The caudolateral neostriatum of the avian forebrain
and its modulation by dopaminergic afferents

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Introduction

One of the long-standing issues that memory research continues to confront is the dilemma of localizing a complex function in an anatomically highly differentiated central nervous system. Is memory a mental function that can be identified within an isolated neural structure or specific groups of neurons? Traditionally, this question has been framed by psychological inquiry. In the last century, clinical observations in patients with focal lesions begun to establish a correspondence between specific symptoms and localized regions of injury, most notably left hemisphere frontal sites with expression of speech (Broca, 1861) and left hemisphere posterior temporal lobe sites with speech comprehension (Wernicke, 1874). These and subsequent cases strengthened the localizationist view, and it was not unreasonable to expect that other mental processes, like memory functions, could similarly be localized to circumscribed areas of the cortex. The classical studies of Lashley (1950) demonstrated, however, that the „engram“, as a single entity, does not exist, but that rather the behavioral effects of brain lesions correlated with the size of the lesion and not their location, with different areas making an essential contribution. Since the time of Lashley, it has become increasingly clear that memory is divisible into different processes and takes heterogeneous forms based on the type of memory (e.g., classical conditioning versus procedural), the content of memory (e.g., episodic versus semantic), the temporal parameters of memory (short term versus long term), and the level of processing (encoding or retrieval).

An important cognitive feature of „higher“1 organisms is their ability to temporarily structure their behaviour and to actively „hold“ in mind information relevant for goal-attainment. Food-hoarding birds for example, must remember a large number (up to 33,000) of caches in which they hide their food (reviewed in Shettleworth, 1983). The information about the location of the caches is certainly stored in long-term memory. In order to effectively retrieve the food, however, the animal must actively remember both the places already visited (and update this information with every new place visited), and must plan a route to the closest location that contains food (c.f. Shettleworth, 1983).

The active retention of information in memory for the guidance of responses in forthcoming situations is embedded in the concept of „working memory“ (Baddeley, 1992). Working

1 The term „higher“ is not used in the sense of „further developed“ with regard to a scala naturae, but to describe the complex abilities of the cognitive system. Therefore, the processes underlying „higher“ cognitive functions show some degree of abstraction and may be engaged in various behaviours. This might be reflected for example in the ability of an organism to rapidly adapt to new situations.
memory encompasses both storage and processing functions, and within cognitive psychology it represents an extension of an earlier concept, short-term memory, a limited-capacity temporary memory store, typified by the model proposed by Atkinson and Shiffrin (1968). Among different species working memory probably takes different forms, and its functions may range from the "simple" active retention of goal-related information, to an import role in (speech)-comprehension, thinking, and planning in primates and humans (e.g. Baddeley, 1992; Kimberg and Farah, 1993; Goldman-Rakic, 1996; Cohen et al., 1997). In order to understand this sentence, for example, a reader must maintain the first half of the sentence in working memory while reading the second half.

In mammals, working memory is closely related to the functions of the prefrontal cortex (PFC) and its innervation by the mesocortical dopaminergic system (see below), and in recent years considerable progress has been made in identifying the cellular elements that participate in working memory in mammals (e.g. Goldman-Rakic, 1996 for review). Important aspects in this quest relate to the specificity of the input-output relationships (i.e. the functional heterogeneity of brain areas, and the laminar and synaptic organizations of these connections), the distribution of receptors, and the physiological properties of the neurons involved.

In birds, a region in the posterior forebrain, called neostriatum caudolaterale (NCL), has recently been shown to be crucially involved in working memory (Mogensen and Divac, 1982, 1993; Gagliardo et al., 1996, 1997; Güntürkün, 1997; Kalt et al., 1999), and other tasks which in mammals target "frontal" executive functions (see below), e.g. reversal learning (Hartmann and Güntürkün, 1998), or response inhibition (Güntürkün, 1997; Aldavert et al., 1999). In addition, the observation that the NCL shows a high dopamine (DA) content (Divac et al., 1985) and a strong innervation by dopaminergic fibers (Waldmann and Güntürkün, 1993; Divac et al. 1994), led to the notion that the NCL might be the avian equivalent of mammalian PFC. In contrast to mammals, however, virtually nothing is known about the anatomical and physiological properties of the circuitry that might subserve higher cognitive functions in non-mammalian species.

The organization of the forebrain of birds and other amniotes (i.e. reptiles) is radically different from that of mammals (see chapter 1 and 4), reflecting at least 250 million years of divergent evolution. A comparison between birds and mammals may thus shed light on the structural and physiological requirements of a proposed canonical circuit involved in working memory, and more specifically, how the avian brain manages to solve these tasks in the absence of the layered isocortex of mammals.
In the studies presented here, I will describe some aspects of the anatomical and physiological organization of the NCL and its modulation by dopaminergic afferents, to gain insights into the neuronal circuitry that may underly working memory and other higher cognitive functions in birds. It should be stressed, however, that within this view no structural homology between the NCL and the PFC is assumed, but that rather a functional analogy between these brain areas may exist with regard to some functions, but not necessarily others as well. The term „homology“ not only implies similar functions of an organ in different species, but also stipulates a common ancestor (Campbell and Hodos, 1970). Functional „analogies“ in contrast may reflect parallel evolution, i.e. similar adaptive trends and corresponding selective pressures (Rehkämper and Zilles, 1991).

For comparison with the results obtained here in birds, the following sections give a brief overview over some of the functions of the PFC and its interaction with the mesocortical dopaminergic system. It is sought to describe the basic neuronal circuitry that underlies these functions (as far as it is known), and specifically its relationship to the layered structure of mammalian neocortex.

The prefrontal cortex - connectivity and areal organization

The prefrontal cortex occupies the rostral pole of the isocortex of mammals. In various mammalian species the PFC extends over increasingly larger areas of brain (e.g. primates > dogs > cats > rats), and its relative growth is larger than that of the other associative cortices, reflecting a phylogenetic development (Fuster, 1989).

The most commonly used hodological criterion to define the PFC in various species is the projection of the thalamic nuc. mediodorsalis (MD) (Rose and Woolsey, 1948; Divac et al., 1978; Goldman-Rakic and Porrino, 1985; Groenewegen et al., 1990). In primates this projection is topographically organized and allows a rough anatomical division of the PFC (figure 1). The dorsolateral PFC (Walkers areas 8, 9, 10, 45, 46; Walker, 1940) receives afferents from the lateral part of the MD (pars parvocellularis), and the orbitofrontal/ventromedial PFC (Walkers areas 11, 12, 13) is innervated by the medial part of the MD (pars magnocellularis) (Goldman-Rakic and Porrino, 1985; Groenewegen, 1988; Groenewegen et al., 1990; Barbas et al., 1991; see Fuster, 1989, for review). However, the definition of the PFC by its afferents from MD remains a matter of debate, as the MD projects
also to brain areas beyond the PFC, and on the other hand the PFC receives substantial innervation from other thalamic nuclei, e.g. the anterior nuclei and the pulvinar (Markowitsch and Pritzel, 1979; Goldman-Rakic and Porrino, 1985; Groenewegen et al., 1990; Barbas et al., 1991). Based on criteria defined by Campbell and Hodos (1970), Kolb (1984) proposed, that in rodents the prelimbic and infralimbic regions of the PFC might be functionally analogous to the dorsolateral PFC in primates (but see Preuss, 1995; Williams and Goldman-Rakic, 1998).

In addition to thalamically relayed input, direct subcortical and limbic afferents reach the PFC from the brainstem tegmentum, the pons, the hypothalamus, and the amygdala (for reviews see Fuster, 1989; Groenewegen et al., 1990). In various mammalian species the PFC has furthermore been shown to receive afferent fibers from the hippocampal complex, the cingulate cortex, and from adjacent areas of the limbic cortex (Goldman-Rakic et al., 1984; Reep, 1984; Pandya and Yeterian, 1990; Conde et al., 1995). In the context of working memory, the connections of the PFC with the MD, the hippocampus, the tegmentum, and the nuc. accumbens (see below) appear to be of particular importance, as they form a unitary functional network, and lesions in these regions result in impairments similar to those observed after lesions of the PFC (e.g. Sakurai and Sugimoto, 1985; Floresco et al., 1997; Seamans et al., 1998; see below).

Sensory input from other cortical areas reaches the PFC via a set of interconnected pathways (for reviews: Jones and Powell, 1970; Pandya and Yeterian, 1990). The primary sensory area of each modality projects first to an adjacent area in parietal, occipital, or temporal cortex (figure 2). This is the beginning of a sequential order of cortical areas that make up a pathway for that modality. Each area in the sequence projects not only to the next in line but also to a discrete area of the frontal cortex, which in turn reciprocates by sending fibers back to the
The fields that constitute the third link in each of the three pathways - namely, parietal area 7 (somatic), temporal area 22 (auditory), and inferotemporal area 21 (visual) - project to the prefrontal cortex and, in addition, to the cortex in the depths of the superior temporal sulcus, which constitutes another multimodal area. Although the termination fields show considerable overlap, especially around the principal sulcus of primates (Area 46), a strong topography of these afferents exists, resulting in a distinct pattern of connections for each of the subdivisions of PFC (primates: Jones and Powell, 1970; Selemon and Goldman-Rakic, 1988; Pandya and Yeterian, 1990; Romanski et al., 1999; rodents: Condé et al., 1995; Reep et al., 1996).

The (reciprocal) connections of the PFC with the posterior parietal lobe\(^2\) can be considered a prototypical example for the organization of cortico-cortical associational fibers. Both, the sources of the afferents and their areas of termination are organized in modules. The largest

\(^2\) In the parasensory posterior cortex visual and somesthetics information are integrated for the representation of the spatial location of an object. The connection between area 7 in the parietal lobe and the PFC is part of a network responsible for voluntary movements, particularly eye-movements (Selemon and Goldman-Rakic, 1988), and the so-called „dorsal visual-stream“ conveys information about the „where“ of an object. In contrast, the ventral visual stream originating in the temporal lobe, relays information necessary for object-recognition, the „what“ of an object (Wilson et al., 1993; Rao et al., 1997; see Romanski et al., 1999, for auditory processing).
number of cortico-cortical projection neurons can be found in layers III, V, and VI (Schwartz and Goldman-Rakic, 1984; Selemon and Goldman-Rakic, 1988). The afferents from area 7 in the parietal lobe terminate in a feedforward pattern mainly in layers I, IV and VI of the PFC, while the feedback from the PFC terminates in layers I and VI, but spares layer IV (Selemon and Goldman-Rakic, 1988). Within the gaps of the ipsilateral connections terminate callosal fibers from the contralateral PFC or parietal cortex. These callosal connections originate from homotopic functional modules and their termination areas show large overlap in the contralateral hemisphere (Schwartz and Goldman-Rakic, 1984; Selemon and Goldman-Rakic, 1988; Berendse et al., 1992).

The overall pattern of cortical afferents and their coarse topography to the PFC can be summarized such, that the less differentiated cortices, particularly structures of the limbic system, innervate the ventromedial (orbitofrontal) PFC, while the „cognitive“, i.e. sensory aspects of a stimulus are processed in the dorsolateral PFC (c.f. Fuster, 1989).

As already indicated, all of the above mentioned connections of the PFC are reciprocal, and the „feedback“ from the PFC largely retains the topography of the original input (Selemon and Goldman-Rakic, 1988; Sesack et al., 1989; Pandya and Yeterian, 1990). Of special importance are furthermore the reciprocal connections of the PFC with areas in the premotor, supplementary motor, and motor cortex (Brodmann´s areas 6 and 8; Brodmann, 1909), as via these efferents the PFC initiates and controls the execution of movements. An exception from the general rule of reciprocity are the projections of the PFC to the basal ganglia. The inputs from the PFC to the dorsal- (caudate-putamen) and ventral striatum (nuc. accumbens) are relayed back only indirectly via the globus pallidus, nuclei in the thalamus, and the substantia nigra, respectively (Alexander et al., 1990; Berendse et al., 1992; Goldman-Rakic, 1995; Parent and Hazrati, 1995). Depending on their precise location of termination, the striatal efferents of the PFC may participate in motoric, associative and limbic aspects of behaviour (Parent, 1990; Goldman-Rakic, 1995; Parent and Hazrati, 1995).

**Microarchitecture of the prefrontal cortex**

In primates, the clearest cytoarchitectonic feature of the PFC is the distinct granular layer IV, that distinguishes the PFC from the remaining frontal cortex; and therefore the PFC has also been dubbed „frontal granular cortex“ (Walker, 1940). In rodents, however, the granular layer is not as clearly developed. Afferent fibers terminate in all six layers of the PFC, but the projections of most brain regions show a distinct laminar pattern within the PFC (c.f. figure 4;
and the example of area 7 above). Synaptic inputs from many other association cortical regions converge in layer I-II, where the apical tuft of the layer III, and V-VI pyramidal neurons extends (Sesack et al., 1989; Berendse et al., 1992; Condé et al. 1995). Inputs to the soma or proximal/basal dendrites of pyramidal cells in layers III and V-VI, respectively, are known to arise from neighboring reciprocally connected cells within the same cortical region (Levitt et al. 1993; Kritzer and Goldman-Rakic, 1995; Pucak et al. 1996; Melchitzky et al., 1998; Gonzalez-Burgos et al., 2000). This reciprocal interaction may give rise to a reverberative ensemble of local neurons. Such local activity, confined within the PFC, may underlie working memory processes, because the PFC must rely on sustained firing to „hold“ information in the absence of continuous presence of previously presented sensory cues (Amit 1995; Goldman-Rakic, 1996; Dürstewitz et al., 1999). In analogy to the columns of primary sensory cortex, clusters of neighbouring cells in the PFC are assumed to form functional modules (Pucak et al. 1996; Melchitzky et al., 1998; Gonzalez-Burgos et al., 2000), which might share the memory for a certain location (Rao et al., 1999), or display similar object selectivity (Rainer et al., 1998b). Local interneurons that contain γ-aminobutyric-acid (GABA) may play a pivotal role in the fine-tuning of reverberating activity within a cortical module (Rao et al., 1999), and/or provide lateral inhibition between modules encoding different features, e.g. opposite spatial locations (Wilson et al., 1994; Rao et al., 1999). GABAergic interneurons also appear to be a major target of the dopaminergic innervation (see below).

**Behavioural neurophysiology of the prefrontal cortex**

*Lesion studies*

Animals that have been subjected to extensive ablation of their PFC show severe impairments in the planning and organization of complex behavioural sequences (Kimberg and Farah, 1993; Mogensen and Holm, 1994). The results from numerous lesion studies in mammals indicate that the disturbance of 3 (interacting) basic functions of the PFC contribute to the impairments observed in these „frontal animals“ (reviewed in Fuster, 1989; Dias et al., 1997).

1 *Focussing of attention:* The animal with prefrontal lesions has a fundamental difficulty in suppressing its attention to irrelevant stimuli and maintaining its attention on relevant stimuli. This distractability is also responsible for hyperreaction to external stimuli, which in turn accounts for a general motor hyperactivity (Fuster, 1989, for review).

2 *Suppression of competing tendencies:* A loss of inhibition of false response tendencies can be seen in tasks in which the preoperative incentive values of the discriminanda are
reversed („reversal-learning”), or in tasks that demand the (successive) discrimination of two stimuli of which one requires a given response, and the other no response at all (Go/No-Go tasks). Especially lesions of the ventromedial PFC impair the animals ability to suppress interference from competing tendencies (Brutkowski, 1964; Sakurai and Sugimoto, 1985; Dias et al., 1997), typically leading to perseveration and a loss of response control (Iversen and Mishkin, 1970).

3 Working memory: As outlined above, working memory describes the process of active retention and/or manipulation of items in short-term memory, required for the guidance of responses in forthcoming situations. Experimentally, impairments in working memory functions can be assessed by variants of the „delayed-response“ or „delayed-alternation“ tasks, which were established in memory research by Jacobsen in the mid-thirties (e.g. Jacobsen, 1935). Permanent or reversible lesions of the PFC (by cooling, electrical stimulation or pharmacological blockade), result in severe impairments in the acquisition and the performance of delay-tasks. The impairments become particularly obvious after lesions of the dorsolateral PFC (around the sulcus principalis), or its homologue area in the rat (Jacobsen, 1935; Sawaguchi and Goldman-Rakic, 1991; Kesner et al., 1996; Fuster, 1989, for review).

In addition to these impairments regarding attention and cognition, changes in the affective and social behaviour are often observed in frontal animals and human patients (see below).

Cellular correlates of working memory

The polysensory character of the PFC indicated from the pattern of converging afferents, is also reflected in the response characteristics of single neurons. Neurons in the PFC are able to encode the behavioural significance of a stimulus across various modalities (Yajeya et al., 1988; Watanabe, 1992). Subsets of prefrontal neurons in the area of the principal sulcus (i) are activated phasically in the presence of a visual stimulus, (ii) are activated tonically during the delay period over which the stimulus is kept on line, or (iii) show phasic reactivation in relation to the initiation of a memory-guided response. Many prefrontal neurons respond in more than one phase of the trial, i.e., during the cue, delay, and/or response periods, that means their activity is related to the subfunctions of registration, memory, and motor control, respectively (Fuster and Alexander, 1971; Fuster, 1973; Funahashi et al., 1989, 1993; Di Pellegrino and Wise, 1991; Wilson et al., 1993; Funahashi and Kubota, 1994; Rao et al., 1997, 1999; Asaad et al., 1998; Rainer et al., 1998a,b; Sawaguchi and Yamane, 1999; see
The sustained delay activity of neurons in the mammalian PFC encodes information relevant to the current behavioural goal (Yajeya et al., 1988; Funahashi et al., 1989; Di Pellegrino and Wise, 1993; Rao et al., 1997; Asaad et al., 1998; Rainer et al., 1998a,b; Sawaguchi and Yamane, 1999). This delay activity has been shown to be content specific with individual neurons encoding e.g. the location of an object in space (Funahashi et al., 1989; Fuster, 1973, Rao et al., 1997); the direction of a prior response (Funahashi et al., 1993), or the identity of an object such as a face or a fruit (Quintana et al., 1988; Wilson et al., 1993; Rao et al., 1997; Rainer et al., 1998b). In addition, the activity of prefrontal neurons is often polarized, exhibiting excitatory responses to targets in the preferred direction and inhibitory responses to targets at nonpreferred directions (Funahashi et al., 1989; Rainer et al., 1998a; Sawaguchi and Yamane, 1999; Rao et al., 1999). Both a disruption of delay activity as well as delay activity coding for the wrong stimulus or response are correlated with subsequent behavioural errors (Fuster, 1973; Quintana et al., 1988; Funahashi et al., 1989). In contrast to neurons in the temporal and parietal cortices that also show delay-activity during a working memory task (Miller et al., 1996; Constantinidis and Steinmetz, 1996), networks in the PFC are able to sustain delay activity even in the presence of interfering inputs (Miller et al., 1996). It has been hypothesized that this unique feature of the PFC is due to a „protective“ action of DA (Durstewitz et al., 1999).

Data from functional imaging studies in humans have spawned the discussion about the functional segregation of the PFC. While all of these studies have demonstrated the participation of the PFC in different task-specific working memory networks (e.g. Cohen et al., 1997; Belger et al., 1998; D’Esposito et al., 1999; Haxby et al., 2000), it remains a matter of debate, whether differential activation of dorsal and ventral areas within the PFC, reflects content-specific or process-specific domains. One view proposes that the dorsal-ventral subdivisions of the PFC might be concerned with spatial and object working memory, respectively. Alternatively it has been suggested that the dorsal PFC is related to the manipulation of objects in memory, while the ventral PFC merely subserves the active retention of this information (Belger et al., 1998; D’Esposito et al., 1999; but see Haxby et al., 2000).

Pathophysiology of the prefrontal cortex in humans

The clinical symptoms following lesions of the PFC in man, most likely also relate to the basic functions of the PFC reviewed for animals above. In humans the deficits following
frontal lesions are categorized with respect to symptoms pertaining to perception and motility, cognition, and affect and emotion (Fuster, 1989).

Perception and Motility. The perceptive abilities of frontal patients are often impaired in as much as they show increased distractability, or, quite contrary, sensory neglect as a specific form of attention deficit. Other attentional or „sensory“ deficits regard to spatial orientation and gaze control (in Fuster, 1989). The motoric deficits of frontal patients depend on the focus of the lesion. Dorsolateral lesions greatly reduce spontaneous motor activity, leading to hypokinesis which ranges from a certain „asponantemy“ to severe mutism (Stuss and Benson, 1986). In contrast, lesions of the orbitofrontal PFC often result in hyperkinesis, and excessive and aimless motility (in Fuster, 1989).

Cognitive Symptoms. The common cognitive motif following lesions of the PFC are deficits in the temporal integration of stimuli and actions (Fuster, 1989). Lesions of the dorsolateral PFC result in disturbances of short-term memory, and the prospective planning of sequences (Milner and Petrides, 1984; Robbins, 1996). In patients, the ability to sequentially organize a task is often tested with the „Tower of London“ (Robbins, 1996), while short-term memory impairments can also be probed with variants of delay-tasks (Milner and Petrides, 1984; Fuster, 1989). Lesions in the orbitofrontal PFC, however, result in the inability to suppress interfering tendencies and result in perseverative behaviour (Fuster, 1989; c.f. also Dias et al., 1997), which can be assessed with the „Wisconsin-Card-Sorting-Test“ (Robbins, 1996).

Affect and Emotion. Symptoms of disturbed affect and emotion can coarsely be divided into two complexes. The so-called „apathetic syndrome“ is characterized by a low awareness, lack of initiative and hypokinesis. Most specific, however, is the generalized blunting of affects and emotional responses, therefore it has also been termed „pseudodepression“ (Stuss and Benson, 1986). The counterpart of this apathy is the „euphoric syndrome“ which results from orbitofrontal lesions, and which along with euphoria is characterized by distractibility, hypermotility, and a disinhibited hunger and sexual drive (Fuster, 1989).

In addition to deficits resulting from lesions of the PFC, multiple lines of evidence suggest that certain PFC regions may be a locus of dysfunction or structural pathology in such mental illnesses as schizophrenia (reviewed in Yang et al., 1999), or obsessive-compulsive disorder (reviewed in Fuster, 1989). The PFC also appears to be markedly affected in Alzheimer’s disease (Hof et al., 1990; Terry et al., 1991). It has been reported for example that one major correlate of cognitive deficiency in alzheimer’s disease is the synaptic loss in the PFC,
contributing about 70% of the strength of the correlation with psychometric scores (Terry et al., 1991).

**The mesocortical dopamine system**

The dopaminergic innervation of the forebrain of mammals and birds is constituted by a small number (~30,000 - 40,000 neurons on each side of the rat brain) of highly collateralized neurons residing in the ventral mesencephalon (e.g. Björklund and Lindvall 1984; Fallon and Loughlin, 1995; Williams and Goldman-Rakic, 1998; c.f. chapter 4 for birds). These mesencephalic cell groups are designated as A8, A9, and A10, according to the nomenclature of Dahlström and Fuxe (1964), and they generally correspond to the DA cells of the substantia nigra (SN, A9), ventral tegmental area (VTA, A10), and the retrorubral area (A8)³ (Porrino and Goldman-Rakic 1982; Berger et al., 1991; Williams and Goldman-Rakic, 1998).

Functionally, different mesencephalic dopaminergic projections exist which have been designated as mesostriatal-, mesolimbic-, or mesocortical DA system, respectively, based on their main targets of innervation (figure 3; Björklund and Lindvall, 1984; Cooper et al., 1996). The mesolimbic DA system projects to various limbic areas, including the amygdaloid complex, hippocampus, septum, nucleus accumbens, and entorhinal cortex. The mesostriatal DA system provides strong innervation to the striatal aspects of the basal ganglia (i.e. caudate-

³ Due to the similarity of their projection, DA cells in the retrorubral field are often grouped with the substantia nigra, pars compacta (Björklund and Lindvall, 1984; Reiner et al., 1994)
putamen), and to a lesser extent to the globus pallidus and tuberculum olfactorium (Björklund and Lindvall, 1984; Cooper et al., 1996).

The organization and extent of the cortical innervation by the mesocortical DA system varies with the species studied (reviewed in Berger et al., 1991). In primates, tyrosine hydroxylase- or dopamine-immunoreactive fibers, originating in a continuum of cells in A8-A10, are found in the motor, premotor, supplementary motor area, parietal, temporal, and posterior cingulate cortices (sensorimotor), in addition to prefrontal, anterior cingulate, insular, piriform, perirhinal, and entorhinal cortices (association). This innervation shows a strong rostro-caudal gradient (also seen in rodents), the innervation being densest in the frontal cortex, particularly in areas 4 and 6 (Björklund and Lindvall, 1984; Berger et al., 1988, 1991; Goldman-Rakic et al., 1992; Williams and Goldman-Rakic, 1993). The rodent mesocortical DA innervation is mainly confined to the limbic cortices, including the prefrontal, anterior cingulate, insular, piriform, perirhinal, and entorhinal cortices (Björklund and Lindvall 1984; Berger et al. 1991). Input to the rodent PFC derives from largely separate populations of dopaminergic neurons located in the SN and VTA, respectively (Swanson 1982; Björklund and Lindvall, 1984; Fallon and Laughlin, 1995).

The laminar organization of the mesocortical DA innervation generally shows a bilaminar pattern, but again with distinct species-differences between rodents and primates (c.f. figure 3). In the rodent PFC, afferents from the medial VTA provide dense DA input to the deep layers V-VI. The considerably weaker DA innervation to the superficial layer I-III originates in the medial SN and the lateral VTA (van Eden et al., 1987; Berger et al., 1991).

Furthermore, on the ground of developmental, morphological, and pharmacological differences (reviewed in Berger et al., 1991), these distinct inputs have been divided into two classes, with class 1 DA neurons innervating the deep cortical layers. There is also evidence that class 1 and 2 neurons show functional differences, as class 1 neurons are more susceptible to the effects of stress (Deutch et al., 1991). It has been hypothesized that the two modes of dopaminergic innervation in the avian forebrain might relate to these two classes of DA neurons in rodents (Wynne and Güntürkün, 1995; c.f. chapter 4).

In the primate granular cortices, the superficial layers I-III are generally innervated even denser than layers V-VI, whereas in the motor and anterior cingulate (agranular) cortices all layers receive a similarly dense dopaminergic input (Berger et al., 1988, 1991; Berger and Gaspar, 1994; Goldman-Rakic et al., 1992; Lewis et al., 1992; Williams and Goldman-Rakic, 1993; Krimer et al., 1997). In the PFC of rats and primates dopaminergic fibers make about 40
- 90% specialized synaptic contacts (~39% in the sulcus principalis of primates, Smiley and Goldman-Rakic, 1993; ~56% in the suprarhinal, and ~93% in the anteromedial PFC of rats, Séguéla et al., 1988; but see Smiley and Goldman-Rakic, 1993 for technical considerations), which are mostly of the symmetric type. The remaining axonal varicosities constitute non-synaptic release sites and probably contribute to the actions of DA via volume transmission (Smiley and Goldman-Rakic, 1993). The most common synaptic target of the DA terminals in the PFC of rodents and primates appear to be the dendritic spines and shafts of pyramidal neurons (van Eden et al., 1987; Goldman-Rakic et al., 1989; Smiley and Goldman-Rakic, 1993). Many of the postsynaptic spines innervated by DA terminals also receive unlabeled asymmetric (putative excitatory) terminals. Through this „triadic” synaptic arrangement DA might both, pre- and postsynaptically, modulate the excitatory afferents to pyramidal cells (van Eden et al. 1987; Séguéla et al. 1988; Goldman-Rakic et al. 1989; Smiley and Goldman-Rakic 1993; c.f. chapter 4 for the situation in birds). However, dopaminergic fibers in the PFC have also been observed to terminate on smooth GABAergic, stellate cells (Goldman-Rakic et al., 1989; Benes, et al., 1993; Smiley and Goldman-Rakic, 1993). In addition, the localization of DA receptors in interneurons and the physiological actions of DA in the PFC strongly implicate a dopaminergic modulation of GABAergic interneurons (see below).

Dopamine receptors in the PFC

Dopamine receptors belong to the superfamily of seven transmembrane spanning G protein-coupled receptors. In recent years, molecular cloning studies have led to a revision of the traditional classification of dopamine receptors into D1 and D2 subtypes simply based on their effects on the adenylyl-cyclase system (Kebabian and Calne, 1979). So far at least 5 different DA receptors (D1 - D5) have been identified. The pharmacological properties in response to „classical“ D1 and D2 receptor ligands, respectively, distinguish a D1-like family (D1 and D5), and a D2-like family (D2, D3, D4) of DA receptors (Seeman and van Tol, 1993; Cooper et al., 1996). In accordance with the classical view, receptors of the D1 family are coupled positively to the adenylyl-cyclase, while D2 receptors inhibit adenylyl-cyclase activity (Kebabian and Calne, 1979; Cooper et al., 1996; Robinson and Caron, 1997).

Insight into the distinct pharmacological profiles and the differential distribution of the DA receptor subtypes are of high clinical relevance for the development of new drug treatments. For example, the therapeutic effects of neuroleptics are mediated via receptors of the D2 family. The blockade of D2 receptors by „typical“ neuroleptics such as haloperidol, however,
often results in side-effects on the extrapyramidal motoric system, leading to tardive
dyskinesia or parkinson-like symptoms. This is related to the fact that D2 receptors are most
abundant in the neostriatum. "Atypical" antipsychotics such as clozapine, on the other hand,
have only a low affinity - only about 10% of the classical neuroleptics - for the D2 receptor,
but a much higher affinity for D3 or D4 receptors, which are highly abundant in the nuc.
accumbens and the PFC. The latter structures and their dopaminergic innervation appear to be
critically involved in both the positive and negative symptoms of schizophrenia (Seeman and
van Tol, 1993).

The best studied effector protein modulated following the activation of DA receptors in
neurons is DARPP-32 (c.f. chapter 5). DARPP-32 is a Dopamine- and cAMP-Regulated
Phosphoprotein of molecular weight 32,000 that serves as a "third messenger" in the
intracellular cascade that follows D1-receptor stimulation (Hemmings et al., 1987a, 1987b,
1995; Fienberg et al., 1998). Via activation of the adenylyl cyclase, D1-receptor action
stimulates cAMP synthesis, which through protein kinase A (PKA) leads to phosphorylation
of DARPP-32. Phosphorylated DARPP-32 in turn inhibits protein phosphatase-1. In striatal
neurons protein phosphatase-1 controls the state of phosphorylation of a wide array of
phosphoproteins, including neurotransmitter receptors (e.g. for α-amino-3-hydroxy-5-
methylisoxazole-4-propionic acid [AMPA] and N-methyl-D-aspartate [NMDA]), ion channels
(e.g. Ca++ channels and voltage dependent Na+ channels), ion pumps (e.g. the Na+-K+-
ATPase), and transcription factors (Hemmings et al., 1995; Nishi et al., 1997; Fienberg et al.,
1998). Conversely, DA, acting on D2-like receptors, through both inhibition of PKA and
activation of calcium/calmodulin-dependent protein phosphatase (protein phosphatase
2B/calcineurin), may cause the dephosphorylation of DARPP-32 (Nishi et al., 1997), enabling
a possible interplay of D1 and D2-like receptors.

The laminar distribution of DA receptors in the frontal cortex largely parallels the bilaminar
pattern of the innervation by dopaminergic fibers. In rodent frontal cortex there is
considerable expression of mRNA for the D1 receptors in deep layers V-VI. A comparatively
lower expression of D2 receptor mRNA is distributed in superficial layers I-III and in deep
layer V (Gaspar et al. 1995; see Yang et al., 1999 for review). In general, in the frontal
cortices of rats and primates, the density of D1 receptors has been estimated to be about five-
to tenfold higher than that of D2 receptors (Lidow et al., 1991; Joyce et al., 1993). In rodent
frontal cortex there is also evidence for a considerable number of D4 receptors, but not for D3
and D5 receptors (Joyce et al., 1993; Ariano et al. 1997, see Yang et al., 1999 for review). In
primate PFC expression of mRNA for all 5 DA receptor subtypes has been found (Lidow et al., 1998). The largest numbers of receptor proteins were found for the D1, D4 and D5 subtypes in pyramidal neurons of superficial layers II-III and deep layers V-VI, with layer V neurons showing a stronger expression of D4 and D5 receptors (Lidow et al. 1991, 1998). Immunoreactivity and mRNA expression for D1, D2 and D4 receptors has also been found in GABAergic interneurons of rat and primate frontal cortex (Mrzljak et al., 1996; Ariano et al., 1997; LeMoin and Gaspar, 1998; Muly et al., 1998).
Physiology of the mesocortical dopaminergic system

Midbrain dopaminergic neurons exhibit a burst of action potentials immediately following unexpected salient events, which include sudden novel stimuli (Schultz and Romo, 1990; Ljungberg et al., 1992), and primary rewards (Ljungberg et al., 1992; Schultz et al., 1995). During the learning-phase of a task, reward-related activation of DA neurons first occurs in response to the (unexpected) primary reward (the UCS). If the UCS becomes predictable, activation of DA neurons „shifts“ to the occurrence of the conditioned stimulus (CS) (Ljungberg et al., 1992), and finally to the occurrence of a cue that signals the CS (Schultz et al., 1993; Schultz, 1998 for review). Schultz and coworkers (Schultz et al., 1995; Schultz, 1998; c.f. also Montague et al., 1996) have argued that the activity of dopaminergic neurons provides an essential signal for reinforcement learning. This model is based on the observation that dopamine neurons report rewards relative to their prediction rather than signaling primary rewards unconditionally. The dopamine response is positive (activation) when primary rewards occur without being predicted. The response is nil when rewards occur as predicted. The response is negative (depression) when predicted rewards are omitted. (Ljungberg et al., 1992; Schultz et al., 1993; Schultz, 1998 for review). The dopamine reward signal is supplemented by reward-processing neurons in the striatum, amygdala, and frontal cortex (Apicella et al., 1991, 1992; Schultz et al., 1992; Schultz, 1998), and these regions provide direct and indirect feedback to the mesencephalic DA neurons via a cortico-striato-tegmental loop (Alexander et al., 1990; Goldman-Rakic, 1995). However, Redgrave and coworkers (Redgrave et al., 1999) have recently proposed another functional interpretation of the activity of DA midbrain neurons. In their view DA release is responsible for reallocating attentional and behavioural resources, in a sense of „switching“ attention to new or salient stimuli.

In accordance with both models, dopaminergic midbrain neurons are activated at the onset of working memory tasks (Schultz et al., 1993) and DA levels in the PFC increase during delay-task performance (Watanabe et al., 1997). In contrast, the behavioural impairments following lesion of the mesencephalic dopamine neurons or the blockade of dopaminergic transmission in the PFC are very similar to those observed after lesions of the PFC itself (Brozoski et al., 1979; Simon et al., 1980; Sokolowski and Salamone, 1994; Sokolowski et al., 1994). Destruction of dopaminergic neurons in area A 10 (Simon et al., 1980) or of dopaminergic fibers within the PFC by 6-hydroxydopamine (6-OHDA) (Brozoski et al., 1979) result in deficits in delay-tasks, and an impairment in response control (Sokolowski and Salamone, 1994). The effect of DA on performance in delay-tasks is mediated by the D1 receptor, and an
optimal level of stimulation by DA appears to be critical for proper functioning (Sawaguchi et al., 1990b; Sawaguchi and Goldman-Rakic, 1991, 1994; Williams and Goldman-Rakic, 1995; Murphy et al., 1996; Sawaguchi, 1997; Zahrt et al., 1997; Arnsten and Goldman-Rakic, 1998; Seamans et al., 1998).

The cellular bases of the effects of DA in the cortex, however, remain enigmatic. *In vivo* extracellular single-unit recording studies have shown that iontophoretically applied DA either increased or decreased *spontaneous* neuronal firing in the neocortex (Bradshaw et al. 1985; Sesack and Bunney 1989; Yang and Mogenson, 1990). Dopamine applied iontophoretically or released by VTA stimulation, suppressed spontaneous, as well as presumed glutamate-mediated mediodorsal thalamic-evoked firing in the rat PFC *in-vivo* (Ferron et al. 1984; Sesack and Bunney 1989; Godbout et al. 1991; Pirot et al. 1992). In contrast, neuronal firing induced by iontophoretic application of acetylcholine or NMDA is enhanced by very low doses of iontophoretically applied DA (Yang and Mogenson 1990; Cepeda et al. 1992).

These findings are in good agreement with the long proposed *neuromodulatory* action of DA. By definition, a neuromodulator, in contrast to the classical neurotransmitters, is not inhibitory or excitatory per se, but when applied in conjunction with other substances it has the ability to alter their actions. The modulatory nature of DA also appears to extend to a voltage- (or „activity-“) dependence of its effects, in that DA has inhibitory effects at hyperpolarized membrane potentials, but enhancing effects at depolarized potentials (see below).

Another important methodological difference that hampers comparison across different *in-vivo* studies may result from the use of anesthetics that block N-methyl-D-aspartate (NMDA) conductances and thus one of the major excitatory effects of DA. Both in the striatum and PFC, DA appears to differentially modulate AMPA and NMDA receptors. Application of DA reduces AMPA currents, but enhances NMDA conductances (Cepeda et al., 1992, 1998; Durstewitz et al., 2000; but see Law-Tho et al., 1994). This in turn is partly due to the different voltage dependence of these currents. Activation of NMDA receptors requires prior depolarization of the membrane, whereas AMPA receptors show no voltage-dependent activation. Similarly *in vitro*, the effects of DA on striatal neurons, acting via the D1 receptor, are inhibitory when the membrane potential is hyperpolarized (which resembles the so called „down“-state of striatal neurons observed *in-vivo*), but are excitatory when the cell is in the depolarized „up“-state (Hernández et al., 1997). There is evidence that in the cortex DA has comparable enhancing effects particularly on highly activated neurons (Sawaguchi et al., 1988, 1990a; Durstewitz et al., 2000). Although DA inhibits spontaneous activity within the
PFC of anesthetized animals (see above), it enhances task-related single-unit activity more than background activity in the behaving animal (Sawaguchi et al., 1988, 1990a).

In the cortex, this „content-specific“ enhancement of neuronal activity results from a complex interplay of ionic conductances that govern the action-potential threshold of pyramidal neurons and regulate the transmission of synaptic input through the apical dendrite (where most of the cell’s input arrives).

In the cortex DA, via its action at the D1 receptor, appears to shift the threshold of the persistent Na⁺ current (I_{NaP}) towards more hyperpolarized potentials (Yang and Seamans 1996; Durstewitz et al., 2000; but see Geijo-Barrientos and Pastore, 1995). Activation of I_{NaP} or its prolonged activation will reduce the threshold for generating an action potential, and render the cell more susceptible for excitatory inputs (Schwindt, 1992; Yang and Seamans, 1996). Dopamine might also act on a slowly inactivating K⁺ current (I_{KS}) that usually delays spike generation and lengthens inter-spike intervals, which in turn suppresses repetitive firing (Schwindt et al. 1988; Kitai and Surmeier, 1993; Yang and Seamans, 1996). A reduction of I_{KS} shortens inter-spike-intervals and reduces accommodation of firing (Yang and Seamans, 1996; Nisenbaum et al., 1998). The concurrent actions of DA on both I_{NaP} and I_{KS} might reduce spike threshold and contribute to an increased firing rate observed in vivo (Sawaguchi et al., 1988; 1990a,b) and in vitro (Cepeda et al., 1992; Yang and Seamans, 1996).

In addition, DA has been shown to exert a differential effect on high-voltage-activated (HVA) Ca²⁺ currents. Dopamine augments the activation of L-type Ca²⁺ channels, which functionally serve to enhance the amplitude and duration of excitatory synaptic inputs (Seamans et al., 1997), thus further increasing the neurons excitability (Hernández-López et al. 1997; Cépeda et al. 1998). Finally, in both cortical and striatal neurons, DA reduces the amplitude of isolated N-type Ca²⁺ currents, which has been interpreted as a „filtering“-mechanisms that permits only strong synaptic inputs (e.g. behaviourally salient stimuli) to reach the soma (Surmeier et al., 1995; Yang and Seamans, 1996). The properties of these conductances modulated by DA are described in more detail in the discussion of chapter 6.

In the mammalian PFC, DA also appears to modulate the activity of GABAergic interneurons and spontaneous GABA release. Dopamine directly depolarizes interneurons in the PFC and enhances spontaneous and evoked IPSPs recorded in pyramidal neurons (Penit-Soria et al., 1987; Godbout et al., 1991; Rétaux et al., 1991; Pirot et al., 1992; Zhou and Hablitz, 1999; Durstewitz et al., 2000). Accordingly, part of the suppressive effects of DA on spontaneous
firing of pyramidal PFC neurons observed in vivo might be due to the action of DA on GABAergic interneurons.

**Aims of the study**

As outlined above, the experiments in this thesis try to unravel the anatomical and physiological properties of a proposed canonical circuit involved in working memory and other executive functions in a non-mammalian species. The NCL of pigeons (*Columba livia*) and chicks (*Gallus gallus domesticus*) serves as a model structure in this quest as behavioural studies have shown its involvement in working memory (e.g. Mogensen and Divac, 1982, 1993; Kalt et al., 1999), reversal learning (Hartmann and Güntürkün, 1998), and response inhibition (Güntürkün, 1997; Aldavert et al., 1999). Furthermore, the strong dopaminergic innervation (e.g. Waldmann and Güntürkün, 1993) and recent evidence from a behavioural study investigating the effects of pharmacological blockade of dopaminergic transmission (Güntürkün and Durstwitz, in press), support the involvement of DA in the executive functions of the NCL.

The studies undertaken here to uncover the cellular bases of these behaviours address two main topics.

1. The functional organization of the NCL.
2. The influence of the dopaminergic system on identified cell types within the NCL.

**The functional organization of the NCL**

The term working memory refers to the control of reactions depending on past experiences and the ongoing stream of sensory information that is currently being experienced (c.f. Baddeley, 1992; Goldman-Rakic, 1995). As exemplified for the PFC above, this „memory for action“ requires (i) the retrieval of learned responses from long-term memory (possibly from memory stores in the hippocampus or posterior cortices), (ii) the simultaneous integration/evaluation of sensory information about the environment (provided by the posterior cortices and the thalamus) for the selection of the appropriate response, and (iii) the initiation of the response. Thus, a clear prerequisite for a structure in non-mammalian species that might subserve working memory is the ability to integrate all available sensory
information and to exert influence over motor and limbic structures. While the available behavioural data (e.g. Mogensen and Divac, 1982, 1993; Hartmann and Güntürkün, 1998) strongly indicate that such sensorimotor integration takes place in the NCL, the anatomical or electrophysiological basis for these computations is unclear. The experiments described in chapter 1 have thus examined the areal termination pattern of afferents from other telencephalic structures to the NCL, by means of anterograde and retrograde pathway tracing techniques. In chapter 2, these findings are complemented by the anatomical description of thalamic input from a multimodal nucleus in the dorsal thalamus. In addition, the organization of efferents from the NCL to (para-)sensory structures, as well as limbic and motoric areas in the avian forebrain, through which the NCL might modulate its own input and initiate motoric responses, respectively, are characterized in chapter 1. Taken together, these anatomical results reveal the nature of polymodal processing within NCL and possible functional subdivisions of the NCL.

The projection from the mediodorsal thalamus to the PFC serves as a hallmark for the definition of the PFC (Rose and Woolsey, 1948; Divac et al., 1978; Goldman-Rakic and Porrino, 1985; Groenewegen et al., 1990), and lesions of the MD result in deficits in delay-tasks comparable to those following lesions of the PFC (Sakurai and Sugimoto, 1985). It has been suggested that in birds the projection from the multimodal nuc. dorsolateralis posterior (DLP) to the NCL might form a functional equivalent to the MD-PFC connection (Güntürkün, 1997). In chapter 2 the nature of short-latency visual evoked potentials in the NCL is examined by extracellular electrophysiological recordings in-vivo, and the effects of lesions to the DLP on visual responses in the NCL are studied. Together with the anatomical data also presented in chapter 2 (see above), the results demonstrate direct thalamic modulation of NCL activity.

To understand the neuronal circuitry that underlies higher cognitive functions in vertebrates, a detailed knowledge about the celltypes and their possible function within the proposed canonical circuit is required. It is axiomatic in neuroscience that the informational output of most neurons is defined by their temporal patterns of action potentials (e.g. McCormick et al., 1985). This notion is implicit in all contemporary models of the function of the brain, making it essential to understand how each neuron transforms its input into output. Intracellular or patch-clamp electrophysiological recordings give the opportunity to determine the intrinsic electrophysiological properties of neurons by measuring the membrane’s response to hyperpolarizing and depolarizing current pulses. The intrinsic membrane properties of
neurons in the mammalian and avian forebrain are not homogeneous, but instead produce several categories of transform characteristics that can be used to categorize cells into certain types (e.g. McCormick et al., 1985; Larkman and Mason, 1990; Kubota and Taniguchi, 1998). In chapter 3 the principal cell types of the chick NCL are characterized both electrophysiologically and morphologically in vitro, using whole-cell patch-clamp recordings in combination with intracellular staining. The distinct firing patterns and axonal connections of the principal neurons can be interpreted with respect to the integrative role they play within the circuitry of the NCL, thus unraveling the „building blocks“ of mental computation within NCL.

The influence of the dopaminergic system on identified cell types within the NCL.
The dopaminergic innervation in the avian telencephalon takes two different, and possibly discrete, forms. The first, which is also predominant in the mammalian cortex (e.g. Williams and Goldman-Rakic, 1993), is characterized by dopaminergic fibers that travel in close vicinity along the somata and dendrites of target-neurons while forming „en-passant“ a large number of bouton-like axonal swellings. The second one, called the „basket“-type, is a peculiar arrangement of dopaminergic fibers in the avian telencephalon. In this case, single fibers densely coil around the somata and initial dendrites of postsynaptic targets, enwrapping them in basket-like structures (Wynne and Güntürkün, 1995; c.f. chapter 4). It can be speculated that this particularly strong dopaminergic innervation also differs functionally from the more diffuse „en-passant“ innervation. The relative frequency of these modes of innervation differs among various brain areas (Wynne and Güntürkün, 1995; chapter 4), but the NCL is characterized by a strong innervation from both types of dopaminergic fiber systems, thus in principle enabling a direct comparison of the two modes of DA innervation.

Chapter 4 provides a review about the dopaminergic innervation of the avian forebrain and discusses anatomical and behavioural findings with regard to the functional organization of the avian brain, and some general implications of these findings for sensorimotor integration and learning. Chapter 5 presents original data also reviewed in chapter 4. This study attempted to identify the cell type that receives dense basket-type DA innervation by colocalizing the dopaminergic fibers4 with postsynaptic markers of neuroactive substances

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4 In fact, immunoreactivity against tyrosine-hydroxylase (TH), which is the rate limiting enzyme in the synthesis of all catecholamines, is used for the demonstration of presumed dopaminergic fibers, as there is a good correspondence between TH immunoreactivity and a direct marker of DA (c.f. chapter 4), comparable to the situation in the prefrontal cortex (Williams and Goldman-Rakic, 1993).
(i.e. glutamate-decarboxylase, as a marker of GABAergic neurons) or receptor proteins (i.e. DARPP-32 as an indicator for D1 receptors). Specifically it was investigated whether GABAergic interneurons are a main target of dopaminergic fibers, and if so, whether the effects of DA might be mediated via the DA D1 receptor as is the case in the PFC (Krimer et al., 1997; Muly et al., 1998).

Finally, the effects of DA on membrane excitability are tested directly in chick NCL neurons in-vitro. In chapter 6, patch-clamp recordings from neurons of all cell types are made (c.f. chapter 3) and changes in the firing patterns in response to DA application are analyzed. The observed changes in action-potential firing provide insight into the mechanisms and substrate-specificity (i.e. cell types and receptors involved) by which DA might enable sustained activity during a delay task. Furthermore, double-labeling of intracellularly filled neurons with dopaminergic fibers is used to identify classes of neuron that receive basket-type DA innervation. The responses of neurons receiving basket-innervation to application of DA are compared to the responses of neurons that receive only en-passant innervation of dopaminergic fibres. Taken together, this will elucidate whether the basket-type innervation selectively innervates a distinct class of neurons, and/or whether the effects of DA exerted through this strong somatic innervation differs qualitatively or quantitatively from the dendritic en-passant innervation.

Taken together, the results show the structural and physiological bases of polymodal sensory integration and motor control in the avian telencephalon, which appear to underlie higher cognitive functions. Furthermore, the functional organization of the mesotelencephalic dopaminergic system and dopaminoceptive elements in the caudal forebrain are studied, which appear to be critically involved in cognitive processing and motor control, both in birds and mammals.
Afferent and efferent connections of the caudolateral neostriatum in the pigeon \textit{(Columba livia)}: A retro- and anterograde pathway tracing study.

Sven Kröner and Onur Güntürkün

ABSTRACT
The avian caudolateral neostriatum (NCL) was first identified on the basis of its dense dopaminergic innervation. This fact and data from lesion studies have led to the notion that NCL might be the avian equivalent of prefrontal cortex (PFC). A key feature of the PFC is the ability to integrate information from all modalities needed for the generation of motor plans. By using antero- and retrograde pathway tracing techniques we investigated the organization of sensory afferents to the NCL and the connections with limbic and somatomotor centers in the basal ganglia and archistriatum. Data from all tracing experiments were compared with the distribution of tyrosine-hydroxylase (TH) immunoreactive fibers, serving as a marker of dopaminergic innervation. The results show that NCL is reciprocally connected with the secondary sensory areas of all modalities and with at least two parasensory areas. Retrograde tracing also demonstrated further afferents from the deep layers of the Wulst and from the frontolateral neostriatum as well as the sources of thalamic input. Efferents of NCL project onto parts of the avian basal ganglia considered to serve somatomotor or limbic functions. Projections to the archistriatum are mainly directed to the somatomotor part of the intermediate archistriatum. In addition, cells in caudal NCL were found to be connected with the ventral and posterior archistriatum, which are considered avian equivalents of mammalian amygdala. All afferents and projection neurons were confined to the plexus of densest TH innervation. Our results show that the NCL is positioned to amalgamate information from all modalities and to exert control over limbic and somatomotor areas. This organization might comprise the neural basis for such complex behaviours as working memory or spatial orientation.

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INTRODUCTION
The neostriatum caudolaterale (NCL) of the pigeon has been compared with the mammalian prefrontal cortex (PFC), due to its dense dopaminergic innervation (Divac et al., 1985, 1994; Divac and Mogensen, 1985; Waldmann and Güntürkün, 1993; Wynne and Güntürkün, 1995), and the behavioural deficits in working memory (Mogensen and Divac, 1982, 1993, Gagliardo et al., 1996, 1997, Güntürkün, 1997), reversal learning (Hartmann and Güntürkün, 1998), and
go/no-go tasks (Güntürkün, 1997) that follow its ablation. Working memory tasks selectively target the control of reactions depending on past experiences and the ongoing stream of sensory information that is currently being experienced, which in mammals is a key feature of the PFC (Goldman-Rakic, 1987; Fuster, 1989). A clear prerequisite for a structure in non-mammalian species that can subserve such functions and may thus be regarded as an analogue of the PFC is the ability to integrate all available sensory information and to exert influence over motor and limbic structures.

