Direct and indirect communication between functionally different regions of the rat striatum
promotoren: prof.dr. H.J. Groenewegen
proef.dr. C.M.A. Pennartz
Cover illustration: photomicrograph of a medium-sized spiny projection neuron in the nucleus accumbens shell. This neuron was recorded \textit{in vivo} and characterized on the basis of their response to hippocampal stimulation and labeled using juxtaacellular application of neurobiotin by Prof. Dr. Jean-Michel Deniau and the author. Inset: high power magnification reveals a part of a dendrite and local axon collateral of the same neuron in a calbindin-D$_{28kDA}$ stained section.

This study was carried out in the laboratory of the Department of Anatomy & Embryology, Research Institute Neuroscience Vrije Universiteit, Graduate School Neurosciences Amsterdam, The Netherlands.
The Poet Donates Her Body To Science

Come in, this skin is for you.
Nothing will ever again be wrapped
in these creases. Break a rib, make a man
of it, place him in Paradise.

See how he does, minus imagine.
Make up my mind

like a bed. Dissect the tumor of dream.
Can you get at a heart? Resuscitate,
carve, or cover it? The beaten urge.
Do not be afraid

of the dark. Come in, with your hard
and inner answer. Come in,

I am all of the above. End this
fruitless scrutiny. Remove your gloves.

Touch. I am the puzzle
you are always doing the borders of.

Feel the facts of me. Lips, hands, eyes
that never specialized

in a single love. Now the wide mouth
of me merges with no body,

the blood that believed in me.
Here is the gift of skin. Here is the back
door to oblivion. Let the dark in-
divisible out.

Christina Davis

Aan mijn ouders

Aan mijn broer
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<td>ABC</td>
<td>avidin-biotin-peroxidase complex</td>
</tr>
<tr>
<td>ac</td>
<td>anterior commissure</td>
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<tr>
<td>Acb</td>
<td>nucleus accumbens</td>
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<tr>
<td>AcbC</td>
<td>nucleus accumbens core subregion</td>
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<td>ACd</td>
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<td>BDA</td>
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<td>CaB</td>
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<td>DTI</td>
<td>diffusion tensor imaging</td>
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<td>ip</td>
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<td>LCN</td>
<td>local circuit neuron(s)</td>
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<td>LH</td>
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<td>LV</td>
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<td>M</td>
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<td>mesencephalon</td>
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<td>ml</td>
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<td>reticular thalamic nucleus</td>
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<td>rostral pole</td>
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<td>caudate-putamen ventral part</td>
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<td>ventral pallidum</td>
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<td>ventral pallidum lateral part</td>
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<td>ventromedial thalamic nucleus</td>
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<td>2D</td>
<td>two-dimensional</td>
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<td>3D</td>
<td>three-dimensional</td>
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Chapter 1

General Introduction
Chapter 1

GENERAL INTRODUCTION

Our brain is the most delicate organ of our body and, at the same time, has the widest range of functions. It receives and processes all external, i.e., visual, auditory, olfactory, gustatory, thermal and tactile inputs, as well as internal information from the locomotor system and the internal organs. As a consequence of this wide range of inputs, the internal processing of information and the output in terms of voluntary movements and the regulation of internal organs, the brain forms the central steering organ for our behavior, thoughts and feelings.

What is the structural basis for such intricate and essential functions? The brain consists of approximately 10-100 billion nerve cells or neurons that together exert the specific functions of the brain, and even more glial cells that support the nerve cells in structural and functional terms. Neurons are specialized in communicating with each other as well as with their target cells or organs. To perform these functions, neurons have long extensions that either receive information, the dendrites, or send information to other neurons, the axons. In principle, in between the receiving and the sending parts of the neuron the cell body of the neuron is located, and it is at the level of dendrites and somata that integration takes place and the output of the neuron is determined. Brain functions are not exerted by individual neurons, but rather by networks of interacting and communicating neurons. A great deal of our understanding of which brain areas ‘talk’ to each other has come from neuroanatomical studies (see box). Structurally, neuronal cell bodies and their dendrites are clustered together, either in layered structures at the external surface of the brain, i.e., the cerebral cortex with its columns, or in clusters or nuclei in deeper parts of the brain. Collections of neurons in the superficial cerebral cortex and in the deeper nuclei are connected through bundles of axons that travel over longer or shorter distances in the brain. Viewed macroscopically, the collections of neurons in cortical or subcortical nuclear structures can be easily distinguished from the bundles of axons: a large percentage of axons are ensheathed by myelin, a fatty substance that gives fiber bundles a light appearance, i.e., the white matter. This contrasts with the darker appearing cortical and nuclear structures, the gray matter. The cerebral cortex can be divided into distinct subregions based upon morphological differences between the neurons in these subregions. Likewise, subcortical nuclei have a different cellular structure on the basis of which they can be morphologically distinguished. To some extent, it can be stated that morphologically distinct cortical subregions of subcortical nuclei support different functions of the brain. However, the
general functions of the brain are not represented in individual nuclei or specific parts of the cerebral cortex. By far, most of the functions of the brain are based on interactions between distinct parts of the cerebral cortex and subcortical nuclei on the basis of intricate neuronal networks established by the axons that connect the various structures. Along this line, morphologically distinct cortical or subcortical structures may participate in various brain functions on the basis of specific axonal connections and the participation in various functionally distinct neuronal networks.

The present study deals with the interconnections between the cerebral cortex and the largest group of subcortical nuclei of the brain, i.e. the basal ganglia. As will be described in more detail below, the entire cerebral cortex, in an ordered point-to-point relationship projects to the basal ganglia, and virtually the entire frontal lobe receives, albeit indirectly, information back from the basal ganglia. The abundance of the basal ganglia projections to the frontal lobe emphasizes the role of the basal ganglia complex in premotor and prefrontal functions, i.e., in motor, cognitive and affective functions.

