas and Martin (2004) speculated that superficial layers explore all possible interpretations of their intra- and inter-areal and subcortical input, while the deep layers restrict the evolving interpretations and decide the output.

It seems therefore that the structural basis of neocortical computations consists of tightly reciprocally interconnected excitatory and inhibitory neurons across the six layers (see section 1.1.1). In brief, layer 4 forwards its main output to the layers 2/3 and somewhat weaker to the deep layers 5 and 6 (Harris and Mrsic-Flogel, 2013). The layer 2/3 provides only minor feedback to the layer 4 but massively projects to layers 5 and 6, which in turn reciprocate the connections to basically all layers (Thomson and Bannister, 2003; Harris and Mrsic-Flogel, 2013). It is interesting to see that our anatomical investigation revealed a similar principle in the avian forebrain. In each studied system, we found that the local circuitry is dominated by reciprocal interconnection of different layers. It is probably a matter of principle whether to refer to avian forebrain regions with the term layer or nucleus but the point is that the connectivity pattern appears very similar in birds and mammals. Therefore it is possible that the mammalian and avian pallia operate with the same computational principles. Based on connectivity as well as genetic markers (Wang et al., 2010; Atoji and Karim, 2012; Dugas-Ford et al., 2012; Suzuki et al., 2012; Chen et al., 2013; Atoji and Karim, 2014; Pfenning et al., 2014; Ahumada-Galleguillos et al., 2015) it is possible to draw a rough speculative analogy between the neocortical and avian pallial layers (see also sections 1.2 and 4.3). In this analogy the layer 4 would correspond to the input regions including the BAS, entopallium, Field L2 and IHA. These input layers are reciprocally connected to the overlying nidopallial and mesopallial layers, the putative analogues to layers 2/3. Similarly to layer 2/3, the nidopallial and mesopallial areas project to output structures, the arcopallium and HA, which provide feedback to other layers and project to subpallial and extratelencephalic targets, analogous to layer 5 and 6 (Figure 53).

As outlined in the introduction (see section 1.1.1) a prominent feature of the mammalian neocortex is its columnar organization. This was impressively demonstrated especially in the somatosensory cortex of rodents (Petersen and Sakmann, 2001; Petersen, 2007). Here,
columns can be defined anatomically by the extent of the layer 4 axons, and functionally by excitation evoked by layer 4 stimulation which remains within these anatomical boundaries. Interestingly, the processes of neurons in layer 2/3 do not respect the boundaries of the column, however, the excitation within this layer elicited by layer 4 neurons seem to be restricted to the column width by GABAergic inhibition (Petersen and Sakmann, 2001).

Strikingly, our data showed that the avian forebrain also contains columnar structures defined by the axons of neurons in the thalamorecipient (including BAS) layers extending to the overlaying layers. This was peculiarly remarkable in the entopallial system of pigeons, as well as in the wulst of the barn owl but was also clearly present in other modalities. Such anatomical columns have been reported previously in the visual and auditory system in chickens (Wang et al., 2010; Ahumada-Galleguillos et al., 2015). Moreover, functional studies using 2-deoxyglucose autoradiography revealed discrete stripes of activity extending perpendicularly across the Field L and CMM (Scheich, 1983; Müller and Scheich, 1985). The discrete stripes represented different sound frequencies (Müller and Scheich, 1985). Newer electrophysiological recordings observed variation in the spectral tuning of the neurons along the same axis in zebra finch (Kim and Doupe, 2011). Functional columns in the avian forebrain have also been suggested based on activity-dependent gene expression (Jarvis et

**Figure 53: Summary and comparison of local circuitry within the avian DVR and wulst and the mammalian neocortex.**

Circuitry defined by reciprocally connected layers can be found in the mammalian neocortex as well in the avian DVR and wulst. The color coding outlines the putative correspondence between the layer 4 of the neocortex and the primary sensory areas (PS) in the avian pallium, the layers 2/3 and meso-nidopallium, and layer 5 and 6 to arcopallium (Arco) and hyperpallium apicale (HA). Note that acropallium is a nucleus located extracolumnary but is presented as an adjacent layer in this picture for clarity and comparative reasons.

Moreover, the functional column can be extended towards the neighboring column in layer 2/3 via activity dependent NMDA-mediated plastic changes (Petersen and Sakmann, 2001).
al., 2013). In accordance with our anatomical results, Jarvis et al. (2013) proposed functional columns both in the wulst as well as in the rest of the forebrain. The latter span the primary sensory areas, the intercalated nidopallial areas and the mesopallium and include also striatal domains. Thus, there is considerable evidence suggesting that the avian pallium perhaps consists of anatomically and functionally defined columns, reflecting one of the hallmarks of the mammalian neocortex. Furthermore, Calabrese and Woolley, (2015) observed a hierarchical processing in the avian auditory circuit from the thalamorecipient Field L2 to the adjacent superficial and deep layers. The auditory neurons in different layers also showed different firing properties remarkably similar to the features found in the mammalian auditory cortex (Harris, 2015; see also section 1.2.2). Taken together, the avian pallium displays features akin to the mammalian neocortex including a laminar and columnar organization of local circuitry, the connectivity pattern and processing strategies.

**4.1.5.2 The physiology and function of local circuits - the interplay between excitation and inhibition**

The astonishing similarities in the anatomical organization and information processing in the avian and mammalian local forebrain circuits suggest that physiological and computational mechanisms performed by these circuits could be similar as well. Analyses of three layered cortices indicate that some basic principles have originated in the past and might have been duplicated and refined in the six-layered neocortex (Shepherd, 2011). The different principles utilized by cortical circuits allow the performance of computations required to represent different stimuli, memorize experiences or retrieve learned associations. One often discussed computational principle is the gain modulation, which might be the most general neuronal principle in the nervous system (Salinas and Thier, 2000). Gain describes the rate in which the firing of a neuron increases with increasing excitatory input, without changes in the receptive field properties or tuning of the neuron (Salinas and Thier, 2000; Isaacson and Scanziani, 2011; Wilson et al., 2012). Let’s consider an orientation selective neuron in the visual cortex as an example. This neuron displays the highest spiking activity to a grating stimulus in a specific orientation and lowest to the orthogonal orientation. Increasing the contrast of the stimulus would increase the firing rate of the neuron independent of the orientation of the stimulus. That means, though the slope of the input-
output relationship (the gain) of the neuron increases, the orientation selectivity of the neuron remains constant at each contrast (Isaacson and Scanziani, 2011; Katzner et al., 2011; Atallah et al., 2012; Wilson et al., 2012). The evidence indicates that a key factor in this mechanism is the inhibition provided by the parvalbumin expressing inhibitory GABAergic interneurons (Isaacson and Scanziani, 2011; Katzner et al., 2011; Atallah et al., 2012; Wilson et al., 2012).

Cortical principles abundant in phylogenetically older cortices such as the three-layered piriform cortex and the reptilian dorsal cortex include the recurrent excitation and feedback and feedforward inhibition (Shepherd, 2011; Fournier et al., 2015). The recurrent excitatory projections have been suggested to amplify the quantitatively weak input from distant areas including the thalamus (Binzegger et al., 2004; Douglas and Martin, 2007a; Shepherd, 2011). Re-excitation is potentially dangerous and needs to be counterbalanced by inhibition. Modern research has shown that the balance between excitation and inhibition can be temporally favored towards excitation via disinhibition, a phenomenon for a long time discussed in models of the basal ganglia circuitry (Herrero et al., 2002; Klinke et al., 2005; Hammond et al., 2007) but which only recently attracted attention within the cortical circuits (Isaacson and Scanziani, 2011; Letzkus et al., 2015). The disinhibition has been linked to associative learning in auditory cortex (Letzkus et al., 2011). Interneurons in layer 1 inhibit parvalbumin neurons in layer 2/3 when an aversive stimulus is applied (foot shock). Pyramidal neurons targeted by the parvalbumin neurons are then disinhibited and show highest activity during paired presentation of a conditioned and unconditioned stimuli. The disinhibition of the parvalbumin neurons in this study was shown to be necessary for the intact fear learning. The same mechanism is also utilized for the expression of the learned behavior. In a nice study, Courtin et al. (2014) showed that the presentation of a conditioned stimulus associated with foot shock inhibits a subset of parvalbumin neurons in the dorsomedial prefrontal cortex of mice. This again disinhibits pyramidal neurons projecting to the basolateral amygdala and synchronizes their firing by resetting the local theta oscillations. The synchronized firing of the pyramidal neurons elicits the conditioned fear response. Thus, disinhibition in neocortical circuits allows both learning and expression of fear behavior.