A number of previous studies have demonstrated afferent input to the lateral neostriatum from visual (Ritchie, 1979; Shimizu et al., 1995), auditory (Wild et al., 1993) and somatosensory (Shimizu et al., 1995) areas, as well as a projection from the multimodal thalamic nucleus dorsolateralis posterior (DLP); (Waldmann and Güntürkün, 1993; Leutgeb et al., 1996). Based on cytoarchitectonic and hodological data, Rehkämper and Zilles (1991) have proposed that the complete posterior neostriatum, including its caudolateral aspect, might represent an area of multimodal integration. Furthermore, in a recent retrograde labeling study focused on the NCL (Leutgeb et al., 1996) it has been shown that the NCL is reached by afferents from all major secondary sensory areas within the pigeon brain. Until now, however, a detailed study of the termination pattern of these afferents and a possible functional segregation within the pigeon’s NCL is still lacking. Retrograde tracing data from a very recent study in chicks (Metzger et al., 1998) revealed distinct and non-overlapping termination zones for the trigeminal, tectofugal, and auditory systems within the NCL. Since retrograde tracing in pigeons (Leutgeb et al., 1996) suggested a substantially different organization of the NCL with largely overlapping sensory compartments, this report describes the afferent and efferent connections of the NCL as studied by retro- and anterograde pathway tracing and compares it with the distribution of tyrosine hydroxylase-immunoreactive fibers.

**MATERIALS AND METHODS**

**Neuroanatomical pathway tracing experiments**

Fifty-seven adult pigeons (*Columba livia*) from local stock provided the data presented here. Treatment of animals conformed to NIH guidelines and specifications of the German Animal Welfare Act. Accordingly, prior to surgery, the animals were deeply anaesthetized with 0.33 - 0.4 ml Equithesin per 100 g body weight. Animals received pressure-injections of either the sensitive antero- and retrograde tracer Cholera Toxin, subunit b (CTb, 1% in A.dest.; List Labs, Campbell, CA) or biotynilated dextran amines as anterograde tracer (BDA, 10,000 molecular weight form, 10% in sodium phosphate buffer, pH 7.3; Molecular Probes, Leiden, The Netherlands). Tracer was delivered through glass micropipettes (tip diameter 15-20 µm) attached to a
nanoliter-injector (World Precision Instruments, Sarasota, FL). Since previous studies (Leutgeb et al., 1996; Metzger et al., 1998) and our own data have indicated that the majority of connections of the NCL are restricted to the ipsilateral hemisphere, most animals received bilateral injections to minimize the number of pigeons used. Stereotaxic coordinates for the injections were determined by using the atlas of Karten and Hodos (1967).

**TABLE 1: Experimental Parameters**

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</table>

1 R, Right side; L, Left side; for other abbreviations, see list

The afferent sources of the NCL were determined by injections of CTb (30 - 54 nl) into medial and lateral parts of the NCL along its rostro-caudal extent, as well as into the directly adjacent neostriatum dorsale (Nd, Bonke et al., 1979; Wild et al., 1993; Wild, 1994). We included Nd in our analysis because the pattern of catecholaminergic innervation suggests that Nd is continuous with NCL (figure 1) and might constitute its auditory subcomponent. To study the areal pattern of afferent input to the NCL, injections of CTb (9 - 80 nl) and BDA (40 - 100 nl) were made into most of the telencephalic regions which by means of retrograde transport had been shown to constitute afferent sources of the NCL. Efferent connections of the NCL to motor and limbic structures were studied by placing injections into the somatomotor and limbic parts of the archistriatum (Zeier and Karten, 1971) as well as into parts of the avian basal ganglia that are comparable to parts of mammalian striatum.

**Immunohistochemical procedures**

Survival times were 3 days for CTb and 4-6 days for BDA. Fifteen minutes prior to perfusion the animals were injected with 1,000 IU heparin and deeply anaesthetized with 0.4 - 0.5 ml Equithesin per 100 g body weight. Pigeons were then perfused transcardially with 200 ml 0.9% saline (40°C) followed by 1,000 ml of 4% paraformaldehyde in 0.12 M phosphate buffer (PB; 4°C, pH 7.4). After perfusion, brains were dissected and postfixed in the same fixative to which 30% sucrose was added, and then transferred to 30% sucrose in
phosphate buffer containing 0.9% NaCl (PBS; pH 7.4) for approximately 18 hours at 4°C. Brains were cut in frontal slices of 40 µm on a freezing microtome and collected in PBS containing 0.01% NaN₃ as a preservative. Representative sections were then processed for the avidin-biotin-conjugate technique (ABC).

Immunohistochemical labeling for Cholera Toxin b: Endogeneous peroxidases were blocked by preincubating slices in a solution of 0.5% H₂O₂. Slices were washed and for immunohistochemistry of CTb free-floating sections were incubated overnight at 4°C in anti-CTb from goat (Jackson, West Grove, PA; 1:20,000) in PBS containing 0.3% Triton X-100 (Sigma, Deisenhofen, Germany). The following steps were carried out at room temperature, separated by three washes in PBS of 10 minutes each. After washing, slices were first incubated for 1 hour in biotinylated donkey anti-goat (Jackson, 1:500 in 0.3% Triton X-100 PBS) for 1 hour and then in the avidin-biotin complex (ABC Elite, Vector Labs, Burlingame, CA; 1:100 in PBS with 0.3% Triton X-100) for 1 hour. Washes in PBS were followed by two additional washes in 0.12 M acetate buffer (pH 6). Staining was achieved by the 3,3'-diaminobenzidine (DAB) technique with heavy-metal amplification (modified from Adams, 1981) by adding HgN₂NiOgS₂ (2.5 g/ 100 ml), NH₄Cl and CoCl₂ (both 40 mg/ 100 ml). After 15 minutes of preincubation the reaction was catalyzed with a solution of 0.5% H₂O₂. The reaction was stopped by rinsing the tissue in 0.12 M acetate buffer and PBS. Slices were then mounted, dehydrated and coverslipped.

Labeling for BDA: Visualization of BDA was identical to that of CTb, except that primary and secondary antibodies could be omitted and slices were directly incubated in ABC, followed by the staining procedure described above. Selected series labeled for BDA or CTb were counterstained with cresyl violet.

Immunohistochemical labeling for tyrosine hydroxylase: To compare the distribution of afferent fibers and retrogradely labeled neurons within NCL with the distribution of putative dopaminergic fibers, several sections were processed for tyrosine hydroxylase (TH). Therefore, additional sections stained for CTb or BDA were either double-labeled with TH, or occasionally, sections adjacent to these were single-labeled for TH. The same basic procedure as for immunohistochemistry of CTb was applied for labeling of TH: After blocking of endogeneous peroxidases and thorough washing, free floating sections were incubated overnight at 4°C in monoclonal mouse anti-TH (Boehringer, Mannheim, Germany) diluted 1:200 in PBS containing 0.3 % Triton X. Slices were then incubated at room temperature in biotinylated rabbit anti-mouse (Chemicon, Temecula, CA; 1:200 in 0.3% Triton PBS) for 1 hour and finally in ABC for 1 hour. Staining was achieved using the DAB-technique as described for CTb. In those cases that were double-labeled, enhancement of the DAB staining by nickel ammonium sulfate was omitted, resulting in a light brown signal for TH which could be clearly distinguished from the black reaction product of the tracers.

RESULTS

Retrograde tracing of afferent sources of the NCL
Our injections of CTb into medial and lateral parts of NCL generally replicated the results of an earlier study (Leutgeb et al., 1996). All injections were confined to NCL as defined by TH and DA-like immunoreactivity (Waldmann and Güntürkün, 1993; Wynne and Güntürkün, 1995;
present study), and extended from the caudal tip of the telencephalon at approximately A 4.25 to rostral A 7.00.

**Telencephalic afferents to NCL**

Areas that were found to project to the NCL included the medial aspect of the hyperstriatum accessorium (HA) throughout its visual (Karten et al., 1973; Shimizu et al., 1995) and somatosensory part (Delius and Benetto, 1972, Wild, 1987b, Funke, 1989; but see Deng and Wang, 1992, 1993), the ectostriatal belt (Ep) surrounding the ectostriatum dorsally and laterally (Karten and Hodos, 1970) and the adjoining lateral neostriatum, the anterior neostriatum overlying the nucleus basalis (neostriatum fronto-trigeminale, NFT, of Wild et al., 1985), and field L1 and L3 of the auditory field L complex. A large number of cells were labeled in the medial part of the intermediate neostriatum (NIM of Veenman et al., 1995b) and the overlying intermediate and medial parts of the hyperstriatum ventrale (HV); part of these neurons probably correspond to the previously described parasensory area in the intermediate
neostriatum, situated between the rostral pole of field L and the caudomedial border of the visual ectostriatum, that receives multimodal input via the DLP (Gamlin and Cohen, 1986; Wild, 1987a, 1994; Funke, 1989; Korzeniewska and Güntürkün, 1990). These cells were predominantly labeled if injections were made into more rostral and medial (including Nd) parts of the NCL.

After injections into caudal NCL, a distinct cell group occupying the remaining medialmost aspect of the NIM between approximately A 8.50 and A 12.50 was labeled. Cells within this area also extended across the lamina hyperstriatica (LH) into the intermediate and medial parts of the HV. These two cell groups roughly followed the cytoarchitectonic borders of the regions Ne 9 or Ne 8 and Ne 3, respectively, as described by Rehkämper et al., (1985). For the reason of simplicity we will refer to them as the medial part of the NIM (NIMm) and the lateral part of the NIM (NIMl), respectively.

In the caudomedial neostriatum (NCm) scattered cells were found dorsal to the lamina medullaris dorsalis (LMD). Another band of cells stretched along the border between the hyperstriatum dorsale (HD) and the dorsal division of the ventral hyperstriatum (HVdv). These cells could be found in most cases, irrespective of the injection site within NCL. In cases that were centered more medially or caudally within NCL this band of cells also extended into a small triangular shaped zone within the vallecula. Finally, in several cases a number of cells were observed in the frontolateral part of the neostriatum (NFL). Injections of CTb into this area did indeed confirm a reciprocal connection with the ventrolateral part of the NCL (data not shown). The large majority of fibers from this part of the anterior neostriatum, however, terminated within the area corticoidea dorsolateralis (CDL) and the area temporo-parieto-occipitalis (TPO) overlying the NCL. Therefore we can not fully exclude that part of the labeling within NFL was attributable to tracer spread into CDL.

In addition to labeling in sensory areas, retrograde transport revealed a number of labelled neurons in the dorsal and ventral parts of the intermediate archistriatum. Cells in the ventral archistriatum were the only telencephalic connection found to project bilaterally onto the NCL. In contrast to Leutgeb et al., (1996) we never observed any labeling within the nucleus taeniae (Tn). Furthermore, a few cells within the ventral striatum just dorsal to the fasciculus prosencephali lateralis (FPL) were also found to project to the NCL.
Figure 2: Schematic representation of retro- and anterograde labeling following injections of Cholera Toxin b (CTb) into various locations within NCL. Retrogradely labeled cells are represented by filled symbols (dots, triangles or squares for the different injection sites) and anterogradely labeled fibers and terminals are represented by short lines. Anterograde labeling is shown only for selected regions. The left and middle column illustrate the topographic ordering of NCL afferents and efferents after injections into the caudalmost NCL (left column) or the far rostral NCL (middle column), respectively. The right column shows an example of an injection into Nd. Labeling after Nd injections was very similar to that observed after tracer deposits into medial NCL. In this and all following figures medial is to the left and dorsal is at the top.
Figure 2 (continued)
In addition to labeling in sensory areas, retrograde transport revealed a number of labelled neurons in the dorsal and ventral parts of the intermediate archistriatum. Cells in the ventral archistriatum were the only telencephalic connection found to project bilaterally onto the NCL. In contrast to Leutgeb et al., (1996) we never observed any labeling within the nucleus taeniae (Tn). Furthermore, a few cells within the ventral striatum just dorsal to the fasciculus prosencephali lateralis (FPL) were also found to project to the NCL. In general, injections into NCL resulted in a similar pattern of retrograde labeling, but regional differences exist. With respect to a mediolateral topography our results resemble those of Leutgeb and coworkers (1996) and are thus not described in detail here. However, we found a strong rostro-caudal topography in the organization of afferent and efferent connections of the NCL. The nature of this topography was further elucidated in anterograde tracing experiments. Figure 2 illustrates the results after two injections into the far caudal and rostral aspects of NCL.

Subtelencephalic sources of afferent input to NCL were observed in the thalamus, midbrain and tegmentum. In the lower brainstem the avian equivalent of the substantia nigra, the nucleus tegmenti pedunculopontinus, pars compacta (TPc), the locus coeruleus (LoC) and the area ventralis tegmentalis (AVT) were labeled. These cell groups have been shown to be immunoreactive for TH and dopamine and contain the sources of dopaminergic afferents to the telencephalon (Waldmann and Güntürkün, 1993; Metzger et al., 1996; present study). The projection from the LoC and some cells in the formatio reticularis was bilaterally organized. In the thalamus retrogradely labeled cells were mainly found in the nucleus subrotundus (SRt) and the DLP. Yet, we also found a projection from the n. dorsointermedius posterior thalami (DIP) and a band of cells that seemed to intersperse between DLP and DIP and also extended ventral to the latter two. Therefore we termed these cells collectively the ventrointermediate area of the posterior nuclei (VIP). Figure 3 shows examples of retrogradely labeled neurons from sensory forebrain areas (A - F) and the sources of thalamic input to NCL following injections of CTb into NCL.

**Anterograde labeling of efferents of the NCL**

The pattern of anterograde labeling resulting from injections into NCL was studied within the basal ganglia and the archistriatum. Fibers showing terminal-like varicosities were found throughout most parts of the archistriatum. For the description of archistriatal subdivisions we adopted the terminology of Wynne and Güntürkün (1995).
Figure 3: Photomicrographs of retrogradely labeled cells resulting from injections of CTb into the NCL similar to those shown in figure 2. Afferents arise from secondary sensory „belt” regions adjacent to primary thalamorecipient areas. See text for details. Retrogradely labeled cells in the telencephalon include the ectostriatal belt (A), field L 1 (B), HA and HD (C), the border between HD and HVdv (D), the presumed parasensory NIMm (E) and the NFT (F). Labeling in the thalamus was most abundant in the DLP and SRt (G). H: Cells in the ventrointermediate area of the posterior nuclei (VIP) labeled after an injection into far lateral NCL. Scale bars are 100 µm (B, H) and 250 µm (A, C - G). See list for abbreviations.
Injections into the very caudal aspect of NCL predominantly labeled fibers in parts of the central (Ai) and ventral parts (Av) of the intermediate archistriatum and in some cases also in the posterior archistriatum (Ap). Injections into more rostral NCL resulted in terminal labeling that was confined to the dorsal and central parts of the intermediate archistriatum. After injections into the caudal NCL we observed terminal-like labeling in the medial lobus parolfactorius (LPO) and the ventral striatum, i.e. the nucleus accumbens (AC) and the bed nucleus of the stria terminalis (BNST), which was not attributable to tracer spread into the piriform cortex (CPi) or the caudal archistriatum. Injections into rostral NCL on the other hand resulted in dense terminal labeling in the lateral aspects of LPO and large parts of the paleostriatum augmentatum (PA). Labeling in the PA was most abundant in its caudal extent. Both termination patterns within the archistriatum and the basal ganglia thus suggest that the anterior NCL may be connected to areas concerned with somatomotor functions, i.e. the dorsal and lateral striatum (Veenman et al., 1995a,b), as well as the dorsal and central archistriatum (Zeier and Karten, 1971), while the caudal aspect of NCL might project to limbic parts of the striatum (Veenman et al., 1995b) and the presumed avian homologue of the amygdala, e.g. the ventral and caudal archistriatum (Zeier and Karten, 1971, Dubbeldam et al., 1997). Figure 4 shows examples of anterograde labeling in the basal ganglia and the archistriatum following injections of CTb into NCL.

Labeling after Nd injections
Labeling after CTb injections into Nd largely paralleled the results after medial NCL injections. A large number of retrogradely labeled cell bodies and fibers could be observed throughout the NIM, which seemed to cluster in two distinct cell groups as also observed after injections into the NCL. Strong labeling was also present in the caudomedial neostriatum and areas L1 and L3 of the field L complex. Furthermore, cells were found in the medial HV and along the border of HV and HD, the caudal and rostral aspects of HA, the vallecula (Va), and the NFL. Finally, somata and terminal-like labeling could be observed in the ventromedial archistriatum and lateral parts of the NCL. Terminal-like labeling in the basal ganglia after Nd injections was restricted to the medial and central parts of the caudal PA. In the thalamus labeling was most prominent in the SRt and DLP. A number of cells was also found within the shell of the nucleus ovoidalis (Ov) and the nucleus semilunaris parovoidalis. In the midbrain AVT, TPc and LoC contained labeled cell bodies. The results following an injection into Nd are summarized schematically in figure 2 (right column).
Figure 4: Photomicrographs of anterograde labeling of NCL efferents to the basal ganglia and archistriatum. A: Labeled fibers in the presumed „limbic” parts of the avian striatum (i.e., nucleus accumbens and medial LPO) after an injection of CTb into far caudal NCL. B and C: Labeling in the presumed „somatomotor” parts of the basal ganglia, the lateral LPO (B) and central PA (C). D and E: Labeled fibers and somata in somatomotor and limbic parts of the archistriatum. Injections into the most caudal NCL label numerous fibers in the central part of the intermediate archistriatum, but also extend into the ventral archistriatum which forms part of the avian amygdala (D). In contrast, injections into the rostral NCL lead to labeling in the dorsal and central parts of the intermediate archistriatum which are considered somatomotoric in function. Cells from most parts of the archistriatum project back to the NCL. The projection from Av is the only bilaterally organized connection of the NCL. Scale bars are 250 µm (A), 50 µm (B), 100 µm (C), and 500 µm (D, E).
Anterograde tracing experiments

Injections into the ectostriatal belt

After injections into the dorsal and lateral ectostriatal belt and the adjoining neostriatum intermedium (NI) underneath the external pallium (Veenman et al., 1995b) a massive projection of labeled fibers was observed to move caudally through the NI and to innervate the anterior and lateral NCL. This projection seemed to be topographically organized and extended in the case of caudal injections posteriorly to about A 5.00. In addition to terminals, a number of retrogradely labeled cell bodies also were found, indicating a reciprocal connection between the NCL and the ectostriatal belt region. As shown in figure 5 a massive reciprocal connection of the Ep exists with the deeper hyperstriatal layers, especially with the lateral part of HV. Cells within HV and the NI or NFL, respectively, were distributed up to far rostrally within the hemisphere. Relatively few cells and fibers were found within the outer rind of the pallium, i.e. the lateral NI (NIL), TPO and CDL, the larger portion of them remaining underneath the border that separates the NI and anterior NCL from the overlying external pallium. Furthermore, in the dorsal and ventral aspects of the rostral archistriatum small clusters of cells were labeled, as well as a number of somata in the paleostriatum primitivum (PP). Within the ectostriatal core labeled cells were found in a narrow area below the injection site. In the thalamus large numbers of neurons were labeled in nucleus rotundus which probably are due to tracer spread into the ectostriatal core.

Injections into field L

In accordance with previous reports (Bonke, et al., 1979; Wild et al., 1993) tracer deposits into the auditory field L complex which were mainly centered on the secondary sensory field L1 lead to massive terminal-like labeling in Nd. Yet, injections of CTb revealed a diffuse projection that also extended further laterally in caudal NCL (figure 6). In the medioventral aspect of NCL also a small number of retrogradely labeled cells could be observed. Field L injections also resulted in retrograde labeling of neurons throughout the HA, NIM and HV, as well as in ventral and intermediate archistriatum. Diencephalic neurons were labeled within shell and core regions of n. ovoidalis and the n. semilunaris parovoidalis.
Figure 5: Results of a 15 nl CTb injection into the ectostriatal belt region, which receives afferents from the ectostriatum, the primary visual forebrain area of the tectofugal pathway. A: Schematic representation of labeling within the caudal telencephalon. B: Photomicrograph showing the innervation of the anterior NCL and retrogradely labeled cell bodies at about A 7.00. Scale bar is 200 µm.
Injections into the hyperstriatum accessorium

After injections into the Wulst that were centered on the medial aspect of the dorsal hyperstriatum accessorium, fibers were found to travel through the lamina frontalis superior (LFS) and further laterally along the border that separates the anterior NCL from the overlying LPO and CDL. Fibers showing terminal-like varicosities remained in close apposition to the ventricle as they moved further caudally through NCL. Neurons that project back to the injected site generally followed the distribution of terminating fibers but extended somewhat ventrally to these. Injections along the complete rostro-caudal extent of the Wulst revealed a topographic pattern of termination within NCL with more caudal injections leading to stronger labeling in the posterior NCL, while afferents from most rostral HA were confined to more anterior aspects of the NCL. This termination pattern may thus reflect the previously described functional segregation of the Wulst into a caudal visual and a rostral somatosensory area. Yet, the projections from HA onto NCL were not restricted to separate termination fields but showed considerable overlap (compare figures 7 and 8).

Injections into all parts of the HA evinced retrogradely labeled cells in the HD, frontolateral neostriatum, field L1, external pallium (CDL and TPO), a caudomedial portion of the area parahippocampalis (APH), and the ventral and central archistriatum. Injections into rostral HA additionally labeled cells in the NIM. In several cases in which tracer spread occurred into the thalamorecipient granular layer of the intercalated nucleus of the HA (IHA), retrogradely labeled cells were observed in the thalamic relay stations of the thalamofugal visual and somatosensory system, respectively: deposits of tracer into the caudal Wulst evinced cells in the dorsal aspect of the lateral geniculate nuclei (GLd; Karten et al., 1973; Güntürkün et al., 1993). Injections into the rostral HA on the other hand predominantly labeled cells in the somatosensory nucleus dorsalis intermedius ventralis anterior (DIVA; Wild, 1987b; Funke, 1989; Medina et al., 1997).

Injections into the neostriatum frontotrigeminale

After injections into the NFT dorsal to the nucleus basalis (Bas), fibers gathered in the tractus fronto-archistriatalis and passed caudally until they terminated within the lateral part of the anterior archistriatum and the rostral and ventral part of NCL. As can be seen in figure 9 the terminal field of this projection occupied a large part of the anterior and lateral NCL and extended caudally up to about A 5.00. Within this dense terminal field a comparatively small number of neurons were found to project back to the injection site. In those cases that showed
Figure 6: Results of a 9.2 nl CTb injection centered onto the secondary sensory field L1 of the auditory field L complex with slight tracer spread into the HV. A: Schematic representation of labeling within Nd and the adjoining parts of NCL. B: Retrogradely labeled neurons in the nucleus ovoidalis, the main thalamic relay of auditory input to the telencephalon, resulting probably from tracer spread into the primary field L2. C: Photomicrograph of labeled fibers in Nd and NCL. Scale bars are 500 µm (B) and 250 µm (C).
no or minimal tracer spread into Bas, retrogradely labeled fibers and cells within Bas were restricted to the area directly ventral to the injection site. Furthermore, cells were labeled in the HV dorsal to the injection site, the anterior archistriatum, along the tractus fronto-archistriatalis, and in medial HA.

**Injections into the neostriatum intermedium, pars medialis**

Injections into the intermediate neostriatum attempted to evaluate whether two separate projections from this area onto NCL exist as indicated by the results from our retrograde tracing experiments reported above. Tracer deposits were thus aimed at the terminal field of the DLP just medially and caudally to the ectostriatum, termed NIMI here, and the medialmost aspect of NI, the NIMm, respectively.

Injections into NIMm consistently produced dense terminal labeling and a considerable number of retrogradely labeled cell bodies in the most caudal aspect of NCL. Fiber bundles left the injection site laterally and travelled caudally within the LH. They reached the caudal neostriatum at the rostral end of Nd where they made massive terminations. The remainder of labeled fibers coursed laterally and caudally within the periventricular roof of NCL. On their way towards the caudal pole of the hemisphere they gradually fanned out towards the midline until they occupied a large proportion of the hemisphere (figure 10).

Small injections that were entirely confined to NIMI resulted in anterograde labeling that was largely restricted to Nd and the dorsal roof of the medial NCL. Labeling in these cases was virtually identical to that seen after field L injections (compare figure 6). However, labeling within the NCL varied considerably with the amount of tracer used. Larger NIMI injections that also extended into one of the adjacent areas HV, NIMm or Ep, led to a much higher number of labeled fibers and somata within NCL, while at the same time the terminal field also expanded further lateral and medial (figure 11). However, the distribution of these fibers did not resemble the terminal fields resulting from injections into NIMm or Ep. In addition, all of these cases showed a similar labeling in the thalamus that was distinct from the pattern observed after NIMm injections (below).

Deposits of CTb into the medialmost NI yielded numerous neurons within the medial HV, dorsal to the injection site, HA, HD, in Ai and Av. Retrogradely labeled cells from Av were also found on the contralateral side. Massive anterograde labeling was seen in medial LPO. Injections into lateral NIM resulted in a similar pattern of labeling which included the
In addition, cells were labeled in field L1 and L3, as well as in large aspects of the caudomedial neostriatum. In these cases labeled fibers were seen in the PA ventral to the injection site, but not within LPO. Labeling in the thalamus further strengthened the notion of

Figure 7: Labeling observed after a 40 nl injection of CTb into the caudal HA. A: Schematic representation of labeled fibers and cell bodies in the dorsal NCL. Injections of BDA revealed a similar amount of anterogradely labeled fibers but only very few retrogradely labeled cells. B: Photomicrograph showing retrogradely labeled cells in the nuclei of the dorsolateral geniculate complex, as could be observed in some cases in which the injection site involved the thalamorecipient intercalated nucleus of the HA. Scale bar is 200 µm.

somatosensory and visual Wulst, medial HV dorsal to the injection site, as well as Av and Ai. In addition, cells were labeled in field L1 and L3, as well as in large aspects of the caudomedial neostriatum. In these cases labeled fibers were seen in the PA ventral to the injection site, but not within LPO. Labeling in the thalamus further strengthened the notion of
Figure 8: Results following a 13.8 nl injection of CTb into the rostral HA. A: Schematic representation of labeled fibers and cell bodies in the NCL. The projection from rostral HA is largely restricted to the anterior NCL. B: Photomicrograph showing retrogradely labeled cells in the somatosensory thalamic n. dorsalis intermedius ventralis anterior resulting from tracer spread into the intercalated nucleus of the HA. Scale bar is 200 µm.
two distinct subareas within NIM: injections into the NIMl confirmed the projection from DLP (Kitt and Brauth, 1982; Gamlin and Cohen, 1986; Wild, 1994) and in addition labeled cells in SRT, the shell of Ov and DIP. In contrast, after tracer deposits into the medialmost NI with slight spread into the overlying HV, but not into PA or LPO, a large number of retrogradely cells were found in the medial part of the dorsal thalamus, namely the n. dorsomedialis posterior thalami (DMP) and DIP, and only very few within DLP.

**Injections into the archistriatum**

One of the most intriguing results of our retrograde labeling experiments was the extremely high number of cells seen in the NCL that project to the archistriatum and the close match between the distribution of these cells and the distribution of TH-immunoreactive fibers (figure 12). After injections of CTb into the anterior two-thirds of the archistriatum that involved the central and ventral parts of the intermediate archistriatum, cells along the whole rostro-caudal extent of the NCL were labeled (figure 13). These cells clustered underneath the dorsal roof of the NCL and gradually fanned out towards the medial neostriatum. This distribution thus follows closely the original description of the NCL based on the distribution of dopaminergic and catecholaminergic fibers (Waldmann and Güntürkün, 1993; Wynne and Güntürkün, 1995; Metzger et al., 1996; present study) and it thus seems that all parts of the NCL, including Nd, project upon the archistriatum.

According to the injection site within the archistriatum this projection showed an anterior to posterior gradient with more rostral archistriatal injections labeling relatively more cells in anterior NCL. Yet, in general, the highest absolute number of cells was seen in the caudalmost aspect of NCL where labeled cells extend far medially and ventrally. There might exist a further parcellation with respect to dorsal and ventral subdivisions of the archistriatum as suggested from the anterograde tracing experiments, yet with our injections which always involved various subdivisions of the intermediate archistriatum we could not confirm this. A different pattern was seen after injections into the posterior archistriatum which is the source of the tractus occipitomesencephalicus, pars hypothalami (HOM) and which together with parts of the ventromedial archistriatum has been suggested to constitute the avian homologue of the amygdala (Zeier and Karten, 1971, Dubbeldam et al., 1997; Davies et al., 1997). In these cases, a small numer of retrogradedly labeled cells could be observed in the periventricular rim of the NCL and Nd (figure 14). Their number increased towards the caudal tip of the hemisphere supporting the notion that the most caudal NCL projects to the limbic/amygdaloid part of the
Figure 9: Results of a 13.8 nl CTb injection into the neostriatum frontotrigeminale overlying the n. basalis. B: Photomicrograph showing the dense anterograde labeling in the rostral and lateral aspects of NCL. C: Detail from B showing the border between NCL and the adjacent medial neostriatum. Scale bars are 500 µm (B) and 100µm (C).
Figure 10: Pattern of labeling observed after a 11.5 nl injection into the medialmost NIM. As shown in A fibers travel from the injection site in a compact bundle caudally within the lamina hyperstriatica until they reach the medialmost NCL from where they gradually fan out to diffusely innervate the caudalmost part of NCL. B, C: Photomicrographs showing the abundant distribution of anterogradely labeled fibers and retrogradely labeled cells in the caudalmost NCL, following injections of biotinylated dextran amine (B) and CTb (C) into the medialmost NIM. D: Retrogradely labeled cells in the posterior thalamus, specifically in DMP. In B and C dorsal is to the right. Scale bars are 100 µm (B, C) and 300 µm (D).
archistriatum. A very large number of cells, however, was labeled in the ventralmost neostriatum surrounding the archistriatum dorsally and caudally. After injections into the central and ventral archistriatum a large number of retrogradely labeled cells was seen in the ventral hyperstriatum and the intermediate neostriatum. Other areas that project to the central archistriatum include the caudomedial neostriatum, medial HA, rostral HD and the vallecula, the field L complex as well as cells within NFT and NFL. Strong terminal-like labeling was seen in LPO and medial PA as well as in HV. Descending fibers leave the intermediate archistriatum through the tractus occipitomesencephalicus (OM), from which they eventually split off to provide massive input to the deep layers of the optic tectum. Terminal-like labeling was furthermore seen in the dorsal thalamus, predominantly in the posterior thalamic nuclei DLP, DIP and SRt, all three of which also contained labeled cell bodies, as well as in the nucleus spiriformis medialis (SPm) and the nucleus intercollicularis (ICo). At midbrain level, terminal-like labeling and retrogradely labelled neurons could be observed in TPC, AVT, and LoC. The OM further descended onto various nuclei in the brainstem (see Zeier and Karten, 1971, Dubbeldam et al., 1997; Davies et al., 1997).

Injections into the posterior archistriatum labeled somata in the mediodorsal HV and along the LFS. The Ap seemed to be reciprocally connected with cells in CDL and APH, as well as the n. accumbens. Within the archistriatal complex, cells in the „limbic“ subcomponents, i.e. the nucleus taeniae and Av, were labeled. The Ap was also found to project heavily onto the BNST and tuberculum olfactorium. Descending fibers of the HOM terminated mainly within the lateral hypothalamus (lHy). In the diencephalon cell bodies were observed in SRt and along the whole track of the HOM. At the level of the midbrain cells could be found within TPC, AVT, and LoC.

Injections into the paleostriatum augmentatum

Injections into medial and central parts of the paleostriatum augmentatum between A 7.75 and A 9.00 labeled neurons along the complete rostrocaudal extent of NCL and Nd. As illustrated in figure 15 these cells could be found throughout the whole depth of the NCL although their number seemed to be highest within or just medially of the tractus archistriatalis dorsalis, at the medial border of the TH-rich caudal neostriatum. A large number of retrogradely labeled cells were seen in the dorsal part of the archistriatum. A smaller bilateral projection further-

Following page - Figure 11: Results of a comparatively large (23 nl) CTb injection into lateral NIM which also extended slightly into HV. See text for details. B: Photomicrograph of retrogradely labeled cells in the thalamus. C: Labeled fibers and cells in the dorsal NCL at about A 6.00. D: Detail showing the border of the densest innervation. Scale bars are 200 µm (B), 500 µm (C) and 50 µm (D).
Chapter 1: Connections of the avian neostriatum caudolaterale

Figure 11
more seemed to originate from portions of the anterior archistriatum. In addition, our injections into PA labeled cells in HV, lateral HD, and the caudal pallium (e.g. NIL, TPO and CD). A few cells were also seen along the needletrack in NI, the caudomedial HV and the NCm. In the thalamus cells were labeled within the lateral „somatic“ area of the dorsal thalamic zone, namely DIP, VIP and to a lesser extent in DLP, SRt, n. suparotundus (SpRt), and nuclei of the ansa lenticularis. Sometimes marked neurons were also observed within the ventrointermediate area of the thalamus (VIA) and n. rotundus (Rt) as well as n. triangularis (T). While labeling within VIA was most likely due to tracer spread into the dorsal pallidum, i.e. PP (Medina and Reiner, 1997), labeling in Rt and T derived from tracer spread into the ectostriatum (Benowitz and Karten, 1976). At the level of the midbrain AVT and TPc also contained a number of labeled cells.
Figure 13: Results from a large (81 nl) injection of CTb into the archistriatum which involved parts of the central and ventral intermediate archistriatum. A: Diagrammatic representation of retrogradely labeled projection cells within NCL. B: Photomicrograph showing cells in the far caudal NCL. C: Retrogradely labeled cell in the NCL following a BDA injection in the intermediate archistriatum colocalized with TH immunoreactive fibers. Scale bars are 1 mm (B) and 50 µm (C).
Injections into the medial lobus parolfactorius

Labeling within NCL after deposits of CTb into medial LPO between A 9.50 and A 11.50 was very similar to that observed after PA injections. Again, retrogradely labeled neurons were distributed diffusely along the medioventral and rostrocaudal extension of NCL, yet cells were predominantly located along the medial border (figure 16). This was also true for two cases in which the injection accidentally was centered onto lateral LPO and PA. Regarding a possible rostrocaudal topography of NCL projections to the basal ganglia, we found a relative larger number of retrogradely labeled neurons projecting to the LPO in caudal NCL while neurons projecting to the PA tended to be located more rostrally and medially. However, as noted...
above, these projections largely overlapped. Labeling in other areas of the telencephalon included the NIM and HV dorsal to the injection site, medial HA and HD, and the area prehippocampalis (APrH). Many cells were also seen in TPO, CDL and up to far rostral levels within NFL, but only few in NIL. In the archistriatum large parts of the Av and Ai were labeled. The projection from the Av seemed to be bilaterally organized. We found that in the thalamus CTb injections which were centered on medial LPO yielded numerous neurons within n. dorsomedialis anterior thalami (DMA) and DMP, and to a lesser extent also in the n. subhabenularis (SHL) and DIP. In the mesencephalon cells were found in AVT, TPc and LoC.

**DISCUSSION**

The main results of this study can be summarized as follows: The NCL has reciprocal connections with the secondary sensory areas of all modalities, as well with at least two parasensory areas in the NIM and the deep layers of HV. The afferents from these areas have diffuse and largely overlapping termination fields within NCL (see figure 17). These connections are restricted to the plexus of the densest innervation by catecholaminergic and presumably dopaminergic fibers as demonstrated here by TH immunoreactivity. Furthermore, all parts of the so defined NCL project onto the basal ganglia and the archistriatum. The importance of these findings for sensorimotor integration in the avian telencephalon and their possible relevance for the evolution of cortex-equivalent structures will be discussed separately in detail below.

*Sensory integration within NCL*

All five secondary sensory areas were found to project to NCL. The organization of these pathways conforms with a general pattern of sensory processing in the avian telencephalon (summarized in Veenman et al., 1995b): the primary receptive fields of subtelencephalic input

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Following pages:

**Figure 15:** Results following an injection of 28 nl CTb into the „somatic“ central PA. A: Schematic representation of retrogradely labeled cells in the caudal telencephalon. The most abundant labeling can be seen in the dorsal part of the archistriatum and the rostral NCL. See text for details. B: Photomicrograph illustrating the location of projection neurons at the medial border of the NCL and within Aidd. C: Photomicrograph of labeling in the posterior dorsal thalamus. In addition to labeled neurons within DIP, cells could often be observed in the VIP, DLP and SRt. Scale bars are 500 µm (B) and 200 µm (C).

**Figure 16:** Projections to the limbic medial striatum. Results of a 22 nl injection of CTb into the medial LPO with no obvious tracer spread into AC or lateral LPO. Labeling in the caudal telencephalon was complementary to that observed after injections in the somatic parts of the basal ganglia. Most abundant labeling was seen in the ventral part of the intermediate archistriatum and the caudal NCL. B: Photomicrograph showing the location of NCL projection neurons (arrows) at the medial border of the tractus archistriatalis dorsalis as well as in Ai and Av in a slice double labeled for TH. The DA shows only weak staining for TH. C: Retrogradely labeled cells in the posterior neostriatum cluster at the medial extent of the NCL. D: Photomicrograph of retrogradely labeled thalamic neurons within DMA.
Figure 15
relay the information to adjacent secondary sensory structures, which in turn project to areas of the external pallium. In the case of the tectofugal visual pathway the ecto striatum is the primary sensory structure (Kondo, 1933, Benowitz and Karten, 1976). From there intratelencephalic projections lead to the ecto striatal belt (Karten and Hodos, 1970; Watanabe et al., 1985). The present study demonstrates in accordance with Leutgeb et al. (1996) that the NCL is the tertiary telencephalic component of the tectofugal complex. This pattern also holds for the other sensory systems. Caudal IHA and the caudolateral component of HD are the primary sensory areas of the thalamofugal visual pathway (Karten et al., 1973), from where projections lead to HA (Shimizu et al., 1995), which by itself projects to NCL (Shimizu et al., 1995, Leutgeb et al., 1996). More rostrally, somatosensory thalamic fibers terminate in HD/HIS and IHA (Delius and Bennetto, 1972, Funke, 1989, Wild, 1997) which in turn project to rostral HA (Wild, 1987b). Field L2 is the primary telencephalic area of the auditory system (Wild et al., 1993), which projects to L1 and L3, from where efferents lead to Nd/NCL (Bonke et al., 1979, Wild et al., 1993, Leutgeb et al., 1996). N. basalis is the primary telencephalic area of the trigeminal system (Schall et al., 1986), which projects to NFT (Wild et al., 1985; Wild and Farabaugh, 1996), from where efferents lead to NCL (Schall et al., 1986, Wild et al., 1985; Wild and Farabaugh, 1996). Thus, the NCL is the tertiary telencephalic component of all sensory modalities examined in the present study. Due to this multimodal input and the broad overlap of terminations, the NCL is a true associative forebrain structure. Additionally, parts of the caudal neostriatum may also have access to olfactory information: after injections into the posterior archistriatum we found a large number of cells in the ventralmost neostriatum overlying the archistriatum. This region has been suggested to be reciprocally connected with the avian piriform cortex and olfactory bulb, which, as their mammalian counterparts process olfactory information (Reiner and Karten, 1985; Haberly and Bower, 1989; Bingman et al., 1994). In concert with the CPi and limbic archistriatum this area might thus be concerned with viscerolimbic functions. However, from our combined immunocytochemical and pathway tracing data it seems that this part of the NC is not a genuine subdivision of the NCL, although it might be ventrally continuous with it. Olfactory information can reach the NCL nevertheless via its connections with the HD. In addition to afferents from the visual dorsolateral thalamus (Karten et al., 1973; Güntürkün and Karten, 1991; Güntürkün et al., 1993) and non-specific input from the mediodorsal thalamus (Bagnoli and Burkhalter, 1983), the HD is also reciprocally connected with the hippocampal formation and the CPi (Bingman et al., 1994; Casini et al., 1986; Shimizu et al., 1995).
The pattern of afferents within NCL might provide clues to the functional architecture of the pigeon’s nervous system. The only two pathways which show no or little overlap within NCL are those from the auditory and the trigeminal systems. Indeed, conditioning paradigms have shown that pigeons have severe constraints to associate auditory signals with food reward in an appetitive learning paradigm in which pecks on a key serve as an operant (LoLordo and Furrow, 1976, Delius and Emmerton, 1978). The same animals are rapidly able to associate in a classical conditioning paradigm the very same auditory signals with a mild shock applied to the body (Delius and Emmerton, 1978). Delius and Emmerton (1978) speculated that a granivorous animal like a pigeon is simply not in need of associating acoustic cues with food objects since grains are mostly silent. However, grains can be quite noisy during pecking due to the direct auditory feedback generated by the impact of the beak on the substrate. Indeed, Delius (1985) could show that pigeons are easily able to learn an auditory tone discrimination in an appetitive paradigm if the acoustic signals are generated by the pecks. Yet, delaying the acoustic feedback by more than 0.5 s abolishes learning. Since the n. basalis not only receives trigeminal projections but also some auditory afferents from the lemniscal nuclei (Delius et al., 1979, Schall et al., 1986, Arends and Zeigler, 1986; Wild and Farabaugh, 1996) it is likely that the specialised auditory-trigeminal associations occurring during pecking are directly processed within the basalis system. Indeed, lesions of the n. basalis eliminate the ability to discriminate acoustic signals generated by the animals own pecks (Schall and Delius, 1991). Thus, it is conceivable that the separation of auditory and trigeminal afferents within NCL is related to the severe constraints of pigeons in associating acoustic and trigeminal stimuli. Both the tectofugal and the thalamofugal systems project to NCL, but their common area of termination seems limited. This fact might be related to differences in visual field representations between the thalamo- and the tectofugal pathway. The retina of pigeons has two areas of enhanced vision (Galifret, 1968, Binggeli and Paule, 1969). One is the central fovea which looks into the monocular lateral field. The other is the so called “red field“ in the dorsotemporal retina with which the animal views stimuli in the inferior frontal visual field (Güntürkün, 1998). The representation of the red field within the GLd of the thalamofugal pathway is extremely sparse, while the central fovea is heavily represented (Remy and Güntürkün, 1991). Thus, the thalamofugal pathway mainly processes stimuli from the lateral rather than the frontal visual field. Accordingly, thalamofugal lesions produce minor deficits when tested with frontal stimuli, but severe deficits when tested with lateral stimuli (Güntürkün and Hahmann, 1998). Contrarily, there is evidence that tectofugal rotundus lesions impair frontal acuity, while
Figure 17: Schematic representation of terminal fields within NCL showing the large overlap of afferents from secondary sensory areas. Note that the afferents from the presumed parasensory areas within NIM, which occupy large parts of the caudalmost NCL are not included in this figure. See text for details.
leaving lateral acuity intact (Güntürkün and Hahmann, 1998). This in turn may be due to the fact that ventral tectal cells, which represent the lower frontal visual field, project heavily onto rotundus, while the contribution of dorsal tectal cells to the tectofugal pathway is limited (Hellmann and Güntürkün, 1997). Thus, thalamo- and tectofugal visual pathways in pigeons seem to differentially represent lateral and frontal vision, respectively. Their largely complementary representation within NCL might thus be related to their differential representation of the visual field. Despite the small overlap of the tecto- and the thalamofugal visual fields within NCL, these domains extensively overlap with the trigeminal and the auditory projections, respectively. The large common territory of the tectofugal visual and the trigeminal system might be related to the specialization of the tectofugal pathway to the lower and frontal visual field (Hellmann and Güntürkün, 1997, Güntürkün and Hahmann, 1998) which within the egocentric space overlaps with trigeminal inputs from the beak. Thus, a common sensory focus of both systems could create a need for common coding which might be accomplished by extensive areas of terminal overlap in an associative structure like NCL. The large overlap between thalamofugal visual and auditory domains might be similarly interpreted. As outlined above, the thalamofugal pathway in pigeons is oriented laterally (Remy and Güntürkün, 1991, Güntürkün and Hahmann, 1998). Like most other birds, pigeons fixate distant objects laterally with their central fovea (Blough, 1971, Martinoya et al., 1981, Bischof, 1988), since their lateral monocular acuity is about twice as high as their frontal monocular one (Hahmann and Güntürkün, 1993, Güntürkün and Hahmann, 1994). Thus, the thalamofugal visual system is more related to distant visual objects and might therefore need common processing with audition, which also is a distance sense.

**Connections of the NCL with the NIM and ventral hyperstriatum**

Our retrograde and anterograde tracing experiments have suggested the existence of two different, albeit continuous, sources of afferents from NIM to NCL. While the NIMl projection innervates the Nd region and the laterally adjacent parts of the NCL, afferents from NIMm terminate abundantly throughout the caudalmost NCL. Both areas receive differential input from the dorsal thalamus (Kitt and Brauth, 1982; Gamlin and Cohen, 1986; Wild, 1987a, 1994; Metzger et al., 1996; present study) and differ in their cytoarchitectonic characteristics (Rehkämper et al., 1985). The NIMl has been shown to receive visual and somatosensory information from telencephalic areas (Funke, 1989; Wild, 1987b, 1994; Shimizu et al., 1995; present study). Since we found retrogradely labeled cells in L1 and L3 after injections into
NIMl, this structure probably also integrates auditory information. In addition, polysensory input reaches the NIMl via DLP (Kitt and Brauth, 1982; Gamlin and Cohen, 1986; Korzeniewska and Güntürkün, 1990; present study).

The NIMm, as defined here, resembles in its location and connectivity the mediorostral neostriatum/hyperstriatum ventrale (MNH) of the chick which has been extensively studied in the context of imprinting (e.g. Metzger et al., 1996; Bredenkötter and Braun, 1997; Gruss and Braun, 1996; for review: Scheich, 1987). Injections into NCL which labeled cells in NIMm also always produced cells in the overlying HV, suggesting a similar translaminar organization of this area in the pigeon as is characteristic for the MNH. Data on the connections of either the MNH or NIMm with telencephalic regions are very limited (Metzger et al., 1996). Our results indicate that the NIMm, as the NIMl, might also receive input from the visual and somatosensory areas of the Wulst. Thalamic input to NIMm originates mainly in the DMP and to a much lesser extent in the DMA, a situation which is reversed for the MNH of chicks (Kitt and Brauth, 1982; Wild, 1987a; Metzger, et al., 1996; present study). The sensory properties of these afferents are not entirely clear, but the nuclei of the dorsomedial thalamus receive input from the hypothalamus and tractus solitarius (Berk and Butler, 1981, Berk and Hawkin, 1985, Arends et al., 1988; Wild et al., 1990) and in turn project to the viscerolimbic striatum and CPi (Metzger et al., 1996; Bingman et al., 1994; Kitt and Brauth, 1982; Wild, 1987a; Veenman et al., 1995a, 1997). Furthermore, the NIMm and to a lesser extent also the NCL of pigeons display strong immunoreactivity for the calcium-binding protein parvalbumin (S. Kröner, unpublished observations), as has previously been shown for the MNH of chicks (Braun et al., 1991a). Parvalbumin is associated with fast spiking GABAergic neurons (DeFelipe et al., 1989; Kawaguchi and Kubota, 1993), and in songbirds it is characteristic of nuclei displaying high metabolic activity, notably all nuclei of the vocal motor system (Braun et al., 1985, 1991b). As outlined above, both areas of the NIM appear capable of multisensory processing. This draws attention to our observation that their afferents terminate predominantly in those aspects of the NCL which receive relatively little input from secondary sensory regions (compare figure 17). Although it is possible that other telencephalic areas which send projections to NCL (e.g. HA or NFT) are also parasensory in nature (Deng and Wang, 1992, 1993; Schall et al., 1986; Wild and Farabaugh, 1996) the nuclei within NIM clearly are a step progressed in an assumed hierarchy of stimulus processing, as they, like the NCL, receive their afferents from secondary sensory structures. It thus seems that parasensory inputs from NIM to the NCL complement the pattern of multimodal integration.
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It should be noted, that our injections into NCL also labeled cells in the HV which based on their location resemble the intermediate and medial part of the hyperstriatum ventrale (IMHV), another area which in the chick has been extensively studied in the context of imprinting and memory formation (Bradley et al., 1985; Davies et al., 1988; review by Horn, 1998). Although we did not target these cells in the anterograde tracing experiments, results from the study of Shimizu and coworkers (1995) suggest that afferents from the HV also terminate abundantly throughout the caudalmost neostriatum (Shimizu et al., 1995).

**Projections to the archistriatum**

We found a continuous band of cells in the NCL which project in a topographically ordered manner onto most parts of the intermediate archistriatum. In addition, the posterior archistriatum receives a weak projection from cells in the caudal- and dorsalmost aspects of the NCL. The connections with the intermediate archistriatum are reciprocally organized (Davies et al., 1997; Leutgeb et al., 1996; Metzger et al., 1998; present study) and bilaterally projecting cells in the Av provide a means of interhemispheric comparison (Wild and Farabaugh, 1996; Metzger et al., 1998; present study). Functionally, the avian archistriatum has generally been divided into two main subdivisions (Zeier and Karten, 1971; Davies et al., 1997; Dubbeldam et al., 1997). These are a somatic sensorimotor part which in the pigeon comprises parts of the anterior and intermediate archistriatum (Aa, Ai, Aidd, Aidv), and a viscerolimbic division that includes the posterior and medial archistriatum, as well as the ventral parts of the intermediate archistriatum (Ap, Am, Av). The sensorimotor archistriatum receives widespread afferent projections from higher order sensory areas of the telencephalon (e.g., Ritchie, 1979; Wild et al., 1985, 1993; Shimizu et al., 1995) and is involved in high-level motor control (Zeier, 1971; Knudsen et al., 1995) and memory function (Knudsen and Knudsen, 1996). Furthermore, via the OM the archistriatum projects to premotor areas of the brainstem (e.g. reticular formation and lateral pontine nuclei) in all birds (Zeier and Karten, 1971; Wild et al., 1985, 1993; Davies et al., 1997; Dubbeldam et al., 1997; present study) and to some specific groups of brainstem motoneurons involved in respiration and vocalization in songbirds (Nottebohm et al., 1976, Wild, 1993). The limbic portion of the archistriatum on the other hand is considered homologous to the mammalian amygdala (Zeier and Karten, 1971; Davies et al., 1997; Dubbeldam et al., 1997). It seems to be crucially involved in such viscerolimbic functions as agonistic behaviour and homeostasis. (e.g. Cohen, 1975; Ramirez and Delius, 1979; Lowndes and Davies, 1995). The archistriatum´s functional segregation is reflected in the
extratelencephalic projections via the OM or HOM, respectively (Zeier and Karten, 1971; Davies et al., 1997; Dubbeldam et al., 1997), and in its connections with limbic or somatic striatum (Veenman et al., 1995b; present study). While the main output from the NCL might be directed to the sensorimotor part of the archistriatum (Leutgeb et al., 1996), especially the caudalmost NCL also appears to be capable of modulating the amygdaloid division of the archistriatum (present results). The very large number of neurons from NCL that project to the archistriatum, and the close correspondence between the position of these output neurons and the densest catecholaminergic innervation, underline the importance of this projection for an understanding of the functions of the NCL. In addition, we believe that the projection from the caudal neostriatum to the archistriatum might serve as a criterion for the delineation of NCL. Given the fact that injections into the archistriatum labeled a continuos band of cells that extended from the ventrolateral NCL up to Nd, we think it reasonable to consider Nd the ‘auditory subcomponent’ of NCL. This view is also supported by the facts that the Nd shares afferents with the adjacent NCL from areas other than the field L complex, shows the same dense dopaminergic innervation and shows a similar organization of efferent connections other than those to the archistriatum.