To be more specific, the basal ganglia consist of four large subcortical nuclear groups in the basal forebrain that are extensively connected, directly or indirectly, with different parts of the cerebral cortex. The organization of projections of functionally different parts of the cerebral cortex, e.g. motor, sensory, associative and limbic, to the basal ganglia forms the neuronal basis for the functional differentiation of the cortical-subcortical connections. The basic principle of the cortical-basal ganglia relationships is essentially based on the parallel processing of information in functional basal ganglia-thalamocortical circuits, in which distinct functions are for the most part maintained, or segregated one from the other (Alexander et al., 1986, 1990; Groenewegen et al., 1987; Wiesendanger et al., 2004). The formulation of this concept of a parallel organization of connections from the (pre)frontal cortex through the basal ganglia and the thalamus back to the (pre)frontal cortex by Alexander and coworkers some 20 years ago has had great impact on the field of preclinical and clinical research on the basal ganglia. Thus, according to this ‘parallel processing concept’ the basal ganglia play an important role in motor functions, as well as in cognitive and affective functions (Delong et al., 1984; Alexander et al., 1986, 1990; Alexander and Crutcher, 1990; Cardinal et al., 2002; Lewis et al., 2003). In line with these diverse functions, the clinical manifestations of disorders of the basal ganglia include movement disorders such as Parkinson’s disease (Lewis et al., 2003) and mood and thought disturbances such as schizophrenia (Hokama et al., 1995), major depression (Husain et al., 1991) and drug addiction (Everitt et al., 1999). The concept of a parallel organization of cortical-subcortical connections, however, leaves entirely open the question of how the different functional streams interact with each other in order to lead to a
coordinated output of the brain (Wise et al., 1996; Redgrave et al., 1999). The results of functional-anatomical studies have indicated that there are several ways in which functionally distinct basal ganglia-thalamocortical circuits may interact with each other, including interactions mediated by corticostriatothalamic loops (Zahm and Brog, 1992; Joel and Weiner, 1994; Groenewegen et al., 1994; O’Donnell et al., 1997) but also by nigrostriatal circuits (Otake and Nakamura, 2000; Haber et al., 2000) and by intrastriatal interactions (Kawaguchi et al., 1990; Heimer et al., 1991). Recent behavioral studies likewise provide specific suggestions for such interactions at the functional level (Parkinson et al., 1999; Corbit et al., 2001; Robbins and Everitt, 2002). The present study elaborates on this issue of communication between parallel circuits. In general terms, the key objective in the present thesis is to investigate in rats some of the possible direct and indirect connections between functionally different parts of the striatum and, consequently, between functionally distinct cortical-basal ganglia circuits.

The present chapter will provide an introduction to the functional-anatomical organization of the basal ganglia and their relationships with the cerebral cortex. Following a survey of the structure and functions of the basal ganglia in general, we will focus on the nucleus accumbens as component of a larger ventral, limbic-related corticostriatal system. In the context of the question of possible direct and indirect connections between functionally different circuits, we will deal with the structure and connections of the nucleus accumbens and its position in the basal ganglia-thalamocortical circuitry. This will provide the background for the experimental chapters in which we approached the question of interactions within the ventral striatum and between basal ganglia-thalamocortical circuits by studying with neuroanatomical tracing methods direct intrastriatal connections and indirect basal ganglia loop interconnections to establish the neuronal substrate for such presumptive interactions.

1. BASAL GANGLIA, STRUCTURE AND FUNCTIONS

The basal ganglia receive input from the entire cerebral cortex and direct their output to the frontal lobe

The basal ganglia are a group of functionally related and strongly interconnected nuclei located in the forebrain and midbrain (Fig. 1). The four nuclei that comprise the basal ganglia are the striatum, the pallidum, the subthalamic nucleus and the substantia nigra. The basal ganglia are the principal subcortical components of a ‘family’ of basal ganglia-thalamocortical circuits that link
General introduction

Fig. 1: Sagittal diagram of the brain illustrating the structural organization of the basal ganglia and their relationship with the thalamus and cerebral cortex.

the forebrain and midbrain with the thalamus and the cerebral cortex. The striatum is the major recipient of inputs to the basal ganglia from the cerebral cortex, amygdaloid complex, thalamus and brainstem. The pallidum and the substantia nigra are considered to be the output nuclei of the basal ganglia as they connect to the thalamus and several brainstem structures.

Concepts of the basal ganglia, their position in the circuitry of the forebrain and their functions have changed considerably in the past decades. Based on (clinical) observations that lesions of the basal ganglia, in contrast to lesions of the pyramidal system, i.e. the descending corticospinal motor pathway, do not result in paresis or paralysis, originally led to the inclusion of the basal ganglia in the so-called extrapyramidal system. In that context, it was assumed that the basal ganglia send their output, next to the corticospinal tract, to the brainstem and spinal cord, in this way influencing the motor system in parallel with the pyramidal system. An important change of this concept was based on findings by Nauta and Mehler (1966), who showed that the major output of the basal ganglia is not to the brainstem but to the thalamus and, consequently, to the cerebral cortex. It was then realized that, at least to a large degree, the basal ganglia exert their influence on the motor system via the pyramidal tract rather than directly via descending pathways (DeLong, 1974; Turner and DeLong, 2000). Therefore, even though still in use, the term extrapyramidal system seems obsolete, at least in association with the basal ganglia.

Following the seminal paper by Nauta and Mehler (1966), it became generally accepted that the basal ganglia, via the ventral anterior nucleus of the thalamus, reach the premotor cortex in the frontal lobe and in this way play a role in the preparatory phases of the initiation of movements. However, at that time the view of the basal ganglia was still very much focused on the caudate-
putamen complex, the globus pallidus and the substantia nigra. Knowledge of the precise organization of the interconnections between the different components of the basal ganglia was still very scarce. Functionally, these large structures in the forebrain were largely coupled to the motor system.

With the introduction of the concept of a so-called ‘ventral striatopallidal system’ next to a ‘dorsal striatopallidal system’ by Heimer and Wilson (1975)(Fig. 2), it became more and more accepted that the basal ganglia are also involved in non-motor, i.e., cognitive and affective (or limbic) functions. Heimer and Wilson (1975) proposed, based on neurochemical and hodological criteria, to include parts of the basal forebrain into the basal ganglia. It was noted by Heimer and Wilson (1975) that there are striking similarities in cytoarchitecture and chemoarchitecture (e.g., activity of the enzyme acetylcholinesterase) between the caudate-putamen complex, the nucleus accumbens and the medium-celled parts of the olfactory tubercle. Likewise, general patterns of afferent and efferent fiber connections were found to be very comparable between these three regions. Thus, whereas the caudate-putamen complex receives major inputs from the neocortex, the nucleus accumbens and medium-celled parts of the olfactory tubercle receive inputs from the prefrontal cortex, the hippocampus, and the intralaminar and midline thalamic nuclei. In parallel to the pallidal and nigral projections from the caudate-putamen complex, Heimer and Wilson (1975) described projections from the nucleus accumbens and medium-celled parts of the olfactory tubercle to an area of the substantia innominata that they identified as a ventral extension of the pallidum and denoted this area as the ventral pallidum. On the basis of these observations, Heimer and Wilson (1975) proposed to designate the striatal areas receiving allocortical input as the ventral striatum and the remaining parts as the dorsal striatum. As a result, Heimer and Wilson (1975) stressed that the nucleus accumbens, the medium-celled parts of the olfactory tubercle and the ventral pallidum are associated with allocortical formations in much the same way as the caudate-putamen complex and globus pallidus are associated with the neocortex, introducing in this way the concept of parallel organized ‘dorsal striatopallidal’ and ‘ventral striatopallidal systems’.

Although this notion of a limbic role of the basal ganglia was not entirely new, in the years that followed non-motor functions of the basal ganglia, both from clinical and basic research, became more and more apparent (Mogenson et al. 1980; Van den Bercken and Cools, 1982; Robbins et al., 1989; Marsden, 1992).