Inhibitory circuits in general play an inevitable role in cortical processing (Isaacson and Scanziani, 2011). Feedforward inhibition is a simple mechanism, which allows cortical
neurons to respond to diversity of input strengths (Pouille et al., 2009). Due to divergent projections of afferent axons targeting cortical neurons an increase in stimulated cortical afferents would result in very steep rise of the number of recruited neurons (Shadlen and Newsome, 1998). This would dramatically restrict the range of input strengths the cortical network could differentiate as already a little bit stronger inputs would saturate the cortical activity. This problem is overcome by the feedforward inhibition which increases with the stronger inputs, limiting the fraction of pyramidal neurons which can reach the spike threshold (Pouille et al., 2009). In this way, cortical networks are able to respond to a wide range of stimuli despite a large variation in the input strengths owed to varying stimuli or ongoing intrinsic activity.

It is important to note that cortical inhibitory neurons comprise many different classes which differ in their microcircuitry and physiological characteristics (Hu et al., 2014, Jiang et al., 2015, Tremblay et al., 2016). Wilson et al. (2012) could show that somatostatin and parvalbumin expressing neurons have distinctive effects on the network properties. They target dendritic and perisomatic regions of the pyramidal neurons, respectively (Tremblay et al., 2016), and form functionally different subnetworks with the pyramidal neurons (Wilson et al., 2012). While the somatostatin interneurons sharpen the orientation selectivity, the parvalbumin interneurons modulate the response gain (Atallah et al., 2012; Wilson et al., 2012). These facts underline the significance of experiments investigating the cell-type specific microcircuitry to understand the computational mechanism utilized by the avian forebrain networks. The presence of feedforward and feedback inhibition as well as the recurrent excitation in reptilian pallium indicates that these principles are not a mammalian innovation but rather common principles which might have evolved in the common ancestor of amniotes. It is therefore reasonable to expect this kind of circuitry properties within the avian pallium. Unfortunately, with regard to a comparison of avian forebrain circuits to neocortical organization, we are still a big step behind. Although, the circuitry defined by in-vitro studies of the avian brain to some extant resembles the excitatory circuits within the neocortical columns, we still completely lack any information on the (inhibitory) microcircuits. In mammals, much knowledge has been acquired about the morphology, electrophysiology, laminar distribution, neurochemistry and synaptic properties of neocortical interneurons (Hu et al., 2014, Jiang et al., 2015, Tremblay et al., 2016), which execute important influence on the excitatory neurons.
Studies in birds showed that neurons in the visual system respond to different shapes, colors and moving stimuli (Xiao et al., 2006; Koenen et al., 2016; Scarf et al., 2016; Stacho et al., 2016) and display enhanced activity to stimuli associated with reward (Verhaal et al., 2012). Neurons in the auditory cortex distinguish between different songs and possibly participate in the auditory memory traces (Bolhuis and Gahr, 2006; Gobes and Bolhuis, 2007; Smulders and Jarvis, 2013; Bolhuis and Moorman, 2015). Furthermore, in line with the above mentioned fear conditioning in mammals (Letzkus et al., 2011), activity in the auditory system of birds is highest when the auditory and aversive stimuli are paired (Jarvis et al., 1995). Although we lack information about the physiological effects of specific local connections within the avian forebrain, the anatomical and functional evidence suggest that the avian pallium could use mechanisms akin to those utilized by the mammalian cortex. These could involve the amplification of signal via recurrent excitatory projections, inhibition counterbalancing the excitation and allowing gain modulation and neuron fine tuning as well as acquisition and expression of associative learning via disinhibition.

### 4.2 Hippocampus

#### 4.2.1 The circuits and topography

Our data showed that the avian hippocampus contains several pathways with reciprocal connections. Moreover, we saw that the pathways run along the transversal axis of the hippocampus and we could clearly distinguish a dorsomedial and a ventrolateral component. We showed that differentiation of the hippocampus into the dorsomedial and ventrolateral partitions starts already in the DL. While DLv projects mainly to the DMvv, the DLD targets the DMD, VM and the medial part of Tr. The projection from DLv to DMvv is consistent with previous observations of neurons in DLv after injections into the DM (Atoji and Wild, 2004). Furthermore, similar to Atoji and Wild (2004) we saw labelled fibers in DLD after injections into DMv. We noticed that these fibers arise mainly from the DMvd and few of them crossed ventrally to reach the DLv. In addition, some fibers coming from the DMvv also reached the DLv. However, our in vitro injections into DLD labelled consistently neurons within the DMD but not DMvd. Thus the projection from DMvd to DLD might be weak, if present at all, and it
is thinkable that fibers running towards the DLd observed after DMvd injections might be labelled bypassing axons of DMd neurons. Thus, the previously described connectivity of DL is consistent with our findings. Moreover, we could disambiguate the afferents and efferents of the dorsal and ventral part of DL.

Previously, projections from DM to Tr and V-shape have been described (Kahn et al., 2003; Atoji and Wild, 2004). We showed that the DMvv and DMd projected to Vl and Trl and Vm and Trm, respectively. The partition of the DM into a dorsomedial and ventrolateral half is supported by previous studies (Kahn et al., 2003; Atoji and Wild, 2004). Kahn et al. (2003) observed that CTB injections placed into or including what we called DMvv labelled many neurons within the Vl, while their medial DMd (our DMd) was interconnected with Vm but not with Vl. Similarly, Atoji and Wild (2004, 2005) suggested a medio-lateral subdivision of the DM. They observed many labelled neurons within the Vl and Vm after injections placed into lateral and medial DM, respectively (Atoji and Wild, 2004). As judged from their figure 6A, their medial CTB injection is clearly placed in what we called DMd. Together, these previous data correspond to the interconnections between the Vm and the DMd and between the Vl and the DMvv found in our study and are consistent with the dorsomedial-ventrolateral topography. The information from the V-shape layers is further conveyed to the Tr. In addition, Vm projections reach also the Vl as has been previously demonstrated both electrophysiologically (Hough et al., 2002) and anatomically (Kahn et al., 2003). Due to the flexure of the hippocampal transversal axis, it is possible that, at least for some (post-flexure) parts of the hippocampus, the medio-lateral topography described in previous studies is identical to our dorsomedial-ventrolateral subdivision. However, we do not exclude the possibility that a medio-lateral difference co-exists within the DMv. For instance, the medial and lateral DMv seem to differ in their connectivity with the CDL (Atoji and Wild, 2005) and possibly also with HD and CPI (Kahn et al., 2003). Thus a medio-lateral topography might be superimposed on the dorsomedial-ventrolateral one.

As described above (see section 1.3.2), the intrahippocampal projections in mammals are highly topographic. Interestingly, gene expression analyzes subdivide the hippocampal components into multiple distinct domains along the septo-temporal (longitudinal) and the transversal axis, which strikingly match the domains based on intra- and extrahippocampal connectivity (Thompson et al., 2008). Moreover, it seems that genetically defined neuronal populations form separate parallel pathways within the hippocampus (Deguchi et al., 2011;
Moser, 2011). Our data showed that the avian hippocampus contains at least two parallel pathways. Whether these pathways are constituted by different genetically defined populations is unknown. The ordered anatomical projections parallel to the hippocampal transversal axis observed in our study also opens the possibility that multiple parallel pathways exist within the avian hippocampus. These could be further investigated with anatomical and electrophysiological studies allowing spatial resolution on single cell level such as intracellular filling and paired patch clump. Further research is needed to see whether the avian hippocampus contains distinct domains defined by specific gene expression patterns including specific combinations of genes related to cell adhesion and axon guidance molecules, ionic conductances and transcriptional regulation (Thompson et al., 2008).