Projections to the basal ganglia

The connectivity, neurotransmitter content, and cytoarchitecture of the avian basal ganglia are highly similar to that in mammals (Karten and Dubbeldam, 1973; Reiner et al., 1984; Veenman and Reiner, 1994; Veenman et al., 1995b). In mammals corticostriatal inputs from association, sensorimotor and limbic cortices project in a segregated manner onto three distinct striatal regions referred to as associative, sensorimotor and limbic striatal territories (Parent, 1990; Parent and Hazrati, 1995). These corticostriatal inputs provide the basal ganglia with exteroceptive sensory information as to the location of objects in space, interoceptive sensory information on relative body position in space, and neural feedback and feedforward information on ongoing and impending body movements (McGeorge and Faull, 1989; Alexander et al., 1990; Romo et al., 1992; Schultz et al., 1992; Aldridge and Berridge, 1998). Based on its palliostratial connections a similar functional segregation has recently been suggested for the avian striatum (Veenman et al., 1995b): ventral striatal structures such as the AC, the BNST, and olfactory tubercle constitute the „limbic“ parts of the avian striatum. These „limbic“ parts also include the medial LPO and lateralmost PA. The remaining dorsal striatal parts (lateral LPO, medial PA) on the other hand are considered sensorimotor in nature. We show here that
all parts of the NCL project to the ventral and dorsal aspects of the striatum. Our combined retro- and anterograde tracing experiments suggest a rostrocaudal topography of these projections, with caudal parts of NCL projecting predominantly onto limbic medial LPO and more rostral parts of NCL projecting to lateral LPO and mainly medial PA (figure 18). The projection onto the limbic striatum had already been indicated by the study of Veenman and coworkers (1995b); but as their injections also involved the CPi and Ap (see their figure 18 and discussion), which also project heavily onto the limbic parts of the avian basal ganglia (Bingman et al., 1994; Veenman et al., 1995b; present study), until now the existence of this connection from the NCL was not entirely clear. In addition to this direct pathway, the NCL is also capable of influencing the ventral basal ganglia via its connection with the limbic archistriatum. The ventral striatum of mammals receives input from limbic cortical regions such as hippocampus, cingulate cortex, amygdala and piriform cortex (McGeorge and Faull, 1989; Alexander et al., 1990), and similar projections have been shown for the avian homologues of these structures (Bingman et al., 1994; Veenman et al., 1995b). In mammals, a further prominent input to the ventral striatum originates in the frontal cortex (Berendse et al., 1992, Sesack et al., 1989; Alexander et al., 1990), and particularly the prefronto-accumbal network plays a crucial role in motivated behaviour (Apicella et al., 1991; Schultz et al., 1992; Floresco et al., 1997).

The demonstration of a projection from caudal NCL onto the AC thus further strengthens the notion that the NCL might be functionally equivalent to the PFC (Veenman et al., 1995b; present study). As in mammals, this projection might provide the sensory information required, e.g. for the evaluation of appetitive and aversive stimuli. In addition to the projection upon limbic striatum we found a prominent projection onto large parts of PA and lateral LPO which comprise the somatomotoric divisions of the avian striatum (Veenman et al., 1995b), providing the NCL with a further direct pathway for movement initiation. It is unclear why the projection from NCL to the somatic PA was not described by Veenman and coworkers (1995b), but it seems possible that this reflects differences in the sensitivity of the different tracers used, or, more likely, that their injections into PA were not centered on those regions in which we found the most abundant anterograde labeling, namely the caudal aspects of the central and lateral PA (compare their figures 5 and 6 with figures 2 and 15 of the present study).

The palliostriatal projections form asymmetric and probably excitatory synapses with spiny striatal neurons (Veenman and Reiner, 1996), a situation comparable to that in mammals (Dube, et al., 1988). Based on these and similar findings large parts of the external pallium, including the NCL and the archistriatum, have been compared with a subpopulation of corticostriatal
projection neurons from layer III and V in the rat (Zeier and Karten, 1971; Veenman et al., 1995b; Akintunde and Buxton, 1992; Cowan and Wilson, 1994). In sum, the NCL is positioned to amalgamate sensory inputs from all modalities and to relay them to the sensorimotor division of the basal ganglia, providing the latter with information that has been further processed and integrated with input from other modalities.

Comparison with vocal control pathways in songbirds:

In songbirds much work has focused on the pathways that control the acquisition and production of learned song. Within oscines, the forebrain vocal system comprises two major pathways that converge on the same premotor nucleus of the archistriatum. The two pathways diverge from a nucleus in the caudolateral neostriatum, the „high vocal center“ (HVC, Nottebohm et al., 1976, 1982). The main descending vocal motor pathway leads from the HVC to the robust nucleus of the archistriatum (RA), which in turn projects onto mesencephalic and medullary nuclei involved in vocalization (Nottebohm et al., 1976, 1982; Bottjer et al., 1989; Vates et al., 1997).

Figure 18: Diagrammatic illustration of the organization of presumed „limbic“ (grey) and „somatic“ (black) pathways from the NCL to the archistriatum and the basal ganglia. All parts of the NCL send projections to both limbic and somatomotor areas, but with a complementary rostral to caudal topography (see text for details and compare figures 15 and 16). The relative contribution of the caudal and rostral NCL to these pathways is indicated by the width of the arrows. In addition to direct connections indirect pathways exist, as limbic and somatomotor parts of the archistriatum also project to corresponding areas within the basal ganglia.
HVC also projects to RA by a second pathway that involves nuclei in the anterior forebrain. This circuit sequentially connects HVC, area X of the LPO; the dorsolateral thalamus, the magnocellular nucleus of the anterior neostriatum (MAN) and RA (Bottjer et al., 1989; Vates et al., 1997). Auditory input is thought to reach both major pathways of the vocal system via HVC and the adjacent „shelf“ region, which receive auditory information from the field L complex and associated regions of the caudal forebrain (Kelley and Nottebohm, 1979; Fortune and Margoliash, 1995; Vates et al., 1996). In nonsongbirds the projection from field L 1 and L3 to the Nd/NCL is similar to this ascending auditory pathway in oscine songbirds (Wild et al., 1993; present study), while the projection from NIMI to the Nd/NCL has been compared with another polysensory projection upon HVC which arises from the nucleus interfacialis (Nottebohm et al., 1982; Wild, 1994; Fortune and Margoliash, 1995). The present demonstration of a prominent input from NIMm to Nd/NCL suggests a correspondence of this pathway with yet another ascending projection onto HVC: in oscine songbirds MAN shares a similar location in the intermediate neostriatum with the NIM. The MAN can also be divided into a lateral (lMAN) and a medial (mMAN) part which receive differential input from the medial portion of the dorsolateral thalamus (DLM) and the DMP, respectively (Bottjer et al., 1989; Johnson, et al., 1995; Foster et al., 1997; Vates et al., 1997), thus resembling the thalamic projections to NIMI and NIMm (Kitt and Brauth, 1982; Wild, 1987a; present study). The mMAN then gives rise to a projection onto HVC and the adjacent „shelf“ region (Nottebohm et al., 1982; Fortune and Margoliash, 1995; Vates et al., 1996, 1997; Foster et al., 1997). A similar recursive pathway through the anterior forebrain also exists in budgerigars: the central nucleus of the lateral neostriatum (NLc) integrates information from other forebrain nuclei and relays it to the premotor neurons of the archistriatum (Brauth et al., 1994; Striedter, 1994; Durand et al., 1997). It has previously been suggested that the HVC complex, as might be true for the NLc of parrots, is concerned not only with translating auditory signals into vocal output but with the integration of multimodal inputs, and that the song system arose from an elaboration of pathways generally present in all birds (e.g. Wild, 1994; Margoliash et al., 1994). The fact that the shelf region adjacent to HVC shares the afferent inputs of HVC (Fortune and Margoliash, 1995; Vates et al., 1996; Foster et al., 1997) further strengthens the notion that HVC represents a specialized nucleus for the control of learned song, while neural populations in the adjacent neostriatum might be concerned with other aspects of multisensory integration and motor control. However, judging from the presently available data important differences seem to exist: the HVC of Passeriformes, or NLc of parrots, respectively, do not receive...
similarly abundant sensory input as described for NCL (e.g. Hall et al., 1993; Brauth et al., 1994; Striedter, 1994; Fortune and Margoliash, 1995; Leutgeb et al., 1996; present study). Furthermore, in pigeons the NCL and the archistriatum have direct reciprocal connections with all sensory and parasensory areas investigated here, and in addition both project onto the striatum (Veenman et al., 1995b; present study).

Comparison with mammalian prefrontal cortex

The PFC of mammals is actually not a single functional region but, rather, a group of anatomically and functionally heterogenous areas (e.g. Goldman-Rakic, 1987). It is commonly thought to involve the dorsolateral PFC, as well as the orbitofrontal and anterior cingulate cortices (Walker, 1940; Akert, 1964; discussed in Preuss, 1995; Condé et al., 1995). These areas participate to different extents in the complex cognitive, limbic and premotor functions that the PFC subserves, depending on their respective afferent and efferent connections (Sesack et al., 1989; Pandya and Yeterian, 1990). Thus, in primates, deficits resulting from damage to the lateral frontal region involve mainly attentional, temporal and integrative functions and are different from changes relating to emotion following orbitofrontal, and motivational changes following medial frontal damage (summarized in Fuster, 1989). Sensory input reaches the PFC via a set of interconnected pathways (for reviews: Jones and Powell, 1970; Pandya and Yeterian, 1990). The primary sensory area of each modality projects first to an adjacent area in parietal, occipital, or temporal cortex. This is the beginning of a sequential order of cortical areas that make up a pathway for that modality. Each area in the sequence projects not only to the next in line but also to a discrete area of the frontal cortex, which in turn reciprocates by sending fibers back to the projecting area. The fields that constitute the third link in each of the three pathways - namely, parietal area 7 (somatic), temporal area 22 (auditory), and inferotemporal area 21 (visual) - project to the prefrontal cortex and, in addition, to the cortex in the depths of the superior temporal sulcus, which constitutes another multimodal area. Although the termination fields show considerable overlap, especially around the principal sulcus of primates, a strong topography of these afferents exists, resulting in a distinct pattern of connections for each of the subdivisions of PFC (primates: Jones and Powell, 1970; Pandya and Yeterian, 1990; rodents: Condé et al., 1995; Reep et al., 1996).
Taken together, the organization of sensory input to the NCL strongly resembles the pattern described above for mammalian PFC (Leutgeb et al., 1996; present study). In addition, it is reciprocally connected with the avian homologue of the amygdala (present study), a connection which is also characteristic for mammalian PFC (Condé et al., 1995; Reep et al., 1996; Sesack, et al., 1989; Barbas and DeOlmos, 1990), and projects to most parts of the somatic and limbic striatum, as well as the sensorimotor archistriatum (c.f. figure 19; Veenman et al., 1995b; Leutgeb et al., 1996). Furthermore, the NCL and PFC share a dense dopaminergic input (Divac and Mogensen, 1985, Waldmann and Güntürkün, 1993, Divac et al., 1985, 1994, Wynne and Güntürkün, 1995), and a functional importance for processes which serve executive functions (working memory: Mogensen and Divac, 1982, 1993, Gagliardo et al., 1996, 1997, Güntürkün, 1997; behavioral flexibility: Hartmann and Güntürkün, 1998; behavioral inhibition: Güntürkün, 1997). With regard to memory functions it has been proposed that the PFC performs a unitary function, that of holding information gathered by the other association cortices „on-line“ in short term memory (Goldman-Rakic, 1987; Kimberg and Farah, 1993). Judging from the anatomical

Figure 19: Diagrammatic summary of the circuitry in which the NCL participates as it emerges from this study. The NCL is reciprocally connected with the secondary sensory of all modalities as well as at least two parasensory areas. It projects to the avian equivalents of the amygdala (Ap, Av) and premotor cortex (Ai), as well as limbic and somatic parts of the avian striatum which taken together by means of their long descending projections exert control over limbic and motoric centers in the diencephalon, midbrain and brainstem.
data and the behavioural studies cited above the NCL seems well capable to occupy the position of such a central executive in the avian brain.

However, at least one important difference hampers comparisons between NCL and PFC: As a common hallmark for the delination of the PFC across mammalian species serve the afferents from the mediodorsal (MD) nucleus of the thalamus (Rose and Woolsey, 1948; Akert, 1964; Preuss, 1995). Thalamic afferents to the NCL arise mainly from DLP and surrounding structures. DLP, however, is very likely not the avian version of mammalian MD. Based on its afferents and electrophysiological properties, DLP was suggested to be equivalent to the posterior complex of nuclei and especially its supragenulate component (Korzeniewska, 1987, Korzeniewska and Güntürkün, 1990). Like the posterior nuclei, DLP is dominated by afferents from the vestibular nuclei (mammals: Mickle and Ades, 1954; birds: Wild, 1988, Korzeniewska and Güntürkün, 1990), dorsal column nuclei (mammals: Feldman and Kruger, 1980, Berkley et al., 1986; birds: Funke, 1989, Wild, 1989, Korzeniewska and Güntürkün, 1990), superior colliculus (mammals: Hicks et al., 1986, Katoh and Benedek, 1995; birds: Gamlin and Cohen, 1986, Korzeniewska and Güntürkün, 1990), and reticular formation (mammals: Hicks et al., 1986; birds: Korzeniewska and Güntürkün, 1990). Amygdalar or hypothalamic afferents to mammalian posterior thalamic nuclei (Jones, 1985) or avian DLP (Korzeniewska and Güntürkün, 1990) have not been reported. Contrary to this pattern, mammalian MD is reported to receive, among others, afferents from the amygdaloid complex, diagonal band of Broca, and different hypothalamic and preoptic structures (Siegel et al., 1977, Sapawi and Divac, 1978, Velayos and Reinoso-Suarez, 1982, Irle et al., 1984, Russchen et al., 1987, Cornwall and Phillipson, 1988, Groenewegen, 1988). Based on this evidence, any general connectional similarity between MD and DLP could be denied. This view results from a comparison between DLP and the whole MD. If, however, DLP is compared with only lateral MD (rats: paralamellar MD portion, Cornwall and Phillipson, 1988, Groenewegen, 1988; cats: intermediate and lateral MD component, Velayos and Reinoso-Suarez, 1982; monkeys: parvocellular and multiform lateral MD portion, Russchen et al., 1987) the picture changes. The lateral MD portion does not receive afferents from the amygdala but is reached by inputs from vestibular nuclei, superior colliculus, and reticular formation. It thus resemble the pigeon's DLP in many aspects. It is the lateral portion of MD in cats and monkeys which heavily projects onto the dorsolateral component of prefrontal cortex (Markowitsch and Pritzel, 1979, Giguere and Goldman-Rakic, 1988, Ray and Price, 1993), which is known to be of prime importance in delay tasks. The paralamellar MD in rats is innervated by deep layers of superior colliculus
and projects to medial precentral cortex (Groenewegen, 1988), an area which has been compared with the frontal eye field in the monkey (Reep, 1984), a caudal portion of prefrontal cortex (Fuster, 1989; Pandya and Yeterian, 1990). But despite these similarities between lateral MD of mammals and avian DLP, important differences also exist. For example, the pigeon's DLP receives a prominent innervation by the cuneatus-gracilis complex and consequently the majority of neurons in this structure respond to somatosensory stimuli either as uni-, bi- or multimodal units (Korzeniewska and Güntürkün, 1990, Wild, 1994), which is clearly different from the anatomical and physiological pattern found in lateral MD. Thus, at the present state of knowledge, the initial suggestion of Korzeniewska and Güntürkün (1990) that the DLP is equivalent to the mammalian posterior complex of nuclei and not to MD still seems to be the most valid concept. Nevertheless the pigeon’s DLP might serve functions similar to MD, since DLP-lesions were shown to disrupt working memory tasks (Güntürkün, 1997).

Veenman and coworkers (1997) have suggested that DLP might be homologous to the intralaminar nuclei in mammals. In our view this seems not very likely since the mammalian intralaminar nuclei receive extensive afferents from medial hypothalamus, preoptic area, lateral septum, bed nucleus of the stria terminalis, ventral subiculum, perirhinal cortex, and amygdala (Price, 1995). Additionally they are characterized by a dense cholinergic input (Tohyama and Takatsuji, 1998), and their lesion effects are well characterized (Orem et al., 1973). A comparable pattern does not exist for DLP (Korzeniewska and Güntürkün, 1990, Güntürkün, 1997, Veenman et al., 1997). The suggestion of Miceli and Repérant (1985) that the avian SRt, which also projects onto NCL, is an intralaminar structure thus seems to be more likely, given the hodological data provided by these authors.

Recently, Metzger et al. (1996, 1998) suggested that the MNH of chicks might constitute the avian PFC. This hypothesis is based on the observation that MNH is characterized by a medium density of dopaminergic afferents (Metzger et al., 1996), a high density of dopaminoceptive neurons (Schnabel et al., 1997), and afferents from the DMA (Metzger et al., 1996). If avian DMA should prove homologous to mammalian MD (Veenman et al., 1997), the hypothesis of Metzger et al. (1996, 1998) would obviously gain weight. Yet, as discussed above, data on the connections of DMA and MNH are limited. However, the DMA is known to project not only onto MNH, but also to the accumbens (Veenman et al., 1997), medial LPO (Székely et al., 1994), and piriform cortex (Bingman et al., 1994), projections which have not been shown for mammalian MD (Groenewegen, 1988, Price, 1995). Given these contradictions in the DMA - MD comparison and the complete lack of functional data on DMA, clearly more information is
needed to clarify the comparison between avian MNH and mammalian PFC. These discussions on the organization of avian associative structures and mammalian PFC demonstrate that simple equivalencies between avian and mammalian prefrontal systems will not hold, even if two entities resemble each other with regard to their anatomical organization and function. However, difficulties in establishing homologous areas are not specific to comparisons between vertebrate classes but also apply to intraclass comparisons, as reflected in the recent discussion about the criteria that delineate prefrontal cortex in rats (Condé et al., 1995; Preuss, 1995).
A polysensory pathway to the forebrain of the pigeon: the ascending projections of the n. dorsolateralis posterior thalami (DLP)

Onur Güntürkün and Sven Kröner

ABSTRACT
Visual evoked potentials (VEPs) in the associative neostriatum caudolaterale (NCL) have shorter latencies than those recorded in other visual forebrain areas. Therefore visual input into NCL probably stems from a subtelencephalic relay. Tracing experiments revealed a projection of the n. dorsolateralis posterior thalami (DLP) into those portions of NCL in which visual, auditory, and somatosensory afferents from intratelencephalic parasensory areas terminate. Since VEPs in NCL are abolished after DLP-lesions, this structure has to be the critical relay. However, DLP also projects to other associative forebrain areas and parts of the basal ganglia. Previous experiments had furthermore revealed that DLP-neurons integrate visual, auditory, and somatosensory inputs. Thus, the DLP-projection onto various associative forebrain areas represents a true polysensory thalamo-telencephalic system.


INTRODUCTION
This study aims to clarify two questions regarding the organization of the avian neostriatum caudolaterale (NCL), an area thought to be equivalent to the prefrontal cortex (PFC). First, are visual evoked potentials (VEPs) that can be recorded in and around NCL due to a projection of the n. dorsolateralis posterior thalami (DLP) onto the caudal forebrain? Second, what are the detailed patterns of projections established by ascending DLP-fibers?

The thalamo- and the tectofugal pathways which terminate in the wulst and the ectostriatum, respectively, constitute the main ascending visual systems in birds. Latencies of VEPs are about 22 ms for the wulst and more than 50 ms for the ectostriatum (Parker and Delius, 1972). Since Güntürkün (1984) could record VEPs with latencies of 15 ms in the caudal neostriatum, and since these VEPs were unaffected by wulst lesions, it was concluded that an independent ‘third primary visual system’ had to exist which terminated in the caudal forebrain. However, the thalamic relay of this pathway remained unknown.

Due to its dense dopaminergic innervation (Divac and Mogensen, 1985, Waldmann and Güntürkün), and its multimodal organization (Metzger et al., 1998, Kröner and Güntürkün,
1999), the NCL was suggested to be comparable to the mammalian PFC. Indeed, behavioral studies could demonstrate NCL-lesions to cause deficits in working memory and behavioral flexibility tasks (Gagliardo et al., 1996, Hartmann and Güntürkün, 1998), similar to the situation in PFC. The main thalamic afferents of NCL are the DLP and cells in the adjacent ventrointermediate area of the posterior nuclei (VIP) (Kröner and Güntürkün, 1999). The DLP has been shown to constitute a polysensory entity in which visual, somatosensory, and auditory afferents converge on single neurons (Korzeniewska and Güntürkün, 1990) and which plays a role in working memory tasks (Güntürkün, 1997). Since the DLP is known to project to NCL (Waldmann and Güntürkün, 1993), the aim of the present study was to clarify if DLP is the missing thalamic link of the ‘third primary visual system’ and whether all parts of NCL receive input from DLP.

**METHODS**

Due to the multitude of approaches methods are described only briefly and we rather refer to studies, in which these are described in more detail.

**Anterograde tracing:** A 2.5% solution of *Phaseolus vulgaris leucoagglutinin* (PHA-L) diluted in 0.1 M phosphate buffer (PB, pH 7.2) was injected in two animals over 30 min into the DLP using pulsed positive DC current of 4.5 µA. After 5 days animals were anesthetized and perfused first with 0.9% NaCl (40°C) followed by 4% paraformaldehyde (PFA) in sodium acetate buffer (pH 6.5) and finally by 4% PFA in sodium borate buffer (pH 9.5). Brains were postfixed in 4% PFA in acetate buffer (pH 6.5) for 1 hour and stored in 30% sucrose in PB at 4°C. Then, 25 µm frozen frontal brain sections were cut and processed for ABC immuno-cytocytochemistry. For details of the staining procedure see Gerfen and Sawchenko (1983).

**Retrograde tracing:** In 12 pigeons, small amounts of Fast Blue (FB) or rhodamine isothiocyanate (RITC), each dissolved at 2% in 1% DMSO in distilled water were injected stereotaxically into NCL or the terminal field of DLP within the neostriatum intermedium medialis (NIM). An additional 16 animals received pressure injections of cholera toxin b (CTb) into the same areas within NCL or NIM. After 2-3 days animals were perfused with 0.9% NaCl followed by 4% PFA in PB (pH 7.2, 4°C). Brains were postfixed between 1 - 24 h (4°C) in the fixative with 30% sucrose added, and cut frontally in frozen sections of 40 µm. Fluorescent tracers were visualized using a fluorescence microscope. For details see Korzeniewska and Güntürkün (1990). Immunohistochemistry for CTb followed the procedure described by Shimizu et al. (1995). In brief, slices were incubated overnight (4°C) in a primary antibody against CTb (1:20.000, containing 0.3% Triton X), followed by a goat biotinylated antibody (1:500 in 0.3% Triton PB; both Jackson) and finally the avidin-biotin complex (1:100; Vector). Staining was achieved by the 3,3’-diaminobenzidine-(DAB) technique.

**Electrophysiology:** Eight adult pigeons were anesthetized with equithesin and four 0.12 mm thick insulated stainless steel wires were implanted under stereotaxic guidance into the DLP-termination zone of the NCL of each hemisphere. Electrodes tips were staggered in 1 mm steps. An uninsulated loop of wire placed under the
scalp served as reference. Free ends of electrodes terminated in a miniature connector glued onto the skull. Recordings were performed in awake animals. A red light diode (665 nm, half-bandwidth: 30 nm, intensity: 0.8 mcd, stimulus duration: 5 ms, interstimulus interval: 2.3 s) was placed 5 mm from the eye contralateral to recording. Potentials from electrodepairs were bandpass filtered between 7 and 120 Hz with a notch filter at 50 Hz, and were averaged over 32 trials. At the end of recordings small radiofrequency lesions were made at electrode tips to histologically verify their locations. In three pigeons a lesion electrode was placed in a second surgery into the DLP ipsilateral to the recording site and thermal coagulations (20 mA, 8 s) were made. NCL-recordings were made a few days before and one week after lesions. For further details see Güntürkün (1984).

RESULTS

Visual evoked potentials (VEPs) with first inflection latencies 16 to 22 ms were recorded from NCL. The area examined reached from A 4.5 to A 6.75 and from L 2.00 to L 8.00. Structure and latencies of VEPs were in close accordance with Güntürkün (1984). To test whether the initial VEP components derived from the DLP-projection onto NCL, the DLP was thermally lesioned (fig. 1a). Postoperative recordings revealed a complete absence of VEPs (fig. 1a).

PHA-L injections were centered on DLP but also slightly encroached upon surrounding VIP, n. dorsointermedius posterior thalami (DIP) and n. dorsomedialis anterior thalami (DMA) (Fig. 1b). Labeled axons were observed to proceed rostrally to the fasciculus prosencephali lateralis (FPL). On their ascending route, fibers made dense terminal endings on neurons of n. dorsointermedius ventralis anterior (DIVA) and n. reticularis superior dorsalis (Rsd). Many labeled axons could also be seen in the „somatic“ parts of the basal ganglia, namely the lateral lobus parolfactorius (LPO) and the medial paleostriatum augmentatum (PA). Some fibers were seen dorsal to the lamina hyperstriatica (LH) in the ventral hyperstriatum (HV), the largest terminal field however extended underneath the LH just medially and dorsally of the ectostriatum in the NIM. Some fibers coursed frontally and terminated on neurons of the lamina frontalis superior (LFS). Others proceeded caudally to NCL, where fibers were seen to be loosely distributed and making terminal endings (fig. 1c,f). These axons mainly concentrated in the caudodorsal aspect of NCL (Waldmann and Güntürkün, 1993).

Following page - Figure 1: A: Diagrammatical summary of the electrophysiological experiments. The left part of the diagramm shows the location of the recording sites within NCL and the extent of the DLP lesion. The right part shows examples of VEPs before and after DLP lesion. B: Injection site of PHA-L in the dorsal thalamus. C: Anterogradely labeled fibers in the NCL following the PHA-L injection shown in B. D: Terminal field of DLP afferents in NIM. E: Retrogradely labeled cells in DLP after a CTb injection into the neostriatal area shown in D. F: Diagrammatic representation of the DLP terminal fields in NIM and NCL. Scale bars are 500 µm (B,D), 50 µm (C), and 250 µm (D). Abbreviations used in the figures: ectostriatum (E), n. dorsointermedius posterior thalami (DIP), n. dorsolateralis posterior thalami (DLP), lobus parolfactorius (LPO), neostriatum caudolaterale (NCL), neostriatum intermedium medialis (NIM), paleostriatum augmentatum (PA), ventrointermediate area of the posterior nuclei (VIP).
The majority of fibers terminated in an area reaching from A 4.75 to A 7.25 and L 4.5 to L 8.5 within NCL (Fig. 1d,f). Only very few fibers extended into far lateral and ventral parts of NCL. Injections of retrograde tracers into NCL caudal to A 5.00 revealed few labeled cells in DLP. Injections placed further rostrally labeled neurons in VIP and throughout the complete rostrocaudal extent of DLP. Injections of retrograde tracers into the dense terminal field of the DLP within NIM resulted in a very large number of cells within DLP (fig 1e). Injections into LFS revealed a small number of labeled cells in dorsal DLP.

DISCUSSION

This study clearly reveals that the DLP projects to a number of forebrain somatic and association areas. It could additionally be shown that the thalamic component of the ‘third visual system’ proposed by Güntürkün (1984) is the DLP, projecting to large parts of NCL. The prime argument to propose an independent third visual system to the avian forebrain had been the latencies of VEPs which were shorter in caudal forebrain then in wulst and ectostriatum (Güntürkün, 1984). DLP integrates besides auditory, and somatosensory afferents also a massive visual input from the tectum. Since DLP projects onto NCL, the VEPs of the caudal forebrain are likely due to ascending DLP-projections. This assumption is clearly supported by the absence of VEPs in NCL after DLP-lesions. In addition to afferents from DLP, NCL integrates further visual input from secondary visual forebrain structures like caudal hyperstriatum accessorium (HA) and ectostriatal belt (Eb) (Metzger et al., 1998, Kröner and Güntürkün, 1999). It is possible that these afferents contribute to the late VEP components. However, DLP-lesions had completely diminished VEPs, including their late components. This might be due to the cellular sensory properties of the structures involved. Korzeniewska and Güntürkün (1990) demonstrated that DLP-neurons had broad receptive fields and responded to simple stimulus properties. Given the possible complexity of reponse properties in secondary visual association areas of the forebrain, the simple diode-flash induced VEPs are likely to activate DLP-, but not HA- and Eb-neurons. The dorsal part of NCL which receives the densest DLP innervation has also been shown to receive auditory input via field L, afferents from visual and somatosensory parts of HA, as well as a projection from cells in the terminal field of the DLP within NIM (Kröner and Güntürkün, 1999). It thus seems that the dorsal NCL is reached by intratelencephalic input from those modalities that are also transported by DLP afferents, and, in addition, those parts of NCL receive an indirect input from DLP via NIM. In sum, the DLP supplies the NCL with both a fast and a slow copy of incoming sensory information from
all main modalities, which within NCL can be further compared with other input from the same modalities that originates in higher sensory areas of the telencephalon.

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Characterization of cell types in the chick neostriatum caudolaterale: 
Electrophysiological and morphological properties.

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ABSTRACT
The neostriatum caudolaterale (NCL), in the chick also referred to as dorsocaudal neostriatal complex (dNc), is a polymodal associative area in the forebrain of birds that is involved in sensorimotor integration and memory processes. In chick it is part of an „imprinting network“, and might serve to integrate the various sensory and emotional components of natural imprinting objects. We have used whole-cell patch-clamp recordings in chick brain slices to characterize the principal cell types of the NCL. Electrophysiological properties distinguished four classes of neuron. The morphological characteristics of these classes were examined by intracellular injection of Lucifer Yellow. Type I neurons characteristically fired a brief burst of action potentials. A number of these cells showed distinct low-threshold transient depolarizations. Morphologically, type I neurons had lar somata and thick dendrites with many spines. Type II neurons were characterized by their repetitive firing pattern from which conspicuous frequency-adaptation was observed. Cells in this class could be subdivided by the presence or absence of a time-dependent inward rectification. Type II neurons also had large somata and thick dendrites with many spines. Type III neurons showed a high-frequency firing with little accommodation and a prominent time-dependent inward rectification. They had thin, sparsely spiny dendrites and extensive local axonal arborizations. Type IV neurons had a longer action-potential duration, a larger input resistance, and a longer membrane time constant than the other classes. Type IV neurons had small somata and short dendrites with few spines. The axons of cells in all spiny cell classes (types I, II, IV) showed similar projection patterns to targets outside the NCL. The classes of neuron described here may play distinct roles in sensorimotor integration and learning.

Submitted

INTRODUCTION
The neostriatum caudolaterale (NCL), in the chick also referred to as dorsocaudal neostriatal (dNc) complex (Metzger et al., 1998), is a multimodal association area in the forebrain of birds (Leutgeb et al., 1996; Metzger et al., 1998; Kröner and Güntürkün, 1999). In the chick it has mainly been studied as part of a network responsible for early learning, specifically imprinting (Metzger et al., 1996, 1998; Bock et al., 1997; Bock and Braun, 1999a,b; Braun et al., 1999), whereas in the pigeon most work has focused on tasks which in mammals invoke „frontal“
executive functions, e.g. working memory (Mogensen and Divac, 1982, 1993, Gagliardo et al., 1996, 1997, Güntürkün, 1997; Kalt et al., 1999), reversal learning (Hartmann and Güntürkün, 1998), response inhibition (Güntürkün, 1997, Aldavert-Vera et al., 1999), and spatial orientation (Gagliardo and Divac, 1993). Anatomically, the NCL seems to be particularly well suited for the association between external stimuli and the animals' behavior, as it integrates information from all modalities and exerts influence over motor and limbic structures. In chicks (Metzger et al., 1998) and pigeons (Leutgeb et al., 1996; Kröner and Güntürkün, 1999) the NCL has been shown to be reciprocally connected with all major secondary sensory areas and at least three further multimodal brain areas (i.e. the intermediate and medial part of the hyperstriatum ventrale, IMHV, the mediorostral neostriatum/hyperstriatum ventrale, MNH, and the archistriatum). Thalamic input reaches the NCL from several nuclei in the dorsal thalamus, including the multimodal thalamic nuclei dorsolateralis posterior (DLP) and subrotundus (SRt) (Korzeniewska and Güntürkün, 1990; Leutgeb et al., 1996; Metzger et al., 1998; Güntürkün and Kröner, 1999; Kröner and Güntürkün, 1999). The NCL's descending projections are directed mainly to limbic and somatomotor regions within the basal ganglia and the archistriatum (Kröner and Güntürkün, 1999). Thus, the NCL represents a nexus of sensorimotor integration in the avian forebrain. In the chick, the NCL has therefore been postulated to be a polymodal associative part of the „imprinting pathway“, in which the various sensory and emotional components of natural imprinting objects are integrated during both, the learning process and memory recall (Braun et al., 1999). While imprinting traditionally is conceptualized as a relatively specialized form of learning in young animals that occurs during a sensitive phase and which has permanent behavioral effects (Lorenz 1935), it shares certain features with other forms of associative learning (Bolhuis, et al., 1990), such as stimulus generalization (Bolhuis and Horn, 1992), stimulus comparison (Honey and Bateson, 1996), and the association of stimuli in time (Bateson and Chantrey, 1972). If we are to explain the mechanisms that underlie the sensorimotor integration and associative learning processes, we need to understand in detail how sensory coordinates are mapped onto motor ones according to the learning history of the animal and its current behavioral goals. Similar to the modules of mammalian cortical structures (Douglas and Martin, 1992), the avian forebrain might be constructed from a relatively small number of principal or canonical circuits, that are repeated in large quantities to achieve parallel computing power. Furthermore, it has been shown in numerous preparations that there is generally good correspondence between a neuron's intrinsic firing pattern and its morphology (e.g. McCormick et al., 1985; Chagnac-Amitai et al., 1990; Larkman and Mason, 1990; Kang and Kayano, 1994; Kasper et al., 1994a). Thus, to further our understanding of the functions of the NCL on a cellular level, it is important to characterize its intrinsic neuronal
Chapter 3: Cell types in the chick neostriatum caudolaterale

organization and the cell types that might be involved in the processing of different aspects of information. In this report, we have used in vitro whole-cell patch-clamp recording in combination with intracellular staining to characterize the principal cell types in the chick NCL electrophysiologically and morphologically.

MATERIALS AND METHODS

Preparation of slices and electrophysiological recording

Fertilized eggs were obtained from a commercial supplier (Sörries Trockels, Mönnesee, Germany) and chicks (Gallus domesticus) were hatched and kept in small groups on a 12:12 hour dark/light cycle. Treatment of animals conformed to NIH guidelines. A total of 29 chicks (2 - 11days post-hatch, median 6 d) were decapitated and the brains were transferred to iced extracellular solution. Coronal slices (350 µm thick) of the caudal telencephalon were cut on a vibratome. Slices were incubated in extracellular solution consisting of (in mM): 119 NaCl, 2.5 KCl, 1 NaH₂PO₄, 26.2 NaHCO₃, 10 D-glucose, 4.5 CaCl₂, and 1.3 MgCl₂, saturated with 95% O₂ /5% CO₂, pH 7.3. Slices were allowed to recover for at least 1 hour, before being transferred to the recording chamber. All recordings were made at room temperature (21 - 23°C) in a submerged slice chamber perfused with extracellular solution.

Whole-cell patch-clamp recordings were obtained in the caudolateral subventricular region of the forebrain, using the blind-patch technique (Blanton et al., 1989). In current-clamp experiments recording electrodes (6 – 8 MΩ resistance) were filled with an intracellular solution consisting of (in mM): 135 K-gluconate, 20 KCl, 2 MgCl₂, 10 N-(2-hydroxyethyl)piperazine-N’-(2-ethanesulfonic acid) (HEPES), 0.1 ethylene glycolbis (β-aminoethyl ether)- N,N,N’,N’-tetraacetic acid (EGTA), 4 Na₂-ATP, and 0.5 Na₂-GTP, and adjusted to pH 7.3 with KOH. In some recordings 3 - 3.5 mg/ml of the dipotassium salt Lucifer Yellow (LY, Sigma, Deisenhofen, Germany) were added to the intracellular solution and neurons were filled by diffusion during 30 - 90 minutes of recording. In a further set of experiments K-gluconate and KCl in the intracellular solution were substituted with 135 mM CsCl and 20 mM tetraethylammonium (TEA) to block potassium channels. Recordings were made using a HEKA (Lambrecht, Pfalz, Germany) EPC-7 patch-clamp amplifier, filtered at 3 kHz, and sampled at 20 kHz. The voltage drop across the pipette (which was usually about 8 - 10 mV) could not be bridge-balanced.

Sampling was done with a Digidata 1200 interface using pClamp 6.0 software (Axon Instruments, Foster City, CA), and data were stored on computer for off-line analysis with the CLAMPFIT module of pClamp. Input resistance was determined from the voltage deflection induced by a hyperpolarizing current pulse in the linear range of the current-voltage relation. Membrane time constants were calculated by fitting a single exponential function to the voltage response to injections of hyperpolarizing currents in the linear range of the voltage response. The percentage sag that occurred in some neurons in response to hyperpolarizing and depolarizing current pulses was calculated as 100 x (V_max - V_end)/V_max, where V_max was the peak voltage deflection and V_end the voltage at the end of the current pulse. Resting membrane potentials were assessed in current-clamp mode 3 - 5 minutes after establishing the whole-cell configuration. Spike duration was measured at the half-maximal amplitude from threshold, and the amplitude and time-to-peak value for the afterhyperpolarization (AHP) which followed the action potential were measured from the equipotential-point on the repolarizing phase.
Data analysis. Data are presented as means ± standard error (SE). Statistical analyses were done using the SPSS 8.0 software package. The differences among cell classes for the various parameters were compared by analysis of variance (ANOVA) and the statistical significance of the differences was examined with a Scheffé test for multiple comparisons. Subsequent comparisons between and within cell classes were done using Student’s $t$ test for unpaired samples.

Histological procedures
Following recording, slices were fixed in 4% paraformaldehyde and 0.2% glutaraldehyde in 0.12 M phosphate buffer (4°C, pH 7.4) for about 15 hours. They were then transferred to a solution of 30% sucrose in phosphate buffer containing 0.9% NaCl (PBS; pH 7.4) and 0.01% NaN$_3$ as a preservative. Slices were resectioned at 70 µm on a freezing microtome and collected in PBS. For immunohistochemistry of LY, endogeneous peroxidases were blocked by preincubating slices in 0.5% H$_2$O$_2$. Slices were then washed in PBS and floating sections were incubated overnight at 4°C in biotynilated anti-LY from rabbit (Molecular Probes, Leiden, The Netherlands; 1:200 working dilution) in PBS containing 0.3% Triton X-100 (Sigma). After washing, slices were incubated for 2 hours in the avidin-biotin complex (ABC Elite, Vector Labs, Burlingame, CA; 1:100 in PBS with 0.3% Triton X-100). Washes in PBS were followed by additional washes in 0.12 M acetate buffer (pH 6). Staining was achieved by the 3,3′-diaminobenzidine (DAB) technique with heavy-metal amplification (modified from Adams, 1981) by adding H$_8$N$_2$NiO$_8$S$_2$ (2.5 g/ 100 ml), NH$_4$Cl and CoCl$_2$ (both 40 mg/ 100 ml). After 20 minutes of preincubation the reaction was catalyzed using 0.3% H$_2$O$_2$. The reaction was stopped by rinsing the tissue in acetate buffer and PBS. Slices were then mounted, dehydrated and coverslipped. Quantative measures of cell morphology were done using a Leica DMR (Leica, Wetzlar, Germany) and the analySIS software package (Soft-Imaging Software, Münster, Germany). Camera lucida reconstructions of filled cells were drawn using a Leitz BioMed with a drawing tube at x 12.5 or x 50 magnification.

RESULTS
Stable whole-cell recordings were obtained from 90 cells in the NCL. Neurons were selected only if they exhibited a resting membrane potential (RMP) more negative than -50 mV and an overshooting action potential. In this voltage range, there was a general lack of spontaneous firing in all NCL cells, however, in voltage clamp recordings spontaneous postsynaptic currents could be observed in most cells, indicating synaptic activity (data not shown). Neurons were classified into 4 distinct types according to differences in their intrinsic firing properties and in the voltage responses to hyperpolarizing current pulses. For 36 of the neurons thus characterized basic morphological features were analyzed after filling with LY. With a single exception in which a second cell was weakly stained, we found no evidence of dye-coupling between cells in the NCL. Dye-coupling results from neuronal signalling via gap junctions and is a phenomenon commonly observed in adult (Connors et al., 1983; Benardo, 1997), but
particularly in developing mammalian (Connors et al., 1983; Kasper et al., 1994b) and avian (Dutar et al., 1998) neurons, where it is believed to be involved in the functional specification of brain areas (Peinado et al., 1993).

**Type I neurons:**

Type I cells displayed a characteristic firing pattern that consisted of a single action potential or a brief burst of spikes. Type I cells represented 34 of the 90 cells characterized.

*Firing properties:* Cells in this class displayed the highest threshold for firing an action potential, especially in relation to their low resting membrane potential (table 1). Type I cells were marked with their tendency to fire a single action potential or 2 - 8 clustered spikes at high frequency at the onset of depolarizing pulses (fig. 1A). The number of spikes in a burst and the duration of the interspike-intervals strongly depended on the current amplitude injected (fig. 1A, C). No repetitive bursting was observed during long (1.1 sec.) depolarizing current pulses. However, in the course of other experiments (data not shown) spontaneous occurring all-or-none bursts were found when fast GABAergic transmission was abolished by adding picrotoxin (100 µM) to the extracellular solution (see below).

Type I cells varied with respect to their behaviour in the subthreshold voltage range: In response to subthreshold depolarizing current pulses 13 type I cells showed prominent low-threshold potentials that were not seen in the remaining neurons (c.f. fig. 1A1 and 1A2). This subset of neurons also differed from the remaining type I cells in that their action potentials had significantly larger amplitudes (60.9 ± 3.3 mV vs. 51.6 ± 2.9 mV) and shorter durations at half maximal amplitude (1.72 ± 0.1 ms vs. 2.0 ± 0.08 ms), the latter being mostly due to shorter rise-times of the action potentials (0.58 ± 0.05 ms vs. 0.74 ± 0.05 ms; Student’s *t* test for unpaired samples; df = 32; all values for *t* > ± 2.1; *p* < 0.05). Further, 6 of the 13 cells that showed these transient „hump“-like depolarizing responses also displayed a complex pattern of afterpotentials that followed the first spikes. These consisted of an initial, fast afterhyperpolarization (fAHP) that was followed by an intercalated depolarizing afterpotential (DAP) and a late medium-duration AHP (mAHP; see fig. 1A1). The remainig type I cells showed only a monophasic AHP (c.f. fig. 1A2).

*Membrane rectification:* Type I cells had low apparent input resistances and intermediate membrane time constants (table 1). In response to hyperpolarizing current pulses that drove the membrane potential more negative than about -80 mV, all type I neurons showed a pronounced fast-activated inward rectification resulting in an upward bend in the current-voltage relation (fig. 1D). Most type I cells also showed membrane outward rectification at the end of the
Figure 1: Intrinsic firing properties and membrane rectification of type I neurons. A: Examples of voltage responses to hyperpolarizing and depolarizing current pulses (-400 - 700 pA in A1 and -300 - 800 pA in A2) in two neurons that showed rapid adaptation of action potential firing. Type I cells typically responded with „bursts“ of action potentials to current pulses of increasing intensity. Both cells show membrane inward rectification to relatively large hyperpolarizing currents and outward rectification to depolarizing currents in the spike-threshold range. The cell in A1 displays a prominent subthreshold „hump“-like potential and a depolarizing afterpotential following single spikes. The cell in A2 shows only monophasic afterhyperpolarizations of comparatively long duration. Note that there is also apparently less membrane rectification in this cell than in the cell shown in A1. B: Camera lucida reconstruction of a type I neuron. The soma and dendrites are drawn in black, the axon collaterals are shown in grey. Scale bar is 50 µM. C: Relationship between the instantaneous spike frequency (as the inverse of inter-spike-interval) and time for different current amplitudes for the cell shown in A1. D: Current-voltage relationship of the cell shown in A. Deviations of the potential from the extrapolated ohmic responses close to resting potential reflect inward and outward rectification, respectively.
current pulse, that was responsible for a downward bend in the I-V plot at depolarized potentials (fig. 1A).

**Morphology:** Of the 34 cells designated as type I cells 15 were successfully recovered and reconstructed. Single or burst firing neurons had large round or fusiform somata (diameter 15 - 22 µm; table 2) and multipolar arranged dendrites. Dendrites were usually long (dendritic field diameters 225 - 320 µm; table 2) and displayed medium to high spine densities (figs. 1B, 2, and 10). The main axon branched near the soma and gave rise to thin collaterals that ramified mainly in a loose local plexus (figs. 1B, 2, and 10). In addition to these recurrent collaterals which displayed numerous varicose-like swellings, often 2 - 5 longer collaterals were observed that projected outside the NCL. These axonal arborizations showed less varicosities and followed one of three general directions: In most cases 1 - 3 collaterals moved ventrally within the plane of the slice to terminate within the underlying archistriatum. Other collaterals travelled through the NCL either dorsomedially, towards the auditory subunit of the NCL, the neostriatum dorsale (Nd), and possibly other sensory forebrain areas, or ventromedially, in the direction of the basal ganglia (c.f. Metzger et al., 1998; Kröner and Güntürkün, 1999). Ventromedially directed arborizations, however, could be followed only over relatively short distances before they left the sagittal plane of the slice.
Type II neurons

Type II cells were characterized by their repetitive firing pattern. They represented 35 of the 90 neurons studied.

Firing properties: Type II had similarly low resting membrane potentials, spike amplitudes and action potential durations, as well as AHP amplitudes and time-to-peak values as type I neurons (table 1). In contrast to type I neurons, however, small somatically injected depolarizing current pulses readily elicited relatively tonic firing in type II cells. Two typical examples of type II cells are shown in figure 3A. Current pulses that depolarized type II cells just above threshold usually evoked a single action potential that often had long latencies. With larger depolarizing currents a pattern of phasic-tonic firing emerged, in which a short burst, was followed by a longer train of action potentials. This train of action potentials showed conspicuous frequency adaptation (fig. 3A lower traces, and C), but the repetitive firing pattern could be sustained at relatively low frequencies (between 5 -15 Hz) over long (1.1 sec) depolarizing current pulses. This firing behaviour was facilitated by a comparatively low action potential threshold (table 1). A number of type II cells (n = 8) showed the same sequence of afterpotentials following an action potential as described for the subset of type I neurons above.

Membrane rectification: Among the four types identified, type II neurons had intermediate input resistances and time constants (table 1). Type II cells differed with regard to the existence of a hyperpolarization-activated time-dependent inward rectification: When hyperpolarizing current pulses were applied to type II cells usually (n = 22) a fast-activated inward rectifying conductance appeared at membrane potentials more negative than about -80mV (fig. 3A1). This inward rectification resulted in a slight upward bend in the hyperpolarized portion of the current-voltage plot when compared with the extrapolated linear portion (fig. 3D). In another 13 neurons, however, a small sag in the voltage response (2.1 - 6.7 % sag) could be seen at potentials more negative than about -80 mV. This delayed inward rectifying conductance tended to push the membrane potential back towards resting values and lead to a rebound overshoot at the termination of hyperpolarizing current pulses (fig. 3A2, 13B). The amplitude of the sag increased with more hyperpolarizing membrane potentials. These cells in addition seemed to possess the same fast-activated inward rectification seen in the remaining type II neurons (above) as their peak voltage responses exhibited inward rectification over the same range of membrane potentials as the time dependent inward rectification (fig. 3D). At depolarizing voltage steps the membrane potential often depolarized more than expected from the ohmic response around resting potential, indicating the contribution of an inward rectification in the voltage range close to spike threshold (see below).
Figure 3: Intrinsic firing properties and membrane rectification of type II neurons. A: Examples of voltage responses to hyperpolarizing and depolarizing current pulses (-300 - 700 pA) in two neurons that showed a phasic-tonic firing of action potentials over long depolarizing current. The cell in A1 displays fast inward rectification to hyperpolarizing current pulses. The cell in A2 shows the same fast inward rectification and additionally a sag in the voltage response to relatively large hyperpolarizing current pulses, resulting in a rebound overshoot at cessation of current pulses. See text for detail. B: Camera lucida reconstruction of a type II neuron. The soma and dendrites are drawn in black, the local axon collaterals are shown in grey. Scale bar is 50 µM. C: Relationship between the instantaneous spike frequency and time for different current amplitudes for the cell shown in A2. After a phasic response the spike frequency rapidly adapts to a steady-state tonic firing. D: Current-voltage relationship of the cell shown in A2. ●, voltage response measured at the end of the current pulse; ▲, voltage at the peak.
During long current pulses the membrane potential sagged back towards rest (4.8 %– 19.4 % sag) resulting in a rebound undershoot at the termination of the current pulse (figs. 3A2, 12B).

Morphology: The gross morphology of type II cells resembled that of type I cells (figs. 3B, 4, 10). They had large oblique somata (diameter 16 - 23µm; table 2) and long, thick multipolar dendrites. In 4 neurons that were thoroughly analyzed, type II cells displayed the highest number of spines, but this value was not significantly different from that obtained for type I neurons (table 2). Dendrites of type II neurons had the largest number of branch points and covered large areas (diameter of dendritic field 229 - 296 µm; table 2). The axons of type II neurons often formed a radial axonal plexus in the vicinity of the soma giving rise to several local ramifications as well as long collaterals that projected to targets beyond the borders of the NCL. As in type I cells, most neurons sent 1 - 3 descending collaterals towards the archistriatum, but also had additional arborizations that seemed to travel ventromedially and dorsomedially (fig. 5), probably making numerous contacts with other cells within NCL on their way.