It seems very likely that the concept of Heimer and Wilson (1975) has inspired Alexander and colleagues (1986) to describe barely a decade later the parallel organization of several basal ganglia-thalamocortical circuits, mostly based on the functional-anatomical organization of the
connections of the basal ganglia with the cerebral cortex and the thalamus in non-human primates. This landmark paper by Alexander et al (1986) has dominated the basal ganglia literature in the past two decades. Alexander et al. (1986) hypothesized that functionally different (pre)frontal cortical areas are involved in closed cortical-subcortical loops that successively include discrete, non-overlapping parts of the striatum, the pallidum, the substantia nigra, the thalamus and the (pre)frontal cortex. The basic design of each circuit is thought to be similar. Thus, each circuit receives input from several functionally related cortical areas that send partially overlapping projections to a restricted portion of the striatum. These striatal regions subsequently send converging projections to the pallidum and the substantia nigra, which in turn project to a specific region of the thalamus. Each thalamic region projects back to one of the frontal cortical areas that
feeds into the circuit, thereby completing the ‘closed loop’ portion of the circuit. In this way, different parts of the basal ganglia are viewed, along with their connected cortical and thalamic areas, as components of a ‘family’ of basal ganglia-thalamocortical circuits that are organized in a parallel manner and remain segregated from one another, both structurally and functionally (Alexander et al., 1986; 1990; Groenewegen et al., 1990). Alexander et al. (1986) tentatively identified five basal ganglia-thalamocortical circuits: a ‘motor circuit’ that includes the dorsolateral part of the striatum and the premotor cortices, an ‘oculomotor circuit’ that involves the dorsomedial part of the striatum and the frontal and supplementary eye fields, two ‘prefrontal circuits’ that include more central parts of the striatum and the dorsolateral prefrontal and orbitofrontal cortices and, finally, a ‘limbic circuit’ that involves the ventral striatum and the anterior cingulate and medial prefrontal cortices.

To summarize, it has become clear from the above description that the notions about the structural and functional relationships between the cerebral cortex and the basal ganglia have recently undergone major changes. The idea of the basal ganglia that send their output to the brainstem and spinal cord to exert a direct influence on the motor system in parallel with the pyramidal system has been profoundly changed into a concept that holds that the basal ganglia convey their influence mainly on the motor system via the premotor and motor cortices to finally involve the pyramidal tract, and only partly through the brain stem and other subcortical outputs. Moreover, not only motor and premotor cortical areas receive an influence from the basal ganglia, but also the prefrontal cortex, involved with cognitive and emotional-motivational functions. Thus, the basal ganglia, at the level of the striatum, receive input from the entire cerebral cortex (including primary and higher order sensory areas; motor, premotor, and prefrontal regions; and limbic cortical areas), and project via their output nuclei and the thalamus to virtually the entire frontal cortex. These observations have led to the idea that the basal ganglia are not exclusively involved in the control of motor functions, but are implicated in motor as much as in non-motor functions.

**Intrinsic circuitry of the basal ganglia and physiological mechanisms of action**

In the previous paragraphs the basic architecture of the parallel, functionally segregated basal ganglia-thalamocortical circuits has been described, indicating that a number of general organizational principles hold for all functionally distinct circuits. With respect to the basal ganglia structures involved in these circuits we have, however, only dealt with the input- and output structures, i.e., the striatum and the pallidum, respectively. In order to understand the contribution of the basal ganglia to brain functions and the basic functional operations that take place in these
structures, it is important to describe in more detail the anatomical and functional characteristics of the other basal ganglia structures, as well those of striatum and pallidum, and their (intrinsic) connections.

As has been mentioned above, the basal ganglia consist of the striatum, the pallidum, the subthalamic nucleus and the substantia nigra. These nuclei, with the exception of the subthalamic nucleus, can be further subdivided into various macroscopic-anatomically recognizable nuclei. Thus, the striatum encompasses the caudate nucleus and the putamen (indicated above as caudate-putamen complex or dorsal striatum), and the nucleus accumbens and medium-celled part of the olfactory tubercle (identified above as the ventral striatum). The pallidum consists of an external segment and an internal segment of the globus pallidus and, in addition, the ventral pallidum as an extension of the pallidum underneath the anterior commissure. There is, however, no clear distinction in the ventral pallidum between an external and internal segment. Finally, the substantia nigra is divided into the dorsally located pars compacta, comprising the A9 dopaminergic cell group, and the ventrally situated pars reticulata. The medially located A10 dopaminergic cell group in the ventral tegmental area, as a consequence of its strong projections to the ventral striatum, is also considered part of the basal ganglia.

As indicated above, the striatum is the major recipient of afferent inputs to the basal ganglia and is therefore characterized as the 'input structure' of the basal ganglia. The strongest inputs to the striatum are derived from the cerebral cortex and these afferents are excitatory since the corticostriatal fibers contain glutamate as their neurotransmitter (e.g. Webster, 1961; Kemp and Powell, 1970; Fonnum et al., 1981; McGeorge and Faull, 1989; Groenewegen et al., 1990). Other excitatory inputs to the striatum come from the midline and intralaminar thalamic nuclei and limbic structures such as the hippocampus and the amygdala. The projections from the cerebral cortical areas (as well as the midline and intralaminar thalamic nuclei) to the striatum are topographically organized. This organization holds both for primate and rat brain. Thus, the dorsolateral part of the striatum receives converging inputs from motor, premotor, and sensory cortical areas as well as the posterior and lateral intralaminar thalamic nuclei. The dorsomedial part of the striatum receives input from (pre)frontal, temporal, and parietal associative cortical areas and, in addition, from the ventrally and medially located intralaminar thalamic nuclei. Finally, the ventral part of the striatum receives input from the hippocampus, the amygdala, and parahippocampal, medial prefrontal and orbitofrontal cortices as well as the midline thalamic nuclei (reviews by Groenewegen and Berendse, 1994; Parent and Hazrati, 1995; Wise et al., 1996; Van der Werf et al., 2002). This
topographical arrangement of corticostriatal and thalamostriatal projections ‘imposes’ a functional subdivision of the striatum into a dorsolateral ‘sensorimotor’, an intermediate or central ‘associative’ and a ventral ‘limbic’ (emotional/motivational) sector. Further inputs to the striatum are derived from the mesencephalon and include the earlier mentioned dopaminergic fibers from the substantia nigra pars compacta and the ventral tegmental area, the serotonergic fibers from the raphe nuclei, as well as the cholinergic fibers of the pedunculopontine nucleus.

The outputs of the basal ganglia originate in the internal segment of the globus pallidus, the substantia nigra pars reticulata, as well as from the ventral pallidum. These output structures mainly project to different ventral and medial nuclei of the thalamus, as well as to the deeper layers of the superior colliculus and the pedunculopontine nucleus more caudally in the mesencephalon (reviews by Groenewegen and Berendse, 1994; Parent and Hazrati, 1995; Wise et al., 1996).