4.2.2 Topography and functional speculations

The mammalian hippocampus can be subdivided into subregions along its longitudinal axis which differ in the connectivity pattern and function (Risold and Swanson, 1996; Kerr et al., 2007; Jones and Witter, 2007; Strange et al., 2014). As outlined above (see section 1.3.2) the different regions of the hippocampus along its longitudinal axis receives input from different portions of the entorhinal cortex along its ventromedial to dorsolateral axis. In turn, these different entorhinal areas receive topographical input from the cingulate cortex (Jones and Witter, 2007). While the ventromedial entorhinal cortex collects input from the infra- and prelimbic cortices, the dorsomedial parts are reached by input from the anterior cingulate and retrosplenial cortices. Therefore it appears that the ventral (temporal) hippocampus is stronger associated (via the entorhinal cortex) to cingulate areas linked to emotion regulation and the dorsal part of the hippocampus is progressively more connected to areas involved in spatial processing (Jones and Witter, 2007). Accordingly, the ventral hippocampus has been implicated in fear response and extinction (Adhikari et al., 2011; Senn et al., 2014; Tovote et al., 2015) whereas the dorsal hippocampus is engaged in spatial processing (Wilson and McNaughton, 1993; Moser and Moser, 1998). It should however be stressed that the different connectivity domains along the hippocampal longitudinal axis are gradient rather than sharp (Strange et al., 2014). Moreover, the ventral hippocampus is also involved in spatial processing, though possibly at a larger spatial scale (Kjelstrup et al., 2008; Geva-Sagiv et al., 2015).
This example shows that various hippocampal domains defined by different connectivity patterns can differ also in functional aspects. Our results spark the question whether the dorsomedial and ventrolateral subdivisions of the avian hippocampus described in our study can be dissociated functionally. This might be difficult to test if the different subdivisions contribute different aspects of the same function. A hint comes from studies of immediate early gene expression in hippocampus in zebra finch (Mayer et al., 2010; Mayer and Bischof, 2012) and pigeons (Brito et al., 2006). It seems that using spatial cues for food location mainly activates the dorsomedial but not the ventrolateral aspect of the hippocampus (Mayer et al., 2010; Mayer and Bischof, 2012). In addition, the dorsomedial part also showed highest activity in fear conditioning in pigeons (Brito et al., 2006). On the other hand, the ventrolateral part showed enhanced activity in female zebra finch listening to directed song of males (Bailey et al., 2002). Thus, these data indicate that the different topographical aspects of the avian hippocampus defined in our study based on connectivity might also have functional significance. However, there are several problems with this conclusion. First, we do not know, whether our findings in pigeons can be one-to-one extrapolated to zebra finch hippocampus. Second, the study of Bailey et al. (2002) does not provide any information about the activity within the dorsomedial part. In addition, functional differences might also exist within the two hippocampal subdivisions (Brito et al., 2006; Mayer and Bischof, 2012). Further research is needed to support a functional distinction of the two topographical subdivisions as well as their different subregions. The situation is further complicated by the fact that the left and right hippocampi seem to utilize different strategies and have distinct contributions to hippocampal dependent functions and possibly also vary across species (Bingman et al., 2005, 2006; Nardi and Bingman, 2007; Kahn et al., 2008; Mayer and Bischof, 2012; Jonckers et al., 2015). For instance, while the left hippocampus seems to be more important for learning of a navigational map in pigeons (Bingman et al., 2005), goal navigation based on geometrical cues in chicks (Mayer et al., 2016) and pigeons (Nardi and Bingman, 2007), the right hippocampus was stronger involved in navigating to food location using spatial cues in zebra finch (Mayer and Bischof, 2012).

4.2.3 Network properties of the hippocampus in birds and mammals

The memory trace has been shown to be represented in neuronal circuits within the mammalian hippocampus (Tonegawa et al., 2015a, b; Poo et al., 2016). It would be
interesting to see whether and how the hippocampal circuits identified in birds contribute to memory storage and encoding. The avian hippocampus has been implicated in acquisition but not necessary in storage of a navigational map (Bingman and Yates, 1992; Bingman et al., 2005; Gagliardo et al., 1999). The fact that hippocampus lesions impair navigation in young but not adult pigeons held in an outdoor aviary was also interpreted in a way that the hippocampus temporarily stores the navigational map memory (Mayer et al., 2013). Several studies showed that the avian hippocampus is involved both in learning and recall of spatial memory based on allocentric cues (Mayer et al., 2010, 2013). Despite some differences, stimulation of neurons in the avian hippocampus can elicit long-term potentiation comparable to that seen in mammals (Margrie et al., 1998). Thus, it is thinkable that the memory engram is represented in plastic hippocampal circuits also in birds.

In mammals, it is thought that episodic memories are consolidated during sleep or quiet wakefulness by reactivating the cell ensembles which were activated during awake state (Wilson and McNaughton, 1994; Nádasdy et al., 1999; Battaglia et al., 2005; Schwindel et al., 2016). For instance, neurons in CA1 which encode similar places in an open-field arena during exploratory behavior increase their cofiring during a subsequent sleep session, while neurons firing at different locations decrease their cofiring (Wilson and McNaughton, 1994; O’Neill et al., 2008). Strikingly, the increase in correlated firing was stronger when novel environments were explored and was stronger for neurons encoding the most visited locations. Moreover, during reactivation the temporal sequences of cell firing are replayed (Nádasdy et al., 1999; Peyrache et al., 2009). The firing of individual neurons is linked to specific phase of the hippocampal theta oscillations, which may be suitable for transferring of the sequences to further regions (Lisman and Jensen, 2013). As the memory trace involves neocortical structures (Squire and Wixted, 2011, Winocur et al., 2010; Cowansage et al., 2014; Xie et al., 2014), replay is also found in other cortical regions and is coordinated with hippocampus (Hoffman and McNaughton, 2002; Ji and Wilson, 2007; Peyrache et al., 2009). Interestingly, similar replay of singing related activity during sleep was reported in a songbirds nucleus involved in song production (Dave and Margoliash, 2000). Here, the activity of the neurons during sleep matched their premotor activity displayed during singing. However, there is no evidence available showing such processes related to spatial representation in the avian hippocampus. Nevertheless, based on the phylogenetically conserved involvement of the hippocampus in spatial representation (Rodríguez et al., 2002;
Mayer et al., 2013; Striedter, 2016), it is possible that avian and mammalian hippocampi utilize to some extent comparable network operations. The place cells, which increase firing rate at a specific location within an environment, are characteristic for the mammalian hippocampus (Moser et al., 2015). Neurons with similar properties, termed location cells, were also described in pigeons (Hough and Bingman, 2004). However, the location cells of birds differ from the mammalian place cells in at least two aspects. First, location cells could only be found in an environment with stable goal locations and their firing fields were often located near the food baited bowls (Hough and Bingman, 2004; Kahn et al., 2008). Second, the reliability scores of the neurons were lower compared to place cells in mammals.