Figure 4: Morphological features of type II cells. A, B: Photomicrographs of two Lucifer Yellow-filled cells that responded with phasic-tonic firing of action potentials in response to depolarizing currents. The arrow in A points to where the axon leaves the cell body. It courses ventrally and ramifies extensively at the bottom of the photomicrograph. The cell in B is the same cell as shown in figure 3B. C: Detail of a type II cell showing a dendrite with numerous spines and part of an axon (top left). Scale bars are 50 µm (A, B), and 20 µm (C).
Type III neurons:
The key features of type III neurons were the ability to fire action potentials at a high frequency and a prominent sag in the response to hyperpolarizing current pulses, as well as a short action potential duration. Type III cells represented 8 of the 90 cells characterized.

Firing properties: In response to suprathreshold depolarizing current pulses all neurons designated as type III responded with regular tonic firing that showed only relatively little frequency adaptation. Thus, type III cells were able to initially fire action potentials at frequencies of about 100 Hz. After several spikes the firing rate usually accommodated to some degree, but sustained firing over long depolarizing current pulses continued at frequencies much higher than found in any other cell class (fig. 6C). These cells also showed the most positive resting membrane potentials, while generally having a very low threshold for the initiation of action potentials (table 1). The duration of action potentials in type III cells was by far the shortest among all classes, which was also reflected by short rise- and fall-times (table 1). The action potential was followed by an AHP that was large in amplitude and had a comparatively

Figure 5: Example of the axonal arborizations of a type II cell within NCL. In addition to recurrent local arborizations most neurons from all spiny cell classes possessed one or several axon collaterals that left the NCL, ventrally towards the archistriatum, ventromedially, in the direction of the basal ganglia, and/or dorsomedially, possibly to provide feedback to sensory and associative forebrain areas. The insert shows the location of the cell in the dorsocaudal neostriatum. Scale bars are 500 µM.
Table 1: Electrophysiological characteristics of classes of NCL neurons (means ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>Type I (n = 34)</th>
<th>Type II (n = 35)</th>
<th>Type III (n = 8)</th>
<th>Type IV (n = 13)</th>
<th>Significant differences*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting potential (mV)</td>
<td>-75.1 (± 0.9)</td>
<td>-73.9 (± 0.9)</td>
<td>-63.5 (± 1.4)</td>
<td>-68.3 (± 1.8)</td>
<td>Type III, IV &gt; type I, II</td>
</tr>
<tr>
<td>Input resistance (MΩ)</td>
<td>210.5 (± 12.1)</td>
<td>241.6 (± 13.1)</td>
<td>458.6 (± 28.6)</td>
<td>568.5 (± 28.7)</td>
<td>Type IV &gt; type III &gt; type I, II</td>
</tr>
<tr>
<td>Membrane time constant (ms)</td>
<td>59.5 (± 2.8)</td>
<td>67.6 (± 5.3)</td>
<td>46.1 (± 6.2)</td>
<td>91.6 (± 8.3)</td>
<td>Type IV &gt; type I, II, III</td>
</tr>
<tr>
<td>Spike threshold (mV)</td>
<td>-25.0 (± 1.2)</td>
<td>-30.3 (± 1.0)</td>
<td>-33.6 (± 1.7)</td>
<td>-27.5 (± 0.7)</td>
<td>Type I &gt; type II, III</td>
</tr>
<tr>
<td>Max. number of spikes (1.1s depolarizing pulse)</td>
<td>3.4 (± 0.5)</td>
<td>10.6 (± 0.9)</td>
<td>46.3 (± 5.9)</td>
<td>7.6 (± 0.8)</td>
<td>Type III &gt; type II &gt; type I, IV</td>
</tr>
<tr>
<td>Spike duration at half amplitude (ms)</td>
<td>1.9 (± 0.07)</td>
<td>1.86 (± 0.1)</td>
<td>0.76 (± 0.1)</td>
<td>2.32 (± 0.1)</td>
<td>Type IV &gt; type I, II &gt; type III</td>
</tr>
<tr>
<td>Rise time (10-90%) ms</td>
<td>0.67 (± 0.03)</td>
<td>0.72 (± 0.04)</td>
<td>0.43 (± 0.06)</td>
<td>1.15 (± 0.07)</td>
<td>Type IV &gt; type I, II &gt; type III</td>
</tr>
<tr>
<td>Fall time (90-10%) ms</td>
<td>1.41 (± 0.1)</td>
<td>1.64 (± 0.05)</td>
<td>0.94 (± 0.1)</td>
<td>1.9 (± 0.08)</td>
<td>Type IV &gt; type I &gt; type III; type II &gt; type III</td>
</tr>
<tr>
<td>Spike amplitude (mV)</td>
<td>54.7 (± 2.3)</td>
<td>51.9 (± 2.0)</td>
<td>49.4 (± 6.7)</td>
<td>45.2 (± 2.3)</td>
<td>–</td>
</tr>
<tr>
<td>AHP amplitude (mV)</td>
<td>-17.8 (± 0.7)</td>
<td>-17.5 (± 0.7)</td>
<td>-20.3 (± 1.7)</td>
<td>-15.8 (± 1.1)</td>
<td>–</td>
</tr>
<tr>
<td>AHP time to peak (ms)</td>
<td>3.4 (± 0.2)</td>
<td>4.1 (± 0.2)</td>
<td>5.6 (± 0.6)</td>
<td>5.8 (± 0.5)</td>
<td>Type III, IV &gt; type I, II</td>
</tr>
</tbody>
</table>

* Significant differences indicated by the use of a Scheffé test for multiple comparisons after analysis of variance (P < 0.05).

long time-to-peak (table 1). Both the amplitude and the time-to-peak could be reduced to a much larger degree than in the other cell types when increased depolarizing currents drove type III neurons to high-frequency tonic firing.

Membrane rectification: Type III cells had comparatively high input resistances but relatively short membrane time constants (table 1). In response to hyperpolarizing current pulses (membrane potential more negative than -80 mV) a prominent sag in the voltage response (8 - 20.4% sag) occurred that was followed by a rebound depolarization at cessation of the current pulse. The time dependent inward rectification and the rebound overshoot increased as the hyperpolarizing current pulses increased. In response to larger negative current pulses the rebound depolarization could initiate action potentials (fig. 6A). Type III neurons also appear to possess an additional fast-activated inward rectifier, as the peak voltage responses exhibited inward rectification over the same range of membrane potentials as the time dependent inward rectification, (fig. 6D).

Morphology: Type III cells were characterized by small fusiform somata (diameter 10 - 14µm; table 2) and thin, aspiny or sparsely spiny dendrites (figs. 6B, 7, 10). In fact, in 3 out of 6 neurons that were successfully reconstructed the dendrites were so thin that they could not be
Figure 6: Intrinsic firing properties and membrane rectification of a type III cell. A: Voltage responses to hyperpolarizing and depolarizing current pulses (-120 - 240 pA). There is a large sag in response to strong hyperpolarizing current pulses, which results in a rebound overshoot that is large enough to trigger spikes. Small depolarizing currents elicit continuous firing at comparatively high frequencies. B: Camera lucida reconstruction of a type III neuron. Scale bar is 50 µM. C: Relationship between the instantaneous spike frequency and time for different current amplitudes for the cell shown in A. There is some frequency adaptation after the first few spikes, but the cell maintains a high-frequency firing without further attenuation over a long current pulse. D: Current-voltage relationship of the cell shown in A. ◯, voltage at peak; ▲, voltage at the end. 
clearly distinguished from the extensive local axonal ramifications. The remaining three cells had relatively few long multipolar dendrites (diameter of dendritic field 227 - 267 µm). The dendrites showed a low number of total branch points and especially few higher order (≥ 4th branches) branchings (table 2). The axon appeared to have a large number of varicosities, and arborized extensively to form a dense plexus of terminals in the vicinity of the soma (fig. 7). Axons of type III neurons could never be followed beyond the borders of the NCL. The diameter of axonal arborizations ranged between 545 - 736 µm and the mean brain area that was covered by these arborizations was 320,400 µm² (s.e. ± 35,503 µm²).

**Type IV neurons:**

The key features of type IV cells are a high input resistance and a long membrane time constant, as well as a long action potential duration. Of the 90 cells characterized, 13 were found to belong to this class.

**Firing properties:** Among the classes identified here, type IV cells had an intermediate RMP and spike-threshold. Their bursting spike pattern resembled that of type I cells (fig. 8), but type IV neurons were able to initially fire a larger number of action potentials (table 1). However, in response to large depolarizing currents the ability of type IV neurons to fire repetitively was markedly attenuated (fig. 8C). A characteristic of cells in this class was the long action potential duration, which was also reflected in the significantly longest rise- and fall-times (table 1). Furthermore, the action potentials of type IV cells showed a prominent progressive spike broadening during repetitive firing.

*Figure 7: Morphological features of type III cells. A: Photomicrograph of a Lucifer Yellow filled sparsely spineous type III cell. B: Detail of the cell shown in A. Through the middle of the photomicrograph runs a long thin dendrite; parallel to this run axon collaterals above and below. C: Part of the local axonal arborizations of another type III cell. Scale bars are 50 µm (A, B), and 20 µm (C).*
Type IV cells had comparatively small monophasic AHPs, but long times-to-peak (table 1). 

**Membrane rectification:** The key feature required for type IV cells was a long membrane time constant (> 60 ms). They also had the highest input resistance (> 350 MΩ) among all classes of cells (table 1) and showed steep current-voltage relationships (fig. 8C). Only at membrane potentials more negative than about -95 mV in most neurons a weak, fast-activated inward

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**Figure 8:** Intrinsic firing properties and membrane rectification of a type IV cell. A: Voltage responses to hyperpolarizing and depolarizing current pulses (-180 - 360 pA). The cell shows large voltage responses to small hyperpolarizing current pulses with relatively little inward rectification. Small depolarizing current pulses readily elicit short bursts of action potentials. B: Camera lucida reconstruction of a type IV neuron. The soma and dendrites are drawn in black, the axon collaterals are shown in grey. Scale bar is 50 µM. C: Relationship between the instantaneous spike frequency and time for different current amplitudes for the cell shown in A. D: Current-voltage relationship of the cell shown in A. The input resistance is high, and the voltage response shows only little inward rectification to large current pulses.
inward rectification became evident (fig. 8D). All type IV cells showed a prominent membrane outward rectification at depolarized potentials.

**Morphology:** Type IV cells were characterized by small, oblique somata (diameter 12 - 16.5 µm; table 2) and multipolar dendrites that possessed few thin spines. Usually, proximal dendrites were short and thin, but sometimes 2 or 3 main dendrites were seen that had thick stems but tapered considerably with distance from the soma (figs 8B, 9). The otherwise spherical form of the dendritic field (diameter 180 - 225µm) was tilted by these dendrites as they also extended further than the others. The dendritic tree of type IV neurons showed relatively few branches and covered the smallest area among the four cell types (table 2). Axonal arborizations of type IV cells were not as extensive as in type I or type II cells, but projections followed the same pattern seen in the other classes of spiny neurons. One to three main collaterals descended ventrally in the direction of the archistriatum, while other collaterals travelled dorsomedially and/or ventromedially within the NCL.

Table 2: Morphological characteristics of classes of NCL neurons (means ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
<th>Significant differences*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 15)</td>
<td>(n = 11)</td>
<td>(n = 6)</td>
<td>(n = 5)</td>
<td></td>
</tr>
<tr>
<td>Soma size (µm²)</td>
<td>217.8 ± 7</td>
<td>252.6 ± 9</td>
<td>121.8 ± 7</td>
<td>142.6 ± 9</td>
<td>Type I, II &gt; type III, IV</td>
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<td>Total dendritic length (µm)</td>
<td>4832 ± 281</td>
<td>5921 ± 354</td>
<td>2228 ± 48†</td>
<td>22212 ± 589</td>
<td>Type I, II &gt; type III, IV</td>
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<tr>
<td>Area of dendritic arborization (µm²)</td>
<td>56449 ± 4251</td>
<td>59845 ± 4294</td>
<td>44412 ± 538†</td>
<td>28782 ± 3133</td>
<td>Type I, II &gt; type IV</td>
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<tr>
<td>Number of branch points</td>
<td>51.7 ± 2.2</td>
<td>61.9 ± 4.6</td>
<td>24.6 ± 1.4†</td>
<td>28.7 ± 0.3</td>
<td>Type I, II &gt; type III, IV</td>
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<tr>
<td>Number of spines (n/10µM) initial segment</td>
<td>1.47 ± 0.59‡</td>
<td>2.91 ± 0.89‡</td>
<td>- †</td>
<td>2.18 ± 0.78‡</td>
<td>-</td>
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<tr>
<td>2nd branching</td>
<td>5.06 ± 0.22‡</td>
<td>6.60 ± 1.08§</td>
<td>0.89 ± 0.32†</td>
<td>2.87 ± 0.20§</td>
<td>Type II &gt; type III, IV; Type I &gt; type III</td>
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<td>3rd branching</td>
<td>5.46 ± 0.35‡</td>
<td>6.89 ± 0.74§</td>
<td>1.32 ± 0.31†</td>
<td>2.83 ± 0.41§</td>
<td>Type II &gt; type III, IV</td>
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<td>4th branching</td>
<td>5.11 ± 0.87‡</td>
<td>6.59 ± 0.70§</td>
<td>0.55 ± 0.02†</td>
<td>2.96 ± 0.55§</td>
<td>Type II &gt; type III, IV; Type I &gt; type III</td>
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<td>5th and 6th branching</td>
<td>5.71 ± 2.32‡</td>
<td>7.23 ± 1.03§</td>
<td>- †</td>
<td>2.82 ± 0.65‡</td>
<td>n.a.</td>
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<td>Direction of axon</td>
<td>ventrally, ventrally, locally ventrally, dorsomedially, and ventromedially</td>
<td>ventrally, dorsomedially, and ventromedially</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Significant differences indicated by the use of a Scheffé test for multiple comparisons after analysis of variance (P < 0.05). † These values could be obtained in only three type III neurons (see text for details). ‡ Spine frequencies were counted in four neurons of each class. n.a.: not enough cases for statistical comparison.
Mechanisms underlying membrane rectification and firing properties in type I and type II neurons:
In a number of neurons the mechanisms underlying membrane rectification and possibly burst generation were studied. Due to the relatively low incidence of recordings that could be obtained from type III and type IV neurons under blind-patch conditions these analyses were restricted to type I and type II cells. In NCL neurons inwardly rectifying properties in the hyperpolarized voltage range seem to depend on K\(^+\) conductances. Blockade of K\(^+\) channels by extracellular CsCl (5 mM) abolished fast inward rectification to hyperpolarizing current pulses in 5 type II neurons which showed rapidly adapting burst firing and 2 regular spiking neurons classified as type II cells. Similarly, in a third type II neuron which showed sag in the voltage response to hyperpolarizing current pulses under control conditions, both the fast and the time-dependent inward rectifying conductances were blocked by CsCl (data not shown).

Rectification in the depolarized voltage range was studied in one type I neuron and two type II neurons. The firing characteristics of these cells were recorded before and after adding 1 µM tetrodotoxin (TTX) to the perfusate (figure 11). As expected for a Na\(^+\) dependent action potential, TTX completely eliminated the generation of spikes in all cells tested. Under control conditions the type I cell showed transient depolarizing „hump” potentials in the subthreshold range which preceeded the generation of spikes (fig 11A, left column). These hump-potentials

Figure 9: Morphological features of type IV cells. A, B: Two examples of Lucifer Yellow filled cells that had high input resistance, small action-potentials of long duration and showed only little inward rectification to hyperpolarizing current pulses. C: Detail Scale bars are 50 µm (A, B), and 20 µm (C).
Figure 10: Comparison of the dendritic morphology and the appearance of local axonal arborizations in the 4 cell types described here. Somata and dendrites are drawn in green, axon collaterals in red. With regard to spine density and dendritic thickness the examples shown here for the type I and type II neurons represent extremes of what appeared to be a continuum. Scale bars are 50 μM.
persisted in the presence of TTX (fig 11A, right column), indicating that they might be mediated by a Ca\(^{++}\) conductance. Like most type I neurons this cell also showed membrane outward rectification, measured at the end of the current pulse, both under control conditions and in the presence of TTX. In the type II neurons there was a small anomalous rectification in the depolarized voltage range under control conditions. The membrane potential tended to ,,sag ,, back towards rest and an undershoot of the potential occurred after cessation of the current pulse (fig. 11B, left column). The underlying current that mediates the inward rectification in the depolarized voltage range is sensitive to TTX blockade. After bath application of TTX depolarizing pulses unveiled activation of an outward rectifying conductance (fig 11B, right column).

**Figure 11:** Membrane rectification in a type I neuron (A) and a type II neuron (B) before (left traces) and after blockade of sodium channels by bath-application of tetrodotoxin (TTX, right traces). A: Under control conditions single spikes were followed by depolarizing afterpotentials in this type I neuron. Application of TTX abolishes the fast action potentials, but small transient depolarizing potentials persist at the beginning of strong depolarizing current pulses. This neuron also showed outward rectification at depolarized potentials under both conditions. B: Under control conditions this type II neuron showed inward rectification and sag in the response to depolarized current pulses, and a voltage undershoot after the cessation of current pulses. Application of TTX abolishes the inward rectification and the undershoot.

In the course of other experiments (data not shown) we had observed spontaneously occurring all-or-none bursts in type I and type II neurons when picrotoxin (100 µM), a Cl\(^-\) channel blocker that eliminates the fast inhibitory effects of GABAergic interneurons, was added to the external solution. In these spontaneous bursts usually 4 - 6 spikes rode on a depolarizing envelope that was followed by an afterhyperpolarization of relatively long duration (tens to hundreds of ms).
(fig. 12). In addition, when depolarizing current steps were applied from hyperpolarized potentials (about -90 mV) both type I and type II cells responded with an increase in the number of spikes, indicating the contribution of a voltage-gated Ca$^{++}$ conductance, that is normally inactive at more positive holding potentials (e.g. Llinás and Yarom, 1981). However, changing the cells holding potential with DC injections never changed the cell’s firing pattern (c.f. McCormick and Feeser, 1990; Livingston and Mooney, 1997).

Taken together, these observations indicated that calcium conductances contribute to the firing behaviour of cells in the NCL. To test this assumption an additional 7 neurons (8 days post-hatch) were recorded under conditions suitable to evoke calcium spikes. Electrodes were filled with CsCl (135 mM) and tetraethylamonium (TEA; 20 mM) to block most K$^{+}$ conductances. Immediately following establishment of the whole-cell configuration, a cells firing pattern was assessed in voltage-clamp. Of the 7 cells tested, 4 cells showed firing characteristics of type I cells, while the remaining 3 neurons maintained repetitive spiking throughout the duration of the depolarizing potential step. Following these measurements TTX (1 µM) was bath applied. When K$^{+}$ conductances were blocked all neurons tested were able to generate slow action potentials in the presence of 1 µM TTX (fig. 13). Such slow action potentials are most likely mediated by Ca$^{++}$ influx (c.f. McCormick and Prince, 1987).

Figure 12: Spontaneous bursts of action potentials occurred in type I and type II cells when GABAergic inhibition was diminished by adding picrotoxin (100 µM) to the extracellular solution. In these bursts 4 - 6 fast action-potentials rode on a slowly developing depolarizing envelope that was followed by a fast afterhyperpolarization (fAHP) and a medium-duration afterhyperpolarization (mAHP), often with an intercalated depolarizing afterpotential (DAP), see insert.

Figure 13: Examples of Ca$^{++}$ spikes that could be evoked in large spiny neurons (n = 7) when K$^{+}$ conductances were blocked by adding tetraethylamonium (TEA; 20 mM) and CsCl (135 mM) to the pipette solution, and sodium conductances were blocked by extracellular tetrodotoxin (TTX; 1µM).
DISCUSSION

We have shown the existence of at least 4 cell types within the NCL of chicks that differ in their intrinsic electrophysiological and morphological properties. In general, there was a good correspondence of physiological and morphological criteria, although the two main cell types, type I and II, could not be clearly differentiated by morphological criteria. Neurons from animals between 2 and 11 days post hatch were studied. As chicks are relatively mature at hatching and capable of independent action most behavioural studies that test the animals ability to learn are undertaken within the first two weeks after hatch. As discussed below, several electrophysiological characteristics that differentiated among neurons between (and within) cell types showed some degree of variation related to age, thus probably reflecting developmental changes. In addition, our finding of 4 main cell types does not exclude the existence of further classes of neurons within NCL.

Cell types:

Type I: Type I neurons possessed a high action potential threshold, particularly in relation to their low resting potential, and they showed prominent outward rectification in response to depolarizing current pulses. These properties indicate that strong, temporally or spatially integrated excitatory inputs are necessary for type I neurons to fire. If sufficiently depolarized, cells in this class preferentially responded with bursts of action potentials. Interestingly, in the mammalian cortex it has also been observed that the threshold of bursting neurons is sometimes higher than that for neurons generating single spikes (Mason and Larkman 1990).

We have used the occurrence of transient „hump“-like depolarizing responses to describe subsets among the neurons which were grouped as type I cells, according to their burst-firing behaviour. Neurons that displayed prominent subthreshold „hump“-potentials and those that did not, differed in several basic parameters, i.e. input resistance, spike amplitude, spike duration and rise time. In mammals these properties have been shown to be particularly prone to developmental changes during early postnatal development (McCormick and Prince, 1987; Kasper et al., 1994b). Thus it is conceivable that the differences among type I neurons reflect at least in part developmental differences. Cells showing transient „hump“-like depolarizing responses were indeed recorded in slices from animals with higher mean ages (7.1 days vs. 5.4 days), a difference that was statistically significant (df = 32; t = -2.7; p < 0.05; Student’s t test for unpaired samples). Similarly, developmental differences might explain the occurrence of a complex pattern of afterpotentials in some, but not all, type I and type II neurons. Single action potentials or bursts were sometimes followed by three distinct afterpotentials: an initial fast, and a late slow after-hyperpolarization, separated by an intercalated depolarizing
afterpotential. In mammalian cortical neurons the two phases of the AHP appear to be due to the activation of Ca\(^{++}\)-dependent K\(^+\) currents (Lancaster and Adams, 1986; Schwindt et al., 1988) whereas the DAP results from the activation of a variety of Ca\(^{++}\) dependent currents (Higashi, 1993; Yang et al., 1996; Haj-Dahmane and Andrade, 1997). The expression of the DAP and the emergence of a tripartionate AHP have been shown to depend on changes during postnatal development (Kasper et al., 1994b). Similarly, in mammals burst firing does not develop before the third postnatal week (Kasper et al., 1994b).

A common factor pertinent to the occurrence of either the „humps“ and the DAPs, as well as the expression of full bursting behaviour in our preparations might be an increase in Ca\(^{++}\) conductances during development. Calcium currents are believed to underlie burst firing in mammalian central neurons (Wong and Prince, 1981; Llinás and Jahnsen 1982; Friedman and Gutnick, 1987; Schwindt and Crill, 1999). Notably, the generation of transient depolarizing potentials by low-threshold Ca\(^{++}\) currents can result in bursts of fast Na\(^+\)/K\(^+\) action potentials (Llinás and Jahnsen 1982; McCormick and Feeser, 1990; Yang et al., 1996).

When the network activity in the slice was increased by diminishing the inhibitory control of GABAergic interneurons, spontaneous burst could be seen in type I and II cells that were clamped at or just below resting potential. In these bursts short-duration action potentials rode on a slowly activated depolarizing envelope, resembling the plateau potentials generated by low-threshold Ca\(^{++}\) currents in mammalian central neurons (Llinás and Jahnsen 1982; McCormick and Feeser, 1990; Yang et al., 1996; Magee and Carruth, 1999). A contribution of a low threshold Ca\(^{++}\) current to the firing behaviour of the large projection neurons was further indicated by the fact that preconditioning hyperpolarization of the membrane potential led to an increased number of spikes in response to a depolarizing current pulse. Also, in addition to high-threshold Ca\(^{++}\) spikes, transient „hump“-like depolarizing potentials similar to those occurring in some type I neurons under control conditions could be evoked in the presence of TTX and TEA. Furthermore, about half of the type I neurons that showed „hump“-like potentials (6 of 13) also showed prominent DAPs following single spikes. As briefly outlined above the expression of DAPs depends either directly on low-threshold Ca\(^{++}\) currents (Zhang et al., 1993; Yang et al., 1996) or the activation of other Ca\(^{++}\) dependent currents (Higashi, 1993; Haj-Dahmane and Andrade, 1997). Functionally, depolarizing afterpotentials appear to be related to a neurons ability to fire bursts of action potentials (Wong and Prince, 1981; Chagnac-Amitai et al., 1990; Mason and Larkman, 1990; Yang et al., 1996; Magee and Carruth, 1999). And it has been postulated that the Ca\(^{++}\) entry resulting from the DAP is responsible for long-term potentiation (LTP) because manipulations that eliminate the DAP also block the induction of LTP (Komatsu and Iwakiri, 1992). While in sum these findings strongly implicate the
contribution of Ca\textsuperscript{++} influx in the emergence of burst firing in type I and type II cells, it should be noted that other components such as a sodium current (Schwindt et al., 1988; Franceschetti et al., 1995; Magee and Carruth, 1999) or outward currents (Schwindt et al., 1988; Kang and Kayano, 1994) might also be involved in the generation of bursts. Taken together, subthreshold potentials appear to serve an important role in sculpturing a cell's final output (Hamill et al., 1991), and their occurrence in the large spiny neurons of type I and II might reflect the integrative role these cells have in combining the diverse multimodal synaptic inputs that reach the NCL from various sensory and associative areas (Metzger et al., 1998; Kröner and Güntürkün, 1999). Furthermore, a neuron's ability to fire bursts of action potentials will probably enhance its integrative role within a network and aid the plasticity of synaptic connections: Burst firing increases the probability to drive the postsynaptic cell beyond spike threshold (Snider et al., 1998). The high frequency firing of action potentials during a burst is thus thought to amplify a neural signal and to synchronize the activity in a population of postsynaptic cells, both temporarily and spatially (Hablitz, 1986; Snider et al., 1998).

Type II: An initial tonic firing and a relatively low action potential threshold that characterize type II neurons may indicate that the firing of these cells is readily elicited by weak excitatory inputs. The occurrence of spontaneous burst in the absence of GABAergic inhibition and the phasic-tonic firing pattern that often emerged with large depolarizing currents indicates that type II neurons will respond strongly but transiently to a brief, new stimulus, yet produce a sustained response to a maintained input. In contrast to the behavior of neurons in the burst firing mode, a pattern of single action potentials can give a more faithful representation of the characteristics of depolarizing inputs. Functionally, the ability of type II neurons to generate a tonic firing mode could also enable these cells to retain informations of their input for a short time period. It is known that NCL-lesions in pigeons cause specific impairments in a variety of delay tasks (Mogensen and Divac, 1982, 1993, Gagliardo et al., 1996, 1997, Güntürkün, 1997, Güntürkün and Durstewitz, in press). In these experiments, the animal has to hold online a specific information provided during a previous cue period to perform a correct response after delay offset. In mammals, neurons in the deep layers of the prefrontal cortex (PFC) have been identified as a cellular substrate for short term memory (see Goldman-Rakic, 1996 for review). When the activity of these cells is recorded in awake rats or primates performing a delayed-response task, a significant enhancement in their firing rate can be observed during the delay (e.g. Fuster and Alexander, 1971; Funahashi et al., 1989; Sawaguchi and Yamane, 1999). Thus, sustained delay activity of PFC neurons may provide the animal with the ability to hold an internal representation of relevant aspects of the external world which is needed for planning or
organizing subsequent responses (Goldman-Rakic, 1996). Neurons with similar delay activities to those recorded from rat or primate PFC have been observed in vivo in the pigeon’s NCL during a delayed auditory-visual Go/No-Go task (Kalt et al., 1999). Like their mammalian counterparts, these cells showed significant alterations of their firing mode during the delay period. It is conceivable that the type II neurons of the present study may hold the information of their synaptic inputs within a recurrent network and thus provide a part of the cellular substrate for the delay activity within the NCL.

At least some type II neurons furthermore showed a small transient sag in the voltage response to hyperpolarizing currents, which is likely to be mediated by a voltage- and time-dependent hyperpolarization activated mixed cationic current called $I_h$ (Pape, 1996; see also discussion of type III below). It has been shown that a time-dependent-inward rectifier produces membrane resonance (Hutcheon et al., 1996). When resonance is present, neurons fire action potentials preferentially to rhythmic (oscillatory) inputs. The membrane resonance produced by $I_h$ can be amplified by a persistent sodium current (Hutcheon et al., 1996). In type II neurons we observed inward rectification in the depolarized voltage range that may be indicative of a persistent sodium current (Stafstrom et al., 1985, Yang et al., 1996). The existence of such a current has already been demonstrated directly in neurons from the caudal forebrain of songbirds which possess similar firing properties and morphological characteristics as the type II neurons described here (Kubota and Saito, 1991; Kubota and Taniguchi, 1998). Thus type II neurons might represent members of a common class of spiny projection neurons in the dorsal forebrain of birds that can respond preferentially to input of a specific pattern.

*Type III:* The electrophysiological and morphological characteristics of the type III cells resemble those of $\gamma$-aminobutyric acid-containing inhibitory interneurons found in the mammalian telencephalon (Schwartzkroin and Mathers, 1978; McCormick et al., 1985; Kawaguchi, 1995); i.e. little or no accommodation of spike frequency, and beaded aspiny dendrites. Type III cells offer a wide-band responsiveness and sustained high-frequency firing if necessary. They are thus able to perform a very reliable input-output conversion, retaining the temporal pattern of their synaptic inputs. These firing characteristics are facilitated by the short rise and fall times of the action potential (table 1). In addition, the relatively pronounced AHPs of type III cells might also contribute to the ability to fire action potentials at high frequencies in that they reduce the accumulation of depolarization-dependent Na$^+$ channel inactivation, which would otherwise reduce action potential amplitudes during long trains of high frequency firing (Hamill et al., 1991). It will also reduce the influx of Ca$^{++}$ into the cell and thus will diminish the effects of Ca$^{++}$-activated K$^+$ channels which otherwise might lead to
prolonged hyperpolarization and spike frequency adaptation (Lancaster and Adams, 1986; Storm, 1990).

A prominent feature in all type III cells was the existence of the strong inward-rectifying current $I_h$ that initiates slow depolarization if the membrane potential has become negative (e.g. by a GABA$_B$ receptor-mediated IPSP or a postspike AHP). In central neurons of mammals $I_h$ is a mixed Na$^+$/K$^+$ current that has been implicated in the determination of the resting potential and the generation of „pacemaker“ potentials. It also serves to decrease the propagation of subthreshold voltage potentials in dendritic trees, thereby regulating the integration of synaptic inputs (McCormick and Pape, 1990; Magee, 1998, 1999; see Pape, 1996 for review). In mammalian cortex GABAergic interneurons are long recognized for such various functions as maintaining the normal integrative properties of the cortex (Sillito, 1984), the control of spread of activity (Chagnac-Amitai and Connors, 1989) and the synchronization of adjacent projection neurons via inhibitory phasing (Cobb et al., 1995; Benardo, 1997), which might impose a rhythm on the activity of the principal neurons, providing a „context“ to the „content“ of their operation (Buzsáki and Chrobak, 1995). In the prefrontal cortex interneurons also appear to posses own sensory or „mnemonic“ fields that enable them to fine tune the delay activity of adjacent pyramidal neurons in a working memory task (Rao et al., 1999). With respect to the extensive local axonal arborizations of each type III neuron these putative interneurons of the NCL presumably also can control a large number of adjacent spiny „principal“ neurons (i.e. types I and II), as is the case in mammalian cortex (e.g. Sik et al., 1995). In sum, given their basic intrinsic electrophysiological and morphological properties type III neurons of the NCL are equipped to play a similarly important role for signal integration as their mammalian counterparts.

**Type IV:** Type IV neurons were characterized by high input-resistances, long time constants and a long duration of action potentials. These electrophysiological properties are also characteristic for developing cortical neurons in mammals (Kasper et al., 1994b; McCormick and Prince, 1987) and possibly birds (Kubota and Taniguchi, 1998). McCormick and Prince (1987) have argued that higher input resistances and longer membrane time constants of immature neurons could increase the magnitude and duration of their responses to imposed synaptic currents. Such cells should also be electrotonically compact, showing relatively little cable attenuation of distal dendritic synaptic inputs, thus compensating for similarly poorly developed synaptic inputs in newborn animals. This is in good agreement with the morphological appearence of our type IV cells identified here which possesed small somata, and short, sparsely spineous dendrites with relatively few branchings.
Similarly, the long duration of action potentials seen in type IV neurons may serve to facilitate the output of developing cells: Activity-dependent spike broadening due to a reduction in specific K$^+$ currents has been correlated with increased efficacy of synaptic transmission onto the postsynaptic target cell (Mercer et al., 1991; Byrne and Kandel, 1996 for review). Similarly, the significantly longer action potentials of developing neurons might result in an enhancement of synaptic transmission onto their postsynaptic cells (Bottjer et al., 1998). An open question remains whether type IV cells constitute a distinct class of late maturing neurons or simply represent developing cells of the other spiny celltypes (types I and II, respectively). However, all physiological and morphological characteristics of type I and II neurons were already present at the earliest ages recorded. Moreover, type IV cells indeed were recorded in slices from animals with the highest median age (7 d post hatch). Bradley and coworkers (1996) have proposed yet another mechanism that may account for developmental and experience-dependent changes in input resistance that occur in neurons of the chicks IMHV. They argue that existing large neurons with numerous spines might be morphologically remodelled to produce cells with significantly higher resistance to render them more susceptible to presynaptic inputs. It has been shown that in the NCL auditory imprinting reduces the spine densities on the medial and distal segments of large multipolar neurons, which correspond most likely to both types I and II of the present study (Bock and Braun, 1999a,b). While the resulting decrease in membrane surface should lead to a decrease in capacitance and an increase of apparent resistance, these changes are probably too subtle to account for the large differences in morphology and electrophysiology observed between type IV neurons and neurons of the other spiny cell classes.

**Connectivity and sensorimotor integration**

An important requirement for sensorimotor integration and associative learning is the ability of a brain structure to combine sensory information from various sources and to affect the animals behaviour either directly or via motoric and limbic structures downstream. Tracing studies *in vivo* have revealed that the NCL receives converging input from the secondary sensory areas of all modalities, as well as from other associative areas which themselves appear to integrate information from more than one modality (Metzger et al., 1998; Kröner and Güntürkün, 1999). Although some degree of regional specialization within NCL might exist, the pattern of afferents strongly indicates that in large areas of the NCL single neurons might receive multimodal input. In the pigeon single unit recordings *in vivo* have shown that neurons in the NCL can encode
aspects of a memory task across modalities (Kalt et al., 1999). The same neurons might be involved in a cell assemblies that „hold“ information over a delay or initiate a motor response via the archistriatum. In the chick Metzger and coworkers (1998) have shown that single neurons within the NCL send axon collaterals to the archistriatum and another multimodal forebrain area, the intermediate, medial part of the hyperstriatum ventrale (IMHV). Our findings provide further insight into the anatomical arrangement that might underlie the integration of various sensory features and the propagation of the NCL´s output to other forebrain areas. Morphologically, single cells in all spiny cell classes of the NCL seem to be able to relay their transformed input not only to neighbouring cells within NCL, but also to sensorimotor and limbic areas downstream, namely the archistriatum and the basal ganglia, and possibly also provide feedback to the sensory areas the original inputs come from. With the exception of the small type IV cells neurons in the NCL had fairly large dendritic fields (see table 2). The extent of the local axon collaterals for the spiny cell classes (types I, II, and IV) can be estimated at a lower limit of about two or three times the area of a given cells dendritic field (c.f. figs. 5, 10). Type III neurons had relatively symmetric axonal fields and their collaterals did not leave the NCL. The diameter of the area covered by axonal arborizations of these cells ranged between 545 - 736 µm (mean area 320,400 µm²). A hypothetical basic „circuit“ consisting of a type III interneuron and two postsynaptic spiny neurons at the rim of the axonal field would thus cover approximately 650,000 µm². Such a basic circuit could thus participate in intra-NCL computations which surpass areas of representation of a single sensory modality. In addition, most spiny cells were seen to send several long collaterals to targets outside the NCL. On their way these collaterals often span the whole medio-lateral and/or dorso-ventral extent of the NCL (c.f. fig. 5). Although the determination of the rostro-caudal extent of these intra-NCL connections remains a critical topic which was constrained by the thickness of the slices used here, it appears that these connections enable a stimulus comparison across most, if not all, possible sensory compartments of the NCL (c.f. Metzger et al., 1998; Kröner and Güntürkün, 1999).

Based on similarities in the pattern of connections and the location in the dorsocaudal forebrain it has been suggested that the Nd, the auditory subunit of the NCL might be related to the sensorimotor „high-vocal center“ (HVC) of songbirds (Wild, 1994; Metzger et al., 1998; Kröner and Güntürkün, 1999). Nucleus HVC is essential for song production and shows selective responses to complex auditory stimuli. Two pathways arise from within HVC that converge on the same premotor nucleus of the archistriatum. The first is a direct projection from HVC to the robust nucleus of the archistriatum (RA), which serves as the primary motor pathway for song production, and can also carry auditory information to RA. The other
projection of HVC begins a pathway through the anterior forebrain that is crucial for song learning but, although active during singing, is not essential for adult song production. In this pathway Area X of the lobus parolfactorius, a motor structure of the basal ganglia, is the first target of the HVC (Nottebohm et al., 1982; Fortune and Margoliash, 1995). In the HVC of zebra finches Area X and RA projecting neurons constitute two separate populations which resemble type I and II neurons of the present study with regard to intrinsic electrophysiological characteristics and morphology (Dutar et al., 1998; Kubota and Taniguchi, 1998). Neurons that project to RA show inward and outward rectification in the subthreshold range (Dutar et al., 1998; Kubota and Taniguchi, 1998) and possibly a strong accommodation in the firing pattern (Dutar et al., 1998; but see Kubota and Taniguchi, 1998), comparable to that seen in at least some type I neurons described here. Area X projecting neurons show a time-dependent inward rectification and regular spiking pattern very similar to that described for type II cells here (Dutar et al., 1998; Kubota and Taniguchi, 1998). However, our finding that almost all spiny neurons of the NCL sent at least one axon collateral in the direction of the archistriatum and sometimes also in the direction of the basal ganglia makes it unlikely that a similar distinction between cell types exists as in the songbird forebrain. These differences might reflect the specialization of the HVC relevant for singing and the need to exert control over specific aspects of singing related motor behaviour. Yet in the songbird brain parts of the lateral neostriatum and the „shelf“ region which adjoin the HVC share most afferents with the HVC (Fortune and Margoliash, 1995; Vates et al., 1996; Foster et al., 1997; Iyengar et al., 1999) and also show a similar pattern of projections (Fortune and Margoliash, 1995; Mello et al., 1998; Iyengar et al., 1999), therefore these areas might resemble the NCL more closely.

In sum, our findings provide a framework for further study into sensorimotor integration and associative learning in chicks and possibly other birds.

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Chapter 4: Dopaminergic innervation of the avian telencephalon

The Dopaminergic Innervation of the Avian Telencephalon

Daniel Durstewitz, Sven Kröner and Onur Güntürkün

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Abstract
The present review provides an overview of the distribution of dopaminergic fibers and dopaminoceptive elements within the avian telencephalon, the possible interactions of dopamine with other biochemically identified systems as revealed by immunocytochemistry, and the involvement of dopamine in behavioral processes in birds. Primary sensory structures are largely devoid of dopaminergic fibers, dopamine receptors, and the D1-related phosphoprotein DARPP-32, while all these dopaminergic markers gradually increase in density from the secondary sensory to the multimodal association and the limbic and motor output areas. Structures of the avian basal ganglia are most densely innervated but, in contrast to mammals, show a higher D2 than D1 receptor density. In most of the remaining telencephalon D1 receptors clearly outnumber D2 receptors. Dopaminergic fibers in the avian telencephalon often show a peculiar arrangement where fibers coil around the somata and proximal dendrites of neurons like baskets, probably providing them with a massive dopaminergic input. Basket-like innervation of DARPP-32-positive neurons seems to be most prominent in the multimodal association areas. Taken together, these anatomical findings indicate a specific role of dopamine in higher order learning and sensory-motor processes, while primary sensory processes are less affected. This conclusion is supported by behavioral findings which show that in birds, as in mammals, dopamine is specifically involved in sensory-motor integration, attention and arousal, learning, and working memory. Thus, despite considerable differences in the anatomical organization of the avian and mammalian forebrain, the organization of the dopaminergic system and its behavioral functions are very similar in birds and mammals.

1. Introduction

The neuromodulatory dopaminergic system of mammals and birds is critically involved in numerous cognitive and behavioral functions, including appetitive and aversive behaviors (Balthazart et al., 1997; Bertolucci-D’Angio et al., 1990b; Deviche, 1984; Salamone, 1992, 1994; Sokolowski et al., 1994), states of arousal and wakefulness (Ferrari and Giuliani, 1993; Ongini, 1993), motor control (Goodman et al., 1983; Rieke, 1981, 1982; Salamone, 1992; Sokolowski & Salamone, 1994; Waddington and Daly, 1993), learning and memory (Beninger, 1993; Gruss and Braun, 1997; McDougall et al., 1987; Schultz et al., 1995), as well as working memory and attention (Güntürkün & Durstewitz, in press; Montaron et al., 1982; Roberts et al., 1994; Sawaguchi and Goldman-Rakic, 1991, 1994; Schultz et al., 1993; Seamans et al., 1998; Zahrt et al., 1997). Since structure and function are tightly coupled in neural systems, information about the neuroanatomical organization of the dopaminergic system is required to unravel the functional role of dopamine (DA) in these various cognitive and behavioral processes. A large body of evidence indicates that the principal function of dopamine is very similar in mammals and birds. Since members of these two classes of vertebrates have highly developed cognitive skills (e.g., Epstein et al., 1984; von Fersen et al., 1990; Lanza et al., 1982; Lubow, 1974) but radically different forebrain organizations, comparative studies may uncover the invariant structural features which enable the dopaminergic system to exert its specific functions.

The present article provides an overview over the structural organization of the dopaminergic system in avian species, compares it to mammals, and discusses behavioral findings and possible functional implications. However, this review will be restricted in two ways: First, almost all studies that dealt specifically with markers of the dopaminergic system in the avian brain (i.e., DA, DA receptors, DARPP-32) have been conducted within the last decade, and the present review will focus mainly on these studies. Older literature on the general distribution of markers of the monoaminergic or catecholaminergic systems are covered in the excellent review by Reiner et al. (1994). Second, most of our knowledge on the dopaminergic innervation of the bird brain derives from studies in pigeons and chicks. However, the few available studies from other avian species make it likely that the results obtained in these avian species generalize to other birds as well.
2. Overview over the neuroanatomy of the avian telencephalon

The avian telencephalon consists of nuclear structures, and mostly lacks the layered organization that is characteristic of mammalian isocortex (Figure 1). This lack of lamination led early investigators to assume that most of the avian telencephalon consists merely of a hypertrophied striatum, and accordingly most forebrain areas were termed with the suffix '-striatum', and this nomenclature remains still in use (Ariëns-Kappers et al., 1936; Edinger, 1903). On the basis of embryological (Källén, 1953, 1962; Kuhlenbeck, 1938; see also Striedter et al., 1998) cytoarchitectonic (Rehkämper et al., 1984, 1985, Rehkämper and Zilles, 1991), hodological (Karten, 1969; Karten and Dubbeldam, 1973; Veenman et al., 1995) and
histochemical (Juorio and Vogt, 1967) criteria, however, it became clear that large parts of the telencephalon could be regarded as pallial, and as such might constitute possible cortex equivalents.

The flow of sensory information in the avian telencephalon follows a common pattern for all modalities: The primary sensory areas which receive subtelencephalic input, relay their information to adjacent secondary sensory structures, which in turn project in parallel to presumed multimodal areas as well as to motor and limbic structures, including the basal ganglia (Figure 2).

As in all amniotes, two main visual pathways convey visual information from the retina to the telencephalon in birds. These are the thalamofugal and the tectofugal pathway. The tectofugal pathway may correspond to the mammalian colliculo-thalamo-extrastriate pathway, whereas the thalamofugal pathway is comparable to the mammalian geniculostriate pathway (review by Güntürkün, 1991). The ectostriatum (E) is the primary sensory structure of the tectofugal pathway (Benowitz and Karten, 1976; Kondo, 1933). From there intratelencephalic projections lead to the surrounding ectostriatal belt (Karten and Hodos, 1970; Ritchie, 1979). The thalamofugal visual pathway consists of a bilateral projection from a retinorecipient nucleus in the dorsal thalamus onto a thin band of granule cells, the intercalated nucleus of the hyperstriatum accessorium (IHA), as well as to the lateral part of the hyperstriatum dorsale (HD), which together constitute the primary sensory areas within the so-called Wulst. The IHA and HD in turn project to secondary sensory structures within the Wulst, mainly to the hyperstriatum accessorium (HA; Karten et al., 1973; Shimizu et al., 1995).

The same organization applies to a part of the somatosensory system which occupies the rostral extent of the Wulst, and that seems to be comparable to the mammalian primary somatosensory cortex (Karten, 1971; Medina et al., 1997): Somatosensory fibers from the dorsal thalamus terminate in the HD and the IHA (Delius and Bennetto, 1972, Funke, 1989, Wild, 1997) which in turn project to the rostral HA (Wild, 1987). Another primary somatosensory telencephalic area is located within the nucleus basalis (Bas). The Bas mainly processes trigeminal information (Schall et al., 1986), but also receives additional somatosensory and auditory input (Arends and Zeigler, 1986; Wild and Farabaugh, 1996; Wild et al., 1997). It relays this information to the overlying frontal neostriatum (NF; Wild and Farabaugh, 1996; Wild et al., 1985).

The main telencephalic area reached by auditory input from the thalamus is the so-called Field L complex in the caudomedial neostriatum (Karten, 1968). Field L2 is the primary sensory...
structure of the auditory system, which projects to adjacent fields L1 and L3 (Bonke et al., 1979; Karten, 1968; Wild et al., 1993).

The secondary sensory areas of all modalities are reciprocally connected with a region in the caudolateral neostriatum, which in the pigeon is referred to as NCL (Kröner and Güntürkün, 1999; Leutgeb et al., 1996; Metzger et al., 1998). Other multimodal areas probably exist in the intermediate aspect of the hyperstriatum ventrale (IMHV) and the intermediate neostriatum (NIM; or mediorostral neostriatum/hyperstriatum ventrale, MNH, in chicks). Both these areas have been studied extensively in chicks in the context of imprinting (Bradley et al., 1985; Bredenkötter and Braun, 1997; Metzger et al., 1996; reviews: Horn, 1998; Scheich, 1987). In addition, a system of "cortico"-striatal projection neurons which occupies the most dorsal and lateral extent of the external pallium (PE; Brauth et al., 1978; Veenman et al., 1995) includes, from rostral to caudal, the lateral frontal neostriatum (NFL), the lateral intermediate neostriatum (NIL), the area temporo-parieto-occipitalis (TPO) and the area corticoidea dorsolateralis (CDL).

Figure 2. Schematic overview of the functional anatomy of the pigeon telencephalon as described in Section 2. Brain areas that are still poorly understood with regards to their connections and functions were left blank. It should be noted, however, that the hyperstriatum intercalatus superior (HIS) probably receives secondary and, to a limited extent, possibly also primary visual input. The hyperstriatum ventrale (HV) is a highly heterogeneous region which probably encompasses multiple polysensory subdivisions.
At the next level of information processing in the avian brain, sensory information is funneled in parallel from both, the tertiary as well as the secondary sensory areas to the basal ganglia and, in addition, to the ventrolaterally located archistriatum (e.g. Bradley et al., 1985; Csillag et al., 1994; Kröner and Güntürkün, 1999; Leutgeb et al., 1996; Metzger et al., 1998; Phillips, 1966; Shimizu et al., 1995; Wild et al., 1985, 1993). Functionally, the avian archistriatum has been divided into two main subdivisions (Davies et al., 1997; Dubbeldam et al., 1997; Zeier and Karten, 1971). These are a somatic sensorimotor part and a viscerolimbic division that is considered to be equivalent to the mammalian amygdala (Davies et al., 1997; Dubbeldam et al., 1997; Zeier and Karten, 1971).

The connectivity, neurotransmitter content, and cytoarchitecture of the avian basal ganglia are highly similar to that in mammals (Anderson and Reiner, 1991b; Karten and Dubbeldam, 1973; Reiner and Anderson, 1990; Reiner et al., 1983, 1984a; Veenman and Reiner, 1994; Veenman et al., 1995). Based on the palliostriatal connections, a functional segregation of the avian striatum into associative, sensorimotor and limbic territories has recently been suggested (Veenman et al., 1995), similar to the situation in mammals (Parent, 1990). The paleostriatum augmentatum (PA) and lobus parolfactorius (LPO) make up the dorsal (somatomotor) striatum. Ventral striatal structures include the nucleus accumbens (Acc), the bed nucleus of the stria terminalis (BNST), and the olfactory tubercle (TO) which constitute the 'visceral-limbic' parts of the avian striatum. The pallidal parts of the avian basal ganglia consist of the paleostriatum primitivum (PP), which is homologous to the globus pallidus of mammals, and the ventral pallidum (VP) which is comparable to the "limbic" ventral pallidum of mammals (Karten and Dubbeldam, 1973; Medina and Reiner, 1997).

The description of the dopaminergic innervation of the avian telencephalon given in chapter 5 will follow the functional classification outlined above. It will turn out that the distribution of DA and DA receptors is closely related to these functional subdivisions. However, certain exceptions exist, as, for example, components of the song system of songbirds subserve sensory as well as motor functions.