Between the input- and the output structures of the basal ganglia various specific intrinsic basal ganglia connections exist that contain different neurotransmitters, express different neurotransmitter receptors and exhibit distinct electrophysiological properties. A functionally important aspect of the intrinsic basal ganglia connections is that they can be categorized into two pathways connecting either directly or indirectly the input- and output structures of the basal ganglia (Albin et al., 1989; DeLong, 1990; Chevalier and Deniau, 1990)(Fig.3). To understand the organization of these so-called ‘direct’ and ‘indirect’ intrinsic pathways that connect the input- and output structures of the basal ganglia, it is important to describe in more detail the cellular architecture and neurochemical content of the striatum.

The striatum consists of two main types of neurons, i.e. medium-sized, densely spiny projection neurons and interneurons (DiFiglia et al., 1976; Wilson and Groves, 1980; Bishop et al., 1982; for an extensive review see Gerfen, 2004)(Fig. 4). The medium-sized spiny projection neurons make up approximately 95% of the neuronal population, while the neurochemically and morphologically heterogeneous group of interneurons represents the remaining 5% of the neurons (Kemp and Powell, 1971; Gerfen, 2004). The medium-sized spiny projection neurons primarily collect the specific incoming information from the cerebral cortex, their dendritic spines being the main target of such inputs, and they integrate these inputs to generate striatal output.

Spiny projection neurons utilize γ-aminobutyric acid (GABA) as their major neurotransmitter (Ribak et al., 1979) but this population of neurons falls apart into two subgroups on the basis of the expression of various neuropeptides and dopaminergic receptor subtypes (Penny et al., 1986). One population of neurons expresses the neuropeptides substance P and dynorphin, as
General introduction

Fig. 3: Diagram illustrating the major input and output connections of the basal ganglia, the independent, interacting ventral and dorsal striatopallidal circuits, feedback pathways, and the ‘direct’ and ‘indirect’ pathways. The cerebral cortex excites the striatum. The striatum inhibits the ventral pallidum (VP), globus pallidus externus (GPe), globus pallidus internus (GPI) and ventral mesencephalon, including de substantia nigra pars reticulata (SNr), substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). When striatal neurons inhibit the GPI (red, direct pathway), this leads to a disinhibition of the corresponding thalamus region and the gating of ascending information to the cortex. Simultaneously, neurons of the GPe are inhibited (blue, indirect pathway), which leads to disinhibition of the subthalamic nucleus (STN) that excites the other neurons in the GPi/SNr, which is thought to prevent any other stream to be disinhibited.

Acb, nucleus accumbens; Amy, amygdala; Hipp, hippocampus; LH, lateral hypothalamus; M, matrix compartment; MD, mediodorsal thalamic nucleus; P, patch compartment; PFC, prefrontal cortex; RTN, reticular thalamic nucleus; VL, ventrolateral thalamic nucleus; VM, ventromedial thalamic nucleus; VP, ventral pallidum; VTA, ventral tegmental area; VPI, lateral ventral pallidum; VPM, medial ventral pallidum; VStr, ventral striatum.

wel as the dopamine D1 receptor subtype. These neurons project to the internal segment of the globus pallidus and substantia nigra pars reticulata, and in this way form a direct link between the striatum and the output nuclei of the basal ganglia. This pathway is in general being referred to as the direct striatal output pathway (Gerfen and Young, 1988; Albin et al., 1989; DeLong, 1990; Gerfen et al., 1990)(see Fig. 3). The second population of neurons expresses the neuropeptide enkephalin as well as the dopamine D2 receptor subtype. These striatal neurons project preferentially to the external segment of the globus pallidus. The neurons of the external segment of the globus pallidus project via GABAergic neurons to the subthalamic nucleus. The subthalamic nucleus, in turn, projects through excitatory, glutamatergic fibers to the internal segment of the globus pallidus and the substantia nigra pars reticulata. This multisynaptic pathway from the striatum to the basal ganglia
output nuclei through the subthalamic nucleus is in general referred to as the indirect striatal output route (Gerfen and Young, 1988; Albin et al., 1989; DeLong, 1990; Gerfen et al., 1990)(see Fig. 3).

The projection neurons in the basal ganglia output structures have the electrophysiological characteristics of being tonically active and in this way exert a tonic inhibitory influence on the thalamus and mesencephalon. Following corticostriatal activation in the direct striatal output pathway, the release of striatal GABA will lead to an inhibition of the tonically active output neurons of the internal segment of the globus pallidus and the substantia nigra pars reticulata, resulting in a disinhibition of their target areas (Chevalier and Deniau, 1990). Following corticostriatal activation in the indirect striatal output pathway, a higher activity in the striatopallidal
projections will lead to the inhibition of neurons of the external segment of the globus pallidus, which themselves are also GABAergic, leading to a disinhibition of the neurons of the subthalamic nucleus. An increased activity of excitatory subthalamic projections to the output neurons of the internal segment of the globus pallidus and the substantia nigra pars reticulata will lead to a stronger activity of these output neurons and hence an increased inhibition of their target areas. If it may be assumed that a higher activity in the basal ganglia-thalamocortical circuits is associated with increased motor or cognitive/behavioral output of the brain, we can conclude that the direct striatal output pathway facilitates, whereas the indirect striatal output pathway suppresses motor, cognitive and emotional behavioral output. As we will discuss later, it has been argued that the indirect pathway is active most of the time to suppress unwanted or inappropriate behaviors, while the direct pathway allows for behavioral selection by stimulating one particular action only every now and then, in a context when this action is wanted and appropriate (e.g. Mink, 1996; Redgrave et al., 1999; Wise et al., 1996).

The subthalamic nucleus not only receives a (tonic) inhibitory input from the external segment of the globus pallidus, but also is projected upon directly by excitatory cortical and thalamic fibers (Gerfen and Wilson, 1996; Feger et al., 1994)(see Fig. 3). This means that the cerebral cortex plays a role in a strong inhibition of the basal ganglia target areas and, thereby, the suppression of motor and cognitive/behavioral outputs.

Via different subtypes of dopamine receptors in the two subpopulations of striatal projection neurons, dopamine has an opposing role on these output pathways of the striatum. As has been mentioned above, the dopamine D1 receptor subtype is primarily localized in neurons that project to the substantia nigra and colocalize with substance P and dynorphin, i.e., the striatal projection neurons of the direct output pathway. Conversely, the dopamine D2 receptor subtype is primarily localized in neurons that project to the external segment of the globus pallidus and colocalizes with enkephalin, i.e., in striatal projection neurons that initiate the indirect output pathway. The functional significance of the segregation of dopamine D1 and D2 receptors expressed by striatal projection neurons of the direct and indirect output pathways has been demonstrated by gene regulation studies (Hong et al., 1978; Young et al., 1986; Gerfen et al., 1991). It was demonstrated that the levels of enkephalin and substance P are oppositely regulated by dopamine. Thus, dopamine depletion resulted in an elevation of enkephalin and a reduction of substance P peptide and mRNA levels in the indirect and direct striatal output pathways, respectively. In contrast, enhanced dopamine neurotransmission resulted in elevated substance P and reduced enkephalin peptide and mRNA levels in the direct and indirect striatal output pathways, respectively. These opposite effects
of dopamine on the peptides in striatal projection neurons were demonstrated to be related to the differential expression of the dopamine D1 and D2 receptor subtypes by the neurons that express these peptides. Using specific dopamine D1 and D2 receptor agonists and antagonists, the differential roles of these two receptor subtypes could be confirmed (Gerfen et al., 1990).