The above mentioned oscillations within the theta band are a prominent feature of the hippocampal network (Buzsáki, 2002, 2003). They are found in all hippocampal subdivisions, are dependent on the cholinergic input from the medial septum (Hangya et al., 2009), and are possibly mainly generated by the recurrent network of the CA3 region and by the entorhinal input (Bragin et al., 1995; Buzsáki, 2002). Pyramidal neurons and interneurons in the hippocampus are phase-locked to these oscillations and the power of high-frequency oscillations (gamma) can also vary as function of the theta cycle (Buzsáki et al., 2003; Buzsáki and Wang, 2012). These mechanisms have been implicated in inter-areal communication as well as sensory and memory processes (Lisman and Jensen, 2013). However, it is unclear whether similar processes are utilized by the avian hippocampus (Rattenborg and Martinez-Gonzalez, 2011). The hippocampus in birds also massively projects to the septum but receives only weak septal input (Atoji and Wild, 2004). Only a single study reported theta oscillations hitherto in birds (Siegel et al., 2000). In this study, the theta oscillations were observed during locomotor activity but also immobility and during feeding behavior and resembled the theta oscillations described in cats, while differed in some aspects from those described in rats. In mammals, theta oscillations also occur during sleep, and together with sharp-wave ripples and thalamocortical spindles are thought to be important for memory consolidation (Nádasdy et al., 1999; Peyrache et al., 2009; Rattenborg et al., 2011; Sadowski et al., 2011). Interestingly, birds also display slow-oscillations during sleep but it remains questionable whether such oscillations represent the same phenomenon in both species and whether theta oscillations in hippocampus are present in birds at all (Berger and Walker, 1972; Walker and Berger, 1972; Rattenborg et al., 2011; Rattenborg and Martinez-Gonzalez, 2011).
Our data show that the avian hippocampus is characterized by several reverberatory circuits. The pathway along the transverse axis of the hippocampus (section 4.2.4), contains strong feedback projections at many stations. Furthermore, the dorsomedial and ventrolateral components of this pathway interact at each level. Interactions between the two parts are further achieved by neurons aligned orthogonally to the transverse axis which extend their dendrites across the hippocampus. As the hippocampus is coated by fiber bundles dorsally and ventrally, the dendrites reaching these fibers most probably sample and integrate the incoming input. These observations are consistent with previous detailed descriptions of hippocampal neurons in pigeons and chicks (Tömböl et al., 2000a,b). Moreover, the data of Tömböl et al. (2000a,b) showed, that hippocampal projection neurons very often give rise to recurrent collaterals extending in both the dorso-ventral and medio-lateral plane, which might contact other hippocampal excitatory and inhibitory neurons. Similarly, the axons of local circuit neurons show dense arborizations along the medio-lateral or dorso-ventral axis. Thus, the avian hippocampus seems to contain a complex intrinsic network which might be able to sustain and modulate the incoming activity. Such recurrent network could be an oscillator producing the observed theta-rhythm (Siegel et al., 2000; Buzsáki, 2002) or it could be able to generate sequences of activity and associate them with the items of the navigational map (Bingman et al., 2005; Cheng, 2013). Moreover, recurrent connectivity is a key element required for attractor dynamics in an attractor neuronal network (Tsodyks, 2005; Leutgeb et al., 2005; Gerstner et al., 2014). The attractor networks provide an elegant theoretical framework how the hippocampus encodes and stores memories, activates representations by receiving only partial cues, and prevents interference between similar representations. An attractor network has several different states (attractors) to which the network dynamic is “attracted”. The states are specific patterns of neuronal activity corresponding to different memories. They can be retrieved by partial cues (pattern completion) and will persist in that state without further inputs due to the recurrent circuitry. The retrieved pattern will remain separate from the other dissimilar patterns (pattern separation).

Besides further anatomical and functional studies, we desperately need electrophysiological recordings from hippocampus during navigation and exploration, especially in environments with stable goal locations (Hough and Bingman, 2004; Kahn et al., 2008; Mayer et al., 2013). Such studies shall provide insights into the coding principles of the avian hippocampal
network and reveal whether similar phenomena to mammalian hippocampus such as replay and remapping can be found in birds (Nádasdy et al., 1999; Colgin et al., 2008; Peyrache et al., 2009; Moser et al., 2015; Schwindel et al., 2016). As the avian hippocampus has been implicated in encoding and disentangling of different environmental configurations (White et al., 2002; Kahn and Bingman, 2009), it would be interesting to see how the firing pattern in different hippocampal areas relates to the environment. Sparse coding in some hippocampal regions, as well as changes in firing rate and/or in the location of the receptive field of neurons between different environments would indicate that the avian hippocampus might be able to represent distinct environments in decorrelated neuronal activity patterns (Leutgeb et al., 2007; Colgin et al., 2008; Schmidt et al., 2012). Such findings would strengthen the argument that the avian hippocampus utilizes similar pattern separation and completion mechanisms as its mammalian counterpart (Nakazawa et al., 2002, 2004; Fenton, 2007; Leutgeb et al., 2007; Schmidt et al., 2012; Herold et al., 2015).

Taken together, the hippocampal networks of birds and mammals may in principle share at least some basic mechanisms, which however might have been differently adapted to environmental challenges in these two species. Further research is needed to acquire a deep understanding of the mechanisms and functional organization of the hippocampal network in birds.
Discussion

Figure 54: Hippocampal pathways.
A: Main hippocampal pathway and its topographical organization in birds. B: Main hippocampal pathway in the arrangement corresponding to the mammalian trisynaptic pathway according to proposed homologies. C: Anatomical connectivity within the mammalian hippocampal complex. D: Anatomical connectivity within the avian hippocampal complex. Note that the connectivity reveals a complex but very similar pattern in both species.
4.2.4 The main hippocampal pathway and its comparison to mammals

The extensive intrahippocampal connectivity with many recurrences offers multiple possible pathways which could be utilized by the avian hippocampus. Previous work suggested a main feedforward circuit constituted by the projection from DL to DM, from DM to Vm and from Vm to VI and then back to DM, which then projects out of the hippocampus (Hough et al., 2002; Kahn et al., 2003; Bingman et al., 2005). Our findings suggest that at least two pathways can be distinguished within the avian hippocampus based on the dorsomedial or the ventrolateral course of their trajectories. The dorsomedial pathway starts in DLd, while the ventrolateral one originates in the DLv. They target the DMd and DMvv, respectively and continue further either directly to Tr or indirectly via the V-shape layers (Vm and VI, respectively). The Tr projects back to the DL (Atoji and Wild, 2004) but also targets the DMd and DMvv, respectively. Moreover, it also projects out of the hippocampus targeting the CDL (Atoji and Wild, 2005). The CDL conveys sensory input to the (lateral) DL and DMv, as well as to the V-shape layers and Tr (Atoji and Wild, 2004, 2005). Since CDL receives direct input from the olfactory bulb and CPi (Patzke et al., 2011, Atoji and Wild, 2005, 2014) and is interconnected with NFL and TPO (Atoji and Wild, 2005) it might support the hippocampus especially with the olfactory and visual information. The thalamic input originates mainly in the DLM and targets the DL and DMv (Casini et al., 1986; Atoji and Wild, 2004, 2005; Figure S11). Moreover, our data indicates the input to DMv might arrive predominantly within its dorsal part which then conveys the information to DMd (Figure S11).

It has been suggested that the DL resembles the mammalian entorhinal cortex, the DM contains features of the CA-regions and the subiculum, while the V-shape layers resemble the dentate gyrus (Atoji and Wild, 2004, 2006; Abellán et al., 2014; Herold et al., 2015; Atoji et al., 2016). Under these circumstances, if we aim to compare the avian feedforward route with the mammalian trisynaptic pathway, we have to slightly rethink the order within the avian pathway. It should still start in DL but the comparable projection would be the one to the V-shape layers. This is consistent with our observation that both VI and Vm receive direct input from the DL (DLv and DLd, respectively). However, these projections seemed to be weaker compared to other targets of DL. The projection of V-shape layers to the DM then resembles the projection of the dentate gyrus to the Ammons horn. With its extrahippocampal projections which includes the hypothalamus, Atoji and Wild (2004) ascribed DM also features of the mammalian subiculum. The Atoji and Wild (2004, 2006) did
not include Tr into the comparison. Since, Tr also receives input from the V-shape (Atoji and Wild, 2004; our data) and share at least some similarities with the Ammons horn (Herold et al., 2014), we think that it could be incorporated into this model. Without claiming any one-to-one homologies between the subregions (which is neither possible nor necessary expectable), these rough comparisons suggest that the main pathway comparable to the mammalian trisynaptic pathway is constituted by DL-V-shape-Tr-DM projection. The fact that DM receives also direct input from DL and is connected to extrahippocampal structures including the thalamic, hypothalamic as well as amygdaloid and olfactory structures, and CDL is consistent with the idea of DM being comparable to both the CA regions and subiculum (Witter et al., 2000; Scharfman, 2007; Aggleton et al., 2010; Arszovszki et al., 2014). The input from CDL to the V-shape differs from the case in mammals as the CDL was compared to the cingulate cortex (Atoji and Wild, 2005). However, CDL might also be comparable to the medial entorhinal cortex (Abellán et al., 2014) which would make the CDL-V-shape projections consistent with the entorhinal input to the dentate gyrus in mammals. Further studies shall show whether and how this whole pathway is recruited in the function of the avian hippocampus.

4.2.5 Did birds evolve a complex circuitry different from the mammalian trisynaptic pathway?

The hippocampus possibly emerged with vertebrates and since then conserved its function in spatial memory (Roth and Westhoff, 1999; Rodriguez et al., 2002; Herold et al., 2015; Hevner, 2016). Gene expression studies uncovered some conserved developmental features and adult gene expression but also some differences (Puelles et al., 2000; Gupta et al., 2012; Abellán et al., 2014; Belgard et al., 2013). Although the hippocampus as a whole is homologous across taxa, it differs markedly between birds, reptiles and mammals (Striedter, 2016). It obviously underwent many changes in the mammalian and reptilian/avian lineages and thus the question arise whether the functionally equivalent and developmentally homologous structures share common circuit properties or whether they evolved dissimilar organization.