3. Origin of dopaminergic fibers in the avian telencephalon: Mesencephalic A8-A10 cell groups

Comparable to the situation in mammals, the dopaminergic innervation of the avian telencephalon arises mainly from three mesencephalic dopaminergic cell populations located in the n. tegmenti pedunculopontinus pars lateralis and the area ventralis tegmentalis of Tsai
(AVT) (Figure 3; Kitt and Brauth, 1986; Metzger et al., 1996; Waldmann and Güntürkün, 1993). The former nucleus is homologous to the mammalian substantia nigra pars compacta (SNC; Brauth et al., 1978; Karten and Dubbeldam, 1973; Kitt and Brauth, 1986; Reiner et al., 1983; Rieke, 1981, 1982), where according to Reiner et al. (1994) the rostro-ventral part comprises the A9 group and the caudo-dorsal part the A8 group. The medial part of the n. tegmenti pedunculopontinus is considered to be equivalent to the mammalian substantia nigra, pars reticulata (SNR; Kitt and Brauth, 1981; Reiner et al., 1983). The avian AVT is homologous to the ventral tegmental area of mammals (A10 group) (Kitt and Brauth, 1981, 1986; Reiner et al., 1983, 1994; Waldmann and Güntürkün, 1993). In the AVT and SNC, intense perikaryal immunoreactivity (ir) for tyrosine hydroxylase (TH) and DA (Figure 3A-C) but not for dopamine-beta-hydroxylase (DBH), noradrenaline (NA), or phenylethanolamine-N-methyl transferase (PNMT) can be observed (Bailhache and Balthazart, 1993; Moons et al., 1995; Reiner et al., 1994; Waldmann and Güntürkün, 1993; Wynne and Güntürkün, 1995). TH is the rate-limiting enzyme in the synthesis of all catecholamines and, with a few exceptions (Smeets and Gonzales, 1990), must be present in dopaminergic neurons. The presence of DBH which converts DA to noradrenaline, or of PNMT which converts noradrenaline to adrenaline, would indicate non-dopaminergic catecholaminergic neurons and fibers. An A8-A10 group has been recognized in many avian species, including zebra finches (Bottjer, 1993; Lewis et al., 1981), quail (Bailhache and Balthazart, 1993; Bons and Olivier, 1986), warbling grass parakeet (Takatsuki et al., 1981), chicks (Metzger et al., 1996; Moons et al., 1994), and pigeons (Kitt and Brauth, 1981, 1986; Waldmann and Güntürkün, 1993; Wynne and Güntürkün, 1995). The SNR, on the other hand, exhibits comparatively little perikaryal but high neuropil labeling for DA (Waldmann and Güntürkün, 1993; Wynne and Güntürkün, 1995), as it is the case in mammals where the SNR mainly contains GABAergic neurons (Fallon and Loughlin, 1995).

From the AVT and SNC, fibers ascend mainly ipsilaterally within two tracts of the mediolateral part of the lateral forebrain bundle (Karten and Dubbeldam, 1973; Kitt and Brauth, 1986; Metzger et al., 1996; Wynne and Güntürkün, 1995). In general, there is substantial overlap in the telencephalic projection zones of the SNC and AVT (Kitt and Brauth, 1986; Metzger et al., 1996; Waldmann and Güntürkün, 1993), although some degree of specialization and topographical organization seems also to be present (see, e.g., Section 5.4.1).
Vice versa, the A8-A10 group receives pallidal inputs from the PP and striatal inputs from the LPO, PA, and Acc (Karten and Dubbeldam, 1973; Kitt and Brauth, 1981; Reiner et al., 1994). By far most of the striatonigral projection neurons co-contain substance P and dynorphin, while the remainder contain enkephalin (Anderson and Reiner, 1990, 1991b; Reiner et al., 1983, 1984b; Reiner and Anderson, 1990). These two apparently disjunctive groups of neurons project to different populations of dopaminergic and overlapping populations of nondopaminergic tegmental and nigral neurons (Reiner et al., 1994; Smeets, 1991), where
substance P-ir fibers make symmetric synaptic contacts predominantly on thin dendrites and spines (Anderson et al., 1991), whereas enkephalinergic fibers contact dopaminergic somata as well (Medina et al., 1995).

Both, D1 and D2 receptor densities seem to be quite low in the pigeon SNC compared to the 'striatal' parts of the avian basal ganglia (Dietl and Palacios, 1988; Richfield et al., 1987). Thus, D2 receptors seem not to play such a prominent role as autoreceptors in avian dopaminergic midbrain neurons (at least not within the local SNC/VTA circuit) as they do in mammals (Brock et al., 1992; Bunney et al., 1987; Cooper et al., 1996; Yung et al., 1995). Furthermore, DARPP-32 neuropil labeling (indicating the presence of D1 receptors, see Section 4.3) but no soma labeling could be detected in the VTA, SNC, and SNR (Figure 3D). This observation agrees well with the mammalian situation (Ouimet et al., 1984, 1992).

4. General features of the dopaminergic innervation of the avian telencephalon

Before getting into a detailed description of the distribution of dopaminergic fibers and dopaminoceptive elements in the avian telencephalon, some general remarks on specific features of the dopaminergic innervation of the avian brain, and on markers of the dopaminergic system seem to be in place.

4.1. 'Basket'- and 'en passant'-type of dopaminergic innervation

The dopaminergic innervation in the avian telencephalon takes two different, and possibly discrete, forms. The first form, which is found also in the mammalian cortex (Oades and Halliday, 1987; Williams and Goldman-Rakic, 1993), is characterized by dopaminergic fibers contacting the somata and dendrites of neurons 'en-passant' while passing through their target region. These dopaminergic axons often travel in close vicinity along the somata and dendrites of target-neurons while forming a large number of bouton-like axonal swellings. The second one, called the 'basket'-type, is a very peculiar arrangement of dopaminergic fibers in the avian telencephalon. In this case, single fibers densely coil around the somata and intial dendrites of postsynaptic targets, enwrapping them in basket-like structures (Figure 4). In some telencephalic regions, this type of innervation can be so dense that unlabeled postsynaptic neurons and their initial dendrites can virtually be seen by labeling of catecholaminergic fibers
alone (Figure 4A, B). These fibers exhibit many varicosities in the vicinity of the soma and proximal dendrites, which have been shown to contain large numbers of round clear vesicles and also a few dense core vesicles (Metzger et al., 1996; Karle et al., 1992, 1994; see 4.4.). Baskets seem to contact predominantly bigger neurons, whereas smaller neurons are more likely innervated en-passant (Wynne and Güntürkün, 1995). However, basket-type structures are not a speciality of the dopaminergic system, as they can also be demonstrated with antibodies against NA and DBH (Moons et al., 1995).

4.2. Tyrosine hydroxylase as a marker of dopaminergic fibers in the avian telencephalon

Many studies have been carried out using THir as a marker of dopaminergic fibers in the avian telencephalon. As noted above, TH is the rate-limiting enzyme in the synthesis of all catecholamines and, with few exceptions (Smeets and Gonzales, 1990), must be present in dopaminergic neurons. In general, the distribution of THir fibers closely follows that of DAir fibers and might be a better indicator for the distribution of dopaminergic fibers than for the much sparser noradrenergic innervation in the avian telencephalon, which predominantly distributes along the VP, septum, HA, and the hippocampal region (Bailhache and Balthazart, 1993; Karle et al., 1996; Moons et al., 1994, 1995; Reiner et al., 1994; Wynne and Güntürkün, 1995). However, there are also some exceptions like the area parahippocampalis (APH) or the n. taeniae (Tn) which are quite high in THir but low in DAir fibers (Wynne and Güntürkün, 1995). Hence, THir might be a good marker of dopaminergic fibers in most but not all telencephalic areas.

4.3. DARPP-32 as a marker of D1-receptors and dopaminoceptive neurons

DARPP-32 is a Dopamine- and cAMP-Regulated Phosphoprotein of molecular weight 32,000 that plays a role as a "third messenger" in the intracellular cascade induced by D1-receptor stimulation (Hemmings et al., 1987a, 1987b, 1995). Via activation of the adenylyl cyclase, D1-receptor action stimulates cAMP synthesis, which through protein kinase A leads to phosphorylation of DARPP-32. Phosphorylated DARPP-32 in turn inhibits protein phosphatase-1, and thus affects the state of various target proteins. In rat striatal neurons for instance, DA affects via phosphorylation of DARPP-32 the gating of Na⁺ and various high-voltaged-activated Ca²⁺ channels (Hemmings et al., 1987a, 1995). DARPP-32 is itself dephosphorylated by Ca²⁺/calmodulin-dependent protein phosphatase 2B (calcineurin), thus opening possibilities for the interaction of glutamatergic transmission and Ca²⁺ influx with
DA-induced effects (Hemmings et al., 1987a, 1995; Nishi et al., 1997). In mammals the distribution of DARPP-32 has been shown to be closely related to that of D1-receptors (Berger et al., 1990; Hemmings and Greengard, 1986; Ouimet et al., 1984; Walaas and Greengard, 1984). In general, this is also the case in the pigeon (Durstewitz et al., 1998) and chick (Schnabel et al., 1997) brain.

Figure 4. Modes of dopaminergic innervation in the avian telencephalon. (A, B) Examples of the ‘basket-type’ of dopaminergic innervation. (C-G) Examples illustrating various densities and relative contributions of en-passant and basket-type innervations. Photomicrographs were taken from the NCL (A, C), PP (B), Aid (D), Ai (E), NIM (F), and Av (G). Scale bars represent 100 µm (C, D, E), 50 µm (F, G) and 25 µm (A, B).
The use of antibodies against DARPP-32 as a marker of dopaminergic structures has the advantage that postsynaptic neural targets of dopaminergic fibers are often labeled to a large extent (Figure 5). In some cases even fine dendritic branches and spines are clearly visible at the light-microscopic level (Figure 5D-F), revealing additional information about the morphological properties of dopaminergic targets which are not apparent using D1 receptor-ir (see Schnabel et al., 1997). The clear perikaryal labeling obtained with DARPP-32-ir also enables morphometric approaches since cell counts can easily be performed.

Figure 5. DARPP-32 immuno-labeling in the pigeon forebrain. (A) Frontal section at level A 9.00, illustrating the different intensities of DARPP-32-positive neuropil staining. (B, C) Most DARPP-32-positive cells displayed only labeling of the soma and initial dendritic segments. (D-F) In some neurons also fine dendritic processes and spines are visible. Photomicrographs were taken from the n. basalis (B), neostriatum frontolaterale (C), ventromedial hippocampus (D), border of ectostriatum and ectostrial belt (E), and medial septum (F). Scale bars represent 250 µm (A), 50 µm (B, D, E), 20 µm (C), 15 µm (F). Scale bars represent 250 µm (A), 50 µm (B, D, E), 20 µm (C), 15 µm (F). Taken from Durstewitz et al. (1998).
4.4. Ultrastructural features of the dopaminergic innervation and of dopaminoceptive neurons

At the ultrastructural level, dopaminergic and THir fibers in the avian basal ganglia and in the neostriatum form many varicosities which often exhibit multiple active zones and contain many round clear small to medium-sized vesicles together with some large dense core vesicles (Karle et al., 1992, 1994, 1996; Metzger et al., 1996), indicating the co-release of DA and neuropeptides as it is the case in mammalian dopaminergic neurons (Cooper et al., 1996; Seroogy et al., 1988a, 1988b). Some of these varicosities (about 50% in the avian basal ganglia according to Karle et al., 1996) show small and flat synaptic specializations, most often of the symmetrical type, but occasionally also asymmetrical ones (Karle et al., 1996; Metzger et al., 1996). Dopaminergic synapses within the neostriatum, LPO, and PA are most often found on thin dendritic shafts (about 50% in the basal ganglia), less frequently on dendritic spines, and occasionally (<20% in the basal ganglia) on thick dendritic shafts or perikarya (Karle et al., 1996; Metzger et al., 1996). Thus, in the vicinity of the soma, unspecialized varicosities seem to prevail. On dendritic spines, dopaminergic synapses are sometimes engaged in 'triadic complexes' with other unlabeled symmetric or asymmetric synapses terminating on the same spine, or where they make axo-axonic contacts on these unlabeled synapses (Metzger et al., 1996). Thus, in the avian brain as in mammals (Calabresi et al., 1987; Hernández-López et al., 1997; Law-Tho et al., 1994; Pralong and Jones, 1993; Yang and Seamans, 1996), DA might control both, synaptic transmission via pre- and/or postsynaptic mechanisms as well as postsynaptic somatic and dendritic membrane properties. Furthermore, both these aspects of neural functioning may be modulated via D1 receptors, as D1 receptor-ir and DARPP-32ir have been observed not only within the dendrites but occasionally also in axons and axon terminals (Schnabel et al., 1997).

5. Distribution of dopaminergic fibers and dopaminoceptive elements in the avian telencephalon

The distribution of dopaminergic fibers is closely related to functional subdivisions of the avian telencephalon. Most prominently, structures of the avian basal ganglia receive by far the densest dopaminergic input, whereas all primary sensory structures seem to be devoid of DA, L-DOPA, DA receptors, and DARPP-32 (Dietl and Palacios, 1988; Durstewitz et al., 1998; Juorio, 1983; Juorio and Vogt, 1967; Metzger et al., 1996; Moons et al., 1994; Schnabel and Braun, 1996; Wynne and Güntürkün, 1995). All other telencephalic areas fall somewhere in
between, however, with clear regional differences. In the following, we will describe the
distribution of dopaminergic fibers and dopaminoceptive elements according to functional
subdivisions, following the pathway of sensory information from the primary sensory to the
motor output structures as outlined in Section 2. Figures 6 to 9 give an overview over the
distribution of dopaminergic fibers, D1 receptors, D2 receptors, and DARPP-32 neuropil and
soma labeling throughout the avian telencephalon. In addition, findings on colocalizations of
markers of the dopaminergic system with other biochemically identified systems will be
discussed.

However, before going into details, it should be mentioned that although the general pattern may
be the same across avian species, some strain and species differences exist. Thus, Divac et al.
(1988) observed an about two-fold higher DA concentration in the posterior telencephalon of
mixed breed than of white Carneaux pigeons, while in the anterior telencephalon the DA
concentration was only slightly higher. Nevertheless, the relative DA concentration in six
telencephalic regions examined remained qualitatively the same in both strains. Similarly,
telencephalic DA concentrations were noted to vary by factors of up to five between chicks, ducks, finches, pigeons, fowls, and quails (Juorio and Vogt, 1967; Juorio, 1983).

5.1. Primary sensory areas

5.1.1. Dopaminergic fibers and projections

Primary sensory areas of the pigeon and chick telencephalon display by far the lowest DAir and L-DOPAir (Figure 10A; Metzger et al., 1996; Wynne and Güntürkün, 1995; Moons et al., 1994), and no projections from the VTA or SNC to these areas have been demonstrated, except for a restricted input to the HD (Kitt and Brauth, 1986). Whereas the E (visual tectofugal), Field L2 (auditory), and the Bas (trigeminal) are devoid of dopaminergic fibers, some DAir fibers are present in the rostral (somatosensory) and caudal (visual thalamofugal) IHA, and lateral HD, the two input laminae of the Wulst. Interestingly, the specific sensory nuclei of the thalamus (e.g., the n. rotundus which projects to the E) also contain little or no THir (Reiner et al., 1994).
Thus, primary sensory processes do not seem to be modulated, at least not to a significant extent, by DA in the adult avian brain.

5.1.2. D1 receptors

Given the virtual absence of a dopaminergic innervation, it comes with no surprise that the D1 receptor density in the primary sensory structures is very low, with the E exhibiting the lowest D1-specific binding in the whole telencephalon (Dietl and Palacios, 1988; Schnabel et al., 1997; Schnabel and Braun, 1996; Stewart et al., 1996). However, as it is the case for DAir labeling, the IHA and HD are higher in D1-specific binding, although still significantly lower than the respective secondary projection zones in the HA and most of the neostriatum (Schnabel et al., 1997).

5.1.3. D2 receptors

In general, D2 receptor densities as measured by [3H]spiperone and [3H]CV205-502 are low and homogeneously distributed throughout the whole avian telencephalon, except for the basal ganglia and the hippocampal complex as discussed below (Dietl and Palacios, 1988; Schnabel and Braun, 1996; Stewart et al., 1996).

5.1.4. DARPP-32

The distribution of DARPP-32ir confirms the extremely low or absent dopaminergic input to the primary sensory structures. The E and Field L2 are largely devoid of DARPP-32ir (Figure 10B; Durstewitz et al., 1998; Schnabel et al., 1997). The IHA and HD display low levels of neuropil labeling and a low percentage of labeled neurons, while, however, scoring in both respects again much lower than the respective secondary sensory zones in the HA and most of the neostriatum (Durstewitz et al., 1998; Schnabel et al., 1997). Hence, primary sensory regions in the avian forebrain could be sharply delineated and differentiated from bordering areas by virtue of their low DARPP-32ir (Figure 5A; Figure 10B).

The only primary sensory forebrain area that might deviate from this general pattern, at least in pigeons, is the trigeminal Bas. Although it is very low in terms of DARPP-32ir neuropil labeling, the pigeon’s Bas contains a high proportion of labeled perikarya, many of them exhibiting clear neuronal properties (Figure 5B; Durstewitz et al., 1998; but see Schnabel et al., 1997, for chicks). This finding might be related to other peculiarities of the Bas and to the specific demands imposed on the neural circuit controlling pecking behavior, in which the Bas
is critically involved (Schall, 1987; Wild et al., 1985). The Bas is the only avian forebrain structure with direct sensory inputs bypassing the thalamus (Schall et al., 1986). Moreover, within the trigeminal neural circuit controlling pecking, sensory signals possibly must be integrated very fast and relayed rather directly to motor outputs. As the dopaminergic system probably plays a central role in sensory-motor integration and learning (see Section 7), the dopaminergic modulation might start earlier in this trigeminal somatosensory circuit than within other forebrain systems. This notion is furthermore underpinned by the fact that the Bas receives auditory and possibly also vestibular input, in addition to trigeminal information (Schall et al., 1986; Schall and Delius, 1986; Wild et al., 1997), and might thus actually be regarded as a multimodal structure. Furthermore, behavioral evidence supports the hypothesis of a dopaminergic modulation within the pigeon Bas: Lindenblatt and Delius (1988) showed that local apomorphine-injections into the Bas induce pecking bouts, while Wynne and Delius (1996) made it likely that Bas lesions decrease pecking fits induced by peripheral apomorphine-injections. However, the high number of DARPP-32ir neurons in the pigeon Bas

Figure 8. Schematic illustration of the distribution of D2 receptors in the avian telencephalon.
is clearly at odds with the lack of D1-receptors and dopaminergic fibers reported so far, as well as with the lack of DARPP-32 in the chick Bas (Schnabel et al., 1997). In this context, it should be pointed out that DARPP-32 is not exclusively involved in the D1-induced cascade but is regulated by other neuromodulator/neurotransmitter pathways as well, e.g. by D2- or NMDA-receptor stimulation via Ca\(^{2+}\) influx (Hemmings et al., 1995; Nishi et al., 1997). Thus, DARPP-32 in the Bas might be linked to intracellular pathways other than the one coupled to the D1 receptor, and differences between pigeons and chicks might exist in this respect.

5.1.5. Colocalizations

Durstewitz et al. (1998) found that glutamate decarboxylase (GAD) ir neurons were never colocalized with DARPP-32 or located in THir baskets throughout the whole pigeon telencephalon, including the primary sensory areas.

5.2. Secondary sensory and multimodal areas

5.2.1. Dopaminergic fibers and projections

In general, the dopaminergic innervation of both, the secondary sensory and tertiary association areas is clearly higher than that of the primary areas (Figures 10, 11). In addition, most of the multimodal areas score higher than the secondary sensory areas due to an increasing rostrocaudal and mediolateral gradient of DAir structures (Figure 6; Metzger et al., 1996; Wynne and Güntürkün, 1995). Thus, in the secondary visual Ep and the trigeminal NF, DA concentration is very low. The HA which includes the secondary projection fields of the thalamofugal visual and the somatosensory system receives a moderate dopaminergic input (Metzger et al., 1996; Moons et al., 1994; Wynne and Güntürkün, 1995).

Among the presumed multimodal forebrain structures, the rostrally located NFL is only moderate and thus lowest in its DA content (Metzger et al., 1996; Moons et al., 1994; Wynne and Güntürkün, 1995), while the medial portion of the lamina frontalis superior seems to be most densely innervated by dopaminergic fibers (Figure 11A), with the fibers arising mainly from the SNC (Bottjer, 1993; Kitt and Brauth, 1986; Wynne and Güntürkün, 1995). High DAir and basket-like structures are also present in the MNH (Figure 11A; Metzger et al., 1996; Moons et al., 1994; Wynne and Güntürkün, 1995), an area that has been implicated in filial imprinting (Gruss and Braun, 1996, 1997). Metzger et al. (1996) also demonstrated a prominent input to these areas from VTA by retrograde tracing, and to a lesser extent from SNC.
Considerable numbers of basket-like structures and dopaminergic fibers are also present in multimodal areas comprising the pallium externum, especially in the TPO, which receives its major input from SNC (Kitt and Brauth, 1986). Within the caudal neostriatum which is generally very high in DA input, the NCL has received special attention because of its presumed equivalency to the prefrontal cortex (PFC) of mammals. This assumption was first derived from behavioral, radioenzymatical, and histofluorescence studies conducted by Divac, Mogensen and Björklund (Divac et al., 1985; Divac and Mogensen; 1985; Mogensen and Divac, 1982). These authors demonstrated that the NCL contains a high DA-to-NA-ratio and an especially high number of catecholaminergic fibers of presumed dopaminergic origin, as it is characteristic for the PFC of mammals (Berger et al., 1988, 1991; Björklund et al., 1978; Divac et al., 1978; Glowinski et al., 1984; Van Eden et al., 1987). Using immunocytochemical methods, Waldmann and Güntürkün (1993) and Wynne and Güntürkün (1995) confirmed that
the NCL could be differentiated from the surrounding caudal neostriatum by its denser dopaminergic innervation and by the higher number of DA\textsubscript{ir} fibers which constitute a basket, while the number of baskets per se does not increase in the NCL (Figure 11B, C; see also Divac et al., 1994). The dopaminergic input to the NCL arises from the dopaminergic midbrain nuclei AVT and SNC (Waldmann and Güntürkün, 1993).

In chicks, a region in the dorsocaudal neostriatum shares the location, connections and dense dopaminergic innervation with the pigeon’s NCL (Metzger et al., 1996, 1998). This tertiary area has also been studied in the context of imprinting (Bock et al., 1997; Schnabel and Braun, 1996). Its dopaminergic innervation is also characterized by a higher occurrence of basket-like structures and by a dopaminergic input from AVT and SNC (Metzger et al., 1996). However, it appears that the area of densest DA\textsubscript{ir} and TH\textsubscript{ir} is shifted somewhat ventromedially from the ventricle compared to pigeons (Metzger et al., 1996; own unpublished observations).

5.2.2. D1 receptors

In general, the distribution of D1 receptors closely mimics that of dopaminergic fibers, although D1 receptors seem to be more homogeneously distributed throughout the entire neostriatum and hyperstriatum than dopaminergic fibers, which seem to be more locally restricted (Figure 7). The HA, HD, HV, and neostriatum contain comparable, medium concentrations of D1 receptors, with the hyperstriatal areas scoring a bit higher than at least the rostral and intermediate neostriatum (Ball et al., 1995; Dietl and Palacios, 1988; Schnabel and Braun, 1996; Schnabel et al., 1997; Stewart et al., 1996). In addition, two obvious tendencies in distribution are apparent (Schnabel and Braun, 1996; Schnabel et al., 1997): First, the number of D1 receptors increases from rostral to caudal in both, hyperstriatal and neostriatal areas. Second, in the neostriatum, the number of D1 receptors increases from medial to lateral. Thus, the NCL and the caudal HV are the telencephalic areas highest in D1-specific binding outside the basal ganglia. This finding also implies that secondary sensory structures in the frontal neostriatum, like the Ep and the NFL, but also the secondary auditory fields L1 and L3, are less dense in D1 receptors than most tertiary telencephalic regions. Hence, in general, and possibly with some species differences (Ball et al., 1995), there is an increase of D1-receptor density proceeding from primary sensory to tertiary multimodal structures. This pattern might be related to an increase in sensory-motor integration and complex learning processes that take place primarily in higher-order structures.
Chapter 4: Dopaminergic innervation of the avian telencephalon

Figure 10. Distribution of dopaminergic fibers and dopaminoceptive elements in primary and secondary sensory areas as exemplified for the auditory Field L complex. (A) The primary sensory area L2 is almost devoid of dopaminergic fibers and (B) of DARPP-32 immunoreactivity, while the surrounding secondary sensory “belt” regions L1 and L3 show considerably more labeling for dopamine and DARPP-32. Scale bars represent 500 µm.

Figure 11. Dopaminergic innervation and DARPP-32 immunoreactivity in higher order sensory and multimodal structures. (A) Distribution of dopaminergic fibers in the rostromedial forebrain. The neostriatum intermedium (NIM) and hyperstriatum ventrale (HV), and possibly also the lamina frontalis superior (LFS), constitute multimodal areas. Note that in the NIM and HV the density of dopaminergic fibers increases slightly from lateral to medial. (B) Localization of the neostriatum caudolaterale (NCL), the presumed prefrontal cortex of birds, in the caudal telencephalon. (C) Detail of the dopaminergic innervation of the NCL and the posterior archistriatum. (D) DARPP-32 immunoreactivity in the caudal neostriatum is evenly distributed at medium levels, but is low in the archistriatum. Scale bars represent 1 mm (B) and 50 µm (A, C, D).
5.2.3. D2 receptors

D2 receptor densities in the neostriatum and hyperstriatum are low and homogeneously distributed (Figure 8; Dietl and Palacios, 1988; Schnabel and Braun, 1996; Stewart et al., 1996). For example, Schnabel and Braun (1996) found the density of D2 receptors to be about one third or less (< 25 fmol/mg) of that of D1 receptors in these areas. Hence, outside the basal ganglia, dopaminoceptive neurons in the avian telencephalon seem to be modulated predominantly via receptors of the D1 class, although it should be kept in mind that even low densities of D2 receptors might play an important physiological role.

5.2.4. DARPP-32

DARPP-32ir is distributed with medium to high densities throughout the entire neostriatum, HA, CDL and TPO (Figure 9; Durstewitz et al., 1998; Schnabel et al., 1997). Thus, DARPP-32ir reaches much higher levels in the secondary and tertiary areas than in the primary sensory structures (Figures 5A, 10B). In addition, Schnabel et al. (1997) reported a rostrocaudal and a mediolateral trend in the DARPP-32 distribution in the chick neostriatum as it has been described for D1 receptors. However, Durstewitz et al. (1998) were not able to discriminate between secondary sensory and multimodal pigeon forebrain structures based on DARPP-32ir neuropil labeling and cell countings, although a slight trend in neuropil labeling was also observed by these authors. Thus, differences between secondary sensory and multimodal areas with regards to their dopaminergic input may not be so apparent in DARPP-32ir as they are in D1 receptor and dopaminergic fiber density.

A relatively high percentage of neurons (up to about 60 %) exhibits DARPP-32ir in the secondary sensory and multimodal areas, about the same as in structures of the basal ganglia and the ventral archistriatum, and significantly higher than in the HD (receiving primary visual input) and the dorsal archistriatum (Figure 11D; Durstewitz et al., 1998). The NCL has already been introduced as the possible avian equivalent of the mammalian PFC, and as such receives a prominent dopaminergic input. However, this structure cannot be differentiated from the surrounding neostriatal areas by DARPP-32 neuropil labeling or by its relative number of DARPP-32-positive neurons (Figure 11D; Durstewitz et al., 1998). In contrast, the percentage of DARPP-32-positive neurons seems even to decrease proceeding from rostral to caudal. Similar discrepancies in the distribution of dopaminergic fibers, D1 receptors and DARPP-32 have also been observed in the mammalian neocortex (Berger et al., 1990; see Section 6.2). One of the most obvious discrepancies between the distributions of DARPP-32ir and D1
receptors, however, is the significantly higher amount of DARPP-32 in the neostriatum than in the HV, while just the opposite is the case for the density of D1 receptors (Durstewitz et al., 1998; Schnabel et al., 1997). Another possible discrepancy is the considerable DARPP-32ir in the CDL where Ball et al. (1995) reported very low concentrations of D1 receptors. These discrepancies might be partly related to the fact that ligand-binding autoradiographic demonstrations of D1 receptors have different properties than the immunocytochemical demonstration of a receptor-related phosphoprotein. Thus, while receptor autoradiography might provide objective means to demonstrate receptor densities, immunocytochemistry reveals more about the morphology and possible colocalizations of the cells under study. However, it should be stressed that DARPP-32 generally is a good marker for the distribution of D1 receptors in avian species as it is in mammals (Berger et al., 1990; Hemmings and Greengard, 1986; Ouimet et al., 1992).

5.2.5. Colocalizations

Figure 12A presents examples from the caudal neostriatum of DARPP-32ir neurons which are located in TH-baskets. A quantitative examination of the number of DARPP-32ir neurons located in TH-baskets showed that this percentage differs considerably between various areas (Durstewitz et al., 1998). The highest percentage (15-30 %) of DARPP-32/TH-basket colocalizations was observed in the multimodal lateral and caudal aspects of the neostriatum, including the NCL, i.e. exactly in those regions, which expressed the highest numbers of D1 receptors according to Schnabel et al. (1997). Less DARPP-32/TH-basket colocalizations were found in the rostral and intermediate neostriatum (5-15 %), including most of the secondary sensory areas. The lowest percentage of DARPP-32/TH-basket colocalizations occurred in the hyperstriatal and hippocampal regions (< 5 %), although the HA is strongly innervated by dopaminergic fibers and exhibits a high concentration of D1 receptors. That some areas are dense in D1 receptors and dopaminergic input but display only few basket-like structures is a further hint to a functional dissociation between an 'en passant'-mode of dopaminergic innervation and the basket-like DA input. According to the functional considerations outlined in Section 6.3, one might speculate that the higher level lateral and caudal neostriatal areas have a special role in synchronizing and coordinating network activity. In the male quail telencephalon, especially within the neostriatum, colocalizations of THir baskets and varicosities and aromatase-ir cells were observed (Balthazart et al., 1998). The enzyme aromatase converts testosterone into 17β-estradiol which is involved in the activation
of male sexual behavior. These colocalizations may thus represent an anatomical basis for the regulation of male sexual behavior by DA (see Section 7.3.) DA probably stimulates aromatase activity and is, vice versa, possibly influenced itself by aromatase (Balthazart and Absil, 1997; Pasqualini et al., 1995).

From a functional point of view, it is of great importance to identify the postsynaptic targets of the dopaminergic innervation. Baskets provide a clear and convenient morphological marker to do this. GABAergic inhibitory neurons constitute one of the prominent potential candidates which might be dopaminergically modulated via baskets. Veenman and Reiner (1994) estimate the fraction of GABAergic neurons to be about 10-12 %, homogenously distributed throughout most of the telencephalon. Antibodies directed against GAD, the enzyme which converts glutamate to GABA, labels approximately the same population of cells, and thus represents a useful indicator for GABAergic neurons (Veenman and Reiner, 1994). Like in other telencephalic structures, throughout the neostriatum and hyperstriatum no GADir neurons were observed that were located in THir baskets (Figure 12B) or were colocalized with DARPP-32 (Figure 12C; Durstewitz et al., 1998). Thus, GABAergic neurons in the pigeon telencephalon might not express D1 receptors and might not receive a strong dopaminergic input. As in addition D2 receptors are very low in these areas, GABAergic neurons possibly do not receive a significant dopaminergic input at all. Hence, an interesting functional conclusion might be that mainly or solely excitatory neurons are the targets of the dopaminergic innervation in the pigeon telencephalon. However, it has to be emphasized that D2 receptors despite their low densities might nevertheless modulate the physiological activity of dopaminoceptive neurons in a significant manner. Thus, it still has to be investigated whether GABAergic neurons express D2 receptors.

5.3. Septum, hippocampal complex, archistriatum

5.3.1. Dopaminergic fibers and projections

Prominent motor and limbic structures in the avian telencephalon include the septum, the hippocampal complex, and the archistriatum. The lateral septum exhibits a high number of DAir, L-DOPAir, and THir baskets and fibers (Bailhache and Balthazart, 1993; Balthazart et al., 1998; Bottjer, 1993; Moons et al., 1994; Wynne and Güntürkün, 1995), whereas the medial septum displays a high density of THir and NAir fibers (Moons et al., 1995) but only moderate to low amounts of DAir and L-DOPAir (Figure 13A). The lateral septum, and especially the ventrolaterally located region, receives most of its dopaminergic input from the VTA, while the
medial septum is more densely innervated by axons from the SNC (Kitt and Brauth, 1986). In the hippocampus (Hp) and APH only a low number of DAir and L-DOPAir structures was detected (Metzger et al., 1996; Moons et al., 1994; Wynne and Güntürkün, 1995), which mainly stem from the SNC (Kitt and Brauth, 1986).

Fig. 12. Fluorescence double-labeling against (A) DARPP-32 and TH, (B) GAD and TH, and (C) DARPP-32 and GAD. The photomicrographs of each pair were taken from the same site in caudal neostriatum of the pigeon telencephalon. Two of three DARPP-32ir neurons (A1) are surrounded by THir baskets (A2). GADir neurons (B1) do not receive input from THir baskets (B2). GADir neurons (C2) do not show DARPP-32 immunoreactivity (C1), although GADir and DARPP-32ir neurons may occur in close vicinity to each other. Arrows mark corresponding positions in each pair. In (C), open and filled arrows indicate positions of DARPP-32ir and some GADir cells, respectively. Scale bars represent 30 µm (A, B), 40 µm (C). Taken from Durstewitz et al. (1998).
Hence, the dense THir in these areas may be mainly due to a noradrenergic innervation, which has also been demonstrated by DBHir and NAir (Bailhache and Balthazart, 1993; Moons et al., 1995; Reiner et al., 1994).

The archistriatum displays a very heterogenous innervation by dopaminergic fibers (Figures 11C, 14A). A particular dense dopaminergic input, reaching levels as high as in the basal ganglia, was described for the most dorsal archistriatum intermedium (Aid), which constitutes part of the sensorimotor archistriatum, as well as for the posterior 'limbic-visceral' archistriatum (Ap) (Bailhache and Balthazart, 1993; Balthazart et al., 1998; Metzger et al., 1996; Wynne and Güntürkün, 1995). This input stems mainly from SNC, and to a lesser extent from VTA (Kitt and Brauth, 1986). However, whereas Metzger et al. (1996) reported the rest of the archistriatum in the chick to be low in dopaminergic structures, Wynne and Güntürkün (1995) demonstrated a relatively high number of dopaminergic structures throughout the whole pigeon archistriatum, with a decreasing gradient of dopaminergic fiber density from dorsal to ventral. The ventromedially located Tn is devoid of DAir, but is relatively high in THir, NAir and DBHir (Bailhache and Balthazart, 1993; Moons et al., 1995). Interestingly, despite the very low DAir fiber density in the ventral archistriatum, there is nevertheless a high number of DAir baskets present in this structure, possibly surrounding the large projection cells which give rise to the descending tractus occipitomesencephalicus (Wynne and Güntürkün, 1995). Projections to the intermediate archistriatum from VTA and SNC have also been demonstrated by Kitt and Brauth (1986).

5.3.2. D1 receptors

In general, the medial and lateral septum seem to be very low in D1 receptor binding, at least in the quail (Ball et al., 1995). However, in accordance with its relatively dense input from SNC (Kitt and Brauth, 1986), the n. commissuralis of Baylé et al. (1974) within the medial septum is relatively high in D1 density (Ball et al., 1995), and this seems also to be the case in the domestic chick as judged from Figure 1 of Stewart et al. (1996). Both, the Hp and the APH are very low in D1-specific binding (Schnabel and Braun, 1996; Schnabel et al., 1997; Stewart et al., 1996). The pattern of $[^3]H$SCH23390 labeling in the archistriatum in general follows that described for dopaminergic fibers above. In particular, the dorsal archistriatum intermedium contains a very high number of D1 receptors, while the density strongly drops in the ventral archistriatum (Dietl and Palacios, 1988; Schnabel and Braun, 1996; Schnabel et al., 1997; Stewart et al., 1996).
5.3.3. D2 receptors
The dorsal Hp and medial APH are the only telencephalic regions besides the basal ganglia (see below) which display considerable amounts of D2 receptors (Schnabel and Braun, 1996; Stewart et al., 1996). As it is the case in the basal ganglia, D2 receptors even outnumber D1 receptors in the avian hippocampal areas.

5.3.4. DARPP-32
The septum is devoid of DARPP-32ir except for the n. commissuralis of Baylé et al. (1974) and a circumscribed region in the lateral septum (Figure 13B), both of which contain DARPP-32ir somata as well as fibers (Durstewitz et al., 1998).

DARPP-32ir neuropil labeling in the Hp is heterogenous, with the medial part being completely blank, while the ventromedial part and the lateral border show considerable neuropil labeling and a high number of DARPP-32ir somata (Durstewitz et al., 1998). These regions probably correspond to the V-shaped region and area 7 of Krebs et al. (1991) and Erichsen et al. (1991), respectively. The APH displays moderate to high levels of DARPP-32ir neurons and neuropil labeling.

With the exception of the Tn, which is virtually devoid of DARPP-32, the pigeon archistriatum is homogenously stained by DARPP-32ir with densities comparable to the neostriatum and the APH (Figure 14B; Durstewitz et al., 1998). Only the ventral but not the dorsal archistriatum in addition contains a high percentage of DARPP-32ir neurons, while just the opposite is the case for the distribution of DA fibers and receptors (Metzger et al., 1996; Wynne and Güntürkün, 1995; see Section 5.3.1). However, the finding of many large DARPP-32ir neurons in the ventral archistriatum is consistent with the relatively high number of dopaminergic baskets in this region (Wynne and Güntürkün, 1995), and may thus further establish a functional dissociation between a ‘basket’- and an ‘en passant’-mode of dopaminergic innervation. In addition, a small, dense population of DARPP-32ir cells was observed at the dorsomedial
border of the intermediate archistriatum (Figure 14B; Durstewitz et al., 1998), lying within the
dorsal archistriatum intermedium dorsale which is also densely innervated by dopaminergic
fibers and is dense in D1-receptor content (see Sections 5.3.1 and 5.3.2). As judged from figure
3 in Schnabel et al. (1997), the situation seems to be comparable in chicks, with quite high
numbers of DARPP-32ir somata and fibers in the dorsal intermediate and most ventral
archistriatum, but low staining in between.

5.3.5. Colocalizations
In the archistriatum, and especially in its ventral component, a large proportion of DARPP-32ir
neurons are located within THir baskets (15-40%; Durstewitz et al., 1998). Since axons which
constitute baskets very likely exert a massive effect on their postsynaptic targets, it is
conceivable that many D1-positive archistriatal cells are to an important extent under
modulatory control of the dopaminergic system. Since in the quails archistriatum intermedium,
THir fibers have also been found to enwrap aromatase-ir neurons, it is possible that D1-
positive archistriatal cells are jointly modulated by DA and steroids and thus play an important
role in the motor control of sexual behavior (Balthazart et al., 1998). In contrast, in the
hippocampal complex, the percentage of DARPP-32ir neurons located in THir baskets is very
low (< 5%), further supporting the notion that most if not all THir baskets in this region may be
of non-dopaminergic and thus probably noradrenergic origin (Moons et al., 1995; Reiner et al.,
1994; Wynne and Güntürkün, 1995). In the archistriatal and hippocampal regions GADir
neurons were never observed to be located within TH baskets or to be colocalized with
DARPP-32 (Durstewitz et al., 1998). Thus, in these regions like in most other telencephalic
areas the avian dopaminergic system might mainly modulate non-GABAergic and thus
excitatory forebrain neurons. The situation may be different in the hippocampal region,
however, where a higher density of D2 receptors has been observed (see Section 5.3.3). In the mammalian cortex, mainly GABAergic interneurons seem to be modulated via D2 receptors, while the activity of pyramidal cells seems to be mainly modulated via D1 receptors (Godbout et al., 1991; Law-Tho et al., 1994; Pirot et al., 1992; Rétaux et al., 1991; Yang and Seamans, 1996). If this would hold true also in the dorsal HP and medial APH, where D2 receptors are even more numerous than D1 receptors, then one might speculate that in the hippocampal areas mainly the network of inhibitory interneurons is modulated by DA, in contrast to the situation in other telencephalic areas.

5.4. Basal ganglia

5.4.1. Dopaminergic fibers and projections

The avian basal ganglia can be subdivided into four major functional regions: 1) the LPO and the PA which together are comparable to the dorsal, somatomotor striatum, 2) the Acc, TO, and BNST, which form the ventral, visceral-limbic striatum, 3) the PP, and 4) the VP, which constitute the dorsal and ventral pallidum, respectively (Karten and Dubbeldam, 1973; Reiner et al., 1984a; Veenman et al., 1995). Whereas the dorsal striatum contains small, densely packed cells, the PP mainly contains sparsely scattered large projection neurons. Comparable to the mammalian situation, anterograde and retrograde tracing studies demonstrated that structures of the avian basal ganglia are the ones receiving the densest input from the dopaminergic midbrain nuclei SNC and VTA, with some degree of topographical organization (Bons and Oliver, 1986; Brauth et al., 1978; Karten and Dubbeldam, 1973; Kitt and Brauth, 1986, Metzger et al., 1996). Thus, the LPO and the rostromedial PA receive input from VTA and the central core of SNC (but see Metzger et al., 1996, in chicks), the medial and caudal PA receive inputs mainly from the medial SNC, and the lateral PA from the lateral SNC (Bons and Oliver, 1986; Brauth et al., 1978; Kitt and Brauth, 1986). Structures of the ventral striatum also receive projections from the SNC, and to a somewhat lesser degree from VTA (Balthazart and Absil, 1997; Kitt and Brauth, 1986). In contrast, notable mesencephalic projections to the PP and n. intrapeduncularis (INP), i.e. pallidal parts of the avian basal ganglia, were not observed (Braith et al., 1978; Kitt and Brauth, 1986; Medina and Reiner, 1997).

The results from tracing studies have been confirmed by immunocytochemical investigations in several avian species using antibodies directed against DA, L-DOPA, or TH (Figure 15;
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Bailhache and Balthazart, 1993; Balthazart et al., 1998; Bottjer, 1993; Karle et al., 1996; Metzger et al., 1996; Moons et al., 1994; Wynne and Güntürkün, 1995). The 'striatal' parts of the avian basal ganglia are characterized by an extremely dense meshwork of dopaminergic fibers (Figure 15A, C), which exhibit many bouton-like axonal swellings. Actually, this meshwork is so dense that basket- and non-basket-types of dopaminergic innervation can hardly be differentiated in these regions, except for the BNST and the Acc, where the innervation is less dense (Karle et al., 1996; Wynne and Güntürkün, 1995). In the Acc, a high number of DAIR (but not THIR) basket-like structures are observed in addition to DAIR and THIR fibers (Figure 13C). In contrast, DA- and TH-positive labeling in the 'pallidal' parts of the avian basal ganglia is comparatively weak (Figure 15A, C; Bailhache and Balthazart, 1993; Balthazart et al., 1998; Bottjer, 1993; Karle et al., 1996; Metzger et al., 1996; Moons et al., 1994; Wynne and Güntürkün, 1995). Moons et al. (1994) were not able to detect any DAIR or L-DOPAIR in the PP and INP in the chick brain at all. However, Wynne and Güntürkün (1995) observed that many unlabeled large neurons in the pigeon PP are enwrapped by basket-like DA-positive structures (c.f. Figure 4B). The light labeling of the PP may thus in part be due to the sparse distribution of the large projection neurons in this area.

Considerable THIR occurs in the VP, which must be largely due to labeling of noradrenergic fibers (Bailhache and Balthazart, 1993; Karle et al., 1996; Moons et al., 1995; Wynne and Güntürkün, 1995). In contrast, by far the most TH-positive elements in the PA and LPO seem to be of dopaminergic origin, as only little DBHIR and NAIR were observed in these regions (Karle et al., 1996; Moons et al., 1995). Furthermore, in the PA and LPO, DAIR and THIR terminals make up for about 15-20% of all synaptic terminals in these areas (Karle et al., 1996). A substantial decrease of DA concentration within the basal ganglia from rostral to caudal has been noted by Juorio and Vogt (1967).

5.4.2. D1 receptors

Autoradiographic D1-receptor binding studies, all using \[^3^H\]SCH23390 as ligand, in chicks (Schnabel et al., 1997; Stewart et al., 1996), pigeons (Dietl and Palacios, 1988; Richfield et al., 1987), Japanese quails (Ball et al., 1995), and European starlings (Casto and Ball, 1994) consistently demonstrated that D1 receptors are most abundant in structures of the avian basal ganglia (Figure 7). However, the absolute binding of \[^3^H\]SCH23390 varied by several orders of magnitude between studies, ranging in the LPO from 3.8 fmol/mg protein (Stewart et al., 1996) to 2800 fmol/mg (Ball et al., 1995). This might partly be due to species and strain
differences (see Divac et al., 1988; Juorio and Vogt, 1967), but as large variations occur even within a given species (e.g., compare studies in chicks of Schnabel et al., 1997, and Stewart et al., 1996), differences in technical procedures are probably the main factor. Nevertheless, the consistent result of all of these studies was an about 5- to 10-fold lower concentration of D1 receptors in the pallidal parts (PP and VP) than in the striatal parts (LPO, PA, BNST, Acc, TO) of the avian basal ganglia.

With regards to subdivisions within the avian striatum, some authors reported higher D1 concentrations in the LPO than in the PA (Ball et al., 1995; Schnabel et al., 1997), while others could not observe notable differences (Dietl and Palacios, 1988; Richfield et al., 1987; Stewart et al., 1996). In addition, Schnabel et al. (1997) reported a decreasing rostro-caudal gradient of D1 receptors in the LPO, in accordance with the rostro-caudally decreasing DA concentration noted above (Juorio and Vogt, 1967; Juorio, 1983). In a developmental study by Schnabel and Braun (1996) in chicks, D1 receptors showed a gradual but non-significant increase during the first post-hatch week. Thus, the D1 receptor system seems to be largely developed in chicks at the time of hatching, consistent with their precocial nature.

![Figure 15. (A, C) Dopamine-immunoreactive fibers and (B, D) DARPP-32 immunoreactivity in the basal ganglia. Scale bar represents 1 mm.](image)
5.4.3. D2 receptors
The density of D2 receptors (Figure 8) in striatal parts of the avian basal ganglia (LPO, PA, BNST, TO, Acc) seems to be even higher (about 1.5-fold) than that of D1 receptors (Dietl and Palacios, 1988; Richfield et al., 1987; Stewart et al., 1996), although this was not consistently found (Schnabel and Braun, 1996; Stewart et al., 1996). The lack of a higher D2 receptor density in the Schnabel and Braun (1996) study is possibly due to a significant developmental increase in the striatal parts of the basal ganglia during the first post-hatch week as reported by these authors. Stewart et al. (1996), on the other hand, found that the relation of D1:D2 receptor densities in the basal ganglia depended on the learning history of the animals, since after one-trial avoidance learning the density of D1- but not D2-receptors was highly increased in the LPO. The density of D2 receptors in the LPO is approximately the same as (Dietl and Palacios, 1988; Richfield et al., 1987; Schnabel and Braun, 1996) or slightly lower than in the PA (Stewart et al., 1996). In contrast, D2 densities are 5- to 10-fold lower in the PP and INP than in the LPO/PA (Dietl and Palacios, 1988; Richfield et al., 1987; Stewart et al., 1996).

5.4.4. DARPP-32
In pigeons (Durstewitz et al., 1998) and chicks (Schnabel et al., 1997), the striatal parts of the basal ganglia exhibit very dense DARPP-32 labeling (Figure 15B, D), consistent with the abundance of D1 receptors in these structures. In addition, and in contrast to the autoradiographic D1-receptor binding studies, the pallidal PP (but not the INP) is as dense in its DARPP-32 labeling as the striatal components (Figure 15B, D). However, DARPP-32-positive labeling in the PP is almost exclusively constrained to fiber labeling, whereas a high number of DARPP-32ir somata shows up in the neighbouring PA and LPO. It is conceivable that the dense neuropil labeling in PP stems from descending D1-positive axons, as it is the case in the globus pallidus of rats. In these animals, D1- and DARPP-32- but not D2-positive fibers which probably belong to the striatonigral and striatoentopeduncular tracts have been described surrounding unlabeled somata and dendrites in the globus pallidus (Ouimet and Greengard, 1990; Yung et al., 1995). Finally, the avian Acc is relatively low in DARPP-32 content (Figure 13D; Durstewitz et al., 1998; Schnabel et al., 1997), in contrast to the high densities of dopaminergic fibers (Wynne and Güntürkün, 1995) and D2 receptors (Dietl and Palacios, 1988), but consistent with the low density of D1 receptors in the quail Acc according to Ball et al. (1995).
5.4.5. Colocalizations

Anderson and Reiner described two major populations of striatonigral projection neurons, one that co-contains substance P and dynorphin and makes up for about 85-95 % of all projection neurons, and the other that contains enkephalin and makes up for only 1-4 % of all projection neurons (Anderson and Reiner, 1990, 1991b; Reiner et al., 1984b; Reiner and Anderson, 1990). Projections from both of these neuronal populations in the medial striatum terminate on DAir and THir, but also - to an about equal degree - on unlabeled cells in the SNC (Anderson et al., 1991; Medina et al., 1995). Vice versa, both populations of striatonigral projection neurons receive input from THir fibers as demonstrated by EM, targeting perikarya, dendritic shafts, and spines (Karle et al., 1992, 1994). Furthermore, a subpopulation of substance P-ir projection neurons (39%) also contained somatostatin, whereas neurons containing only somatostatin or neuropeptide Y were not observed to project to the SNC (Anderson and Reiner, 1990). These findings demonstrate that in the avian like in the mammalian basal ganglia (Song & Harlan, 1994; Takagi, 1986) subpopulations of striatal output neurons which interact with the dopaminergic midbrain neurons could be differentiated by means of the neuropeptides co-released by these neurons.

Reiner and Anderson (1990; Reiner et al., 1994) suppose that both, substance P containing and enkephalin containing neurons are also colocalized with GABA. However, until now there is no convincing evidence confirming this hypothesis. According to immunocytochemical studies, the percentage of GADir and GABAir neurons in the avian basal ganglia seems to be low (< 15 %), even after pre-treatment with colchicine, and no significant regional variations could be observed throughout the entire telencephalon (Durstewitz et al., 1998; Veenman and Reiner, 1994). If this pattern should hold true in the light of other methods such as in situ hybridization, this would stand in striking contrast to what is known from the mammalian basal ganglia, where the vast majority of neurons are of the medium spiny type which utilizes GABA (Chesselet et al., 1987; Kita and Kitai, 1988; Surmeier et al., 1988). This apparent difference between the mammalian and avian basal ganglia might also explain why no colocalizations of GAD and DARPP-32 could be detected in the PA by Durstewitz et al. (1998). In contrast, in the mammalian striatum, the very high percentages of GABAergic and DARPP-32ir neurons per se implicate a high degree of overlap (Anderson and Reiner, 1991a; Ouimet et al., 1984, 1992). However, it has to be pointed out that the extremely dense meshwork of THir and DAir fibers and the very high concentration of DARPP-32 in the avian basal ganglia make the study of possible colocalizations at the light-microscopic level extremely difficult if not impossible in
For two other reasons the apparent lack of DARPP-32/GAD colocalizations in the paleostriatum does not exclude that GABAergic neurons in these regions are dopaminoceptive: First, Veenman and Reiner (1994) reported a subpopulation of small GABAergic neurons not detected by GAD immunochemistry. Second, as discussed above, D2 receptors are even more abundant than D1 receptors in the avian basal ganglia, so that the possibility remains that GABAergic cells in these regions are equipped with receptors of this class. Hence, the issue whether GABAergic neurons in the avian basal ganglia receive a significant dopaminergic input awaits further investigation.