In summary, at the striatal level, dopamine appears to facilitate transmission along the direct output pathway and inhibit transmission along the indirect output pathway, these two opposite effects being mediated by D1 and D2 receptors, respectively (Gerfen and Wilson, 1996). Striatal dopamine levels are thought to largely determine the balance between the direct and indirect output pathways and, consequently, the degree of basal ganglia output. An imbalance between the activity in the direct and indirect output pathway, as a result of either higher or low striatal dopamine levels, is thought to account for a comparable effect on the thalamic and brainstem targets. Higher levels of striatal dopamine are associated with facilitation of movements and cognitive/behavioral acts, whereas low levels of striatal dopamine are correlated with a paucity of movements and cognitive/behavioral acts.

Considering the various interconnections between the different basal ganglia components and the fact that extrinsic cortical projections can also reach the subthalamic nucleus directly, thus bypassing the striatum, Bolam et al. (2000) have proposed to view these indirect connections are part of an indirect modulatory ‘network’ rather than an indirect striatal output pathway. This ‘network’ view of the indirect connections between the input and output structures of the basal ganglia is much in line with the variety of outputs of the basal ganglia and the supposed role of the basal ganglia on a big repertoire of motor and behavioral functions.

**Functions of the basal ganglia**

The functions of the basal ganglia are as yet not completely understood but, as argued above, these functions must be considered in the context of their close association with the cerebral cortex, i.e., their inclusion in the basal ganglia-thalamocortical circuitry. In very general terms, it has been suggested that the basal ganglia may play an important role, in close association with the (pre)frontal cortex, in selecting an appropriate motor or behavioral output in a particular context (Wise et al., 1996; Mink, 1996; Redgrave et al., 1999). The link between ‘context’ and ‘motor or behavioral’ output stresses the important aspect of convergence and integration of sensory, motor/behavioral and mnemonic (~ past experience) information in the basal ganglia. This integration of perceptive and executive functions has its neuronal substrate within the basal ganglia most clearly at the level of the striatum where there is strong convergence of corticostrial
projections originating from various functionally different cortical areas. Yet, as a consequence of the topographical arrangements of the corticostriatal projections (see above), there appear to be different sectors of the striatum that are involved in different functional aspects of the basal ganglia. In the dorsolateral part of the striatum, convergence of inputs from sensory and motor cortices takes place leading to an involvement of this part of the striatum in stimulus–response associations. In case such stimulus-response associations have been well-established and are sustained even in the absence of continued reinforcement, such contextually elicited motor sequences or behavioral procedures may also be indicated as ‘habits’. Habit formation has been attributed to the basal ganglia since many years (Packard and Knowlton, 2002; White and McDonald, 2002). In the medial and ventral parts of the striatum, convergence of inputs from prefrontal cortical areas with the amygdala and hippocampus takes place representing contextual information and information related to the emotional value of environmental cues, respectively. The medial and ventral portions of the striatum, therefore, are thought to be involved in complex behavioral procedures depending on emotional/motivational and mnemonic aspects (Setlow, 1997; Devan and White, 1999). In line with this, the medial and ventral sectors of the striatum have been indicated to guide behavior using stimulus-reward associations (Robbins and Everitt, 1996). Dopamine has been shown to play an important role in the establishment and the maintenance of the various types of association at the level of the striatum. Despite this variety of basal ganglia functions, the neuronal mechanism of the selection process that leads to the appropriate response to a particular stimulus might be rather universal throughout the striatum (Hikosaka, 1994; Robbins and Everitt, 1996; Packard and Knowlton, 2002; Schultz, 2002).

It may be clear from the foregoing that the basal ganglia are thought to play a major role in the process of selecting the most wanted or appropriate motor or behavioral output in a given situation. However, the question of what the exact neuronal substrate is for this selection process that takes place in the basal ganglia can at present not be fully answered. The above-described architecture of the intrinsic connections within the circuitry, i.e., the direct and indirect striatal output pathways, may provide some clue. These opposing parallel pathways may play a role in adjusting the magnitude of the inhibitory output of the internal pallidal segment to the thalamus in order to facilitate or suppress the expression of movements (Alexander et al., 1990; DeLong, 1990). Thus, an increased output from the internal segment of the globus pallidus slows or prevents movements whereas a decreased output from the internal pallidum increases movement. However, although these mechanisms expressed by the two intrinsic pathways connecting the input- and
output structures of the basal ganglia may explain in rather crude terms the selection of movements or behavioral acts to be carried out, more intricate processes must take place to fully explain the complex repertoire of our behaviour. Various theories have been proposed to provide an explanation for the neuronal mechanisms, either at the macrocircuit or the (striatal) microcircuit level (e.g., Pennartz et al., 1994; Mink, 1996; Redgrave et al., 1999).

An attractive hypothesis of the selection mechanism in the dorsal, motor system-innervated striatum is given by Mink (1996). According to Mink (1996), the tonically active inhibitory output of the basal ganglia acts as a ‘brake’ on competing motor programs and the disinhibitory output of the basal ganglia acts as an ‘acceleration’ on desired motor programs (Mink, 1996). Thus, according to this hypothesis, when a desired movement is to be initiated by a certain motor program, the basal ganglia output neurons projecting to competing motor programs increase their firing rate, thereby increasing inhibition and applying a ‘brake’ on those other motor programs. The selected movements are in this way enabled and competing postures and movements are prevented from interfering with the one selected. In the selection hypothesis of Mink (1996), the subthalamic nucleus plays an important role. To be more specific, when one makes a voluntary movement, that movement is initiated by mechanisms in the prefrontal, premotor, supplementary motor, and primary motor cortices, as well as in the cerebellum. Initially, the cerebral cortical areas send an excitatory signal to the subthalamic nucleus. The subthalamic nucleus projects in turn to the internal segment of the globus pallidus and provides an excitatory drive on the internal pallidal neurons. This increased activity of the internal segment of the globus pallidus causes inhibition at the level of the thalamus and the brainstem. This mechanism may lead to the suppression of the competing thalamocortical and brainstem motor programs. In parallel to this pathway, the cerebral cortical areas send a signal to the striatum. This cortical input is translated by the striatal integrative circuitry to a focused, context-dependent output that inhibits specific neurons in the internal segment of the globus pallidus. The inhibitory striatal input to the internal segment of the globus pallidus is slower, but more powerful, than the excitatory input from the subthalamic nucleus. This results in a decreased activity of the internal segment of the globus pallidus that in turn selectively disinhibits the desired thalamocortical and brainstem motor program. The indirect striatal output pathway from the striatum to the internal segment of the globus pallidus via the external segment of the globus pallidus and the subthalamic nucleus results in further focussing of the output. In the most general sense, this concept by Mink (1996) provides a selection mechanism for surround inhibition (the “brake” of competing motor programs) and center excitation (the “acceleration” of the desired motor program). This general principle of selection of the desired program and
inhibition of competing programs at the level of the output structures may also be applied to the other domains of the basal ganglia.