The mammalian unidirectional trisynaptic pathway has been long considered to be the main hippocampal circuit. The unidirectionality and exclusivity of this pathway clearly contrast the organization of the avian hippocampus. Although a main pathway can be outlined in the
avian hippocampus based on anatomical and few electrophysiological data, we found that this pathway contains many alternative routes as well as recurrent feedback loops. Are these apparent discrepancies the result of 300 million years of independent evolution which led to the establishment of divergent hippocampal circuits and mechanisms? To answering this question, we first need to have a closer look on the intrahippocampal connectivity in mammals to better understand the extent to which the circuitry differs between the two species.

In mammals, alternative pathways, intrahippocampal feedback projections and the circuitry of the CA2 region have received much less attention and have not been included in the standard trisynaptic model of the hippocampus circuitry (van Strien et al., 2009; Jones and McHugh, 2011). In the standard model, the information from the entorhinal cortex arrives in the dentate gyrus and is forwarded to the CA3 region and from there to CA1. However, the projections of the entorhinal cortex can bypass the dentate gyrus and reach all three CA regions directly (see also section 1.3.1). This opens the possibility of using disynaptic, trisynaptic, or even quadrisynaptic pathways from EC to CA1 (Chevaleyre and Siegelbaum, 2010; Piskorowski and Chevaleyre, 2012; Kohara et al., 2014). For instance, the entorhinal afferents were shown to activate CA2 neurons which in turn excite the CA1 region, thus forming a powerful disynaptic pathway (Tamamaki and Nojyo, 1993; Chevaleyre and Siegelbaum, 2010). Moreover, contrary to previous reports, the granule neurons of the dentate gyrus also projects to the CA2-field which provide an alternative trisynaptic pathway from the entorhinal cortex to the CA1 region via the dentate gyrus and the CA2 (Kohara et al., 2014). Since CA2 also receives input from CA3, the feedforward hippocampal pathway can involve four synapses and run across all its subdivisions (Ishizuka et al., 1990; Chevaleyre and Siegelbaum, 2010; Piskorowski and Chevaleyre, 2012). However, the projection from CA3 to CA2 seems to be dominated by a strong feedforward inhibition (Chevaleyre and Siegelbaum, 2010) and was therefore suggested to modulate the balance between the disynaptic and the classical trisynaptic pathway (Jones and McHugh, 2011). Thus, there are various alternative pathways to the classical one which involve different numbers of synaptic steps and may be selectively recruited by different aspects of behavior or during different phases of the theta cycle (Jones and McHugh, 2011; Kitamura et al., 2015).

Furthermore, the unidirectional nature of the classic trisynaptic pathway is not supported by the anatomical and physiological data either. First, all three CA subdivisions have been
shown to project back to the dentate gyrus (Ishizuka et al., 1990; Li et al., 1994; Scharfman, 1994, 2007; van Strien et al., 2009). The feedback projection of CA3 to the dentate gyrus was implicated in models of sequence learning (Lisman, 1999; Lisman et al., 2005) and pattern separation (Myers and Scharfman, 2011), and might also play a role in epileptic seizures (Scharfman, 2007). Second, the CA regions are interconnected among each other. For example, both CA1 and CA2 project back to CA3 (Ishizuka et al., 1990; Mercer et al., 2007; van Strien et al., 2009). Third, the subiculum which projects to the entorhinal cortex also sends pronounced feedback connections back to CA1 (Sun et al., 2014; Xu et al., 2016). In addition, not only CA1 but also CA3 was shown to project to the subiculum (Li et al., 1994) and the information can reach the entorhinal cortex also from CA2 (Rowland et al., 2013). Last but not least, although it was most impressively demonstrated for CA3 (Wittner et al., 2007), all hippocampal regions (DG, CA1-3) recurrently innervate themselves (Ishizuka et al., 1990; van Strien et al., 2009; Piskorowski and Chevaleyre, 2012).

Taken together, the connectivity and information transfer within the mammalian hippocampus is by far not unidirectional and certainly not restricted to one pathway. Coming back to the phylogenetical question from the beginning of this section, the data on the hippocampal connectivity argue against the emergent of a massively divergent circuitry in birds and mammals. If we look behind the well-established framework of the mammalian trisynaptic pathway, we suddenly end up with a network strikingly less dissimilar to the network we found in our study. In both species, the hippocampal circuitry is characterized by several possible pathways with various numbers of synaptic steps and recurrent feedback projections. Furthermore, the presence of topographically separated pathways in both mammals and birds indicate that such topographical organization might be an advantageous principle of the hippocampal anatomy (Deguchi et al., 2011). Precise topography and discrete circuits within the hippocampus might enable a detailed representation of different environments which can be mapped on uncorrelated place maps (Colgin et al., 2008; Moser, 2011).

However, a conclusion about the degree of similarity between the hippocampi of the two species is still not possible. In birds, we lack a detailed understanding of the circuitry as can be revealed by intracellular filling of neurons. We further need electrophysiological investigations of acute hippocampal slices to establish different pathways and to understand their dynamics. To comprehend the circuitry, we also have to identify and characterize the
inhibitory network within the hippocampus. Last but not least, with in vivo electrophysiology and optogenetic methods we shall figure out the coding properties of the network and dissociate the function of the different subdivisions and pathways.

4.3 What did we learn about the evolution of the forebrain?

Our data contribute to the recent evidence indicating that the local circuitry of the avian sensory pallium is organized in a laminar and columnar fashion and resembles in some aspects the circuitry found in the mammalian neocortex (Wang et al., 2010; Ahumada-Galleguillos et al., 2015; Calabrese and Woolley, 2015). Furthermore, we also showed that the connectivity pattern within the hippocampus displays features similar to the mammalian hippocampus. What does this tell us about the evolution of the forebrain? The pallia of birds and mammals are homologous as whole (Reiner et al., 2004) but perhaps with the exception of the hippocampus and the wulst, the remaining pallial structures (nidopallium, mesopallium and parts of the arcopallium) can not clearly be homologized to the neocortex as different pallial sectors contribute to their development (Fernandez et al., 1998; Striedter et al., 1998; Puelles et al., 2000; Medina and Abellán, 2009). It could therefore be concluded that the neocortical circuits in mammals and the pallial circuits in the DVR of birds evolved independently. However, they converged to similar architectures possibly due to the necessity of reciprocal networks (and perhaps their laminar and columnar arrangement) for complex computations (Johnson, et al., 2000; Tsodyks, 2005; Leutgeb et al., 2005; Douglas and Martin, 2007a; Shepherd, 2011; Dockendorf and Srinivasa, 2013; Gerstner et al., 2014; Crockett et al., 2015). This would also indicate that there might be limited degrees of freedom in the circuitry organization for complex sensory processing.