In the VP and the BNST of the male quail brain, aromatase-ir cells were found to be located in THir baskets, and THir varicosities were found in close vicinity of almost all of these cells (Balthazart et al., 1998). In fact, due to the particular high concentration of both of these markers in the VP and BNST, these structures might be major loci of interaction between catecholaminergic systems and forebrain steroid hormone activity.

5.5. Dopaminergic innervation of song nuclei in song birds

The brain of song birds contains several sexually dimorphic nuclei involved in the song system (Nottebohm et al., 1976, 1982) which are adapted to the specific sensorimotor requirements of song perception and production. In the context of the dopaminergic system, these specific nuclei deserve a special discussion, because, interestingly, they receive a much denser dopaminergic input as measured by THir and catecholamine histofluorescence than the surrounding tissue (Bottjer, 1993; Lewis et al., 1981; Sakaguchi and Saito, 1989; Soha et al., 1996). Among these nuclei are a nucleus in the dorsolateral LPO, the so-called Area X, the robust n. of the archistriatum, the lateral magnocellular n. of the anterior neostriatum, the n. interfacialis, and the high vocal center located in the caudal neostriatum. In addition to the higher THir exhibited by these nuclei compared to the surrounding areas, a higher density of D1-specific binding has been demonstrated in area X of male starlings compared to the surrounding LPO (Casto and Ball, 1994). Furthermore, a dense input to area X from VTA and SNC has been shown by retrograde tracing (Lewis et al., 1981). In addition, the sexual dimorphism of the song nuclei is also reflected in their dopaminergic innervation as these nuclei in male birds which sing receive a much denser dopaminergic input than in the non-singing females. In contrast, sex differences with regards to DA receptor densities have not been observed in non-singing birds like quails (Ball et al., 1995).

What makes these nuclei even more interesting in terms of their dopaminergic innervation is that
the density of the dopaminergic input to these nuclei as well as DA levels and turnover rates are clearly correlated with the phases of song learning. Thus, in young zebra finches (< 30 d), THir in the song nuclei is even less dense than in the surrounding tissue (Sakaguchi and Saito, 1989; Soha et al., 1996), while a strong developmental increase occurs with the onset of the sensorimotor phase of song learning around day 35. In accordance with these findings, Harding et al. (1998) observed highly significant increases in DA levels and turnover rates in the song nuclei within the LPO, the n. robustus of the archistriatum, the lateral magnocellular n. of the anterior neostriatum, and the n. interfacialis in the neostriatum in zebra finches between postnatal days 35-55, which strongly declined again at day 90. A similar peak around day 35 at least in DA turnover was observed in the auditory Field L. Thus, the dopaminergic system probably has a prominent role in song learning and production that might be related to the requirement that sensory and motor aspects have to be integrated over time during song learning and production (see 7.6).

6. Comparative aspects of the dopaminergic system in mammalian and avian species

From the description above, readers familiar with the dopaminergic system of mammals may have recognized that the avian dopaminergic system shares many important organizational features with its mammalian counterpart. Like in mammals (Berger and Gaspar, 1994; Björklund and Lindvall, 1984; Fallon and Loughlin, 1995; Swanson, 1982), the dopaminergic innervation of the avian telencephalon arises mainly from a small population of dopaminergic midbrain nuclei in the VTA and SNC, where only a small percentage of fibers crosses into the contralateral hemisphere (Metzger et al., 1996; Kitt and Brauth, 1986). The following description focuses on some very prominent and interesting similarities and differences in the dopaminergic innervation of the telencephalon of birds, rodents, and primates.

6.1. Basal ganglia

Like the avian 'striatum', the caudate-putamen in rats and primates receives the densest dopaminergic fiber input and displays the highest concentrations of D1- as well as D2-receptors (Ariano, 1997; Björklund and Lindvall, 1984; Brock et al., 1992; Camps et al., 1990; Joyce et al., 1993; Ouimet et al., 1984; Yung et al., 1995). In addition, in mammals and birds the caudate-putamen, or dorsal striatum, is higher in its dopaminergic innervation, DARPP-32 content, and DA receptor density (at least in D2-specific binding; Richfield et al., 1987) than
the n. accumbens/ventral striatum (Ball et al., 1995; Brock et al., 1992; Durstewitz et al., 1998; Hemmings and Greengard, 1986; Joyce et al., 1993; Karle et al., 1996; Yung et al., 1995; Wynne and Güntürkün, 1995). In contrast, 'pallidal' structures in both amniote classes are much weaker innervated. Furthermore, despite the lower D1 receptor densities in pallidal compared to striatal structures, a high amount of DARPP-32 is present in the mammalian globus pallidus (Hemmings and Greengard, 1986; Ouimet et al., 1984, 1992) and its avian equivalent PP (see 5.4.4). However, as discussed for the PP in Section 5.4.4, the high DARPP-32ir in the globus pallidus of rats is almost exclusively due to neuropil labeling, in contrast to the high number of DARPP-32ir neurons in the striatum (compare Figure 5B in Durstewitz et al., 1998, to Figure 8 in Ouimet et al., 1984), and may stem from intense staining of descending striatonigral and striatopallidal fibers. Finally, a rostro-caudal gradient in DA receptor densities and DA concentration as observed in birds (see Section 5.4) has also been noted in mammals (Bockaert et al., 1976; Boyson et al., 1986; Richfield et al., 1987; Tassin et al., 1976).

However, some differences between birds and mammals are also apparent. First, direct comparisons between pigeons, rats, and cats revealed that the concentration of D1 receptors in the avian 'striatum' is about 5- to 10-fold lower, and that in the avian 'pallidum' is about 20-fold lower than in the mammalian striatum (Dietl and Palacios, 1988, Richfield et al., 1987). In contrast, the density of D2 receptors in the pigeons 'striatum' is only about half of that in the mammalian striatum, while, however, this difference is larger for the pallidum (Dietl and Palacios, 1988, Richfield et al., 1987). Second, the first point implies that the ratio of D1:D2 receptors is different in the avian and the mammalian basal ganglia. Whereas in mammals D1 receptors are much more prevalent than D2 receptors (Joyce et al., 1993), the opposite seems to be the case in birds (see 5.4.3). As D1- and D2-receptors are linked to different G-proteins and influence adenylate cyclase activity in opposite ways (Hemmings et al., 1987b; Robinson and Caron, 1997), the different D1:D2 receptor ratios may imply important differences in basal ganglia function between birds and mammals.

The possible lack of DARPP-32/GAD colocalizations in the pigeon PA has already been discussed (Section 5.4.5), and has been related to other particularities of the avian basal ganglia, namely to the fact that the number of GAD- and GABA-positive neurons in the avian basal ganglia as assessed by immunocytochemical techniques (Durstewitz et al., 1998; Veenman & Reiner, 1994) seems to be much lower than in the mammalian basal ganglia (Kita and Kitai, 1988; Surmeier et al., 1988).
6.2. Cortex

Like in birds, in rodents and primates the dopaminergic input to cortical areas is clearly weaker than that to the striatum, and the densities of dopaminergic receptors and of DARPP-32 sharply decline outside the basal ganglia (Ariano, 1997; Berger et al., 1988, 1990, 1991; Berger and Gaspar, 1994; Björklund and Lindvall, 1984; Brock et al., 1992; Hemnings and Greengard, 1986; Joyce et al., 1993; Ouimet et al., 1984, 1992). In the frontal cortices of rats and primates, the density of D1 receptors has been estimated to be about five- to tenfold higher than that of D2 receptors (Joyce et al., 1993; Lidow et al., 1991), whereas this ratio seems to be in the range of 3:1 throughout most of the bird telencephalon (Dietl and Palacios, 1988; Schnabel and Braun, 1996; Stewart et al., 1996). In contrast, in the avian Hp the density of D2 receptors outnumbers that of D1 receptors (Stewart et al., 1996). In the mammalian Hp, the D1:D2 ratio seems to be at least lower than in the neocortex although in general D1 receptors may still be more numerous (Dewar and Reader, 1989; Joyce et al., 1993). However, D1 and D2 receptors distribute differentially across hippocampal layers (Köhler et al., 1991).

With respect to the functional organization of the dopaminergic innervation of the avian and mammalian 'neocortex', strong equivalencies can be observed. Like in birds, the primary visual, auditory, and somatosensory cortices in mammals are only weakly innervated by dopaminergic fibers, at least much weaker than the respective secondary areas (Berger et al., 1988, 1991; Berger and Gaspar, 1994; Joyce et al., 1993). These areas also express lower levels of DARPP-32 (Berger et al., 1990). In addition, layer IV in the granular cortices in rats and primates, which is the major target zone of specific thalamic input, lacks a significant dopaminergic input (Berger et al., 1988, 1991; Berger and Gaspar, 1994; Joyce et al., 1993; Phillipson et al., 1987).

With respect to the 'higher order' neocortical areas, considerable differences between rodents and primates have been pointed out by Berger and coworkers (Berger et al., 1991; Berger and Gaspar, 1994). In general, the dopaminergic innervation of the primate neocortex is more widespread than that of the rat neocortex, and may thus compare better to the dopaminergic innervation of the avian neostriatal and hyperstriatal areas. In both, rodents and primates, the prefrontal areas and the anterior cingulate cortex are densely innervated (Berger et al., 1991; Berger and Gaspar, 1994; Joyce et al., 1993). Therefore, the dense dopaminergic innervation of the NCL in pigeons was taken as an indication that this multimodal area may be comparable to the mammalian PFC (Divac et al., 1985; Divac and Mogenson, 1985; Waldmann and Güntürkün, 1994; Wynne and Güntürkün, 1995). However, the PFC of mammals is not a
homogeneous structure. In primates, only the orbitofrontal and ventrolateral portions receive a dense dopaminergic input, while the dorsolateral part is lower in this respect (Berger et al., 1988; Lewis et al., 1992; Williams and Goldman-Rakic, 1993), and is also very low in DARPP-32 content in adult animals (Berger et al., 1990). In rats, however, the medial prefrontal areas, in particular the pre- and infralimbic region, which are assumed to be equivalent to the primate dorsolateral PFC, are the neocortical areas highest in DA fiber density (Berger et al., 1991; Berger and Gaspar, 1994; Joyce et al., 1993). In fact, this was one of the reasons for Preuss (1995) to question the equivalency between the rat pre- and infralimbic cortex and the primate dorsolateral PFC. Similar subdivisions of the avian 'prefrontal cortex' were not identified yet. Given the dense dopaminergic input of the NCL, one would compare this area to the prelimbic/infralimbic area of rats, or to the orbitofrontal or ventrolateral PFC of primates. On the other hand, the percentage of DARPP-32ir neurons in the NCL was lower than in other, more rostrally located neostriatal structures, reminiscent of the relatively low DARPP-32ir in the adult primate dorsolateral PFC (Berger et al., 1990). The NCL would also compare better to the primate dorsolateral PFC with respect to its functional characteristics, as both areas seem to be involved especially in spatial working memory (Funahashi and Kubota, 1994; Gagliardo and Divac, 1993; Gagliardo et al., 1996; Goldman-Rakic, 1988; Güntürkün, 1997; Mogenson and Divac, 1982, 1993; Wilson et al., 1993; but see Petrides, 1995).

Furthermore, in primates, in contrast to rats, the premotor and motor cortices are very densely innervated by the dopaminergic system (Berger et al., 1991; Berger and Gaspar, 1994; Joyce et al., 1993). Insofar, pigeons and chicks compare better to monkeys than to rats (see also Reiner et al., 1994), as major parts of the avian 'motor cortex', in particular the dorsal archistriatum intermedium, also receive a rich dopaminergic input. The comparison holds also for the distribution of DARPP-32ir neurons: Despite its exceptionally high dopaminergic input, the primate motor cortex displays only a sparse distribution of DARPP-32ir neurons (Berger et al., 1990), similar to the very low number of DARPP-32ir neurons throughout most of the intermediate archistriatum (Durstewitz et al., 1998). In addition, Berger et al. (1990) pointed out a mismatch between the distribution of D1 receptors and DARPP-32ir neurons in the primate motor cortex similar to that described for the dorsal archistriatum (Sections 5.3.2 and 5.3.4).

Finally, moderate to high numbers of dopaminergic fibers are present in the secondary sensory and association areas of the primate neocortex (Berger et al., 1988, 1991; Berger and Gaspar,
1994). In this respect, the dopaminergic innervation of the avian telencephalon might also be more similar to that of primates than to that of rats.

6.3. Laminae-specific distribution of dopaminergic fibers

An additional feature of the dopaminergic innervation of the mammalian neocortex is its laminae-specific distribution. In the rat medial and orbital (lateral) prefrontal areas, the deep layers V-VI are the major targets of dopaminergic fibers (Berger et al., 1991; Björklund and Lindvall, 1984; Joyce et al., 1993). In the primate granular cortices, the superficial layers I-III are generally even more densely innervated than the deep layers V-VI, whereas in the motor and anterior cingulate (agranular) cortices all layers receive a dense dopaminergic input (Berger et al., 1988, 1991; Berger and Gaspar, 1994; Goldman-Rakic et al., 1992; Lewis et al., 1992). As outlined in Section 2, a cortical lamination is absent in the avian brain. Nevertheless, based on hodological, histochemical, and functional criteria, some authors have compared subdivisions of the avian telencephalon to specific laminae of the mammalian neocortex. It was argued that the primary sensory areas (E, IHA,, Field L2, Bas) are equivalent to the thalamic input layer IV of the respective neocortical areas (extrastriate and striate visual, auditory, somatosensory), and the secondary sensory belts surrounding these primary areas and the HD to layers II-III (Karten and Shimizu, 1989; Reiner and Karten, 1983; Veenman et al., 1995). Furthermore, the sensorimotor archistriatum, the HA, structures of the PE, and possibly the NCL, which either give rise to the major descending pathways or directly project onto the sensorimotor avian striatum, have been identified with the neocortical output layers V-VI. This conception would again render the dopaminergic innervation of the avian brain more similar to that of primates than to that of rats, as both, the 'deeper layers' and the 'superficial layers' of the avian 'neocortex' would be high in DA and DARPP-32.

From a functional perspective, another interesting parallel between birds and mammals might lie in the fact that DA-baskets seem to contact predominantly bigger neurons in the avian brain (Ø > 15 µm), whereas smaller neurons are more likely innervated en-passant (Wynne and Güntürkün, 1995). Likewise, in the mammalian neocortex, the bigger pyramidal neurons reside in the deeper layers and are thus the ones probably most heavily innervated by DA fibers within their deep proximal and, in primates in addition, upper layer distal apical dendrites. Moreover, the bigger pyramidal cells are the ones which most likely exhibit repetitive oscillatory bursting-characteristics in the neocortex (Mason and Larkman, 1990; Yang et al., 1996a), an electrophysiological feature that - theoretically - could be exclusively due to their bigger
somata and dendrites (Mainen and Sejnowski, 1996). Thus, dopaminergic baskets may innervate neurons in the avian telencephalon with specific functional characteristics as dopaminergic fibers possibly do in the mammalian neocortex.

6.4. Ultrastructural features and postsynaptic targets of the dopaminergic innervation

With respect to the postsynaptic targets of the dopaminergic input to the mammalian neocortex, pyramidal cells seem to be the predominant dopaminceptive population, and most DARPP-32ir neurons are of the pyramidal type (Berger et al., 1990; Goldman-Rakic et al., 1989; Smiley and Goldman-Rakic, 1993). Thus, the finding that GADir neurons in the avian telencephalon do not express DARPP-32 and are never located in THir baskets (Durstewitz et al., 1998), on a first glance, fits well into the mammalian schema. However, dopaminergic fibers in the PFC have also been observed to terminate on smooth, presumably GABAergic, stellate cells (Smiley and Goldman-Rakic, 1993), and the activity of GABAergic neurons in the mammalian PFC has been shown to be modulated by DA in vivo and in vitro (Penit-Soria et al., 1987; Pirot et al., 1992; Rétaux et al., 1991; Yang et al., 1997). The dopaminergic effect on GABAergic activity can be antagonized by D2- but not by D1-receptor blockers (Godbout et al., 1991; Pirot et al., 1992; Rétaux et al., 1991, but see Yang et al., 1997), although D2 receptors are present in much lower densities in the mammalian neocortex than D1 receptors (see above). Hence, GABAergic neurons may be affected mainly via D2 receptors, and this might also be the case in the avian brain. Thus, despite their low densities, D2 receptors might play an important functional role also in the avian telencephalon, and further investigation of this subject is certainly very important.

Dopaminergic fibers in the avian telencephalon also have most of their ultrastructural features in common with their mammalian counterparts. In both animal classes, dopaminergic synapses are relatively small, are mainly of the symmetric type, contact predominantly dendritic arbors and spines, and sometimes converge with other unlabeled synapses on the same spine (i.e., form triadic complexes) (Goldman-Rakic et al., 1989; Karle et al., 1996; Metzger et al., 1996; Séguéla et al., 1988; Smiley and Goldman-Rakic, 1993; Yung et al., 1995; see 4.4). In addition to a specialized synaptic release-mode, much of the neuromodulator seems to be released via unspecialized axonal varicosities in both amniote classes (Cooper et al., 1996).

In conclusion, the general pattern of the distribution of dopaminergic fibers and receptors in the avian telencephalon, as well as the laminar and biochemical characteristics of the dopaminergic target neurons, are quite similar to that of mammals. Some specific differences
seem also to exist, but may partly be due to the fact that our knowledge about DA receptor subtypes in the avian brain is still incomplete. Finally, considerable differences in the cortical organization of the dopaminergic system have also been observed within the class of mammals.

7. Behavioral studies and functional implications

From the neuroanatomical features of the dopaminergic innervation in the avian brain, some functional clues could be derived. For example, the fact that primary sensory areas are devoid of DA, while higher sensory, associative, and motor areas that have a direct link to structures of the avian basal ganglia receive a dense dopaminergic input and are high in DA receptors makes it likely that DA plays a special role in sensory-motor integration and associative learning.

In fact, the dopaminergic system in mammals is implicated especially in motor, associative, and higher order functions like aversive and appetitive learning, and working memory (Beninger, 1993; Salamone, 1992, 1994; Sawaguchi and Goldman-Rakic, 1991, 1994; Schultz et al., 1993, 1995; Seamans et al., 1998; Sokolowski et al., 1994; Zahrt et al., 1997). Although much less is known about the involvement of DA in behavioral and cognitive functions in birds, from the studies in avian species that do exist so far a similar functional involvement of the dopaminergic system as in mammals is apparent. The next sections will deal with these studies.

7.1. Dopaminergic modulation of (unconditioned) motor functions

Dopamine in the avian brain has been shown to be critically involved in motor functions. Rieke (1980, 1981) observed that unilateral kainic acid lesions of the paleostriatum, or its source of dopaminergic input, the SNC, in pigeons induced persistent turning in one direction, postural problems, arhythmic movements, and head or whole body tremors. These behavioral dysfunctions were reproduced by unilateral injections of GABA agonists into the SNC (Rieke, 1982). Furthermore, the turning behavior elicited by unilateral GABA-agonistic injections into the SNC or 6-hydroxydopamine (6-OHDA) destruction of dopaminergic neurons in this area could be enhanced by apomorphine but was suppressed by haloperidol administration (Rieke, 1982; Yanai et al., 1995).

Apomorphine has also been shown to facilitate stereotypic pecking bouts in a dose-dependent manner, alone or induced by feeding stress, and the avian basal ganglia is a likely site for these dopaminergic effects (Cheng and Long, 1974; Deviche, 1985; Goodman et al., 1983; Nisticò and Stephenson, 1979; Rieke, 1982; Zarrindast and Amin, 1992). In contrast, DA antagonists
like haloperidol, chlorpromazine, or clozapine inhibit behavioral stereotypies (Cheng and Long, 1974; Goodman et al., 1983; Kostal and Savory, 1994), and could cause sedation and tonic immobility that could be reversed by paleostriatal but not by neostriatal lesions (Sanberg and Mark, 1983). The effects of apomorphine and amphetamine on pecking behavior can furthermore be antagonized by specific D1- (SCH23390) as well as by high doses of specific D2-receptor blockers (sulpiride), with a combination of both being most effective, while D1- or D2-specific agonists enhance apomorphine induced pecking (Dehpour et al., 1998; Zarrindast and Amin, 1992; Zarrindast et al., 1992; Zarrindast and Namdari, 1992). Low doses of sulpiride, however, enhanced pecking, presumably by blocking D2 autoreceptors, and low doses of apomorphine, on the other hand, might reduce pecking also via an autoreceptor mechanism (Deviche, 1985). It is important to point out that DA antagonists like haloperidol do not prevent all aspects of motor behavior although they reduce pecking bouts and turning, and may lead to sedation. Hence, even after haloperidol administration, Goodman et al. (1983) observed pigeons engaging in some normal behaviors like feeding, and Rieke (1982) was still able to elicit orienting and escape responses, including flying, by stressful stimuli.

Stereotypic motor behavior and hyperactivity on the one hand as induced by supranormal DA receptor stimulation or DA activity, and sedation on the other hand as induced by, e.g. neuroleptic drugs, are also typically observed in mammals including humans (Bo et al., 1988; Clark and White, 1987; Gessa et al., 1985; Le Moal and Simon, 1991; Ridley, 1994; Sokolowski and Salamone, 1994; Waddington and Daly, 1993; Wickens, 1990). However, like in birds, behavioral reactions to intense or significant stimuli may be left intact after DA depletion or subnormal DA receptor stimulation in the basal ganglia (Salamone, 1992). Thus, Salamone (1992) concluded that DA is not that much involved in motor processes per se but more in sensory-motor integration that requires novel motor sequences or in processes that extend significantly in time (see also Taylor and Saint-Cyr, 1995). DA might also be especially important for motor behavior that is driven by internal states without being directly controlled by external stimuli.

Lindenblatt and Delius (1988) demonstrated that apomorphine-induced pecking bouts could also be elicited by local injections into the Bas, and could be reversed by 6-OHDA injections into this structure. However, since dopaminergic fibers and receptors seem to be lacking in the Bas, the behavioral effects of DA-agonistic drugs might also be attributed to ligand diffusion into the adjacent PA and PP. Consistent with this interpretation, apomorphine injections into the Bas also produced contralateral turning (Lindenblatt and Delius, 1988), a typical symptome of
altered DA activity in the avian basal ganglia (Rieke, 1981, 1982). Wynne and Delius (1996) reported that apomorphine-induced pecking could be reduced by lesions of the Bas, thus demonstrating that the Bas is involved in apomorphine-induced stereotypies. However, this result does not necessarily imply that the Bas is in fact a site of apomorphine action. Apomorphine might increase pecking rates via actions in the basal ganglia, while Bas lesions could reduce pecking rates independently. On the other hand, the finding of Durstewitz et al. (1998) that the Bas had high number of DARPP-32ir neural cell bodies may hint to some anatomical basis for the apomorphine induced pecking bouts. Hence, the neuroanatomical site of the apomorphine-induced pecking bouts remains unclear.

7.2. Dopaminergic involvement in arousal and wakefulness

Ferrari and Giuliani (1993) found that DA in the chick brain might also participate in the regulation of states of arousal and wakefulness. Selective D2 agonists induced hypomotility and sleep-like states. However, for some D2 agonists (like lisuride) or the mixed D1/D2 agonist apomorphine, this effect reversed at higher doses, leading to behavioral excitation and increased spontaneous pecking instead. Similar dose-dependent patterns were also observed in rodents, where the sedative effects of the drugs were attributed to predominant stimulation of presynaptic D2 autoreceptors at lower doses (Ferrari and Giuliani, 1993; Ongini, 1993). In accordance with this interpretation, microinfusion of apomorphine into the rat VTA, but not into the Acc, caudate n., or PFC, induces behavioral and EEG signs of sleep, which could be antagonized by sulpiride (Bagetta et al., 1988a, 1988b). In general, in mammals dopaminergic drugs increase alertness, wakefulness and exploratory activity, with accompanying indications in the EEG, while lesions of the dopaminergic system, DA antagonists, or subnormal DA activity after preferential D2 autoreceptor stimulation have the opposite effect (Bagetta et al., 1988a, 1988b; Gessa et al., 1985; Montaron et al., 1982; Ongini, 1993). Dopaminergic effects on arousal and wakefulness on the one hand, and on behavioral excitation and stereotypies (Section 7.1) on the other, may be mediated by the same neural mechanism (Section 7.6).

7.3. Dopaminergic modulation of appetitive and consummatory behavior

DA in the mammalian brain is well known to regulate aspects of food and water intake (Bertolucci-D’Angio et al., 1990a, 1990b; Cooper and Al-Naser, 1993; Wilson et al., 1995), and of sexual behavior (Pfaus and Phillips, 1989). Likewise, apomorphine and DA administration in pigeons reduces food consumption (Deviche, 1984; Ravazio and Paschoalini,
1992), possibly because apomorphine and DA could substitute for the rewarding value of food. Alternatively, the low doses of apomorphine used by Deviche (1984) might have reduced food intake by very much the same mechanism that has been proposed for apomorphine-induced sedation (see Section 7.2), i.e. primarily via D2 autoreceptors. In fact, Deviche (1984) noted that the doses of apomorphine used in his study were too low to elicit pecking bouts, or even inhibited pecking.

Apomorphine also reduces appetitive and consummatory aspects of sexual behavior in the male quail (Absil et al., 1994; Castagna et al., 1997). These apomorphine-induced effects are probably due to D2-receptor stimulation as D2 agonists reduce both appetitive and consummatory sexual behavior, while D1 agonists do just the reverse, i.e. increase both aspects of sexual behavior (Balthazart et al., 1997). Again, these actions of the dopaminergic system may be related to a rewarding function of DA which has been proposed by several authors (Schultz et al., 1993, 1995; see Beninger, 1993; see Salamone, 1992, 1994; Wickens, 1990; Wickens and Kötter, 1995). According to studies in rats, part of the dopaminergic effects on food consumption and sexual behavior might be regulated by dopaminoceptive hypothalamic nuclei (Cooper and Al-Naser, 1993). However, additional structures are probably also involved since increases of DA metabolites during food consumption or upon presentation of appetitive stimuli have also been observed in the Acc, medial PFC, and in the amygdala in rats (Bertolucci-D’Angio et al., 1990b; Wilson et al., 1995).

The ‘reward theory’ of DA function partly derived from studies on intracranial self-stimulation and self-administration of dopaminergic drugs (Fibiger, 1978; Le Moal and Simon, 1991; Wise, 1978; Yokel & Wise, 1975). Self-administration of cocaine was also observed in pigeons, where it could be antagonized by haloperidol (Winsauer and Thompson, 1991), pointing to a similarly ‘rewarding’ action of DA in the avian as in the mammalian brain.

7.4. Dopaminergic modulation of learning and conditioned behavior

Dopamine in the avian telencephalon is involved in various forms of learning. Stewart et al. (1996) found that after a one-trial taste aversion avoidance learning, D1- but not D2-specific binding increased highly significantly in the LPO. A role for DA in parts of the striatum, namely in the Acc, in passive avoidance training (and, more generally, in aversive situations) is also well established in mammals (Beninger, 1993; Bertolucci-D’Angio et al., 1990a, 1990b; Salamone, 1994). In an in vivo microdialysis study, Gruss and Braun (1997) demonstrated decreased levels of the DA metabolite homovanillic acid in the MNH after auditory imprinting
in young chicks, while significant increased levels were observed after exposing the chicks to handling stress. Stressful situations induce increases of DA metabolites also in rats in the Acc and medial PFC (Abercrombie et al., 1989; Bertolucci-D’Angio et al., 1990b). The studies by Soha et al. (1996) and Harding et al. (1998) reported in Section 5.5 suggest that DA is additionally involved in the sensorimotor stage of song learning in zebra finches as the postnatal development of the innervation of various forebrain song nuclei by THir fibers as well as DA levels and turnover in these nuclei are correlated with this period. A direct involvement of DA in learning mechanisms is made likely by a study by Lindenblatt and Delius (1987) who showed that apomorphine (and thus probably DA) might serve as a positive reinforcer when coupled with a conditioned stimulus. Furthermore, DA antagonists like haloperidol reduce the rate of conditioned responding without affecting discrimination performance (Tombaugh, 1981), while conditioned responding of young chicks for heat reinforcement was shown to be enhanced especially after combined D1- and D2-agonist injection (Dose and Zolman, 1994). Finally, following apomorphine administration, chicks were found to be unable to suppress formerly rewarded but now punished responses, while this can be reversed by haloperidol pretreatment (McDougall et al., 1987).

Although these studies show that DA is somehow involved in various learning processes, the nature of the underlying mechanism is still far from clear. Thus, often it may not be easy to decide whether the DA-induced effects can be attributed to a specific role of DA in learning, to its motivational effect, to its role in motor behavior, or to its impact on attention and arousal (see Salamone, 1992). This is especially true when DA agonists are administered systemically, thus acting on many structures. For example, McDougall et al. (1987) hinted to the fact that apomorphine in their experiments generally enhanced the response rate but that this effect may have been obscured due to a ceiling effect as long as the response was associated with reward only. Only when punishment was introduced, the behaviorally exciting effect of apomorphine might have become apparent. Thus, although it is interesting to note that increased pecking rates prevail even in the presence of punishment, the main effect of apomorphine may not be to inhibit response suppression learning but to induce behavioral excitation.

7.5. Dopaminergic modulation of working memory

A failure to inhibit inadequate responses as demonstrated by McDougall et al. (1987) is also often observed after lesions of the rat and primate PFC, or lesions of its dopaminergic afferents (Dias et al., 1997; Fuster, 1989; Sokolowski and Salamone, 1994). A failure to suppress
irrelevant response options may also play a role in the inability of pigeons with NCL lesions -
depending on the extent to which the NCL was destroyed - to perform a reversal learning
(Hartmann and Güntürkün, 1998) or a go/no-go task (Güntürkün, 1997). In addition, lesions of
the NCL or its main source of thalamic afferents, the n. dorsolateralis posterior thalami, lead to
diminished delayed alternation performance (Gagliardo et al., 1996; Güntürkün, 1997;
Mogensen and Divac, 1982, 1993). Tasks which involve a delay are thought to be indicative of
working memory functions, as in these tasks an animal has to actively hold information in
memory for the guidance of responding in forthcoming situations (Fuster, 1989). Reduced
delayed alternation performance is also a characteristic deficit observed after PFC lesions in
mammals (Fuster, 1989). With regards to the transient nature of the spatial working memory
deficits after NCL lesions, the NCL compares better to the pre-/infralimbic region of rats than
to the dorsolateral PFC of primates, where lesions lead to more serious and longer-lasting
working memory deficits (Preuss, 1995).

DA and D1 receptors within the PFC are well known to be involved in working memory
functions in primates and rats (Brozoski et al., 1979; Müller et al., 1998; Sawaguchi and
Goldman-Rakic, 1991, 1994; Seamans et al., 1998; Simon et al., 1980; Zahrt et al., 1997). Also
in pigeons, blockade of dopaminergic transmission or receptors by systemic administration of
various neuroleptic drugs like chlorpromazine and clozapine has been shown to decrease
delayed matching to sample performance in a dose-dependent fashion (Picker and Massie,
1988; Watson and Blampied, 1989). Interestingly, both these drugs increased accuracy when
administered at low doses, possibly via predominant actions on D2 autoreceptors at these doses
(Picker and Massie, 1988).

Only recently our laboratory has gathered evidence that D1 receptors specifically in the pigeon
NCL might be involved in response inhibition and working memory. Thus, Diekamp et al.
(1998) presented evidence that local D1 receptor blockade by SCH23390 in the NCL impairs
go/no-go performance. After local SCH23390 application, pigeons tended to respond to all
stimuli irrespective of their reward value. Güntürkün and Durstewitz (in press) trained pigeons
in a labyrinth task which had a spatial working memory and a non-spatial reference memory
component. At the beginning of a daily session pigeons were randomly placed at one of two
starting positions in a labyrinth with 24 chambers, in which 12 arms contained red cups that
were never baited, while the remaining 12 chambers contained white cups that were baited only
at the onset of a session. Thus, as a reference memory component of the task, pigeons had to
learn to discriminate red from white cups as only the latter ones contained food pellets.
Moreover, they had to learn never to return to an arm visited previously as each white cup only contained food once at the onset of a session. This was the working memory component of the task as pigeons mentally had to keep track of all arms already visited, or - complementary - had to keep in mind all arms still worth visiting. After local SCH23390 injections into the NCL, pigeons had no problems with their reference memory, but were significantly impaired compared to pigeons which received saline control injections in the working memory part. In addition to true working memory errors, which were defined as re-entrances into arms after the animal had already visited one or more other arms, there was also a significant increase in 'perseveration' errors. Perseveration in this task was defined as an immediate re-entrance into an arm, most often due to the fact that a pigeon stayed in an arm and tried to access the same food cup a couple of times in close temporal succession. Perseveration is also a well known phenomenon in prefrontal mammals and patients with prefrontal disorders or lesions (Dias et al., 1997; Iversen and Mishkin, 1970; Fuster, 1989; Milner and Petrides, 1984).

7.6. The possible function of dopamine in sensory-motor processes

Summarizing Sections 7.1-7.5, the dopaminergic system in birds - consistent with the anatomical data - plays a role in motor functions, in arousal, in learning, and probably in working memory, while DA levels do not change with visual experience (Davies et al., 1983) and DA receptor antagonists, in general, do not seem to affect sensory discrimination per se, although they might interfere with sensory-motor integration and instrumental responding (Güntürkün and Durstewitz, in press; Mogensen and Divac, 1993; Tombaugh, 1981). Thus, in the avian telencephalon the dopaminergic system is involved in very much the same motor and cognitive functions as it is in mammals, supporting the notion of a tight link between structure and function in the nervous system. That is, the similar structural/anatomical organization of the dopaminergic system in birds and mammals seems to give rise to a similar functional organization, despite the facts that the evolution of birds and mammals diverged more than 200 million years ago and that the organization of avian telencephalic areas is quite different from that of mammals.

As already noted in Section 7.4, some of the behavioral functions of DA might be difficult to discern. According to Salamone (1992), the apparent dopaminergic involvement in motor functions and in motivation might actually refer to common neural processes, related to sensory-motor integration evolving in time, and to instrumental or 'intentional' behavior. To unravel the possible function of DA in various motor and cognitive processes, and to explicate the detailed
neural mechanisms by which DA achieves these functions, knowledge about DA-induced manipulations of neural and synaptic biophysical properties is essential. However, to our knowledge, until now nothing is known about dopaminergic modulation of electrophysiological and biophysical properties of neurons in avian forebrain regions, neither in vivo nor in vitro.

In the PFC of rats, DA enhances a persistent Na\(^+\) current (Gorelova and Yang, 1997; Shi et al., 1997; Yang and Seamans, 1996), reduces a slowly inactivating K\(^+\) and a dendritic high-voltage-activated Ca\(^{2+}\) current (Seamans et al., 1997; Yang and Seamans, 1996; Yang et al., 1996b), reduces synaptic currents through AMPA and NMDA channels (Law-Tho et al., 1994; Pralong and Jones, 1993), and increases spontaneous activity of GABAergic interneurons (Godbout et al., 1991; Penit-Soria et al., 1987; Pirot et al., 1992; Rétaux et al., 1991; Yang et al., 1997). It could be shown by computational modeling that DA by inducing these biophysical changes could act to stabilize active neural representations, and to protect them against interfering stimulation and noise (Durstewitz et al., 1999). That is, DA ensures that delay-activity of PFC neurons in working memory tasks, which is believed to represent the active holding of goal-related information (Funahashi and Kubota, 1994; Fuster, 1989; Goldman-Rakic, 1995), is sustained until the goal has been achieved. The finding that mainly or exclusively excitatory neurons are equipped with D1 receptors (see Section 5) fits well into this functional schema, as, at least in mammals, the D1 receptor-mediated effects on pyramidal cells are the ones contributing most to the stabilizing effect of DA (Durstewitz et al., 1999).

The proposed function of DA may not just be important in working memory situations, but in fact in any sensory-motor process, where a representation of the current goal-state of the movement has to be kept upright and to be compared with incoming sensory information. Indeed, sustained delay-activity has also been observed in the primate prefrontal and motor cortices (Di Pellegrino and Wise, 1991), the striatum (Apicella et al., 1992), and the posterior parietal cortex (Constantinidis and Steinmetz, 1996), which all receive a dense dopaminergic input in primates. In addition, as pointed out by Salamone (1992), temporally extended sensory-motor processes and sequences are especially susceptible to interference with the dopaminergic system while brief responses are less easily disrupted. Sustained delay-activity may be furthermore important in various, especially operant, learning processes where a temporal gap between related stimuli has to be bridged in order to detect the relation. Thus, various forms of learning, selective attention, working memory, and sensory-motor integration may all depend on the stability (and thus maintenance) of neural representations, which might be critically regulated by DA. Hence, some of the seemingly very different effects that DA excerts
in different anatomical structures may in fact be related to the same basic neural mechanism. However, active maintenance of representations may be less important in primary sensory processes, and this could explain why primary sensory areas are not or only weakly innervated by DA in birds as well as in mammals.

The here proposed function of DA might also explain the peculiar finding that many DARPP-32ir neurons show up in the Bas, although it is a primary sensory center. The Bas is the only region of the avian telencephalon that receives direct sensory inputs without thalamic relay, and it is involved in a forebrain circuit for the control of pecking where sensory and motor signals have to be integrated within extremely short time spans. Thus, the Bas might be directly involved in sensory-motor processes where sustained delay-activity plays an important role.

In conclusion, the structural and functional organization of the telencephalic dopaminergic system in birds and mammals suggests that DA might have a common function across animal classes and possibly within different telencephalic regions. DA seems to be particularly involved in sensory-motor and associative processes, where it might act to stabilize and sustain neural activity related to behavioral or motor goal states, or to linkage of temporal discontingent stimuli.

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The Dopaminergic Innervation of the Pigeon Telencephalon: Distribution of DARPP-32 and Co-occurrence with Glutamate Decarboxylase and Tyrosine Hydroxylase

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Abstract
Dopaminergic axons arising from midbrain nuclei innervate the mammalian and avian telencephalon with heterogeneous regional and laminar distributions. In primate, rodent, and avian species, the neuromodulator dopamine is low or almost absent in most primary sensory areas and is most abundant in the striatal parts of the basal ganglia. Furthermore, dopaminergic fibers are present in most limbic and associative structures. Herein, the distribution of DARPP-32, a phosphoprotein related to the dopamine D1-receptor, in the pigeon telencephalon was investigated by immunocytochemical techniques. Furthermore, co-occurrence of DARPP-32-positive perikarya with tyrosine hydroxylase-positive pericellular axonal ‘baskets’ or glutamate decarboxylase-positive neurons, as well as co-occurrence of tyrosine hydroxylase and glutamate decarboxylase were examined. Specificity of the anti-DARPP-32 monoclonal antibody in pigeon brain was determined by immunoblotting. The distribution of DARPP-32 shared important features with the distribution of D1-receptors and dopaminergic fibers in the pigeon telencephalon as described previously. In particular, DARPP-32 was highly abundant in the avian basal ganglia, where a high percentage of neurons were labeled in the ‘striatal’ parts (paleostriatum augmentatum, lobus parolfactorius), while only neuropil staining was observed in the ‘pallidal’ portions (paleostriatum primitivum). In contrast, DARPP-32 was almost absent or present in comparatively lower concentrations in most primary sensory areas. Secondary sensory and tertiary areas of the neostriatum contained numbers of labeled neurons comparable to that of the basal ganglia and intermediate levels of neuropil staining. Approximately up to one third of DARPP-32-positive neurons received a basket-type innervation from tyrosine hydroxylase-positive fibers in the lateral and caudal neostriatum, but only about half as many did in the medial and frontal neostriatum, and even less so in the hyperstriatum. No case of colocalization of glutamate decarboxylase and DARPP-32 and no co-occurrence of glutamate decarboxylase-positive neurons and tyrosine hydroxylase-basket-like structures could be detected out of more than 2000 glutamate decarboxylase-positive neurons examined, although the high DARPP-32 and high tyrosine hydroxylase staining density hampered this analysis in the basal ganglia.

In conclusion, the pigeon dopaminergic system seems to be organized similar to that of mammals. Apparently, in the telencephalon, dopamine has its primary function in higher level sensory, associative and motor processes, since primary areas showed only weak or no anatomical cues of dopaminergic modulation. Dopamine might exert its effects primarily by modulating the physiological properties of non-GABAergic and therefore presumably excitatory units.

INTRODUCTION

The mesotelencephalic dopaminergic system of mammals (Berger et al., 1991; Björklund and Lindvall, 1984; Joyce et al., 1993) and birds (Kitt and Brauth, 1986; Moons et al., 1994; Waldmann and Güntürkün, 1993; Wynne and Güntürkün, 1995) consists of a relatively small population of dopaminergic midbrain neurons in the ventral tegmentum and substantia nigra (cell groups A8, A9 and A10) which project diffusely to various limbic, motor and association areas. Primary sensory structures receive much weaker innervation (Berger et al., 1991; Berger et al., 1988; Joyce et al., 1993; Lidow et al., 1986; Moons et al., 1994; Wynne and Güntürkün, 1995). In mammals, the most prominent telencephalic target areas of the dopaminergic innervation are parts of the basal ganglia (caudate-putamen, n. accumbens), the prefrontal cortex, and the olfactory, entorhinal and anterior cingulate cortex. There are considerable differences in the distributions of dopaminergic fibers in the cortex of rodents and primates (Berger et al., 1991). For example, in primates the cortical motor areas (supplementary motor, premotor and motor cortex) are also densely innervated whereas in rats they are not. In addition to these area-specific differences in dopaminergic intensity, differential patterns of laminar distribution have also been observed (Berger et al., 1991).

The dopaminergic system plays an important role in instrumental learning and working memory (Apicella et al., 1992; Baunez et al., 1995; Beninger, 1993; Brozoski et al., 1979; Salamone, 1992; Sawaguchi and Goldman-Rakic, 1994; Schultz et al., 1995; Shu et al., 1988; Sokolowski and Salamone, 1994; Sokolowski et al., 1994). For example, blockade of D1-receptors in the primate prefrontal cortex produces severe deficits in a spatial delayed response task (Sawaguchi and Goldman-Rakic, 1994). There is evidence that dopamine is more involved in the acquisition of instrumental responses than in their performance (Beninger, 1993). Dopaminergic midbrain neurons are active in new situations where reward or reward predicting stimuli occur (Ljungberg et al., 1992; Schultz and Romo, 1993; Schultz et al., 1993). However, after acquisition of the operant, stimulus-triggered activity disappears in most dopaminergic units. In accordance with these data, Montaron et al. (1982) showed that lesions of the ventral tegmentum in the cat disrupt periods of explorative behavior and focal attention. Also, the 35-45 Hz EEG rhythms normally associated with these states disappear in ventral tegmentum-lesioned animals. Computational models of dopaminergic function suggest that dopamine may enable fast learning in new or unexpected and biologically significant situations by suppressing interference with previously learned associations and aiding ‘relearning’ of connections. (Durstewitz and Güntürkün, 1996).
Although extensive data have been accumulated on the anatomy and functional relevance of the dopaminergic system, detailed comparative information concerning the neural architecture within which dopaminergic fibers interact with other chemically identified systems is lacking. To gain further insight into the structural basis of dopaminergic function, we extended previous studies on the dopaminergic innervation of pigeon telencephalon, which demonstrated substantial similarities to mammals (Divac et al., 1985; Divac et al., 1994; Juorio and Vogt, 1967; Kitt and Brauth, 1986; Moons et al., 1994; Waldmann and Güntürkün, 1993; Wynne and Güntürkün, 1995). Here, the distribution of DARPP-32 throughout the pigeon telencephalon was analyzed and estimates of the percentage of DARPP-32-positive (DARPP-32+) neurons in selected telencephalic regions are provided. Furthermore, co-occurrence of DARPP-32, tyrosine hydroxylase (TH) and glutamate decarboxylase (GAD) was investigated. DARPP-32 is a dopamine- and cyclic AMP-regulated phosphoprotein ($M_r = 32,000$), which is closely associated with cells expressing the dopamine D1-receptor (Berger et al., 1990; Hemmings et al., 1984; Quimet et al., 1992; Qimet et al., 1984; Walaas and Greengard, 1984). The D1 receptor is the principal dopamine receptor subtype in mammalian cortex (Joyce et al., 1993) and bird telencephalon outside the basal ganglia (Dietl and Palacios, 1988). It has been implicated in working memory and associative learning (Beninger, 1993; Sawaguchi and Goldman-Rakic, 1994). TH, the rate-limiting enzyme in the synthesis of catecholamines, is present in catecholaminergic neurons and fibers. A special feature of the dopaminergic innervation of pigeon telencephalon is the presence of ‘basket’-like structures (Veenman and Reiner, 1994; Wynne and Güntürkün, 1995) in which dopaminergic fibers densely coil around single somata and thereby provide a massive input to them (Metzger et al., 1996). Baskets make possible the observation of postsynaptic targets of dopaminergic innervation at the light microscopic level. We therefore investigated co-occurrence of DARPP-32+ neurons and TH baskets. More interestingly from a functional perspective, we determined whether GABAergic neurons, as assessed by GAD-immunoreactivity, receive this type of dense catecholaminergic input. In addition, colocalization of GAD and DARPP-32 was analyzed, which would indicate the expression of D1-receptors in GABAergic neurons.

**EXPERIMENTAL PROCEDURES**

*Animals and tissue preparation.* A total of 14 adult pigeons (*Columba livia*) from local stock were used. Animals were injected with 1,000 IU heparin 15 min prior to perfusion and deeply anaesthetized with 0.3 - 0.4 ml Equithesin (Güntürkün et al., 1993) per 100 g body weight. Pigeons were then perfused intracardially with 400 ml 0.9 % saline (40°C) followed by 1,000 ml of a fixative consisting of 4 % paraformaldehyde in 0.12 M
phosphate buffer (PB; 4°C, pH 7.4). After perfusion, brains were dissected and stored for one hour in the same fixative to which 30 % (w/v) sucrose was added, and then transferred to 30 % (w/v) sucrose in PB for 12 h at 4°C. Brains were cut in frontal slices of 35-40 µm and collected in PB containing 0.05 % (w/v) NaN₃ as a preservative. Representative sections were then processed either for a peroxidase-antiperoxidase (avidin-biotin-conjugate, ABC, or Vector VIP) or the indirect immunofluorescence technique. Treatment of animals conformed to the specifications of the German law for the prevention of cruelty to animals.

**Immunocytochemistry: Avidin-biotin-peroxidase procedure.** For the ABC technique, slices were treated according to the following procedure: Free floating sections were incubated overnight at 4°C in anti-DARPP-32 (C24-6a) from mouse (Hemmings and Greengard, 1986) (working dilution 1:10,000) or anti-TH from mouse (Boehringer; working dilution 1: 200) in phosphate buffer containing 0.9 % (w/v) NaCl (PBS; pH 7.4) and 0.3 % (v/v) Triton X-100 (Sigma). The following steps were carried out at room temperature, separated by three washes in PBS of 10 min each. Slices were preincubated in 10 % (w/v) bovine albumin fraction V (Serva) or sheep (Sigma) serum in PB. After washing, slices were incubated in the secondary antibody directed against mouse from sheep (Boehringer) diluted 1:200 in PBS containing 0.3 % Triton X-100 for 1 hour. After three washes, slices were put for 1 h in Vectastain ABC (Vector) in PBS and 0.3 % Triton X-100. Three washes in PBS were followed by two additional washes in 0.12 M acetate buffer (pH 6). Staining was achieved by the 3,3'-diaminobenzidine-(DAB) technique with heavy-metal amplification (Adams, 1981; Shu et al., 1988) by adding H₈N₂NiO₈S₂ (2.5 g/ 100 ml), NH₄Cl and CoCl₂ (both 40 mg/ 100 ml) or the VIP-(Vector) technique, yielding a black or violet signal, respectively. For labeling with DAB, 400 mg/ 100 ml β-D-glucose were added to the solution. After 15 min of preincubation the reaction was catalyzed with 100-200 U/ mg glucose-oxidase (Sigma, type VII). In some cases, instead of β-D-glucose and glucose-oxidase, a solution of 0.3% H₂O₂ was used to catalyze the reaction. Finally, slices were washed three more times for 5 min in 0.12 M acetate buffer and two times in PBS. For labeling with VIP, 110 µl of reagent 1 of the VIP Substrate Kit were added to 50 ml PBS and mixed well. Then 70 µl of reagent 2 were added and mixed well. Finally 70 µl of reagent 3 were added and mixed well. The reaction was started by a 0.3% H₂O₂ solution. Washing of slices in PBS stopped the reaction. Slices were then mounted and coverslipped.

**Immunocytochemistry: Indirect immunofluorescence procedure.** For indirect immunofluorescence, the following basic procedure was used: Slices were incubated overnight at 4°C in the first primary antibody in PBS. For DARPP-32 and TH, but not for GAD, 0.3 % (v/v) Triton X-100 was added; Triton X-100 yields poorer staining with GAD-antibodies. As described above, slices were washed three times (per 10 min) in PBS, preincubated in 10 % (w/v) bovine serum albumin, and then washed three more times. Slices were then incubated for 1 h with fluorescence dye-coupled secondary antibody and the reaction was stopped by three washes in PBS. This procedure was repeated for the second primary antibody. The primary antibodies were diluted as follows (the respective dilutions of the secondary antibodies are given in brackets): anti-DARPP-32 from mouse 1:1,000 (1:200), anti-TH from mouse (Boehringer) 1:100 (1:50), anti-GAD from rabbit
(Chemicon) 1:500 (1:100). Fluoresceine FITC, Texas Red TRSC and Rhodamine LRSC or TRITC labeled donkey antibodies directed against mouse or rabbit IgG (all Jackson) were used as secondary antibodies. In some cases, the following procedure was used to amplify the DARPP-32, TH, or GAD immunosignal: Instead of the respective fluorescence dye-coupled secondary antibody, a biotinylated secondary antibody directed against mouse IgG from sheep (Boehringer) was used. Slices were then incubated in avidin-coupled fluoresceine or Texas Red (avidin DCS; Vector) for 1h, followed by incubation in a biotinylated tertiary antibody directed against avidin (anti-avidin D; Vector) for 1 h, and again fluoresceine or Texas Red avidin DCS for 1h. All these steps were carried out in PBS with Triton X-100 omitted, and were separated by three washes in PBS of 10 min each.