Interestingly, in a recent review Redgrave et al. (1999) put forward another theory, in which the basal ganglia is hypothesized as a hierarchical selection device. This hypothesis states that when multiple sensorimotor systems seek simultaneous access to a final common motor path, selections at various functional levels are required. For instance, selections between competing systems to decide the general course of action are a first requirement. The sensory processing of an unexpected event that is represented by separate cortico-basal ganglia thalamo-cortical loops as well as by loops connecting subcortical sensorimotor structures with the basal ganglia, converge and compete in the ventral limbic domain of the striatum. Then, the winning system may selectively prime command systems, at the level of the intermediate or central associative domain of the striatum, capable of specifying appropriate patterns of coordinated behavioral acts in the context of the current aim. These striatal neurons are sensitive to experimental context. Multi-dimensional contextual afferents are likely to originate in the cerebral cortex and limbic structures, such as hippocampus, amygdala and thalamus. The final choice will be made at the level of the dorsolateral sensorimotor domain of the striatum, where patterns of appropriate motor activity that can deliver the currently selected action will be specified. At this level, motor-related projections from the motor cortex and subcortical sensorimotor structures (e.g. superior colliculus) reach the striatum directly (via the cortex) or indirectly (via the thalamus), which are likely to provide the striatum with a running multi-dimensional record (or motor efference copy) of commands related to ongoing goals, actions and movements (cf also Redgrave and Gurney, 2006). This ‘system-level hypothesis’ implies a temporal relationship between striatal activation, with ventral striatal activity preceding dorsal striatal activation.

In the previous paragraphs, two important concepts of selection mechanisms at the level of the basal ganglia-thalamocortical loops have been described. Both theories have emphasized the importance of the striatal integrative circuitry to be another source for selection in the basal ganglia. In the feedback inhibition model by Groves (1983), the striatum is imagined as a lateral inhibitory network and cortical inputs compete for control over basal ganglia outputs. This process is mediated by GABAergic synaptic transmission between medium-sized spiny projection neurons. This general principle of selection and competition has provided several new avenues for exploring striatal dynamics and, in addition, inspired several authors to present a lateral inhibition model at the level of both the dorsal (Wickens et al., 1991, 1995; Plenz and Aertsen., 1996; Fukai and Tanaka, 1997; Beiser and Houk, 1998; Suri and Schultz, 1998) and ventral striatum (Pennartz et al., 1994).
Pennartz and colleagues launched the ‘ensemble hypothesis’, which states that the functions of the nucleus accumbens are based on the organization of collections or ‘ensembles’ of striatal neurons that function as parallel-distributed units with distinct functions in guiding different types of behavior and may be variably active in different behavioral situations. Which ensembles of neurons become active and provide an output of the nucleus accumbens, depends upon the patterns of convergence of active glutamatergic, excitatory inputs from cortical, hippocampal, thalamic and amygdaloid origin, in combination with the dopaminergic input from the ventral mesencephalon and, in addition, on the intrinsic circuitry of the nucleus accumbens. Such a network of functionally competing neuronal ensembles may be able to subserve input selection functions and provide appropriate outputs. A prediction from this theory is that there exists a form of lateral inhibition between the ensembles to account for their competitive interrelationships.

To summarize, the three different basal ganglia domains, i.e., dorsolateral sensorimotor, intermediate or central associative and ventral limbic, may be viewed as part of three independent, interacting macrocircuits that entertain different parts of the frontal lobe. Within these macrocircuits, smaller (micro)circuits can be recognized that subserve specific functions within the broader domain. Several mechanisms within this (micro)circuitry support a role for the basal ganglia in regulating motor and behavioral functions by selectively promoting desired and suppressing unwanted neuronal programs to be expressed. The striatum appears to have a specific role in the selection mechanisms that take place in the basal ganglia.

Theories about basal ganglia function have always been driven by our knowledge about the medium-sized spiny projection neurons of the striatum. At the center of these theories lies the question of how, precisely, medium-sized spiny projection neurons process cortical input. The ‘ensemble hypothesis’ of Pennartz et al. (1994) has been formulated in the context of the functions and the circuitry of the nucleus accumbens, the main part of the ventral striatum. Our interest in the present study was to determine to what extent the functional-anatomical organization of the microcircuitry of the nucleus accumbens would lend support for the ‘ensemble hypothesis’. We were further interested in the way in which different basal ganglia-thalamocortical macrocircuits interact with each other, in particular in the context of the question how limbic, emotional-motivational aspects might influence cognitive and motor function. But before we can formulate our specific research questions, it is necessary to deal in more detail with the structure, functions and fiber connections of the nucleus accumbens. These are the subjects of the following paragraphs.
2. FUNCTIONAL ANATOMY OF THE VENTRAL, LIMBIC STRIATUM

Definition of the nucleus accumbens

The striatal area with a longstanding association with non-motor functions is the nucleus accumbens. The first description of this area was provided by Meynert (1872), who described the ventral part of the striatum in relation to the lateral ventricle as “…. the substance of the caput of the corpus striatum, folded around it like a gutter, invests the external (ventricular) surface of the septum pellucidum …. forming the nucleus septi pellucidi”. Ganser (1882) extended this view by adding several details. He stated that the nucleus septi pellucidi could be distinguished from the septum pellucidum on the basis of its structure and fiber connections. The presently accepted term “nucleus accumbens” was given by Ziehen (1879). He described the area as an extension of the adjacent caudate nucleus, ventral to the anterior commissure, and distinct from the underlying olfactory tuberculum. He stated: “I will provisionally designate it as nucleus accumbens”. The Dutch comparative neuroanatomist Ariëns Kappers (1908) wrote in dealing with the nucleus septi pellucidi (Meynert, 1872; Ganser, 1882) and the nucleus accumbens (Ziehen, 1879): “Perhaps it is best to combine both names and to speak of nucleus accumbens septi”. As a consequence, the literature reveals a nucleus accumbens of Ziehen, a nucleus accumbens of Kappers, and even a nucleus accumbens of Herrick. Herrick (1926) included the nucleus accumbens in the olfactory system by introducing the term “olfacto-striatum”.