However, the ubiquity of reciprocally interconnected circuits in the forebrain (our data) and the presence of the same cortical connectivity features also in the reptilian dorsal cortex (Shepherd, 2011; Fournier et al., 2015; Naumann et al., 2015) suggest that the cortical principles might be phylogenetically very old. It is reasonable to assume that certain circuitry motifs were established already in the stem amniote and the genetic mechanisms for their development might be therefore conserved (Cordery and Molnár, 1999; Aboitiz, 2011; Suzuki and Hirata, 2012; Aboitiz and Zamorano, 2013; Luzzati, 2015). It is then a valid
hypothesis that certain pallial cell types and their circuits in birds and mammals might be homologous (Karten, 2015; see section 1.2). In this line of reasoning, the stem amniote ancestor might have possessed a simple pallium which could be transformed through evolutionary changes into the Wulst and DVR in birds and the neocortex in mammals (Reiner, 1993, 2013). Differential upregulation of certain developmental genes in birds and mammals led to enlargement of ventral and lateral sectors in birds and dorsal pallial sectors in mammals (Aboitiz, 2011; Aboitiz and Zamorano, 2013). These facts are consistent with the findings that the dorsal cortex and the DVR in some reptiles share similar architecture and form a continuous structure as is particularly evident in the tuatara, a primitive lizard-like reptile (Butler, 1994; Reiner and Northcutt, 2000; Reiner et al., 2005). What is notably interesting is a three laminar arrangement spanning the dorsal cortex and DVR of the tuatara (Reiner and Northcutt, 2000). If tuatara indeed represents a primitive condition of reptilian forebrain then this has important implications for our reasoning. It indicates that a clear lamination was a feature of the forebrain in the stem amniote ancestor which became much less obvious in the DVR of reptiles and almost completely absent in the avian brain. We now know that despite a nuclear appearance, the avian pallium also possesses layered organization (Wang et al., 2010; Ahumada-Galleguillos et al., 2015; our data). It therefore could be that the lamination in birds was not omitted but became histologically less obvious perhaps during the rapid increase of neurons within the ventral and lateral pallial sectors (Aboitiz and Zamorano, 2013; Olkowicz et al., 2016; Sayol et al., 2016; Güntürkün et al., 2017). On the other hand, in mammals, the changes in developmental genes favored an enlarged dorsal pallium (Aboitiz and Zamorano, 2013) but here the lamination was retained and the number of layers was duplicated (Shepherd, 2011; Luzzati, 2015). Several factors might contributed to the retention of a clear lamination including enhanced reelin expression which led to straight projections of radial glia fibers from the ventricular to the pial surface and to a bipolar shape of migrating neurons (Nomura et al., 2008). The duplication of three layers might have borne several advantages. The perhaps most important is the enormous increase in computational capacities due to dense interconnection of several cellular layers with each layer theoretically being able to function as a semi-independent unit (Reiner, 1993; Shepherd, 2011).

Investigations of the turtle dorsal cortex corroborate the idea of phylogenetically conserved intrinsic connectivity patterns and physiology (Kriegstein and Connors, 1986; Prechtl, 1994;
Cordery and Molnár, 1999; Larkum et al., 2008; Shepherd, 2011; Naumann et al., 2015). However, they also indicate that certain common properties of pallial organization of birds and mammals might have evolved independently. The turtle cortex lacks a prominent columnar organization, granular neurons resembling the mammalian layer 4, and layer2/3 neurons with interareal and interhemispheric projections (Reiner, 1993; Reiner et al., 2005). Instead, the reptilian pyramidal neurons are comparable to deep layers of neocortex (Reiner, 1993). It was therefore suggested that cell types typical for cortical layers 2-4 evolved independently in birds and mammals (Reiner et al., 2005). However, a subset of turtle pyramidal neurons with extracortical targets does express genes characteristic for mammalian layer 2/3 neurons (Suzuki and Hirata, 2014) and layer 4 markers were found in thalamorecipient areas of the turtle cortex (Dugas-Ford et al., 2012). Thus, it seems that genetically different cell types existed in the common ancestor of birds and mammals but were comprised in one cellular layer. It could be that certain subpopulations of neurons became separated, omitted their extratelencephalic projections and acquired intrapallial targets instead independently in birds and mammals. The division of one cellular layer into several interconnected layers might have concomitantly entailed the formation of columns in both species (Figure 55).

![Figure 55: Comparision of reptiles to birds and mammals and the evolutionary scenario.](image)

The reptilian pallium contains one dense cell layer in which the principal neurons receive thalamic input and project to the brain stem as well as to each other. Although the neurons resemble the layer 5/6 neurons, recent genetic studies showed that layer 2/3 and 4 markers are also expressed in subsets of these neurons. This opens the possibility that layer 2/3 and 4 neurons did not evolved de-novo and independently in birds and mammals but demerged from the ancestral cellular layer which already contained genetically differentiated cell types intermingled in one layer. In birds and mammals, the distinct cell populations became segregated into different layers. They maintained their reciprocal connectivity, but the layer 2/3 and 4 neurons lost their extratelencephalic projections. Instead, layer 2/3 neurons acquired distant intrapallial targets. Due to the spatial separation of the cell types the reciprocal connectivity between the principal neurons runs in the vertical plane with axons forming anatomical columns.
Recurrent excitatory networks organized into laminar and columnar fashion seem to be a favorable neurobiological principle as it was also proposed to exist in fishes (Ito and Yamamoto, 2009; Trinh et al., 2016). Future studies should elucidate how this kind of architecture, once tough to be unique to neocortex, is widespread among different vertebrate species. If this is a feature of brains in many different vertebrate classes, one could speculate that a genetic program for laminar and columnar organization is highly conserved and it is suppressed or modified in some species.
5. Concluding remarks

We set out to shed light on the local connectivity in sensory systems and the hippocampus of the avian forebrain. We departed from the assumption that functionally equivalent forebrain structures of mammals and birds could show similarity in their connectivity principles. The similarities could result from a comparable evolutionary pressure on sensory signal processing and restricted neurobiological solutions for complex neuronal network computations. On the other hand, basic network principles and organization could also have been inherited from a common ancestor. The latter could be the case especially in structures which are present in both taxa and originate in equivalent pallial sectors during embryogenesis. We found that the avian hippocampus, for the most part agreed to be homologous to the mammalian one (Atoji and Wild, 2006; Herold et al., 2015; Striedter, 2016), displays possibly a main pathway based on the anatomical strength of the connectivity which can be alternated and bypassed by many alternative connections. As an important feature occurred the topography and recurrent nature of the circuits. Due to extensive reciprocal connections between different hippocampal subdivisions the hippocampus comprises many tentative reverberatory circuits. We pointed out that these characteristics are also prominent features of the mammalian hippocampus and therefore conclude that mammals and birds share similar hippocampal networks despite divergent histological appearance. We also note that any detailed conclusions about the similarity of the networks are premature given the missing physiological, functional, and microcircuitry studies of the avian hippocampus. Similarly, the avian wulst, which has been homologized to (parts of) the neocortex (Puelles, 2001; Reiner et al., 2005), showed a connectivity pattern remarkably comparable to the neocortical one.

A hot topic however, is the organization of the circuitry within the DVR. The question of homology of DVR to the mammalian forebrain has stimulated the biggest dispute in the comparative neurobiology (Bruce and Neary, 1995; Striedter, 1997; Karten, 1969, 1997; Puelles et al., 2000; Puelles, 2001; Butler and Molnár, 2002; Puelles and Medina, 2002; Reiner et al., 2005; Butler et al., 2011; Puelles, 2011; Puelles et al., 2016). We confirmed and extended recent studies showing that sensory system within the DVR are organized in a laminar and columnar fashion and utilize reciprocal connectivity in sensory processing (Wang
et al., 2010; Ahumada-Galleguillos et al., 2015). Likewise, the circuitry bears similarities to the neocortex and seems to process sensory information in way akin to the neocortex (Calabrese and Woolley, 2015).

Recent developmental studies showed that nidopallial and mesopallial subdivisions are continuous with the layers of the wulst based on expression of specific genetic markers during the development and in the adulthood (Jarvis et al., 2013; Chen et al., 2013). These studies contradict the standard distinction of the wulst and DVR based on their dorsal and ventrolateral pallial origins, respectively (Puelles et al., 2000; Medina and Abellán, 2009; Puelles, 2011) and open new perspectives on claims of homology (Chen et al., 2013). The main message of these studies, the continuity of nidopallial and mesopallial sectors with the hyperpallium, further corroborates the idea that the mammalian neocortex and the avian pallium evolved from a common structure, which was not differentiated into dorsal pallium and DVR. Mammals and birds independently increased the number of their neurons through changes in the neurogenesis rate (Nomura et al., 2013; Olkowicz et al., 2016; Güntürkün et al., 2017) which was accompanied by segregation of genetically distinct subpopulations of neurons into separate layers (Dugas-Ford et al., 2012; Wang et al., 2010; Suzuki and Hirata, 2014; Ahumada-Galleguillos et al., 2015; our data). The neurons of superficial layers omitted their projections to extrathalencephalic targets but retained reciprocal connectivity with each other which formed the anatomical basis of pallial columns in birds and mammals. Our data together with recent studies favor the core assumption of Karten’s hypothesis that specific cell types and circuits within the avian pallium have a long phylogenetic history and are therefore homologous to the specific cell types and circuits of the mammalian neocortex.
6. Future studies

The bulk of knowledge on the mammalian cortical circuitries shows that a detailed understanding of cell-specific excitatory and inhibitory microcircuitry is indispensable for unraveling the neurobiology of behavior. Decades of anatomical studies of the avian brain yielded a quite comprehensive picture of its connectivity (Shanahan et al., 2013; see also section 1.2 and 1.4). Future research should now apply modern methods including optogenetics, viral tracings and two photon imaging to pinpoint the function and dynamics of discrete neuroanatomical circuits. For instance, such studies could disentangle the contributions of the entopallio-nidopallial and the entopallio-mesopallial circuits to different aspects of visual processing and categorization. Recordings from various visual areas and simultaneous optogenetic manipulations of neurons in MVL projecting to the entopallium could also unravel their possible modulation of stimulus-driven visual activity. Combination of anatomical tracing and two-photon imaging could be used to uncover functional organization of areas which still remains “terra incognita” such as the temporo-parieto-occipital area or CDL.