For immunofluorescence the following filters were used: Olympus BH - IB block with additional short-pass emitter filter G520 (FITC). Olympus BH-G with additional long-pass exciter filter EO 530 (TRITC/LRSC). Chroma exciter HQ 577/10 with dichromic beamsplitter Q 585 LP and emitter HQ 645/75 (TRSC).

In general, fluorescence methods yielded poorer staining with fewer cells labeled compared to the avidin-biotin-peroxidase techniques.

**Quantitative analysis.** To estimate the percentage of DARPP-32+ neurons in different brain regions, consecutive sections of 35 µm were stained alternately for DARPP-32 as described above or Nissl-stained with cresyl violet. 20 anatomical structures, which are listed in the results section, were selected due to functional considerations. For each of these structures, a center region was defined as illustrated in Fig. 3, divided into thirds, and from each third one sample area was drawn randomly. These sample areas had a size of $108 \times 87 \mu m^2$, which was the maximum size processed by our image analysis system (analySIS, Münster) using a $100 \times 2.5 \times 1.6$ magnification. All DARPP-32 labeled neurons in this area were counted. By use of landmarks, the corresponding area at the same location was selected in the succeeding Nissl-stained section, and here also all neurons were counted. DAB sections were shrunk by a factor of approximately 1.43 compared to Nissl preparations and cell countings were adjusted accordingly. The percentage of DARPP-32+ neurons in a region was estimated by dividing the corrected mean DARPP-32+ count by the respective mean of Nissl-stained neurons.

For calculating percentages of DARPP-32+ neurons located in TH-baskets, we used sections with TH+ fibers labeled with DAB, and DARPP-32+ neurons labeled with VIP, with anti-TH used first. As both antibodies are derived from mouse, cross-reactions might be expected. However, since TH labels only fibers in the telencephalon, with the sole exception of a number of cell bodies in the olfactory bulb, (Wynne and Güntürkün, 1995) staining patterns of DARPP-32 and TH could easily be separated. Proceeding in steps of 500 µm from rostral A 13.0 to caudal A 4.5, a frame of size $95 \times 80 \mu m^2$ was moved across the whole latero-medial and dorso-caudal extent of the telencephalon with a constant step size of 800 µm in vertical and horizontal direction. All labeled neurons within the area enclosed by the frame were checked for being enwrapped by a TH-basket. The same basic procedure was applied to the investigation of GAD/TH and GAD/DARPP-32 colocalizations. However, in these analysis, indirect immunofluorescence was used, since double labeling against GAD and TH with ABC produced a high background such that the GAD signal could not be well discriminated.
**Control experiments.** For GAD, a blocking experiment was performed. Slices were incubated in the primary antibody together with the GAD antigen (10^{-3} M, Worthington) and then processed according to the ABC-technique. This resulted in an unspecific light brown staining. The specificity of the antibody directed against DARPP-32 was assessed by immunoblotting (see below). As controls for the fluorescence double-labeling studies, in different trials either one of the two primary or one of the secondary antibodies was omitted. In all of these cases, no specific staining or no signal at all could be detected for the respective target protein.

**Immunoblotting.** Samples of pigeon brain cerebellum and neostriatum were rapidly dissected and frozen. Frozen brain samples were thawed and homogenized in 1% (w/v) sodium dodecyl sulfate (SDS) and 10 µg/ml leupeptin (Chemicon). Aliquots (100 µg of total protein determined by the bicinchoninic acid method (Smith et al., 1985) with bovine serum albumin as standard) were subjected to SDS/polyacrylamide gel electrophoresis (SDS/PAGE) on gels cast from 13% (w/v) acrylamide and transferred to 0.2 µm nitrocellulose membrane (Schlicher and Scheull) as described (Hemmings and Adamo, 1997). Immunoblotting with anti-bovine DARPP-32 antibody C24-6a (Hemmings and Greengard, 1986) at a 1:500 dilution was performed as described (Hemmings and Adamo, 1997) using prestained molecular weight markers (Amersham).

**RESULTS**

**Immunoblotting**

The specificity of the antibody to DARPP-32 was demonstrated by the selective labeling of a single protein of M_{r} = 32,000 in pigeon neostriatum (Fig. 1). No immunoreactive protein was detected in the cerebellum by immunoblotting or by immunocytochemistry.

**Distribution of DARPP-32**

The antibody directed against DARPP-32 stained a number of neurons to a large extent so that fine dendritic processes with spines were clearly visible (Figs 2D-F, 4D). More often, however, only somata with the first segments of stem dendrites were labeled (Fig. 2B-C). Neurons of various sizes and shapes were stained, with soma diameters ranging from about 5 - 25 µm. Neurons also differed considerably in their degree of staining intensity.

Telencephalic regions differed with respect to the ratio of soma to neuropil staining. Whereas, for example, in the paleostriatum primitivum the neuropil was densely stained and neurons seemed to be selectively spared (Fig. 5B), in the n. basalis there was almost no neuropil staining while a large number of somata were clearly DARPP-32+ (Fig. 4A). To acknowledge the different contributions of neuropil and soma labeling, telencephalic subdivisions were classified according to two different schemes with four categories each. The results are shown in Fig. 3.
Chapter 5: Distribution of DARPP-32 in pigeon telencephalon

The first categorization was based on neuropil staining intensity. Structures were defined as "1" when being virtually devoid of any labeling, as "2" for a light neuropil staining, as "3" for considerably stronger staining, and as "4" for extremely dense staining (Fig. 2A). The second categorization was based on the percentage of DARPP-32+ neurons. Percentage estimates were obtained as described in Experimental Procedures for a subset of 20 areas, selected for functional reasons.

Figure 1. Characterization of the specificity of anti-DARPP-32 monoclonal antibody C24-6a by immunoblotting. Samples of pigeon cerebellum (left 2 lanes) and neostriatum (right 2 lanes) were dissected from the brain, homogenized in SDS, subjected to SDS/PAGE (13% acrylamide), and electrophoretically transferred to a nitrocellulose membrane as described in Experimental Procedures. The nitrocellulose membrane was labeled with a 1:500 dilution of monoclonal antibody C24-6a, followed by 1:500 rabbit anti-mouse IgG and 125I-labeled protein A, and analyzed by autoradiography. Pigeon DARPP-32 (Mr = 32,600) is indicated by the arrow. Samples contained 100 µg of total brain protein.
Figure 2. DARPP-32 immuno-labeling in the pigeon forebrain. (A) Frontal section at level A 9.00 according to the pigeon brain atlas of Karten and Hodos (1967), illustrating the four categories of DARPP-32+ neuropil staining (see text). Paleostriatum augmentatum (PA) and paleostriatum primitivum (PP), displaying the densest neuropil labeling, were classified as ‘4’, ectostriatum (E), showing almost no labeling, as ‘1’, neostriatum (N) as ‘3’, and hyperstriatum ventrale (HV) as ‘2’. (B, C) Most DARPP-32+ cells displayed only labeling of the soma and initial dendritic segments. (D-F) In some neurons, however, also fine dendritic processes and spines were visible. Photomicrographs were taken from the n. basalis (B), neostriatum frontolaterale (C), ventromedial hippocampus (D), border of ectostriatum and ectostriatal belt (E), and medial septum (F). Scale bars represent 250 µm (A), 50 µm (B, D, E), 20 µm (C), 15 µm (F).
Primary sensory forebrain areas included in our analysis were the ectostriatum of the visual tectofugal, (Benowitz and Karten, 1976) the hyperstriatum dorsale pars lateralis of the visual thalamofugal, (Karten et al., 1973) field L2 of the auditory, (Bonke et al., 1979) and n. basalis of the trigeminal system (Schall et al., 1986). The intercalated nucleus of the hyperstriatum accessorium, a further termination area of the visual thalamofugal pathway, (Güntürkün and Remy, 1990) was not included in the analysis since percentage estimates of labeled neurons could not be obtained reliably in this extremely thin structure. The secondary sensory forebrain areas included in our analysis were the ectostriatal belt, which receives afferents from the ectostriatal core, (Ritchie, 1979) the hyperstriatum accessorium as the projection zone of the lateral hyperstriatum dorsalis, (Shimizu et al., 1995) field L1, which receives afferents from L2, (Wild et al., 1993) and the neostriatum frontale, pars trigeminal, which is innervated by the primary trigeminal n. basalis (Wild et al., 1985). Multimodal (tertiary) areas were the neostriatum caudolaterale as defined by Waldmann and Güntürkün (Veenman and Reiner, 1994) with its afferents from ectostriatal belt, (Ritchie, 1979) hyperstriatum accessorium, (Shimizu et al., 1995) L1, (Rehkämper and Zilles, 1991) and neostriatum frontale trigeminal (Wild et al., 1985). Three further multimodal structures, which comprise the pallium externum of Veenman et al., (1995) were included in our analysis: the neostriatum frontolaterale as defined by Shimizu et al (1995), with its afferents from hyperstriatum accessorium/hyperstriatum dorsale (Shimizu et al., 1995) and ectostriatal belt; (Ritchie, 1979) the temporo-parieto-occipital area, and the area corticoidea dorsolateralis. The latter two are known to receive visual afferents from hyperstriatum dorsale (Shimizu et al., 1995) and ectostriatal belt, (Veenman et al., 1995) as well as trigeminal afferents from neostriatum frontale trigeminal (Dubbeldam and Visser, 1987). The archistriatum intermedium with its descending projections to various brainstem structures was considered as a motor output area (Zeier and Karten, 1971). Limbic structures included in our analysis were the archistriatum ventrale and archistriatum posterior (Veenman et al., 1995; Zeier and Karten, 1971). The lobus parolfactorius, the paleostriatum augmentatum, and the paleostriatum primitivum were analyzed as parts of the avian basal ganglia (Karten and Dubbeldam, 1973; Reiner et al., 1984; Veenman et al., 1995). The description of DARPP-32 distribution follows this functional classification. Figure 3 depicts the topographical distribution of neuropil staining density and the DARPP-32+ neuron percentage classification.
Primary sensory areas. Primary sensory regions showed the lowest DARPP-32 immunoreactivity of all telencephalic areas (Figs 3, 4). The ectostriatum and field L2 appeared almost completely blank, containing only few broadly scattered cells (Fig. 2A; Figs 4B, D). The lateral hyperstriatum dorsale had more neuropil staining and labeled neurons, but was considerably lower in both respects compared to most secondary sensory and tertiary areas, particularly its secondary projection zone, the hyperstriatum accessorium (Fig. 4C).
primary sensory area of the trigeminal system, the n. basalis, was an exception. Although it showed very light neuropil staining, it actually contained the highest percentage of DARPP-32+ neurons of all selected areas (Figs 2B, 4A).

**Secondary sensory areas.** The secondary sensory areas of the visual-tectofugal (ectostriatal belt), visual-thalamofugal (hyperstriatum accessorium) and auditory (L1) system ranked clearly higher in both neuropil labeling and the relative number of DARPP-32+ neurons when compared to the respective primary sensory areas (Fig. 3). As mentioned above, the trigeminal system was an exception with the neostriatum frontale trigemina
tale having a much denser neuropil staining than the n. basalis, but containing a lower percentage of labeled neurons than the latter one (Fig. 4A). In general, secondary structures displayed approximately equal relative numbers of DARPP-32+ neurons. Only the caudally located field L1 scored lower than the more rostral structures.

Figure 4. DARPP-32 immunoreactivity in primary and adjacent secondary sensory areas of the pigeon telencephalon. (A) The trigeminal n. basalis (Bas) displays a high number of DARPP-32+ neurons, although neuropil staining is very low in this area. (B) Primary auditory Field L2 shows almost no labeling in contrast to the adjacent secondary areas. (C) Hyperstriatum dorsale (HD), from which the lateral portion receives thalamic input and is part of the primary thalamofugal visual system, shows clearly less soma and neuropil labeling than its secondary projection zone, the hyperstriatum accessorium (HA). (D) Single DARPP-32-positive cell in the otherwise unstained ectostriatum (E), surrounded by the ectostriatal belt and the neostriatum. Scale bars represent 500 µm (A-C), 200 µm (D).
Tertiary areas. The tertiary structures neostriatum frontolaterale, area temporo-parieto-occipitalis, corticoidea dorsolateralis, neostriatum caudolaterale, and the caudal neostratum were stained homogeneously at medium to high densities. Based on the intensity of neuropil labeling as well as on the percentage of DARPP-32+ neurons, these areas could not be differentiated from the secondary sensory structures (Fig. 3). Among the tertiary regions, the more rostral areas of the neostratum (neostriatum frontolaterale, area temporo-parieto-occipitalis, corticoidea dorsolateralis) showed more DARPP-32+ neurons than the caudal components (neostriatum caudale, neostriatum caudolaterale).

Motor and limbic structures. According to neuropil staining intensity, structures of the avian basal ganglia (paleostriatum augmentatum, paleostriatum primitivum, lobus parolfactorius) were most heavily labeled and could not be distinguished from each other (Figs 2A, 3, 5A). Lobus parolfactorius and paleostriatum augmentatum also contained a high percentage of DARPP-32+ neurons, whereas in the paleostriatum primitivum only a very small number of cells was labeled (Fig. 5B). On the basis of cell counting alone, no difference between lobus
parolfactorius/paleostriatum augmentatum and secondary or tertiary areas could be observed (Fig. 3). The n. accumbens and bed nucleus of the stria terminalis, according to the definition of Veenman et al., (1995) also showed a considerable number of labeled cells.

Neuropil labeling in the archistriatum was homogeneous, comparable to the neostriatal regions and considerably lower than in the basal ganglia (Figs 3, 5C). The ventral portions of the ‘motor’ as well as of the ‘limbic-visceral’ archistriatum demonstrated a high percentage of DARPP-32+ neurons (Fig. 3). In contrast, in the dorsal parts of the archistriatum mainly neuropil was labeled, with exception of a small dorsomedical rim of labeled perikarya (Fig. 5C).

Other limbic structures in the telencephalon of the pigeon include the hippocampal system and the septum. Neuropil labeling in most regions of the hippocampus was comparable to that of the archistriatum and higher sensory and multimodal areas of the neostriatum. Also, a high number of DARPP-32+ neurons was observed in the ventromedial area and at the lateral border, which probably correspond to the V-shaped region and area 7 of Krebs et al. (1991) and Erichsen et al. (1991) (Fig. 2D). The medial border, however, which is part of the ventromedial and inferior dorsomedical region as defined by these authors, was found to be completely blank. In the septum, DARPP-32 immunoreactivity strongly paralleled the distribution of TH+ fibers. Only two restricted portions of the lateral and ventromedial septum, the latter probably corresponding to the n. commissuralis of Baylé et al., (1974) contained numbers of DARPP32+ cells, while the whole septum demonstrated very low levels of DARPP-32+ neuropil staining (Fig. 5D).

**Co-occurrence of DARPP-32 and tyrosine hydroxylase baskets**

‘Baskets’, which consist of fibers densely coiling around single perikarya and initial dendritic segments, represent a striking feature of catecholaminergic innervation in avian telencephalon (Fig. 6A, B). As illustrated in Fig. 6C-E and Fig. 7A, a number of DARPP-32+ neurons receive input from TH+ baskets. To obtain estimates of the percentage of DARPP-32+ neurons located in TH-baskets, a total of 3692 neurons were examined. Analysis of several regions were pooled and classified into one of three categories. Because of the extremely dense meshwork of TH+ fibers and the very high DARPP-32 abundance in regions of the avian basal ganglia (paleostriatum augmentatum, paleostriatum primitivum, lobus parolfactorius), reliable countings were impossible here and these areas were not included in the analysis.
Chapter 5: Distribution of DARPP-32 in pigeon telencephalon

The highest percentage of co-occurrence was seen in the multimodal lateral and caudal neostriatum (area temporo-parieto-occipitalis / corticoidea dorsolateralis / neostriatum caudale / neostriatum caudolaterale: 15 - 30 %). This value was lower in the intermediate medial and frontal neostriatum (including ectostriatal belt / neostriatum frontolaterale / neostriatum frontale trigeminale: 5 - 15 %). The lowest percentage of co-occurrences was observed in the hyperstriatal and hippocampal regions (hyperstriatum accessorium / area parahippocampalis / hippocampus: < 5 %), although these areas were rich in DARPP-32+ neurons. Also, many TH-baskets containing no DARPP-32-labeled neurons were observed throughout the telencephalon. Forebrain regions differed with respect to the number of fibers constituting single baskets. Baskets in the caudal neostriatum consisted of a large number of axonal loops surrounding

Figure 6. TH+ fibers and DARPP-32+ neurons in the pigeon caudal neostriatum. (A, B) TH+ baskets consist of axons densely coiling around single perikarya and initial dendritic segments, so that neurons virtually become visible by the TH+ signal alone. (C-E) VIP-stained DARPP-32+ neurons enwrapped by DAB-stained TH+ fibers. Scale bars represent 30 µm (A), 20 µm (B, D, E), 50 µm (C).
perikarya and initial dendrites, while TH+ fibers in the frontal neostriatum coiled only a few times around single somata (Waldmann and Güntürkün, 1993).

**Co-occurrence of glutamate decarboxylase and tyrosine hydroxylase baskets**

To examine whether GAD+ neurons receive TH-basket-type input, 861 GAD+ neurons and 636 TH-baskets throughout the telencephalon were inspected. As demonstrated in Fig. 7B, no co-occurrence was found. However, in the lobus parolfactorius, paleostriatum augmentatum and paleostriatum primitivum reliable counting could not be obtained due to the dense net of catecholaminergic fibers in these regions.

**Co-localization of glutamate decarboxylase and DARPP-32**

To investigate the possible colocalization of DARPP-32 and GAD antigen, a total of 939 DARPP-32+ and 1149 GAD+ neurons throughout the telencephalon were examined. No colocalization was observed. Fig. 7C shows examples of neurons in the neostriatum, labeled for GAD and DARPP-32, respectively. However, in the basal ganglia the possibility of colocalization cannot be excluded with certainty, since the high DARPP-32 staining density in these regions hampered the analysis.

**DISCUSSION**

The present study describes the distribution of DARPP-32 in the avian telencephalon and provides a classification of telencephalic regions in terms of their neuropil staining intensity and DARPP-32+ neuron numbers. Additionally, colocalization of GAD-and DARPP-32-labeled cells and their co-occurrence with TH-labeled axonal baskets were investigated.

**Distribution of DARPP-32**

The strongest DARPP-32 immunoreactivity was observed in parts of the avian basal ganglia, namely the paleostriatum augmentatum, lobus parolfactorius and paleostriatum primitivum, which are regarded as equivalent to the mammalian caudate-putamen and globus pallidus, respectively (Veenman et al., 1995). In the paleostriatum augmentatum/lobus parolfactorius both fibers and somata were densely labeled. In these areas, TH+ and DA+ fibers have been shown to contact predominantly shafts and necks of dendritic spines, (Karle et al., 1996) suggesting possible sites for dopaminergic receptors. The strong staining in the paleostriatum primitivum on the other hand resulted almost exclusively from neuropil labeling (see Fig. 5B).
This clear difference in somatic labeling between the paleostriatum augmentatum/lobus parolfactorius and paleostriatum primitivum corresponds to results obtained with other markers of the dopaminergic system in mammals and birds: the abundance of dopaminergic

Figure 7. Fluorescence double-labeling against (A) DARPP-32 and TH, (B) GAD and TH, and (C) DARPP-32 and GAD. The photomicrographs of each pair were taken from the same site in caudal neostriatum of the pigeon telencephalon. Two of three DARPP-32+ neurons (A1) are surrounded by TH+ baskets (A2). GAD+ neurons (B1) do not receive input from TH+ baskets (B2). GAD+ neurons (C2) do not show DARPP-32 immunoreactivity (C1), although GAD+ and DARPP-32+ neurons may occur in close vicinity to each other. Arrows mark corresponding positions in each pair. In (C), open and filled arrows indicate positions of DARPP-32+ and some GAD+ cells, respectively. Scale bars represent 30 µm (A, B), 40 µm (C).
fibers as well as of D1 and D2 receptors is highest in the caudate-putamen and the paleostriatum augmentatum/lobus parolfactorius of the mammalian and avian basal ganglia, but is relatively low in the globus pallidus and the paleostriatum primitivum, respectively (Ball et al., 1995; Berger et al., 1991; Brock et al., 1992; Joyce et al., 1993; Moons et al., 1994; Richfield et al., 1987; Schnabel and Braun, 1996; Stewart et al., 1996; Wynne and Güntürkün, 1995; Yung et al., 1995). The dense neuropil labeling in the paleostriatum primitivum may stem from descending D1-positive axons, as in the globus pallidus of rats where DARPP-32- and D1- but not D2 receptor-positive axons have been described surrounding unlabeled perikarya and dendrites (Quimet and Greengard, 1990; Yung et al., 1995). These axons probably belong to the descending striatonigral and striatoentopeduncular tract (Yung et al., 1995).

Besides the basal ganglia, several other structures of the avian motor and limbic system showed medium-to-high DARPP-32 immunoreactivity, namely the archistriatum and the area parahippocampalis/hippocampus. According to Zeier and Karten, (1971; 1973) the anterior two thirds of the archistriatum (archistriatum pars dorsalis and archistriatum intermedium) correspond to the mammalian motor cortex, whereas the archistriatum posterior and pars ventralis have been compared to the mammalian amygdala. In the present study, neurons in the ventral parts of the motor as well as of the limbic archistriatum were strongly DARPP-32 labeled while in the dorsal parts only neuropil was labeled. This finding stands in contrast to the pattern described for dopaminergic fibers in the archistriatum by Wynne and Güntürkün, (1995) who found DA baskets, presumably innervating somata, especially in those archistriatal portions where no or few DARPP-32 cells were labeled. D2 receptors cannot account for the observed mismatch, since these are known to have extremely low densities outside the basal ganglia (Dietl and Palacios, 1988; Richfield et al., 1987; Stewart et al., 1996). However, differences regarding the dopaminergic innervation and the distribution of dopamine receptors also exist in motor output areas of various mammalian species. In primates, the motor cortex receives an exceptionally strong dopaminergic innervation (Berger et al., 1991; Björklund and Lindvall, 1984; Williams and Glodman-Rakic, 1993) but displays only a sparse distribution of DARPP-32 labeled cells and contains only medium densities of D1 receptors (Berger et al., 1990; Cortés et al., 1989; Lidow et al., 1989; Lidow et al., 1991; Quimet et al., 1992; Richfield et al., 1989).

DARPP-32 soma and neuropil labeling revealed a general pattern of low or no staining in primary and much stronger labeling in secondary and multimodal association areas (see Fig. 3). This is in good agreement with studies on the distribution of dopaminergic fibers and D1
receptors in birds (Dietl and Palacios, 1988; Moons et al., 1994; Wynne and Güntürkün, 1995). It is also in accordance with data from rodents and primates, where the density of D1-receptors and dopaminergic fibers is lowest in primary sensory but higher in secondary and association cortices (Berger et al., 1991; Berger et al., 1988; Joyce et al., 1993; Lewis et al., 1986).

The n. basalis is a notable exception from the rule outlined above. Although showing weak neuropil labeling, it contains a high percentage of DARPP-32+ neurons (see Fig. 4A). This observation contrasts with results for other primary sensory regions and could be linked to other peculiarities of the pigeon's trigeminal system. The n. basalis receives, among other afferents, direct input from the n. sensorius principalis nervi trigemini and projects through other forebrain areas to rhombencephalic structures related to pecking (Schall, 1987; Schall et al., 1986; Wallenberg, 1903; Wild et al., 1985). It is thus the only avian forebrain structure with sensory afferents bypassing the thalamus. The projection via the n. basalis, neostriatum frontale trigeminale and archistriatum constitutes a trigeminal circuit for the control of pecking(Schall, 1987; Schall, 1989; Zeigler, 1976). Within this circuit, sensory signals and motor outputs probably have to be integrated within extremely short time spans. Therefore, dopaminergic modulation (see general discussion) might start earlier in this system than in other sensorimotor circuits.

The presence of DARPP-32+ cells within the n. basalis contrasts with a number of previous anatomical studies. According to Dietl and Palacios (1988) the levels of D1 receptors in the n. basalis are negligibly low. Kusunoki (1969) could also not reveal monoaminoxidase activity in the n. basalis, and Wynne and Güntürkün (1995) were unable to detect dopaminergic fibers in this structure. In contrast to these anatomical results, behavioral data make it likely that dopaminergic modulation exists within this nucleus. Apomorphine-induced pecking fits are accompanied by an increase of glucose uptake in the n. basalis (Delius, 1985) and intracranial apomorphine injections into this structure induce stereotypical pecking bouts (Lindenblatt and Delius, 1988). Therefore, n. basalis was thought to be a central element of apomorphine triggered pecking stereotypies and to possess dopamine receptors (Delius, 1985). This last assumption was strengthened by the finding of Wynne and Delius (1996) that n. basalis-lesions significantly reduce pecking fits after apomorphine injection. The present finding of a prominent group of DARPP-32+ cells in the n. basalis does not fit easily with the chemoanatomic results gathered up to now but may provide an anatomical clue to behavioral data.

According to biochemical, hodological and behavioral results, the neostriatum caudolaterale has been suggested as an avian equivalent of the prefrontal cortex (Divac and Mogensen, 1985;
Divac et al., 1985; Divac et al., 1994; Güntürkün, 1997; Mogensen and Divac, 1982; Mogensen and Divac, 1993; Reiner, 1986; Waldmann and Güntürkün, 1993; Wynne and Güntürkün, 1995). The dopaminergic innervation of the neostriatum caudolaterale is among the strongest in the pigeon telencephalon, (Divac et al., 1994; Waldmann and Güntürkün, 1993; Wynne and Güntürkün, 1995) just as it is in the mammalian prefrontal cortex (Berger et al., 1988; Joyce et al., 1993; Lewis et al., 1986; Williams and Goldman-Rakic, 1993). The percentage of DARPP-32+ neurons in the neostriatum caudolaterale, as revealed in the present study, is lower than in the secondary sensory areas ectostriatal belt or neostriatum frontale trigemina; while this pattern is reversed for the innervation by dopaminergic fibers in these regions (Wynne and Güntürkün, 1995). Again, this finding parallels data from mammals: The dorsolateral prefrontal cortex of adult monkeys also displays a low number of DARPP-32+ neurons despite its dense dopaminergic innervation (Berger et al., 1990).

**DARPP-32 as a marker for D1-receptors**

In rodents and primates the distribution of DARPP-32 has been found to parallel the distribution of cells possessing D1 receptors (Berger et al., 1990; Hemmings and Greengard, 1986; Quimet et al., 1992; Quimet et al., 1984; Walaas and Greengard, 1984). Receptors of the D1-family are positively coupled to adenylyl cyclase activity and can thus stimulate cAMP synthesis (Kebabian and Calne, 1979). This in turn leads to phosphorylation of DARPP-32 via cAMP-dependent protein kinase, resulting in inhibition of phosphatase-1 (Hemmings et al., 1984; Hemmings et al., 1987). DARPP-32 acting as an intracellular “third messenger” is thus involved in mediating the D1 receptor dependent effects of dopamine.

Key features of the distribution of dopaminergic fibers and D1-receptors in avian, such as sparing of primary sensory areas or dense innervation of structures of the basal ganglia, (Ball et al., 1995; Dietl and Palacios, 1988; Moons et al., 1994; Richfield et al., 1987; Stewart et al., 1996; Waldmann and Güntürkün, 1993; Wynne and Güntürkün, 1995) could also be observed in the distribution of DARPP-32. There are some exceptions, however Richfield et al. (1987) and Stewart et al. (1996) demonstrated higher D1-specific binding in hyperstriatum ventrale than in neostriatum, while this pattern is reversed with DARPP-32. Mismatches in the distributions of DARPP-32 and D1 receptors have also been noted in mammals, e.g. in primate motor cortex (Berger et al., 1990). In general, however, these distributions overlap to a high degree, and DARPP-32 thus seems to represent a useful marker for dopaminceptive cells expressing D1 receptors within the mammalian as well as within the avian telencephalon.
It has been noted that glia may also contain DARPP-32 (Berger et al., 1990; Quimet et al., 1984). Thus, the cell counting analysis presented here may contain overestimations. However, most DARPP-32+ cells observed were larger than glia cells (Ø > 6 µm) or expressed clear neuronal properties like dendritic elements (see Fig. 2B-F). Also, in Nissl-counterstained preparations, very few DARPP-32+ cells were observed resembling glia cells. The estimation error due to glia staining therefore was probably very low in our preparations.

Co-localization of glutamate decarboxylase with DARPP-32 and co-occurrence with tyrosine hydroxylase

GAD is relatively homogeneously distributed throughout the pigeon telencephalon (Domenici et al., 1988; Veenman and Reiner, 1994). Veenman and Reiner (1994) report that the distributions of GAD+ and GABA+ cells overlap almost completely, and they estimate the fraction of GABAergic neurons to be about 10-12% in the neo- and hyperstriatum, showing only slight regional differences. According to the present results, GAD+ neurons do not express DARPP-32 and do not receive input from TH-baskets. Therefore, GABAergic cells, at least in the avian ‘neocortex’, do not seem to be the target of the dense type of dopaminergic innervation and probably do not express D1 receptors. Yet the possibility remains that these cells express receptors of the D2 receptor family. In mammalian neocortex, there is indeed electrophysiological evidence that GABAergic neurons may be modulated by dopamine via D2 receptors (Pirot et al., 1992; Rétaux et al., 1991). However, receptor autoradiography studies in birds have shown that, with exception of the basal ganglia, the abundance of D2 receptors in the telencephalon is very low (Dietl and Palacios, 1988; Richfield et al., 1987; Schnabel and Braun, 1996; Stewart et al., 1996). Thus, GABAergic cells in the pigeon telencephalon might not, or at least not to an important extent, be innervated by dopamine. Therefore presumably excitatory neurons are the main target of dopaminergic afferents. This interpretation would also be consistent with data from mammalian frontal cortex, where dopaminergic axons mainly, but not exclusively, (Smiley and Goldman-Rakic, 1993) synapse on presumably glutamatergic pyramidal cells, (Berger et al., 1991; Goldman-Rakic et al., 1989; Smiley and Goldman-Rakic, 1993) which also constitute the major cell population expressing DARPP-32 (Berger et al., 1990).

In the pigeon basal ganglia also no colocalization of GAD and DARPP-32 was detected. In contrast, GABAergic neurons in mammalian caudate-putamen express D1-receptors and very likely also DARPP-32 (Joyce et al., 1993; Quimet et al., 1992;Yung et al., 1995).
discrepancy may be explained in terms of another difference between avian and mammalian basal ganglia. The avian basal ganglia contain only 5-10% GAD+ cells (Veenman and Reiner, 1994) as opposed to the vast majority of medium spiny neurons in mammalian basal ganglia, which are supposed to utilize GABA (Groves et al., 1995). Yet, for several reasons we cannot exclude the possibility that some GAD/DARPP-32 colocalizations went unnoticed in the present study. First, as noted in the results section, colocalizations were difficult to analyze in the avian basal ganglia due to the high DARPP-32 neuropil staining. Second, it is conceivable that some GABAergic cells were indeed colocalized with DARPP-32 but were not labeled by GAD immunohistochemistry, since Veenman and Reiner (1994) report a subpopulation of small cells which show GABA but not GAD immunoreactivity. Third, the methods used in the present study may have been not sensitive enough to detect all GAD+ cells. However, although Veenman and Reiner (1994) note that pre-treatment of pigeons with colchicine enhances GAD perikaryal labeling, the percentage of GAD+ cells in the paleostriatum as estimated by these authors (5-10%) seems to be in the same range as in the present study.

In conclusion, the present results make it likely that dopamine in the pigeon's forebrain exert its effects primarily by changing physiological parameters of non-GABAergic and thus presumably excitatory units, although this conclusion has to be treated with caution with respect to the avian basal ganglia.

Co-occurrence of DARPP-32 and tyrosine hydroxylase

In the present study, a large number of DARPP-32+ neurons localized in TH-baskets have been observed, although the majority of labeled perikarya did not receive this type of dense catecholaminergic innervation. The highest percentage of DARPP-32/TH co-occurrences was found in the lateral and caudal parts of the neostriatum and thus in parts of the neostriatal association areas. In contrast to primary and secondary sensory areas, these association areas are also known to have a direct link to the avian basal ganglia (Veenman et al., 1995). The finding that there are many 'empty' TH-baskets could be due to noradrenergic fibers, and TH-baskets of non-dopaminergic origin have indeed been demonstrated (Wynne and Güntürkün, 1995). Alternatively, neurons expressing other than D1 dopamine receptor subtypes could also be the target of the basket-type input.
General discussion

In birds, dopaminergic innervation of the telencephalon with clear regional differences arises from the midbrain nuclei tegmenti pedunculopontinus pars lateralis and area ventralis tegmentalis, comparable to the projections from the mammalian substantia nigra pars compacta and area ventralis tegmentalis, respectively (Kitt and Brauth, 1986; Waldmann and Güntürkün, 1993). Our study shows that dopaminergic innervation of the pigeon telencephalon is comparable to that of mammals in three important respects. First, DARPP-32, and therefore presumably the D1 receptor, is most abundant in parts of the avian basal ganglia and is present in medium to high concentrations in most other telencephalic secondary sensory, associative, limbic and motor regions. Second, the primary auditory and visual areas show no or only weak DARPP-32 labeling. Third, GAD+ neurons are not colocalized with DARPP-32 and do not receive TH-basket input. Thus, dopamine presumably mainly innervates excitatory forebrain units in pigeons, a situation at least partly comparable to mammalian neocortex (Berger et al., 1991; Gaspar et al., 1995; Goldman-Rakic et al., 1989; Smiley and Goldman-Rakic, 1993).

What are the possible functional implications of the present findings and the fact that a similar organization of the dopaminergic innervation exists in mammals? The sparing of most primary sensory regions by markers of dopaminergic innervation and the medium to high levels in most other telencephalic structures imply that dopamine has its most prominent role in higher level sensory and motor processes. Based on the findings by Schultz and coworkers, (Apicella et al., 1992; Ljungberg et al., 1992; Schultz and Romo, 1990; Schultz et al., 1993; Schultz et al., 1995) who showed that dopaminergic midbrain neurons are activated in new and unexpected situations, and computational studies, (Durstewitz and Güntürkün, 1996) one may infer that dopamine allows for rapid strengthening of couplings between higher sensory representations, motor outputs, and the reward value of the behavior. By stabilizing such new configurations, the dopaminergic system may enable the organism to develop predictions of behavioral consequences (Montague et al., 1996; Schultz et al., 1995). This type of fast and highly flexible learning may not be that important in primary sensory regions for two reasons. First, most structural modifications in these areas occur during pre- and early postnatal development. In contrast, in adult primary sensory structures there may be need for only slight incremental modifications of, e.g., receptive field properties. In line with this assumption, considerable amounts of DARPP-32 are present in the visual cortex of infant monkeys but not in that of adults (Berger et al., 1990). Second, only higher elaborated sensory representations may participate in those associations reinforced by the dopaminergic system because only these representations...
may stand in a unique relationship to relevant aspects of the situation. In contrast, there may be too many interpretational possibilities for activity patterns in primary sensory areas. Therefore, these patterns possibly cannot become directly coupled to certain motor functions but must first be resolved by higher processes.

In cortex, dopamine has been shown to inhibit spontaneous and evoked activity in vivo, (Bernardi et al., 1982; Ferron et al., 1984; Godbout et al., 1991; Sesack and Bunney, 1989; Verma and Moghaddam, 1996) to reduce excitatory synaptic transmission, (Law-Tho et al., 1994; Pralong and Jones, 1993) and to shift the probability of long term modifications in favor of long term depression as opposed to long term potentiation (Chen et al., 1995; Law-Tho et al., 1995). Computational modeling suggests that dopamine, by these physiological mechanisms, suppresses the recall of previously learned associations during learning and thereby enables the fast formation of new couplings (Durstewitz and Güntürkün, 1996). This hypothesis implies that dopaminergic fibers mainly innervate excitatory neurons and that these cells are the biggest subpopulation expressing dopaminergic receptors. This prediction is consistent with the present findings that GABAergic units do not express DARPP-32 and are not targets of a strong dopaminergic input.

A remaining question concerns why only a certain fraction of DARPP-32+ neurons receives the dense basket-type catecholaminergic input. It is conceivable that these neurons serve special functions in the innervated neural networks. One hypothesis derives from the proposed role of dopamine in fast associative learning (Durstewitz and Güntürkün, 1996). Fast learning presumably requires the respective neurons or cell populations to be synchronously active for some time to allow for long term synaptic modifications (Singer, 1993). The dopaminergic system may be involved in keeping new patterns active by inducing oscillatory processes (Montaron et al., 1982). In the avian telencephalon, the strong basket-type dopaminergic input may terminate on those excitatory cells which can acquire pacemaker-like characteristics in learning situations. Thereby, dopamine in the avian brain may exert some control over the long term formation of associations between certain situations and profitable behaviors. Of course, these notions are highly speculative and clearly more evidence is needed. However, our assumptions may guide further research on the possible functions of dopamine in learning in different species.
Effects of dopamine on the excitability of neurons in the chick NCL

INTRODUCTION
In the previous chapters the importance of the NCL for sensorimotor integration and „frontal“ executive functions has been outlined repeatedly. Most of the evidence that supports the involvement of the NCL in working memory (Mogensen and Divac, 1982, 1993, Gagliardo et al., 1996, 1997, Güntürkün, 1997; Kalt et al., 1999), reversal learning (Hartmann and Güntürkün, 1998), response inhibition (Güntürkün, 1997, Aldavert-Vera et al., 1999), and spatial orientation (Gagliardo and Divac, 1993) stems from lesion studies. More recent studies have begun to unravel the cellular mechanisms that underlie these functions of the NCL. Electrophysiological single-unit recordings in awake pigeons which performed a delayed Go/NoGo task, have elucidated the contribution of neurons in the NCL to response inhibition and working memory (Kalt et al., 1999). In this study single neurons showed an increase in their firing frequency relative to baseline conditions during the delay period of the task. Neuronal activity changes also signalled the onset of the stimulus or the delivery of the reward. Some neurons furthermore seemed to code the behavioral significance of the stimulus, such that the neuronal activity, either an increase or a decrease in spike frequency, was statistically different between Go and NoGo trials (Kalt et al., 1999).

In the prefrontal cortex (PFC) of mammals, task-related electrical activity is modulated by dopamine (DA), mainly via the D1 receptor (Sawaguchi et al., 1988, 1990a,b; Williams and Goldman-Rakic, 1995). Dopaminergic midbrain neurons are activated at the onset of working memory tasks (Schultz et al., 1993) and DA levels in the PFC increase during delay-task performance (Watanabe et al., 1997). Blockade of the dopaminergic input to the PFC or of dopaminergic D1 receptors in the PFC disrupt delay-task performance (Brozoski et al., 1979; Simon et al., 1980; Sawaguchi and Goldman-Rakic, 1994; Seamans et al., 1998). Similarly, in a behavioural study in the pigeon, blockade of D1 receptors in the NCL by a competitive antagonist (SCH-23390), strongly impaired performance in a working memory task (Güntürkün and Durstewitz, in press).

One concept regarding the functions DA might play during working memory, is to increase the signal-to-noise ratio and to stabilize neural representations (Sawaguchi et al., 1990a,b; Durstewitz et al., 1999, 2000). This stabilizing effect on the network could enable sustained
task-related activity, even in the presence of interfering input (Durstewitz et al., 1999), which in mammals appears to be a unique feature of the PFC (Miller et al., 1996).

In this chapter I will present preliminary data on the effects of DA on the firing behaviour of chick NCL neurons recorded in-vitro, using both whole-cell and perforated patch-clamp techniques. The electrophysiological findings are complemented with morphological data from double-labeling experiments, in which neurons that were intracellularly filled during whole-cell recordings were immunohistochemically stained and colocalized with labeling of tyrosine-hydroxylase, as a marker of the dopaminergic innervation. It can be assumed that the basket-like innervation provides a means by which the dopaminergic system could selectively modulate a specific class of neurons, and/or that the strong somatic innervation renders these neurons particularly susceptible for the effects of DA. The results show that DA can increase the excitability of NCL neurons and profoundly alter their firing pattern, which in turn might sustain neuronal delay activity during delay-task performance. However, the responses of neurons, which were anatomically shown to receive basket-like dopaminergic innervation, to DA application did not differ from those that received only „en-passant” innervation, thus suggesting that the different modes of dopaminergic innervation exert no qualitatively different influence over their target neurons.

MATERIALS AND METHODS

Preparation of slices and electrophysiological recording: For preparation of brain slices a total of 32 chicks (5 - 12 days post-hatch, median 7 days) were decapitated and the brains were rapidly transferred to iced extracellular solution. Coronal slices (350 µm thick) of the caudal telencephalon were cut on a vibratome. Slices were incubated in extracellular solution consisting of (in mM): 119 NaCl, 2.5 KCl, 1 NaH2PO4, 26.2 NaHCO3, 10 D-glucose, 4.5 CaCl2, and 1.3 MgCl2, saturated with 95% O₂ /5% CO₂, pH 7.3. Slices were allowed to recover for at least 1 hour, before being transferred to the recording chamber. All recordings were made at room temperature (21 - 23°C) in a submerged slice chamber perfused with extracellular solution.

Whole-cell and perforated-patch recordings were made from neurons in the caudolateral subventricular region of the chick forebrain, using the blind-patch technique (Blanton et al., 1989). Electrodes (6 – 8 MΩ resistance) were filled with an intracellular solution consisting of (in mM): 135 K-glucosate, 20 KCl, 2 MgCl2, 10 N-(2-hydroxyethyl)piperazine-N’-(2-ethanesulfonic acid) (HEPES), 0.1 ethylene glycolbis β-aminoethyl ether)-N,N,N’,N’-tertraacetic acid (EGTA), 4 Na2-ATP, and 0.5 Na2-GTP, and adjusted to pH 7.3 with KOH. In some recordings 3 - 3.5 mg/ml of the dipotassium salt Lucifer Yellow (LY, Sigma, Deisenhofen, Germany) were added to the intracellular solution and neurons were filled by diffusion. Perforated-patch recordings were made by adding nystatin to the pipette solution (final concentration was 500 µg/ml, 0.3 % DMSO). The membrane of a cell-attached patch is permeabilized by nystatin in the patch pipette, thus providing electrical continuity.
between the pipette and the cytoplasm of the cell without the loss or alteration of cytoplasmic compounds necessary for the maintenance of the effects under study.

Recordings were made using a HEKA (Lambrecht, Pfalz, Germany) EPC-7 patch-clamp amplifier, filtered at 3 kHz, and sampled at 10 kHz. The voltage drop across the pipette could not be bridge-balanced. Sampling was done with a Digidata 1200 interface using pClamp 6.0 software (Axon Instruments, Foster City, CA), and data were stored on computer for off-line analysis with the CLAMPFIT module of pClamp.

Experimentally produced changes in membrane potential \( V_m \) during current-clamp recordings were compensated with a bias current to maintain \( V_m \) at baseline levels.

The apparent input resistance \( R_i \) was monitored continuously by applying brief (50 ms) hyperpolarizing current pulses before each depolarizing current step. The amplitude of these hyperpolarizing current pulses (10 - 50 pA) was adjusted for each cell to be in the linear range of the current–voltage relationship, as estimated from the cells response to a series of depolarizing and hyperpolarizing current injections.

**Experimental paradigm:** To study cell excitability, action potentials were evoked by 1.1 sec-long depolarizing current injections with various amplitudes (100 - 800 pA). The amplitude of the current used for statistical comparisons of drug effects was adjusted to be in the mid-range between the current that depolarized the neuron just above spike-threshold and the close-to-maximal stimulation. In the majority of cells the time course of drug action and wash-out of the effect was measured continuously by applying 5 current steps (at 0.2 Hz) every 30 or 45 seconds. After baseline measurements of action-potential number and \( R_i \), dopamine (1 - 50 µM) was bath applied for approximately 1 minute. Two gravity-fed perfusion lines were used to change solutions. Both control and drug ACSF were continuously oxygenated throughout experiments.

The dopamine D1-receptor agonist SKF-38393, a D1 antagonist (SCH-23390), and the D2-receptor agonist (quinpirole) were prepared as stock solutions and stored frozen until diluted to the final concentration. In a number of experiments DA stock solutions were prepared in a similar way and ascorbate (3 mM) was added to the solution to reduce oxidation. In all other experiments DA solutions were prepared at the desired final concentration immediately before application. Dopaminergic agonists and antagonists were obtained from Research Biochemicals (Natick, USA). Only one neuron experiencing drug treatment was used from each brain slice. In several instances different agonists were applied successively to one cell to test its differential response to various pharmaka. Usually, either a D1 or D2 receptor agonist (SKF-38393, or quinpirol) were followed by a single application of DA. In these cases only the response to the agonist that was first applied was used for statistical analysis. In all cases, only cells with a resting potential of at least -60 mV and stable baseline responses were given drug treatments.

**Analysis and statistics.** For each cell, the number of action potentials evoked by depolarizing current steps was quantified by averaging the number of spikes produced in 5 consecutive traces before, at the end of, and approximately 5 minutes after drug application. The input resistance \( R_i \) served as a measure for the stability of a given recording. Thus, cells that showed a non-reversible change of input resistance larger than 15 % were excluded from analysis to control for possible unspecific changes in recording conditions. Furthermore, for cells that showed a change in the number of evoked spikes during a given drug treatment, only those that exhibited a reversible effect (a washout of at least 50% of the effect) were used for analysis. The effects of DA
or its agonists on the number of evoked action potentials were expressed as the percentage change relative to baseline conditions to account for the variation in the absolute number of spikes which could be evoked in neurons of different cell types (c.f. chapter 3).

Comparisons of drug effects across concentrations relative to control recordings were made using univariate analyses of variance (ANOVA). Subsequent tests for statistical significance of effects were done using Student’s t test for unpaired samples. For multiple comparisons the alpha-niveau was Bonferroni-adjusted. „Within-group“ comparisons used a Student’s t test for paired samples where noted.

**Histological procedures**

Following recording, slices were fixed in 4% paraformaldehyde and 0.2% glutaraldehyde in 0.12 M phosphate buffer (4°C, pH 7.4) for about 15 hours. They were then transferred to a solution of 30% sucrose in phosphate buffer containing 0.9% NaCl (PBS, pH 7.4) and 0.01% NaN₃ as a preservative. Slices were resectioned at 70 µm on a freezing microtome and collected in PBS. For double-labeling immunohistochemistry of LY and tyrosine-hydroxylase (TH), endogenous peroxidases were first blocked by preincubating slices in 0.5% H₂O₂ for 30 minutes. Slices were then washed in PBS and floating sections were incubated overnight at 4°C in a monoclonar anti-TH antibody from mouse (Boehringer; working dilution 1: 200) in PBS containing 0.3 % Triton-X (Sigma, Deisenhofen). The following steps were carried out at room temperature, separated by three washes in PBS of 10 min each. After washing, slices were incubated for 2 hours in the secondary antibody directed against mouse from horse (Vector Labs, Burlingame, CA), diluted at 1:200 in PBS containing 0.3 % Triton-X. After washing, slices were incubated for 2 hours in the avidin-biotin complex (ABC Elite, Vector Labs; 1:100 in PBS with 0.3% Triton X-100). Washes in PBS were followed by additional washes in 0.12 M acetate buffer (pH 6). Staining was achieved by the 3,3'-diaminobenzidine (DAB) technique with heavy-metal amplification (modified from Adams, 1981) by adding HgN₂NiO₈S₂ (2.5 g/ 100 ml), NH₄Cl and CoCl₂ (both 40 mg/ 100 ml), which yields a black signal. After 20 minutes of preincubation the reaction was catalyzed using 0.3% H₂O₂. The reaction was stopped by rinsing the tissue in acetate buffer and PBS. The same staining procedure was then repeated for labeling of LY. Therefore, slices were incubated overnight at 4°C in a monoclonar biotinylated anti-LY antibody from rabbit (Molecular Probes, Leiden, The Netherlands; 1:200 working dilution) in PBS containing 0.3% Triton X-100 (Sigma). After washing, slices were incubated for 2 hours in the avidin-biotin complex (1:100 in Triton-PBS). Washes in PBS were followed by additional washes in acetate buffer. Labeling of LY was again achieved by incubating slices in DAB, but without addification of heavy-metal compounds, thus resulting in a brown staining product. The reaction was stopped by rinsing the tissue in acetate buffer and PBS. Slices were then mounted, dehydrated and coverslipped. Camera lucida reconstructions of filled cells were drawn using an Olympus BH-2 with a drawing tube at x 125 magnification.

**RESULTS**

A total of 84 neurons that met the criteria specified in Materials and Methods were analyzed. All neurons could be assigned unequivocally to one of the 4 cell classes described in chapter 3
by their responses to depolarizing and hyperpolarizing current pulses. Of these 84 neurons, 37 were characterized as type I neurons by their burst firing pattern of action potentials, and 33 showed a regular or phasic-tonic firing, characteristic of type II neurons. Five cells displayed the high-frequency firing typical of type III interneurons, and the remaining 9 cells were classified as type IV neurons by their burst firing of action-potentials and the absence of prominent inward rectification.

![Figure 1: The effects of dopamine application in the 4 major celltypes of the chick NCL. Dopamine at 10 µM could largely increase the number of spikes evoked by a constant current injection in the three spiny cell types (types I, II and IV, shown in A, B, and D, respectively), but not in type III interneurons (C). The enhancement of neuronal excitability by dopamine appeared to be independent from changes in the passive properties of the cells.](image-url)
**Effect of dopamine application on neuron excitability**

The effect of DA on the excitability of NCL neurons was studied by measuring changes in spike number in response to 1.1-sec-long depolarizing current injections. The effect of DA did not vary with the recording technique used, therefore, for comparison of dopaminergic effects across concentrations data obtained during whole-cell and perforated-patch recordings were pooled. In control recordings ($n = 9$), neurons that were recorded under the same conditions and over comparably long periods as experimentally-treated cells (at least 20 minutes), showed only a slight decrease of $4.5 \pm 4\%$ in the number of evoked spikes. This change from baseline was neither reversible nor significant ($df = 8; t = 1.05; p > 0.05; \text{Student's } t \text{ test for paired samples}$).

Application of DA reversibly increased the number of spikes in 32 of 43 neurons tested (74%). However, the effect of DA differed with the cell-type studied. Dopamine increased the number of evoked action potentials in neurons of all spiny cell-classes (i.e. types I, II, and IV), but not in type III interneurons (figure 1). Thus, application of dopamine increased the number of spikes in 13 of 16 type I neurons, in 14 of 17 type II neurons, and in 4 of 5 type IV neurons. In contrast, in 4 of 5 type III neurons tested, application of DA resulted in no change in the number of evoked spikes, or in a small decrease, similar to that observed in controls. In one neuron there was a small increase of 11% (9 vs. 8 spikes). To account for these differences in responsivity, type III neurons were excluded from further analysis of drug effects.