New ideas concerning the nucleus accumbens awaited the classical paper of Heimer and Wilson (1975)(see Fig. 2), as introduced above, in which they launched the concept that the nucleus accumbens and the medium-celled parts of the olfactory tubercle together belong to the ventral striatum. Thus, the nucleus accumbens is presently being viewed as a nuclear mass in the rostroventral part of the ventral striatum bordered medially by the septum and ventrally by the olfactory tuberculum. The afferents from the allocortex (Heimer and Wilson, 1975) kept the association of the nucleus accumbens with limbic parts of the brain alive. This view has led to many suggestions about the possible functional role of the nucleus accumbens. In a landmark paper by Mogenson and colleagues (1980)(Fig. 5), the nucleus accumbens was conceptualized as a functional interface between two major systems in the brain: the ‘limbic system’, which is responsible for the effect of motivational states and emotions, and on the other hand the ‘motor system’, which effects behavioral actions. Thus, the nucleus accumbens came to be viewed as the key site for gating motivational and other emotional signals, in order to convert them into adaptive motor responses, while dopamine subserved a role as the neural facilitator for that transaction. Access to motor
circuits was considered to occur via the globus pallidus. More recent (immuno)histochemical and connectional studies, to be outlined in the next paragraphs, have shown that the outflow from the nucleus accumbens, instead of exiting via a single efferent route, follows multiple, parallel pathways to different centers in the forebrain and midbrain (e.g. Groenewegen and Russchen, 1984; Zahm and Heimer, 1988). Therefore, an important question in this regard is which mechanisms play a role in selecting between these functionally different input-output channels of the nucleus accumbens. As mentioned above, there are several theories that deal with these selection mechanisms, for example the ‘ensemble hypothesis’ of Pennartz et al. (1994) or the ‘system-level hypothesis’ by Redgrave et al. (1999) of a ventral-to-dorsal progression of information transfer. But before we elaborate further in more detail on these theories, it is necessary to specify the
heterogeneous structure of the nucleus accumbens and its input-output relationships. This will be described in the next paragraphs.

The nucleus accumbens: a highly compartmentalized structure

As has been mentioned above, the striatum has a rather homogeneous cytoarchitectonic structure in which the dominant cell type is the medium-sized spiny projection neuron. Unlike the cerebral cortex, it lacks an apparent cytoarchitectonic feature like a laminar organization. The ventral striatum in principle has a cytoarchitectonic structure much comparable with the rest of the striatum (exceptions will be described below). However, it has been recognized already more than 30 years ago that neuro- and histochemically the striatum is a heterogeneous structure. Ragsdale and Graybiel (1978) revealed the so-called striosome-matrix system in the primate dorsal striatum. On the basis of a differential activity of the enzym acetylcholinesterase, weakly stained striosomes and a darkly stained matrix were recognized. Since this discovery, a host of markers have been described to identify the compartmental structure of the striatum (for review, see Graybiel, 1990).

Whereas in the dorsal striatum a bicompartmental structure exists, the ventral striatum has been shown to exhibit a much more complex compartmental structure. The complexity of the nucleus accumbens must be described at two levels. First, the nucleus accumbens falls apart into two larger subregions, denoted the shell and core. Second, within these larger subregions smaller compartments can be identified. Thus, using the patterns of Timm staining, acetylcholinesterase activity and cholecystokinin immunoreactivity as markers, Zaborszky and colleagues (1985) recognized an inner core region and outer crescent-shaped shell subregion in the nucleus accumbens. Subsequent studies have revealed that the distinction between the shell and core subregions of the nucleus accumbens can be demonstrated most clearly with an antibody against the calcium binding protein calbindin-D$_{28}$ KDA (CaB) in rodents, monkeys and humans (Voorn et al., 1989, 1994a, 1996; Zahm and Brog, 1992; Jongen-Rêlo et al., 1994; Meredith et al., 1996; Haber and McFarland, 1999; Brauer et al., 2000; Prensa et al., 2003). Thus, the core subregion consists of rather densely packed cells that express intense CaB immunoreactivity, and comprises the area around the anterior limb of the anterior commissure that merges dorsally with the caudate-putamen complex. The distribution of CaB has a patch-like appearance similar to that in the dorsal striatum (Herkenham et al., 1984; Voorn et al., 1989; Jongen-Rêlo et al., 1993, 1994)(Fig. 6). Lightly stained areas, in general referred to as the patch compartment or, in short, the patches, stand out against the intense CaB-immunoreactive ‘background’ generally referred to as the matrix compartment or
Fig. 6: Photomicrograph of a transverse section through the rat striatum immunostained for the calcium binding protein calbindin-D$_{28K}$. Regional differences in calbindin expression are present throughout the striatum. These differences allow for the delineation of the nucleus accumbens shell and core, but do not allow for the delineation of dorsal and ventral striatum. Arrows point out to heterogeneities in the dorsal striatum consisting of the low immunoreactive calbindin compartments, the so-called ‘patch compartments’. Scale bar, 1mm. ac, anterior commissure; AcbSh, nucleus accumbens shell; AcbC, nucleus accumbens core; CPu, caudate-putamen; ICjM, major island of Calleja; LS, lateral shell; LV, lateral ventricle; OT, olfactory tubercle.

simply the matrix. Patches are relatively large in the rostral part of the core and become smaller in the more caudal part of the core.

The shell subregion is composed of more loosely arranged cells that are lightly to moderately immunoreactive for CaB in its medial to ventrolateral parts, respectively (Voorn et al., 1989; Meredith et al., 1989; Zahm, 1989; Groenewegen et al., 1991; Zahm and Brog, 1992; Jongen-Rêlo et al., 1994). The lightly CaB-stained area indicated as the shell forms a crescent shape medially, ventrally and laterally to the core. In addition, the shell also rostrally curves around the core and this rostral part of the shell is by some authors considered a third subregion of the nucleus accumbens (Heimer et al., 1991; Zahm and Heimer, 1993) and has been referred to as the rostral pole.

Whereas the differential distribution of CaB immunoreactivity marks the shell and core subregions of the nucleus accumbens, as well as the patch-matrix compartments in the core, the
staining intensity of other histochemical markers varies both within the shell and core. These neurochemical substances include among others substance P, enkephalin, neurotensin, naloxone, and dopamine (Voorn et al., 1989; Jongen-Rêlo et al., 1993, 1994). In the rostrolateral part of the core, for instance, relatively large ‘patches’ of intense CaB and enkephalin-immunoreactivity correspond with similarly shaped areas strongly immunostained for naloxone and lightly stained for dopamine and substance P. In the same region, a lightly stained ‘matrix’ for CaB and enkephalin overlies a similar shaped area of intense substance P-immunoreactivity. Caudally, in the border region of the shell and core, small, elongated cell clusters in Nissl-stained sections exhibit almost no CaB, enkephalin, dopamine, substance P and neurotensin-immunoreactivity but a dense immunoreactivity for naloxone. At the most caudal level, in the dorsomedial part of the shell, the so-called cone-shaped area is weakly stained for CaB and naloxone but enriched in enkephalin, dopamine, substance P and neurotensin-immunoreactivity.