Another important question regards the morphology and physiology of individual pallial neurons. The pyramidal neurons in reptiles possess multiple apical dendrites capable of similar physiological processes comparable to mammalian pyramidal cells (Larkum et al., 2008; Shepherd, 2011). While mammalian pyramidal neurons evolved single apical dendritic branch morphology with basal dendrites and oblique branches (DeFelipe and Fariñas, 1992), birds seem to favored multipolar dendritic trees (Reiner et al., 2001; Wang et al., 2010; Ahumada-Galleguillos et al., 2015; our data). As the apical dendrites seem to perform complex physiological contributions to the signal processing in both reptiles (Larkum et al., 2008; Shepherd, 2011) and mammals (Larkum et al., 2009; Murayama et al., 2009; Shepherd, 2011; Manita et al., 2017), the questions arise which advantage the avian dendritic morphology has and whether birds retained similar or evolved different dendritic physiology. The morphological and physiological differences between neocortical and avian pallial neurons could have an important impact on extracellular signatures of neuronal activity as measured for instance by the local field potentials as these seem to be often
dominated by neurons with apical dendritic structure (Linden et al., 2010, 2011; Einevoll et al., 2013; Reimann et al., 2013).
7. References


References


Manger, P.R., Elston, G.N., Pettigrew, J.D. (2002). Multiple maps and activity-dependent representational plasticity in the anterior Wulst of the adult barn owl (Tyto alba). The European Journal of Neuroscience, 16, 743–750.


8. Appendix

8.1 Supplementary figures

Figure S1: Injections into MD. Section left depicts the injection site as well as the magnified area on the right. Injections into MD labelled neurons in NI as well as descending fibers running toward the NI and entopallium.
Figure S2: MD and MV are reciprocally connected with each other.
A: Injections into ventral mesopallium labelled fibers and neurons in the dorsal mesopallium, indicating a reciprocal connection between ventral and dorsal mesopallium. B: Magnification of the region marked in (A). Black arrows and arrowheads point to examples of retrograde labelled neurons and anterogradely labelled fibers, respectively. C: Another example of fibers terminating in MD (arrowheads) after MVL injection. D: Similarly, injections into MD labelled many varicosity-rich fibers terminating in MVL. Scale bars =200 µm in A; 50 µm in B; 20 µm in C-D.
Figure S3: Injection sites into the NCL of the animal shown in Figure 15 and Figure 17.  
A: Sagittal section showing the location of the CTB injection into the visual-tectofugal part of the NCL.  
B: A more medial section in the same hemisphere of the same animal, showing the injection placed into the auditory NCL.
Figure S4: *In vitro* tracing in the visual-tectofugal system of the barn owl.
Injections into the entopallium labelled few neurons in NI and several fibers running across NI towards the MVL. Scale bars = 100 µm in A.
Figure S5: Injections sites NCL and arcopallium.  
A: CTB injection into the trigemino-recipient area of the NCL.  
B: CTB injection into the anterior arcopallium. Scale bar = 1000 µm.
Figure S6: In vitro tracing in the auditory system of the barn owl.
A: Strong interconnection between the L2 and CMM was revealed by injection into L2. Interestingly, the fibers seemed to be organized into bundles. B: Fibers and few retrogradely labelled neurons were also observed in L3. Scale bars = 100 µm.
Figure S7: Cytoarchitecture in the mesopallium and HD of the owl.

A: Nissl stained neurons in the mesopallium of the barn owl. Note that neurons tend to form clusters consisting of small granulate neurons concentrated around one (often bigger) neuron. B: In contrast, the HD is devoid of such arrangements and contains bigger neurons with low density of granulate neurons in-between.
Figure S8: Striatal projections of primary sensory areas.

A: Fibers from BAS turned ventrally and run downwards across the striatum. B: The fibers built several collaterals and varicosities within the striatum. C: Similarly, also injections into the Field L2 also labelled fibers running across the striatum. D: Magnification of the labelled fibers in striatum coming from L2. E: Entopallium also sent fibers towards the striatum. F: Magnification of fibers within the lateral striatum. Scale bars = 500 µm in A, C and E; 20 µm in B, D and F.
Figure S9: Injection into the medial DMv including DMd.
A: Slice counterstained with Nissl showing the injection site. The injection included the medial part of DMvd and the lateral aspect of the DMd. In this case, fibers were observed, which run across the central Tr. B: Magnification of the central Tr region. Fibers contained varicosities and appeared to contact neurons in Tr (black arrows). Together with the other injections these data indicate the projections of DM to the rest of the hippocampus are topographical with a mediolateral and dorsoventral gradient. C: The same slice as in Figure 44A. D: Magnification of a neuron extending its dendrites (black arrows) up to the periventricular border. Scale bars = 500 µm in A and C; 20 µm in B and D.
Figure S10: Summary of all in vivo injections into the hippocampus.
The injections were grouped into DMv injections, DMd+medial DMv+Tr, and V-shape injections. The case T-113 does not fit to any of these groups and is the only in vivo case with an anterior DLD injection. Schematic drawings on right site represent the hippocampus from caudal A 5.00 (top left) to rostral A 8.50 (bottom right). The colored dots represent retrogradelly labelled neurons after CTB injections. Each case is represented by one color corresponding to the color of the circle depicted in the pictures of the injection sites left.
Figure S11: Thalamic input to the hippocampus.
The thalamic input originates mainly in the DLM. Some neurons were also found in SPC. The majority of the thalamic neurons projected to DMv. Less retrogradely labelled neurons were observed in the V-shape group and only few neurons were usually labelled in the DMd+Tr+medial DMv group. Furthermore, comparing the cases T-111, T-400 and T-417 suggests that the major input reach the DMvd.
8.2 List of figures

Figure 1: Structure of the mammalian and the avian forebrain ......................................................... 3
Figure 2: The neocortical excitatory circuit .......................................................................................... 4
Figure 3: Ascending auditory system in pigeons ............................................................................... 10
Figure 4: Visual pathways in the pigeon ....................................................................................... 12
Figure 5: Ascending somatosensory systems in the pigeon ............................................................ 13
Figure 6: Topographical organizations of the trisynaptic pathway along the transversal axis .......................................................................................................................... 20
Figure 7: The avian hippocampus ................................................................................................... 25
Figure 8: Transversal section of the avian telencephalon ............................................................... 34
Figure 9: Entopallium connectivity .................................................................................................. 35
Figure 10: Different types of neurons in NI ....................................................................................... 36
Figure 11: Spiny neurons in NI ......................................................................................................... 37
Figure 12: Dorsal and ventral aspects of the entopallium project to MVL ........................................ 38
Figure 13: NI is interconnected with MVL ...................................................................................... 39
Figure 14: MVL injections confirmed the connectivity between NI and entopallium and MVL ........................................................................................................................................... 40
Figure 15: NCL projecting neurons in NI located in-between the bundles ........................................ 41
Figure 16: Mesopallium projects to dorsal and ventral entopallium ................................................ 42
Figure 17: Neurons in NI forward the visual information to the NCL ............................................... 42
Figure 18: Sagittal section of the avian telencephalon ...................................................................... 44
Figure 19: BAS targets both the ventral and dorsal mesopallium .................................................. 45
Figure 20: BAS connectivity ............................................................................................................. 46
Figure 21: Dorsal BAS projections .................................................................................................. 47
Figure 22: MFV is interconnected with NFT and BAS ......................................................................... 48
Figure 23: NFT injections labelled BAS neurons ............................................................................... 49
Figure 24: NFT forwards the information to NCL and arcopallium ................................................. 50
Figure 25: The auditory system of the pigeon ............................................................................... 51
Figure 26: Connectivity within the auditory system ......................................................................... 52
Figure 27: Output of the auditory system to the arcopallium ............................................................ 53
Figure 28: Fibers running to the CMM and their collaterals ............................................................. 53
Figure S4: In vitro tracing in the visual-tectofugal system of the barn owl .................. 151
Figure S5: Injections sites NCL and arcopallium ............................................................ 152
Figure S6: In vitro tracing in the auditory system of the barn owl ............................... 153
Figure S7: Cytoarchitecture in the mesopallium and HD of the owl ............................ 154
Figure S8: Striatal projections of primary sensory areas ............................................... 155
Figure S9: Injection into the medial DMv including DMd .............................................. 156
Figure S10: Summary of all in vivo injections into the hippocampus ............................ 157
Figure S11: Thalamic input to the hippocampus ............................................................. 158