![Figure 2: The effect of dopamine at various concentrations.](image)

In contrast to control recordings, in which a small decrease in the number of evoked spikes occurred during prolonged recording, application of dopamine consistently increased the firing of spiny NCL neurons. The change in the number of action potentials was significantly different ($p < 0.01$) from changes in controls at 5 µM and 10 µM dopamine. At higher concentrations the effect of dopamine did not saturate, but rather diminished, resulting in an inverted U-shape of the dose-response curve.
The increase in spike number induced by DA across the range of concentrations studied (i.e. 1, 5, 10, 20, and 50 µM; \( N = 38 \)) was significantly different from changes observed in controls (df = 46; \( F = 3.7; p < 0.01; \) ANOVA). When grouped according to the concentration of DA applied, significant increases in the spike number compared to control recordings occurred only at concentrations between 5 and 10 µM DA, indicating a dose dependence of the effect of DA (figure 2). In 5 µM DA the mean number of spikes increased by 74.5 ± 9.7 % (df = 12; \( t = -8.9; p < 0.01, \alpha\)-adjusted; Student’s \( t \) test for unpaired samples) and in 10 µM DA by 112.5 ± 22.6 % (df = 24; \( t = -5.1; p < 0.01, \alpha\)-adjusted; Student’s \( t \) test for unpaired samples). The effect of DA did not saturate, but rather decreased with higher concentrations, resulting in almost no change in the number of spikes at 50 µM DA (\( n = 4 \); c.f. figure 2). This finding is in agreement with an inverted U-curve that has been used to describe the range of optimal dopaminergic activation (Williams and Goldman-Rakic, 1995; Murphy et al., 1996; Zahrt et al., 1997). The effect of DA usually developed within 3 minutes and peaked about 5 - 10 minutes after the end of bath-application (figure 3). The effect was long lasting, sometimes persisting up to about an hour, depending on the concentration of DA that was applied.

![Figure 3: Example for the time course of dopamine-induced enhancement of cell excitability in a type IV neuron.](image-url)
To examine possible mechanisms by which DA might regulate the excitability of NCL neurons, the near-threshold firing behaviour of 20 neurons that had received DA applications at doses shown to effectively increase the number of spikes (i.e. 5 and 10 µM) was compared with changes in controls. The effects of DA on the latency and the threshold of the first spike were studied, and in addition the change in the duration of the first inter-spike-interval was analyzed. The results show that application of DA led to a negligible reduction in the latency of the first spike (mean changes -6.5 ± 3.8 % for DA, and +4.3 ± 6.1 % for controls; df = 27; t = 1.50; p > 0.05; Student’s t test for unpaired samples). In current-clamp recordings the threshold for generation of an action-potential varied considerably over the duration of the experiment (a change of +1.69 ± 1.4 mV in controls); application of 5 or 10 µM DA, however, resulted in a small shift of the spike-threshold towards hyperpolarized potentials (-1.14 ± 0.72 mV), and this change showed a statistical trend when compared to controls (df = 27; t = 1.98; p = 0.058; Student’s t test for unpaired samples). Dopamine had its strongest impact on the duration of the first inter-spike-interval, which usually occurred at more depolarized potentials than the first spikes. Application of DA significantly shortened the first inter-spike-interval when compared to changes in control recordings (mean changes -23.5 ± 4.4 % for DA, and +32.6 ± 14.4 % for controls; df = 27; t = 4.89; p < 0.01; Student’s t test for unpaired samples).

Application of DA had little effect on the passive membrane properties of NCL neurons. There was only a negligible overall reduction in input resistance (-2.66 ± 1.4 %) after DA application compared to controls (df = 46; F = 0.36; p > 0.05; ANOVA).

In addition to its effects on cortical pyramidal neurons in mammals, DA has been shown to modulate the cell excitability of GABAergic interneurons (Penit-Soria et al., 1987; Retaux et al., 1991; Pirot et al., 1992; Zhou and Hablitz, 1999). Because spontaneous synaptic input can strongly modulate the passive properties (i.e. input resistance) of neurons (Paré et al., 1998), I tested whether DA might increase the excitability of NCL neurons by decreasing spontaneous inhibitory synaptic transmission. Therefore, in 6 principal neurons the GABA\textsubscript{A} receptor blocker picrotoxin (100 µM) was added to the extracellular solution before the application of 10 µM DA. In the presence of picrotoxin, DA still produced a significant increase in the number of spikes of 82.6 ± 26.5 % (df = 13; t = -3.24; p < 0.05; Student’s t test for unpaired samples). This result indicates that the effect of DA on evoked spikes is not mediated indirectly by a decrease in spontaneous inhibitory synaptic transmission.
Pharmacology of dopaminergic enhancement of excitability

To determine which DA receptor subtype might mediate the observed changes in spike number, the effects of selective agonists of either the D1 or D2 family of DA receptors were studied. In the same line of evidence the effect of DA was challenged by a specific antagonist to dopaminergic D1 receptors (figure 4).

Application of the D1 receptor agonist SKF-38393 mimicked the effects of DA in that it significantly increased the number of spikes, both at 10 and 25 µM (df = 23; F = 5.75; p < 0.01; ANOVA; figure 4A, D). The mean increase in the relative number of action potentials was 79.3 ± 19.1 % compared to control recordings. At 10 µM (n = 7), SKF-38393 led to an increase in the number of spikes of 98.9 ± 33.9 % (df = 14; t = -3.02; p < 0.05, α-adjusted; Student’s t test for unpaired samples), while at 25 µM (n = 8) the increase was 67.4 ± 24.3 % (df = 15; t = -3.04; p < 0.05, α-adjusted; Student’s t test for unpaired samples).

Conversely, when 10 µM DA was applied approximately 5 min after bath application of the D1 receptor antagonist SCH-23390 (45 µM), the change in the number of action potentials was not significantly different from control recordings. In the presence of SCH-23390, DA (n = 5) had only a small (mean increase 16.0 ± 21.3%), non-significant effect on the number of evoked spikes (df = 12; t = -0.94; p > 0.05). Bath application of SCH-23390 for 5 - 10 minutes alone, following 5 minutes of baseline measurements, had no effect on the number of evoked spikes (df = 4; t = 0.87; p > 0.05; Student’s t test for paired samples; figure 4B, D).

The D2 receptor agonist quinpirole (n = 6) had no effect on the excitability of NCL neurons. Bath application of 25 or 50 µM quinpirole resulted only in a small, non-significant decrease in the number of action-potentials by 1.53 ± 1.5 % (df = 13; t = -0.57; p > 0.05; Student’s t test for unpaired samples), similar to that observed in controls (figure 4C, D). In 3 of the 6 neurons 10 µM DA was applied subsequently to the establishment of the quinpirole effect (at least 20 minutes after application of quinpirole). In all 3 cells DA application led to an increase in the number of spikes (mean 74.8 ± 41%), showing that these cells were not generally unresponsive to dopaminergic transmission (figure 4C). This result supports the assumption drawn from the anatomical data reviewed in chapter 4, that D2 receptor mediated mechanisms have only relatively little, if any, influence on neurons in the caudal forebrain of birds. Taken together, these data suggest that DA modulates cell excitability in the NCL via activation of D1 type receptors by a mechanism that appears to be independent of input resistance.
Figure 4: Pharmacology of the enhancement of cell excitability. Activation of the D1, but not the D2 receptor, mimicked the effects of DA in that it led to a significant increase in evoked action potentials. A: Example of a regular spiking neuron in which the selective D1 agonist SKF-38393 was bath applied. B: Dopamine D1 receptor blockade by application of the competitive D1 receptor antagonist SCH-23390 before and during application of dopamine results in no change in the number of spikes in this neuron, and only in a small non-significant increase across all neurons tested (c.f. D). C: Application of the selective D2 receptor agonist quinpirole has no effect on cell excitability in this type I neuron, and in all neurons tested (c.f. D). Application of DA in the same neuron, however, could largely increase the number of spikes in a burst, demonstrating that this neuron was not generally insensitive to dopaminergic stimulation. D: Overview of the effects of different DA receptor agonists and antagonists on cell excitability compared to control recordings.
Cell type specificity

One aim of the present experiments was to determine whether the response to DA in cells that receive the basket-type dopaminergic innervation differ from those that receive only "en-passant" innervation of their dendrites. Type I and type II neurons are the most plausible candidates to receive basket-type innervation, as baskets preferentially innervate neurons with large soma sizes (c.f. chapter 4) and the anatomical data presented in chapter 5 also had shown that GABAergic interneurons do not receive dopaminergic basket input.

In addition, spikes are unitary events and I had expressed the effects of DA as the percentage change occurring between baseline and drug condition. Thus, although the spike number was averaged across 5 consecutive sweeps, this procedure might have led to a significantly larger statistical increase in type I neurons, as they have a low basal firing rate and might therefore "profit" more from small variations in the number of spikes. However, a comparison of the effect of 5 - 10 µM DA on type I and type II neurons, respectively, showed no significant effect of cell type. Dopamine increased the mean number of spikes in type I cells ($n = 10$) by $120 \pm 20.7\%$, and in type II neurons ($n = 11$) by $73.5 \pm 23.1\%$ ($df = 19; t = 1.77; p > 0.05$; Student’s t test for unpaired samples). This result shows that DA appears to modulate the large principal cell types in the NCL in the same manner.

Morphology

It was attempted to identify the class of neurons that receives basket-type dopaminergic input by colocalizing Lucifer Yellow-intracellularly filled neurons with tyrosine-hydroxylase containing fibers by means of double-label immunohistochemistry.

In 15 slices in which neurons were filled with Lucifer Yellow, TH-labeling was sufficiently good enough to examine the catecholaminergic innervation of the soma in detail. Of these 15 neurons 12 showed only few TH immunopositive fibers in the vicinity of their soma or initial dendrites, making no, or seemingly only "random" contact. The remaining 3 neurons received catecholaminergic input from TH-labeled fibers showing numerous varicose-like swellings in close apposition to the soma and the initial segments of dendrites, resembling the basket-type innervation (figure 5). All of these 3 neurons were characterized as type I neurons by their burst firing pattern of action potentials. In each of these neurons application of DA ($n = 2$) or SKF-38393 ($n = 1$) resulted in a marked increase in the number of spikes. This finding shows that burst-firing neurons in the NCL appear to be a main target of the dense basket-like somatic innervation, however, due to the small sample size, it would
Figure 5: Anatomy of the dopaminergic innervation of electrophysiologically characterized neurons that were shown to be responsive to dopamine application. Neurons were intracellularly filled with Lucifer Yellow (LY) during recording, and double-labeled immunohistochemically for LY and tyrosine-hydroxylase (TH) as a marker of dopaminergic fibers. A: Camera lucida reconstruction of the basket-type innervation of a type I neuron by TH-positive fibers (red). For reason of clarity only the proximal innervation by dopaminergic fibers is shown. The axon of the cell is shown in light green. B, C: Photomicrographs of the cell shown in A. D: Another example of a neuron that received numerous TH-positive varicosities in close apposition to the soma and initial dendrites (arrows). E: Photomicrograph of a cell that did not receive basket-like innervation. Although the number of labeled fibers is generally lower than in C (due to differences in staining quality), several basket-like structures can still be seen (arrows; c.f. also F). Furthermore, there is a large number of varicose-like swellings in close apposition to the shafts and spines of the cells dendrites. F: Detail of the TH-labeling shown in E. The arrow points to a basket-like structure that coils around the soma of an unlabeled neuron. Scale bars are 25 µM in A, B, D, F, and 50 µM in C, E.
be premature to conclude that they constitute the only cell class receiving basket-innervation. Moreover, the observed changes in the spike-pattern following DA application in neurons innervated by basket-forming dopaminergic fibers did not differ qualitatively nor quantitatively from cells that received diffuse “en-passant” innervation to their dendrites and somata. Thus, it appears that the different modes of dopaminergic innervation exert no qualitatively different influence over their target neurons.

**DISCUSSION:**

The data presented here, suggest that DA modulates cell excitability in the NCL via activation of D1-type receptors. The enhancement of membrane excitability appears to be independent of changes in input resistance, and an indirect effect on GABAergic transmission. The latter was inferred from direct recordings of putative GABAergic fast-spiking interneurons, and by the absence of an effect of the Cl⁻ channel blocker picrotoxin on the DA-induced increase in projection neurons.

The effect of DA appeared to be dose-dependent, being maximally in a narrow range at concentrations of 5 - 10 µM DA in the sample of neurons recorded here. This finding is in agreement with numerous studies which have demonstrated that an optimal degree of activation of the dopaminergic system is necessary for the active retention of information, or the planning of motor sequences in working memory (Sawaguchi and Goldman-Rakic, 1991; Williams and Goldman-Rakic, 1995; Murphy et al., 1996; Sawaguchi, 1997; Zahrt et al., 1997; Arnsten and Goldman-Rakic, 1998).

Similarly to the present results in birds, in mammals an enhancement of cell excitability in vitro, or an increase in task-related activity in vivo, have been shown to depend on activation of D1-like receptors (Sawaguchi et al., 1988, 1990,b; Sawaguchi and Goldman-Rakic, 1991, 1994; Williams and Goldman-Rakic, 1995; Yang and Seamans, 1996; Shi et al., 1997; Sawaguchi, 1997; Kita et al., 1999). It should be noted, however, that several other studies have observed an inhibitory action of DA on pyramidal cells (Geijo-Barrientos and Pastore, 1995; Gulledge and Jaffe, 1998; Zhou and Hablitz, 1999; Geijo-Barrientos, 2000), and a possible involvement of D2 receptors in this depressant effect has been reported (Gulledge and Jaffe, 1998).

One possible explanation for these conflicting results is a differential, and possibly also time-dependent activation of D1- and D2-type receptors by DA, which might result in exactly
opposite effects (Zheng et al., 1999). Thus, activation of D2-like receptors may require higher concentrations of DA than those needed to activate D1-like receptors, as DA has a higher affinity for D1-like receptors than for D2-like receptors when measured at the high affinity state of the receptors (Seeman and Van-Thol, 1993). Furthermore, in the neocortex there are much more D1-like receptors than D2-like receptors (e.g. Lidow et al., 1991; Joyce et al., 1993), a situation also seen in the dorsal forebrain of birds (Schnabel and Braun, 1996; see chapter 4). In addition, the depressant effects of DA may be time-dependent and/or related to receptor desensitization. Thus, studies in PFC neurons that utilized long (5 min) DA or D2-like agonist applications, noted a decrease of input resistance and a concurrent reduction in the number of evoked action-potentials (Geijo-Barrientos and Pastore, 1995; Gulledge and Jaffe, 1998; Zhou and Hablitz, 1999). In contrast, the short duration application time (about 1 min.) used in the experiments presented here, served to minimize rapid D1 receptor desensitization (Jarvie et al. 1993; Ng et al., 1995). Thus, for a given depolarizing input, time-dependence (desensitization) or concentration-dependence of the DA effect might in principle account for the different results obtained in various studies. In this view, the D2-like receptor may induce an „early“ transient suppression of neuronal excitability, which \textit{in-vitro} becomes only evident after desensitization of the D1 receptor (Geijo-Barrientos and Pastore, 1995; Gulledge and Jaffe, 1998), whereas D1/5 receptor activation may induce a „late“, prolonged (tens of minutes) enhancement of neuronal excitability in response to depolarizing inputs (Yang and Seamans, 1996; Shi et al., 1997; present study). However, the cellular basis for a possible D2 receptor mediated effect in the cortex is not known. More importantly, in the present experiments no depressant action of DA, or an involvement of D2 receptors in the mediation of the dopaminergic effects was found. The ionic mechanisms by which DA exerts its effects in the frontal cortex following stimulation of the D1 receptor are much better studied. By reviewing some of these findings I hope to reveal some parallels and differences regarding the possible effects of DA in mammalian cortical neurons and cells of the NCL. However, voltage-clamp studies into the effect of DA on isolated currents in NCL neurons are necessary to directly study the ionic mechanisms by which DA appears to modulate cell excitability.

In the cortex DA, via its action at the D1 receptor, appears to shift the threshold of the persistent Na$^+$ current (I_{NaP}) towards more hyperpolarized potentials (Yang and Seamans 1996; Gorelova and Yang, 1997; but see Geijo-Barrientos and Pastore, 1995; Geijo-Barrientos, 2000). This current is responsible for inward rectification in the depolarized voltage range and serves important functions in setting the firing threshold for repetitive spike firing (Schwindt, 1992) and signal amplification (Stuart and Sakmann, 1995). The existence of such a current in neurons
of the bird’s caudal forebrain was shown in the zebrafinch HVC (Kubota and Saito, 1991), and indicated by a TTX-sensitive inward rectification in NCL neurons (chapter 3). The tendency of DA to lower the spike threshold and to prolong repetitive firing in NCL neurons, might reflect an action of DA on the persistent Na⁺ current.

In contrast, the observed reduction in the duration of the first inter-spike-interval in the present study might result from an action of DA on K⁺ currents, e.g. the Ca²⁺ activated K⁺ current termed **IₐHₚ**, which is involved in spike-frequency adaptation (Madison and Nicoll, 1982; Storm, 1990), or the slowly inactivating K⁺ current (**Iₖₛ**) that is responsible for membrane outward rectification in the depolarized voltage range (Schwindt et al. 1988; Kitai and Surmeier, 1993; Yang and Seamans, 1996). The latter will functionally counteract sustained membrane depolarization and suppress repetitive spike firing. A strong outwardly rectifying conductance was also seen in the spiny projection neurons of the NCL (see chapter 3). Thus in the NCL, DA might shorten inter-spike-intervals and reduce accommodation by inactivating an outwardly rectifying K⁺ conductance, as has been shown in the PFC and striatum (Yang and Seamans, 1996; Nisenbaum et al., 1998).

The concurrent actions of DA on both, **IₐNaP** and **Iₖₛ**, have been proposed to reduce spike threshold and to contribute to the increased firing rate observed *in vivo* (Sawaguchi et al., 1988; 1990a,b) and *in vitro* (Cepeda et al., 1992; Yang and Seamans, 1996; Shi et al., 1997; Yang et al., 1999). In the present study, the facilitatory effects of DA could be seen most clearly at membrane potentials considerably above threshold, i.e. in response to large depolarizing current pulses. This might indicate a dopaminergic modulation of ionic currents that are maximally active at depolarized potentials, such as high-voltage activated (HVA) Ca²⁺ channels of the L, P, and N type (Sayer et al., 1990; Seamans et al., 1997; but see Durstewitz et al., 2000). High-threshold Ca²⁺ channels are activated by suprathreshold synaptic inputs that elicit spike firing in the soma and which then backpropagates into the dendritic arbor (Stuart and Sakmann, 1994; Spruston et al., 1995). Dendritic high-voltage-activated Ca²⁺ currents are likely to participate in multiple functions that are important for burst firing mechanisms, sustaining repetitive firing, and enduring forms of changes in synaptic plasticity (Yang et al., 1999). The existence of Ca²⁺ currents that are capable of initiating slow Ca²⁺ spikes has also been shown in neurons of the NCL (c.f. chapter 3), but the voltage-dependence of these currents was not studied.

In neurons of the PFC and the striatum DA has been shown to activate D1/5 receptors on pyramidal dendrites to directly attenuate the amplitude of high-voltage-activated Ca²⁺ spikes, or to augment the duration of a dendritic HVA Ca²⁺ plateau, respectively (Surmeier et al., 1995; Yang and Seamans, 1996; Hernández-López et al., 1997; Yang et al., 1998). At moderate
concentrations, DA application results in a weak suppression of N-type Ca$^{++}$ channels, that cluster in the distal dendrites (Westenbroek et al., 1992). This action of DA may serve to „sharpen” incoming depolarizing synaptic signals (from layer I–II inputs) arriving at the distal dendrite, such that only sufficiently strong depolarizing inputs are propagated to the soma. Large suppression of N-type Ca$^{++}$ channels, however, may „uncouple” the distal dendrites, preventing distal signals from reaching the soma (Yang and Seamans, 1996; Yang et al., 1999). This latter action may help to explain in part why dopamine has often been attributed as having an “inhibitory” or “suppressive” effect on spontaneous firing or activities evoked by specific inputs in the PFC\(^1\). At the same time DA augments the duration of a Ca$^{++}$ plateau, which is mediated by L-type Ca$^{++}$ channels that cluster in the soma and proximal dendrites of pyramidal neurons (Westenbroek et al., 1990; Yang et al., 1998). Functionally, activation of L-type Ca$^{++}$ channels may enhance the amplitude and duration of subthreshold excitatory synaptic inputs (Seamans et al., 1997), and the dopaminergic modulation of L-type Ca$^{++}$ currents has been shown to increase the excitability of striatal neurons to NMDA application and depolarizing current injections (Hernández-López et al. 1997; Cepeda et al. 1998). Through its action on L-type Ca$^{++}$ channels, DA will thus complement the increased cell excitability caused by the enhancement of I$\text{NaP}$, eventually leading cortical neurons to sustained firing (Yang et al., 1999). Both, L-type Ca$^{++}$ channels (Westenbroek et al., 1990; Seamans et al., 1997), and the persistent Na$^+$ conductance (Westenbroek et al., 1989; Stuart and Sakmann, 1995) are found predominantly in the soma and basal dendrites of cortical neurons, thus enabling a differential regulation of distal vs. proximal inputs by DA, as originally proposed by Yang and Seamans (1996). While distal signals may be inhibited through the actions of DA on N- and P-type Ca$^{++}$ channels, proximal inputs may be facilitated by dopamine’s enhancement of I$\text{NaP}$ and L-type currents.

In the mammalian PFC, the network-effects of DA also appear to involve a modulation of GABAergic interneurons. Dopamine directly depolarizes interneurons in the PFC and enhances spontaneous and evoked IPSPs recorded in pyramidal neurons (Penit-Soria et al., 1987; Godbout et al., 1991; Rétaux et al., 1991; Pirot et al., 1992; Zhou and Hablitz, 1999; Durstewitz et al., 2000; but see Law-Tho et al., 1994). Accordingly, the suppressive action of DA on spontaneous firing of PFC neurons \textit{in vivo} is blocked by previous application of a GABA antagonist (Pirot et al., 1992), suggesting that DA acts through interneurons in the PFC to reduce spontaneous activity of pyramidal neurons. In the NCL, however, a significant contribution of

\(^{1}\) This effect of DA might also in part explain the downward slope of the dose-response curve at high concentrations of DA (inverted U-shape).
GABAergic interneurons is highly unlikely, as in the present study DA did not modulate the firing-pattern of presumed GABAergic fast-spiking interneurons, and its actions on spiny principal neurons were unaffected by depressing spontaneous inhibitory transmission.

It is important to note that in vivo the effect of DA within the PFC is determined by the activity level of PFC neurons and the specific inputs that are driving this activity. Although DA inhibits spontaneous activity within the PFC of the anesthetized rat (Ferron et al., 1984; Mantz et al., 1988; Pirot et al., 1992), it enhances task-related single-unit activity more than background activity in the behaving primate (Sawaguchi et al., 1988, 1990a). DA (but not VTA stimulation) also enhances responses evoked by hippocampal stimulation in the anesthetized rat and use-dependent changes in synaptic efficacy in the hippocampal-PFC pathway that may be associated with learning (Doyère et al., 1993; Jay et al., 1995). Similarly, in vitro the effects of DA are voltage-dependent, favouring excitatory actions (e.g. via $I_{\text{NaP}}$, HVA-Ca$^{2+}$, or NMDA currents), when the membrane already is in a depolarized state (Cepeda et al., 1998; Durstewitz et al., 2000). Thus, DA may enhance task- or learning-related activity relative to spontaneous or background activity within the PFC, and very likely also in the NCL. In the context of a delay-task, the DA-induced increase in action-potential firing might augment the reliability of transmission and thus provide the stability needed to hold online goal-related information in a recurrent network.

The cellular basis by which DA in the NCL could achieve such a selective enhancement of task-related inputs or reverbarating activity in a local network, remains unclear. One working model assumed that basket- and non-basket-type innervation are dichotomic modes of dopaminergic innervation which control different postsynaptic targets, or which strongly differ in the degree of modulation of cell excitability. From the present results, burst firing neurons of type I appear to be a main target of the dopaminergic basket-type innervation. Cells in this class display a high threshold for action-potential generation and may thus require strong spatially or temporarily summated inputs to fire. On the other hand, their burst of action-potentials results in a faithfull transduction onto their target cells (Snider et al., 1998). An increase in the phasic response of type I neurons will effectively drive more postsynaptic cells and „bind“ them in a network. Enhanced firing of bursting neurons by DA could therefore establish a „pacemaker“-activity in a network.

However, neurons from the other cell types in the NCL were modulated in the same way by DA, and there was no obvious difference in the size of the response between cells receiving basket and non-basket innervation. In addition, due to the small sample size of neurons that
could morphologically be shown to receive basket-type innervation, it should not be excluded that members of other cell-types also receive basket-type dopaminergic innervation. In any way, the observed enhancement of firing in regular-spiking neurons of the type II, will also strongly support sustained firing in a reverberating network, and thus functionally might enable active retention of information in the NCL.
General discussion

The experiments presented in this thesis provide an attempt to delineate an associative region in the forebrain of birds, which has been shown to be involved in executive functions, such as working memory and response control. The input- and output relations of the neostriatum caudolaterale (NCL) with telencephalic and thalamic structures were studied with pathway tracing techniques (chapters 1 and 2), and the direct sensory projection from the dorsal thalamus was tested in-vivo (chapter 2). The principal celltypes of the NCL were described physiologically and anatomically in-vitro (chapter 3), and the results give hints about their possible roles in a proposed canonical circuit. Furthermore, the relationship of the dopaminergic innervation with immunohistochemically or electrophysiologically identified elements of this circuit was analyzed at the light-microscopic level (chapters 5 and 6). Finally, the effect of dopamine or its receptor-specific agonists on the excitability of NCL neurons were studied in-vitro (chapter 6).

Elements of a proposed circuit involved in working memory and executive functions

Fuster (1989) has proposed that the temporal organization of behaviour is at the heart of prefrontal cortex functions. The cognitive operations that underly this „memory for action“ are essentially targeted by the logic of delay tasks: „If earlier that, then now this“. This logic requires a dynamic process of internal transferring of information across time and of cross-temporal matching, the contents of which must be constantly changing as behaviour evolves, especially in situations in which the outcome of the behaviour is yet not fully predictable, e.g. when learning a new task (Fuster, 1989).

The PFC seems essential for three cognitive functions necessary to mediate contingencies across time, (i) a temporally retrospective function of short-term memory (working memory), (ii) a temporally prospective function of anticipatory set or preparation (planning and foresight), and (iii) a protective function of interference control (inhibition of false response tendencies).

Behavioural studies have shown that the NCL serves at least two of these „prefrontal“ functions. Lesions of the NCL show behavioural impairments in a variety of working memory tasks (Mogensen and Divac, 1982, 1993, Gagliardo et al., 1996, 1997, Güntürkün, 1997).
Similarly, the role of the NCL in interference control has been tested with Go/NoGo tasks and reversal-learning (Hartmann and Güntürkün, 1998; Güntürkün, 1997, Kalt et al., 1999). The prospective coding functions of the NCL have not been studied yet, but Kalt and coworkers (1999) observed neurons that increased their firing towards the end of the delay, which, in accordance to the findings in primates, has been interpreted as „reward expectancy“ or a preparatory motor function of these cells. Therefore, it is highly likely that the anticipation of events and the preparation for them, also takes part in the NCL.

As briefly outlined in chapter 1, the temporal organization of behaviour requires to bridge temporally separate elements of action and sensorium. The anatomical data presented in chapters 1 - 3 strongly suggest that the NCL may serve such a synthetic function in the perception-action cycle. Similar to the PFC of mammals, the NCL receives converging afferents from various telencephalic and thalamic areas that relay input from the major sensory modalities of somesthesia, vision, and audition (chapters 1 and 2). At least the intratelencephalic projections are reciprocal which might allow the NCL to control its own input. The pattern of subtelencephalic projections is not known and need to be studied with more sensitive anterograde tracers. In addition, the NCL is reciprocally connected with the avian homologue of the amygdala (chapter 1), a connection which is also characteristic for the PFC (Condé et al., 1995; Reep et al., 1996; Sesack, et al., 1989; Barbas and DeOlmos, 1990), and projects to most parts of the somatic and limbic striatum, as well as the sensorimotor archistriatum (chapters 1 and 3). Via the projections through the basal ganglia and the archistriatum, the NCL does not only exert indirect influence over motor structures in the brainstem (Wild, 1993; chapter 1), but may also control its own dopaminergic innervation from the substantia nigra and AVT.

In the context of working memory tasks it is generally assumed that items from long-term memory relevant to the task at hand are retrieved from stores outside the PFC. Functional imaging studies in humans, and electrophysiological recordings in animals performing delay tasks indeed show time- and domain-dependent activation within distributed networks related to the PFC (Cohen et al., 1997, Belger et al., 1998; Chafee and Goldman-Rakic, 1998). It is reasonable to believe that such a gross anatomical arrangement of different sensory-specific networks also exists between the NCL and other associational or sencondary sensory forebrain areas in the avian brain. This is inferred from the sheer necessity to save memory „storage“, and the fact that both, at the areal level (chapter 1), and at the single cell level
a large number of cells were seen to project back to sensory areas in the anterior forebrain. The reciprocal connection of the NCL with virtually all of its sources of afferents suggest the existence of multiple distributed networks within the avian forebrain. One such functional network probably involves the connection between the NCL and the parasensory area in the neostriatum intermedium (NIM, or MNH in chicks). This area sends a massive projection to large parts of the NCL and in turn receives considerable feedback from the NCL (chapter 1). Both forebrain areas receive a distinct innervation from the polymodal DLP (chapter 2) and the termination field of the DLP within NCL completely overlaps with the termination area of the NIM (c.f. figs. 10, 11 in chapter 1, and fig 1f in chapter 2).

The exact homologue of the DLP remains unclear (c.f. detailed discussion in chapter 1), functionally, however, the DLP-NCL projection has been implicated in the proper execution of working memory tasks (Güntürkün, 1997), similar to the MD-PFC projection in mammals (Sakurai and Sugimoto, 1985; Fuster, 1989 for review). It is therefore not unreasonable to speculate that the DLP-NIM projection also conveys information pertinent to these tasks. The NIM receives afferents from areas in the Wulst and archistriatum, and its projection onto NCL could complement the parallel input the NCL receives from these areas. Via the projections of the NCL and NIM to the basal ganglia and the archistriatum, this loop through the forebrain could also influence activity in the dorsal thalamus (including DIP and DLP) and lower mesencephalic areas (e.g. the optic tectum, or AVT; c.f. fig. 13 in chapter 1).

In the PFC of mammals, working memory is manifest in the activity of delay-related neurons that are activated after offset of the cue and continue firing throughout the duration of the delay (e.g. Fuster, 1973; Sawaguchi and Yamane, 1999). Most cellular models of working memory assume that the activity of these neurons reflects reverberating activity in a cell assembly that codes for the given memorandum (Amit, 1995; Goldman-Rakic, 1996; Durstewitz et al., 1999; 2000). In the PFC, reciprocal horizontal projections between neurons in layers III, and V-VI, respectively, connect discrete „columns“ within the same cortical region, which are assumed to form functional modules (e.g. Goldman-Rakic, 1996; Melchitzky et al., 1998; González-Burgos et al., 2000). The local interaction between these cells provides a neural basis for positive feedback circuits in the PFC that may play a role in sustaining neural activity (Amit 1995; Goldman-Rakic, 1996; Durstewitz et al., 1999, 2000).

In the pigeon, Kalt and coworkers (1999) have recently shown that neurons in the NCL can similarly enhance their firing throughout the delay period of a delayed response task to „hold“ information after the offset of the sensory cue. It can be assumed that at least part of the
sustained activity results from reverberating activity within the NCL. In accordance with this view, simultaneous recordings from two NCL neurons during a working memory task show a significant correlation of task-related activity (Thomas Kalt, personal communication).

The electrophysiological analysis of cell types *in-vitro*, allows the description of certain distinct features (e.g. firing pattern, or membrane time constant) that determine their integrative properties and the impact on other cells (chapter 3). However, the conclusions that can be drawn from this analysis about a cell’s role within a functional circuit are rather limited. This is due to the fact that *in-vivo* the cell’s role might be determined much more by the properties of the network, than by the properties of the cell itself.

In the NCL, spiny principal neurons of the type II, and smooth type III interneurons showed sustained firing over a wide range of frequencies and throughout long depolarizing current pulses. Thus, in principle, the firing patterns of these cells could represent a neural substrate for active memory retention on the single cell level. Furthermore, Durstewitz and coworkers (2000) could show, that in a network of the PFC stimulus-specific recurrent activity can be maintained at frequencies below 20 Hz, which is in the range of the steady-state firing frequency of type II neurons (c.f. chapters 3 and 6).

However, in contrast, the firing frequency of single NCL neurons *in-vivo* is not only highly irregular (as might also be the case in cortical neurons), but furthermore it appears that a large portion of neurons which show delay-related activity, respond with bursts of action potentials (Thomas Kalt, personal communication). Thus, reverberating activity in an ensemble of burst-firing neurons (i.e. cell types I and IV\(^1\)) also seems to make a major contribution to delay activity. As bursting neurons showed strong accommodation of firing and were not able to generate repetitive bursts in response to continued depolarization, phases of refractory inhibition, most likely provided by feedback from the type III interneurons, are also essentially to produce the pattern observed *in-vivo*.

Functionally, burst firing increases the probability to drive the postsynaptic cell beyond spike threshold (Snider et al., 1998). The high frequency of action potentials during a burst will thus amplify the neural signal and synchronize the activity in a population of postsynaptic cells (Hablitz, 1986; Snider et al., 1998). Therefore, burst firing pattern of type I cells might provide for reliable transmission within an active cell assembly, which is needed for the stability of the mnemonic representation.

\(^1\) As discussed in detail in chapter 3, neurons of type IV are likely to represent immature stages of other spiny cell types, and therefore they are not considered here further.
Modulation by the dopaminergic system

As discussed in detail in chapters 4 and 5, the organization of the mesotelencephalic DA projections and the regional distribution of dopaminoceptive elements is highly similar in mammals and birds, despite approximately 250 million years of divergent evolution.

As also shown in chapter 4, the structural similarities extend to functional similarities with regard to the control of sensorimotor and associative processes, that are controlled by the dopaminergic system.

In mammals, the integrity of the active short-term memory trace appears to be critically dependant on an optimal level of DA receptor stimulation. Both, prefrontal DA depletion (Brozoski et al., 1979; Simon et al., 1980) and excessive stimulation of DA receptors (Murphy et al., 1996; Sawaguchi, 1997; Zahrt et al., 1997; Arnsten and Goldman-Rakic, 1998), disrupt performance on delay tasks. Similarly, the administration of high doses of D1 antagonists into the PFC also impair performance on delay tasks and decrease delay-period activity of PFC neurons (Sawaguchi et al., 1990b; Sawaguchi and Goldman-Rakic, 1994; Williams and Goldman-Rakic, 1995; Zahrt et al., 1997), however, application of low doses of D1 antagonists or DA into the PFC increases delay-correlated activity of PFC neurons relative to background activity (Sawaguchi et al., 1988; 1990a; Williams and Goldman-Rakic, 1995; Müller et al., 1998). Thus, too much or too little DA may be detrimental to cognition.

Thus, one major finding of the present studies is that DA also profoundly alters the excitability of NCL neurons, and that the observed increase in the number of spikes was strongly dose dependent. Moreover, this dose dependency was best described with an inverted U-curve, demonstrating also that under physiological conditions in birds, as is the case in mammals, an optimal level of DA might be required for the changes in neuronal excitability to take place (chapter 6).

Together with the data by Kalt and coworkers (1999) and the voltage-dependence of the DA effect (discussed in chapter 6), one can speculate that in-vivo the mesencephalic DA signal to the NCL enhances the excitability of active cell assemblies, which might result in recurrent activity that is strong enough to carry the memory trace over the delay.

So far, there exists only one study that has examined the contribution of the DA system on working memory in birds (Güntürkün and Durstewitz, in press). In support for the hypothesis outlined above, the results show a severe impairment in working memory functions, following the blockade of D1 receptors by injections of high doses of SCH-23390 into the NCL. Therefore, D1 receptor mediated enhancement of neuronal firing might be a requirement for the
active retention of memory contents both in mammals and birds. To test the validity of this assumption, it could be attempted to pharmacologically alter the delay-related activity of NCL neurons through the iontophoretic application of D1 receptor antagonists, or supranormal doses of DA (c.f. Sawaguchi and Goldman-Rakic, 1991; Williams and Goldman-Rakic, 1995).

However, a general, non-specific increase in activity will not produce a stable representation in short-term memory. As indicated above, the voltage- (i.e. activity-) dependance of the DA effects (c.f. discussion in chapter 6) might serve to enhance only those cell assemblies that are currently activated. Yet, the question remains, how the representation of memory items within the recurrent activity might be protected from other interfering stimuli.

In mammals, the bilaminar pattern of prefrontal cortical DA innervation might be an inherent aspect of the dopaminergic modulation of working memory. In the PFC, the innervation by dopaminergic fibers is densest in the upper layers I-II and V-VI (c.f. figure 4 in the introduction). The arrangement of long-range associational and local input, respectively, shows a somewhat parallel pattern. Synaptic inputs from other association cortical regions mainly arrive in layers I-II, where the apical tuft of the layer III, and V-VI pyramidal neurons extends (Sesack et al., 1989; Berendse et al., 1992; Condé et al. 1995), and, as outlined above, local synaptic connections that contact the soma and proximal dendrites arise from neurons from within the same lamina.

As discussed in detail in chapter 6, DA might modulate distal signals in a manner different from its effect on proximal inputs. In sum, DA acting on pyramidal neurons of the PFC (i) restricts the effects of inputs to the apical dendrites of these neurons by attenuating the dendritic HVA Ca\(^{++}\)-mediated amplification of such inputs (Westenbroek et al., 1992; Surmeier et al., 1995; Yang and Seamans, 1996); and (ii) increases cell excitability through its actions on conductances that are located mainly in the soma and proximal dendrites (Yang and Seamans, 1996; Yang et al., 1996b). Thus, one effect of DA might be to selectively attenuate distal EPSPs relative to proximally arriving EPSPs, resulting in a differential „selection“ of synaptic inputs (Yang et al., 1996b; see also Law-Tho et al., 1994).

In the absence of a layered structure that enables the spatial separation of functionally distinct inputs, another mechanisms is needed to „switch“ between inputs from long range associational fibers and from intrinsic connections. In the avian brain, the dichotomic modes of DA innervation might serve this function.
Figure 1: Summary of the anatomical data presented here, and the modulatory actions of dopamine on identified neuronal elements in the NCL. The input-output relations of the NCL have been described in chapter 1 - 3. Note that for reason of clarity not all possible afferent and efferent projections are depicted. However, neurons from all spiny cell types (i.e. types I, II, IV [not shown]), but not smooth interneurons (type III), project to the same targets outside NCL. In addition, it can be assumed, that neurons of all cell types receive the same afferent input. The assignment of DARPP-32 immunoreactivity to certain cell types is inferred from a comparison of the morphology of DARPP-32 positive neurons described in chapter 5 with those identified in chapter 3, and this is complemented by the reaction of these cell types to the application of DA and the D1 receptor agonist SKF-38393. Part of the intrinsic connections of the NCL can be inferred from the pattern of local axonal collateralizations of different cell types (c.f. chapter 3). However, a detailed description of the intra-NCL connections is still lacking. The insert shows a hypothetical arrangement through which the dopaminergic system could achieve a spatial selection of functionally distinct synaptic inputs. Local synaptic connections that arrive at the soma of neighbouring cells might be enhanced relative to synaptic inputs from sensory areas arriving at the distal dendrites. In accordance with the data presented in chapter 6, a type I neuron is shown to receive basket-type innervation. For the model proposed, however, it is not relevant whether a specific cell type receives basket-innervation, and there is evidence that projection neurons of the other cell types also receive basket-innervation.

There are several working hypotheses regarding the role of the dopaminergic basket-type innervation that have been tackled by the studies presented here. One assumption might have been that the basket-innervation provides a means for the dopaminergic system to selectively target a certain class of neurons, e.g. GABAergic interneurons, cells with distinct firing patterns, or distinct axonal connections. Alternatively, it might have been thought that the strong somatic basket innervation exerts a qualitatively or quantitatively different influence on the
target cell, in the sense that it provides a higher concentration of DA, which in turn might result in opposite or quantitatively different effects than comparatively lower concentrations of DA (e.g. inverted U shape of effects).

Clearly, our understanding about the functional subtypes of NCL neurons is still too limited to unequivocally rule out any of these hypothesis. However, from the data provided in chapter 6 no obvious qualitative or quantitative difference in the response to DA was evident for neurons that received strong somatic basket input.

So what is the functional importance of the two modes of dopaminergic innervation then? As indicated above, I would like to propose that baskets provide a means for spatial selection of functionally distinct synaptic inputs. A differential effect of DA on distinct inputs might be achieved if a specific class of connections, e.g. local connections between NCL neurons, make synapses preferentially on the somata and initial dendrites of neighbouring cells. Such a hypothetical arrangement is shown in the insert in figure 1. For this model it is not necessary that all intrinsic connections of NCL neurons selectively make contacts only with the somata of other NCL neurons. Dopamine might still enhance these inputs relatively more if only a fraction of intra-NCL connections synapses on the somata of neighbouring cells. For the differential effects of DA to take place it is merely critical to assume that a larger number of proximal inputs originates from intra-NCL projections relative to long-range sensory inputs. Also, the large number of baskets found in the NCL is not an indicator for a larger number of intrinsic connections. Other areas in the avian forebrain might possess a similarly large number of local interconnections, but the functions of these circuits may not depend as much on dopaminergic modulation as it appears to be the case for the NCL.

In the NCL, however, the proposed „input-selection“ effect of DA could account for reverberating activity between NCL neurons, and a concurrent protection of this memory trace against interfering stimuli. This effect of DA could be brought about by several mechanisms. The easiest model to assume is that the distribution of ionic conductances across the cell membrane of NCL neurons varies in a manner similar to that found in mammalian neurons, so that DA will attenuate distal („sensory“) inputs through dendritic HVA Ca\(^{++}\) channels, and will enhance proximal inputs via its actions on \(I_{\text{NaP}}\) and L-type currents. Indeed it appears, that such ionic „compartments“ exist not only in neocortical neurons that extend long apical dendrites. For example, striatal (Surmeier et al, 1995) and cerebellar (Usowicz et al., 1992) neurons show a similar distribution of N- and P-type currents in the dendritic segments, and a predominance of L-type currents at the soma and proximal dendrites. Alternatively, DA could
have dose-dependent effects on these neurons, as DA released from baskets will probably faster reach significant concentrations at a large number of receptors. However, such an effect would have went undetected in the experiments presented in chapter 6, in which DA was bath applied, and the recordings were made (most likely) only from the somata of NCL neurons.

Support for a differential dendritic vs. somatic action of DA may come from the observation by Schnabel and coworkers (1997), who found that the majority of D1 receptor immunoreactivity was localized to synaptic and extrasynaptic sites in spines and dendrites, but that in addition some neurons also showed dense labeling of the somatic cytoplasm.

To test the ideas outlined above, anatomical and electrophysiological studies may be devised. Clearly, an electronmicroscopic analysis of the synaptic types and the subcellular distribution of intra-NCL connections is required. To this end, either small injections of an anterograde tracer confined to the NCL might be utilized, or the axonal arborizations of single, intracellularly labeled neurons might be studied. Functionally, these connections could also be analyzed by paired recordings from neighbouring cells in NCL, and the effect of DA on the synaptic weight may be compared relative to DA-induced changes in synaptic inputs from areas outside the NCL. Furthermore, direct recordings from dendrites of NCL neurons and pharmacological isolation of single ionic currents could address the issue whether DA exerts its effects via a differential modulation of specific conductances.

In sum, the data presented here describe the organisation of a polymodal associative structure in the avian forebrain, and they provide a structural and physiological framework in which sensorimotor integration and working memory may take place. Furthermore, based on a comparison with the prefrontal cortex of mammals, it appears that functional similarities also necessitate some structural similarities. Thus, the localization of a cognitive function within a neuronal circuit, and a further understanding of the circuit’s structural and physiological requirements may ultimately help to identify the building blocks of mental computation in various species.
Abbreviations used in the text and figures

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>6-OHDA</td>
<td>6-hydroxydopamine</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid</td>
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<tr>
<td>AC or Acc</td>
<td>nucleus accumbens</td>
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<td>Aidv</td>
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<td>DA</td>
<td>dopamine</td>
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<td>DARPP-32</td>
<td>dopamine- and cAMP-regulated phosphoprotein, $M_r = 32,000$</td>
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<td>fasciculus prosencephali lateralis</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>GAD</td>
<td>glutamate decarboxylase</td>
</tr>
<tr>
<td>GLd</td>
<td>dorsolateral geniculate nuclei</td>
</tr>
<tr>
<td>HA</td>
<td>hyperstriatum accessorium</td>
</tr>
<tr>
<td>HD</td>
<td>hyperstriatum dorsale</td>
</tr>
<tr>
<td>HIS</td>
<td>hyperstriatum intercalatus superior</td>
</tr>
<tr>
<td>HOM</td>
<td>tractus occipitomesencephalicus, pars hypothalami</td>
</tr>
<tr>
<td>Hp</td>
<td>hippocampus</td>
</tr>
<tr>
<td>HV</td>
<td>hyperstriatum ventrale</td>
</tr>
<tr>
<td>HVA</td>
<td>high-voltage-activated (Ca$^{++}$ currents)</td>
</tr>
<tr>
<td>HVdv</td>
<td>hyperstriatum ventrale dorso-ventrale</td>
</tr>
<tr>
<td>HVvv</td>
<td>hyperstriatum ventrale ventroventrale</td>
</tr>
<tr>
<td>ICo</td>
<td>nucleus intercollicularis</td>
</tr>
<tr>
<td>IHA</td>
<td>nucleus intercalatus of the hyperstriatum accessorium</td>
</tr>
<tr>
<td>IMHV</td>
<td>intermediate and medial part of the hyperstriatum ventrale</td>
</tr>
<tr>
<td>I$$_{KS}$</td>
<td>slowly inactivating potassium current</td>
</tr>
<tr>
<td>I$$_{NaP}$</td>
<td>persistent sodium current</td>
</tr>
<tr>
<td>INP</td>
<td>nucleus intrapeduncularis</td>
</tr>
<tr>
<td>ir</td>
<td>immunoreactive</td>
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<tr>
<td>K$^+$</td>
<td>potassium</td>
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<td>L1</td>
<td>field L1</td>
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</tr>
<tr>
<td>LH</td>
<td>lamina hyperstriatica</td>
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<tr>
<td>LPO</td>
<td>lobus parolfactorius</td>
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<tr>
<td>MNH</td>
<td>mediorstral neostriatum/hyperstriatum ventrale</td>
</tr>
<tr>
<td>n.</td>
<td>nucleus</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
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<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>NA</td>
<td>noradrenaline</td>
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<tr>
<td>Na++</td>
<td>sodium</td>
</tr>
<tr>
<td>NC</td>
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</tr>
<tr>
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</tr>
<tr>
<td>NCm</td>
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</tr>
<tr>
<td>Nd</td>
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<tr>
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<tr>
<td>NFL</td>
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<tr>
<td>NFT</td>
<td>neostriatum fronto-trigeminale</td>
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<tr>
<td>NI</td>
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<tr>
<td>NIM</td>
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</tr>
<tr>
<td>NIMI</td>
<td>neostriatum intermedium medialis, pars laterale</td>
</tr>
<tr>
<td>NIMm</td>
<td>neostriatum intermedium medialis, pars mediale</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>OM</td>
<td>tractus occipitomesencephalicus</td>
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<tr>
<td>Ov</td>
<td>nucleus ovoidalis</td>
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<td>PA</td>
<td>paleostriatum augmentatum</td>
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<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
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<tr>
<td>PNMT</td>
<td>phenylethanolamine-N-methyl transferase</td>
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<tr>
<td>PP</td>
<td>paleostriatum primitivum</td>
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<tr>
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<tr>
<td>VIP</td>
<td>ventrointermediate area of the posterior nuclei</td>
</tr>
<tr>
<td>VP</td>
<td>ventral pallidum</td>
</tr>
<tr>
<td>Rt</td>
<td>nucleus rotundus</td>
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<td>SAC</td>
<td>stratum album centrale</td>
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<tr>
<td>SCI</td>
<td>stratum cellulare internum</td>
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<tr>
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<tr>
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<td>substantia nigra, pars compacta</td>
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<tr>
<td>SNR</td>
<td>substantia nigra, pars reticulata</td>
</tr>
<tr>
<td>SPC</td>
<td>nucleus tractus septomesencephalici</td>
</tr>
<tr>
<td>SPL</td>
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<tr>
<td>T</td>
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<td>TH</td>
<td>tyrosine hydroxylase</td>
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<td>Tn</td>
<td>nucleus taeniae</td>
</tr>
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<td>TO (chapter 1)</td>
<td>optic tectum</td>
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<tr>
<td>TO (chapter 4)</td>
<td>olfactory tubercle</td>
</tr>
<tr>
<td>TPc</td>
<td>nucleus tegmenti pedunculopontinus, pars compacta</td>
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<tr>
<td>TPO</td>
<td>area temporo-parieto-occipitalis</td>
</tr>
<tr>
<td>TSM</td>
<td>tractus septomesencephalic</td>
</tr>
<tr>
<td>Va</td>
<td>vallecula</td>
</tr>
<tr>
<td>VIA</td>
<td>ventrointermediate area of the thalamus</td>
</tr>
<tr>
<td>VTA</td>
<td>ventral tegmental area (A10); also see AVT</td>
</tr>
</tbody>
</table>
LITERATURE


Wernicke, C. (1874) *Der aphasische Symptomemkomplex* (Cohn and Weigart, Breslau, Germany).


Wild, J.M. (1994) Visual and somatosensory inputs to the avian song system via nucleus uvaeformis (Uva) and a comparison with the projections of a similar thalamic nucleus in a nonsongbird (*Columba livia*). *J. Comp. Neurol.* **349**:512-535.


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Lebenslauf

Sven Kröner

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Tag der mündlichen Prüfung 29. 05. 2000

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Stipendien

Okt. 1996 Stipendium der G.A. Lienert Stiftung:
Erklärung


Ich habe diese Dissertation weder in dieser, noch in irgendeiner anderen Form bereits vorgelegt. Ich habe darüberhinaus bislang auch keine andere Dissertation vorgelegt.

Bochum, den 27. 03. 2000

Sven Kröner