8.3 List of chemicals

3,3’Diaminobenzidine (Sigma)                      Isopropanol (Merck)
96% ethanol (Sigma-Aldrich)                        Isotonic sodium chloride solution 0.9%
Acetic acid (Merck)                                (Braun)
Ammonium chloride (Riedel de Häen)                Ketamine (Ketavet)
Ammonium nickel sulfate (Riedel de Häen)          Magnesium sulphate (J.T. Baker)
Chloralhydrat (ACROS ORGANICS)                    Magnesium chlorid (J.T. Baker)
Cobalt(II)chloride hexahydrate (Merck)            Neutral red (Fluka)
Cresylviolet acetat (Sigma)                       Paraformaldehyde (Prolabo)
Dipotassium hydrogenphospate (Merck)              Pentobarbital (Sigma)
Dolorex (MSD)                                     Propandiol (Merck)
DPX (Fluka)                                       Rimadyl (Pfizer)
Ethanol (Merck)                                   Sodium acetate (J.T. Baker)
Fluoromount (SouthernBiotech)                     Sodium chloride (J.T. Baker)
Gelatine(Merck)                                   Sodium dihydrogenphosphate (J.T. Baker)
Glucose oxidase from Aspergillus niger (Sigma)    Sodium hydroxide (Merck)
Heparin (Rotexmedica)                             Sodium hypochloride (J.T. Baker)
Hydrochloric acid (Merck)                         Sucrose (AppliChem)
Hydrogen peroxide(J.T. Baker)                     Triton X-100 (Sigma)
Isoflurane (Abbott)                               Tyrothricin (Tyrosur)

Xylene (J.T. Baker)  \(\beta\)-D-glucose (Sigma-Aldrich)

Xylocain (AstraZeneca)

**Normal Serum**

Normal goat serum (Vector Laboratories)
Normal rabbit serum (Vector Laboratories)
Normal horse serum (Vector Laboratories)

**Primary antibodies:**

Anti-CtB antibody from goat (Calbiochem)
Anti-CtB antibody from rabbit (Sigma)

**Streptavidin**

Alexa-488 Streptavidin (Invitrogen)
Alexa-594 Streptavidin (Invitrogen)

**Secondary fluorescence antibodies:**

Alexa-488 anti-rabbit from donkey (Invitrogen)
Alexa-488 anti-rabbit from goat (Invitrogen)
Alexa-594 anti-goat from donkey (Invitrogen)

**Kits:**

Elite Vectastain ABC – Kit anti-rabbit (Vector laboratories)
Elite Vectastain ABC – Kit anti-goat (Vector laboratories)
Elite Vectastain ABC – Kit anti-mouse (Vector laboratories)

**Tracers:**

Choleratoxin subunit B (Sigma)
Biocytin (Santa Cruz Biotechnology)
8.4 Abbreviations

Aivm – ventromedial part of the intermediate acropallium
Arco - arcopallium
BAS – nucleus basorostralis pallii
CA 1-3 – Cornu ammonis (Ammon’s horn)
1 - 3
CDL – area corticoidea dorsolateralis
CMM – caudomedial mesopallium
CN – cochlear nuclei
CP – cortex piriformis
DCN - dorsal column nuclei
DG – dentate gyrus
dHA – dorsal HA
DIVA - nucleus dorsalis intermedius
ventralis anterior
DL – dorsolateral part of the hippocampus
DLD – dorsal DL
DLM - nucleus dorsolateralis anterior
thalami, pars medialis
DLv – ventral DL
DM – dorsomedial part of the hippocampus
hippocampus
DMA - nucleus dorsomedialis anterior
thalami
DMd – dorsal DM
DMv – ventral DM
DMvd – dorsal portion of DMv
DMvv – ventral portion of DMv
DVR – dorsal ventricular ridge
E - entopallium

Ed – dorsal E
Ev – ventral E
ex-IHA – external part of IHA
FPL – fasciculus prosencephali lateralis
GLd – lateral geniculate nucleus, pars dorsalis
GP – globus pallidus
HA – hyperpallium apicale
HD – hyperpallium densocellulare
HI – hyperpallium intercalatum
HP - hippocampus
IHA – interstitial part of the hyperpallium apicale
in-IHA – internal part of IHA
LFM – lamina frontalis suprema
LFS – lamina frontalis superior
LL – lateral lemniscal nucleus
LM – lamina mesopallialis
LPS – lamina pallio-subpallialis
LSt – lateral striatum
M - mesopallium
MD – dorsal mesopallium
MFD – frontodorsal mesopallium
MFV – frontoventral mesopallium
MFV-in – internal layer of MFV
m fz – medial fiber zone
MLd - nucleus mesencephalicus lateralis, pars dorsalis
MSt – medial striatum
APPENDIX

MVL – ventrolateral mesopallium
MVL-ex – external layer of MVL
MVL-in – internal layer of MVL
NCL – nidopallium caudolaterale
NCM – caudomedial nidopallium
Nd – dorsal nidopallium
NFL – nidopallium frontolaterale
NFT – nidopallium frontotrigeminale
NI – intermediate nidopallium
NIL – lateral part of the intermediate nidopallium
NIMl – medial intermediate nidopallium, pars lateralis
NIMm – medial intermediate nidopallium, pars medialis
NL – nucleus laminaris
nRt – nucleus rotundus
OM – tractus occipitomesencephalicus
OS – superior olivary nucleus
Ov – nucleus ovoidalis
pfz – paraventricular fiber zone
PrV - principal sensory trigeminal nucleus
PS – primary sensory areas
QF – quintofrontal tract
Sub - subiculum
TPO – temporo-parieto occipital area
Tr – triangular part of the hippocampus
Trl – lateral part of Tr
Trm – medial part of Tr
vHA – ventral HA
VI – lateral V-shape layer
Vm - medial V-shape layer
VS – vestibular nuclei

Other abbreviations:
DAB - 3,3 diaminobenzidine tetrahydrochloride
PBS - phosphate buffer saline
PBST - phosphate buffer saline with 0.3% Triton X-100
PFA – paraformaldehyde
M - molar concentration
ml - milliliter
pH - potentia hydrogenii
Ich möchte mich ganz herzlich beim Prof. Dr. Onur Güntürkün bedanken, dass er mir die Möglichkeit gegeben hat in seinem Labor jahrelang an diversen Projekten zu arbeiten. Ich habe viel gelernt, vieles erlebt und viele Leute kennen gelernt und hatte die Gelegenheit in einem außerordentlich netten Umfeld zu arbeiten. Danke sehr! Danke für die jahrelange Betreuung und ständige Motivation.

Weiterhin danke ich Herrn Prof. Dr. Onur Güntürkün und Herrn Prof. Dr. Sen Cheng für die Gutachtung dieser Arbeit.

Bedanken möchte ich mich auch beim Herrn Prof. Dr. Harald Luksch und Dr. Tomás Vega-Zuniga für den warmen Empfang beim Besuch in ihrem Labor und deren nette Hilfe und Unterstützung bei der Etablierung der in vitro Tracingtechnik. Dank geht auch an Dr. Jorge Mpodozis und Dr. Patricio Ahumada-Gallguillos für deren Freundlichkeit, Hilfe und Tipps für die Nutzung der Biocytin-Kristalle.


Danke liebe Janet für Dein Verständnis und jahrelange Unterstützung.

This research was conducted within and funded by the SFB 874.
ERKLÄRUNG


Bochum, den

__________________________________

Martin Stacho