In conclusion, the subdivision of the nucleus accumbens in a shell and core region, the presence of cell clusters and a cone-shaped area in the shell as well as of patch-matrix configurations in the core, all suggest that the basic structure of the ventral striatum is far more heterogeneous than considered on the basis of cytoarchitectonical characteristics alone. As will be outlined below, this heterogeneous, compartmental structure of the nucleus accumbens is also reflected in the organization of its afferent and efferent connections. This observation will lead to the tentative conclusion that the cellular and histochemical compartmental structure of the nucleus accumbens ‘marks’ the existence of largely segregated input-output channels which may have a relevance for the functional concept of ensembles (Pennartz et al., 1994; Groenewegen et al., 1999b).

**Relationships of afferents and efferents with shell and core**

Numerous studies have shown that the differential distribution patterns of the inputs and outputs of the nucleus accumbens are strongly related to its subregional structure. The major sources of excitatory inputs to the shell and core are the prefrontal cortex, the hippocampus, the basal amygdaloid complex, and the midline and intralaminar nuclei of the thalamus (Groenewegen et al., 1987; Zahm and Heimer, 1990; Berendse and Groenewegen, 1990; Berendse et al., 1992a; Brog et al., 1993; Haber et al., 1995; Wright et al., 1996; Totterdell and Meredith, 1997; Groenewegen et al., 1999a, b; Voorn et al., 2004)(Fig. 7). The major supply of inhibitory inputs to
the shell and core are derived from the ventral pallidum (Zahm and Heimer, 1990; Heimer et al., 1991; Usuda et al., 1998), whereas the major sources of monoaminergic inputs reach the shell and core from the dopaminergic ventral tegmental area (A10), medial substantia nigra (A9) and retrorubral (A8) cell groups, the serotonergic neurons of the dorsal and median raphe nuclei, and the cholinergic neurons of the pedunculopontine nucleus (Nauta et al., 1978; Beckstead et al., 1979; Brog et al., 1993; Haber et al., 2000; Hasue and Shammah-Lagnado, 2001). The major target areas of the shell and core include ventral pallidal, hypothalamic and mesencephalic centers (Nauta et al., 1978; Groenewegen and Russchen, 1984; Heimer et al., 1991).
Afferent connections

Within the nucleus accumbens, various afferent systems have specific patterns of termination that show different degrees of overlap and segregation in different parts of the nucleus accumbens shell and core (Berendse et al., 1988; McGeorge and Faull, 1989; Berendse et al., 1992a; Wright et al., 1996; Brown et al., 1998; Groenewegen et al., 1999a, b; Van der Werf et al., 2002)(Fig. 7). For instance, the hippocampal (subicular and CA1 regions) and the parahippocampal (medial and lateral entorhinal areas) afferents dominate in the medial shell (Groenewegen et al., 1987; Brog et al., 1993; Totterdell and Meredith, 1997; Mulder et al., 1998). Whereas the ventral subicular fibers are predominantly directed toward the medial shell, the dorsal part of the subiculum sends fibers to rostrolateral and rostroventral parts of the nucleus accumbens, in particular its shell subregion (Groenewegen et al., 1999).

Afferents from different nuclei of the basal amygdaloid complex terminate in different parts of the nucleus accumbens (Wright et al., 1996). Whereas the nuclei of the caudal basal amygdala complex (that include the parvicellular, accessory and magnocellular basal amygdaloid nuclei) send fibers predominantly to the medial shell, it targets, in addition, the core. The nuclei of the rostral basal amygdala complex (that include the magnocellular basal amygdaloid nucleus) send fibers to the lateral shell and, additionally, to the core. The mid-rostrocaudal part of the basal amygdaloid complex (that includes the accessory basal amygdaloid nucleus) issues fibers predominantly to the core.

A similar topographical organization is found in the afferents from the midline and intralaminar thalamic nuclei (Brog et al., 1993; Berendse and Groenewegen, 1990). The midline paraventricular nucleus of the thalamus has a strong projection to the medial and lateral shell. The intermediodorsal and central medial thalamic nuclei, project heavily to the core.

The projections from different parts of the prefrontal cortex to the nucleus accumbens are also topographically arranged. The infralimbic and ventral prelimbic areas project to the medial shell. The lateral shell receives predominantly input from the ventral agranular insular area. The dorsal prelimbic and dorsal agranular insular areas project primarily to the core (Berendse et al., 1992a; Gerfen, 1989). Finally, the dopaminergic A10 cell groups in the medial and lateral parts of the ventral tegmental area project to the medial and lateral shell, respectively. The retrorubral A8 cell group projects exclusively to the lateral shell, whereas the dopaminergic A9 cell group in the medial substantia nigra projects in the nucleus accumbens predominantly to the core (Brog et al., 1993; Beckstead et al., 1979; Berendse et al., 1992a).

Efferent connections
Chapter 1

The nucleus accumbens projects in a topographical manner to the most medial part of the globus pallidus, the ventral pallidum, the entopeduncular nucleus, and the medial part of the substantia nigra pars reticulata. In addition, the nucleus accumbens has a number of projections that are not common to most other striatal areas, such as those to the lateral septum, the bed nucleus of the stria terminalis, the lateral and medial hypothalamus, the ventral tegmental area, the dorsal part of the substantia nigra pars compacta, the retrorubral field, and the ventrolateral part of the periaqueductal gray (Nauta et al., 1978; Groenewegen and Russchen, 1984; Heimer et al., 1985; Haber et al., 1990; Heimer et al., 1991; Arts and Groenewegen, 1992; Berendse et al., 1992b, Zahm and Brog, 1992; Groenewegen et al., 1994; Usuda et al., 1998). These non-classical projections originate primarily in the caudomedial shell.

The ventral pallidum is the major structure that mediates the output of the nucleus accumbens. The ventral pallidum is subdivided, with respect to its neurochemical composition, into dorsolateral, ventromedial and ventrolateral parts (Zahm, 1989; Groenewegen et al., 1993). As demonstrated by immunohistochemical staining, both enkephalin- and substance P-positive fibers have been identified in the ventral pallidum of rodents, monkeys and humans (Haber and Nauta, 1983; Haber and Watson, 1985; Haber et al., 1990; Groenewegen et al., 1996; Heimer et al., 1999). There is, however, no clear distinction in the ventral pallidum between an external and an internal segment, when enkephalin- and substance P-positive fibers are taken as markers for the external and internal segments of the globus pallidus, respectively. It turned out that in the ventral pallidum the two neuropeptides are largely intermingled (Groenewegen et al., 1993). The only part of the ventral pallidum that can be best compared with the external segment of the globus pallidus is the dorsolateral subcommissural part, which exhibits the strongest enkephalin-immunoreactivity and is reciprocally connected with the subthalamic nucleus (Maurice et al., 1997; Groenewegen et al., 1990). This part of the ventral pallidum is predominantly innervated by the nucleus accumbens core subregion (Heimer et al., 1991). On the other hand, the ventromedial and ventrolateral parts of the ventral pallidum are predominantly innervated by the nucleus accumbens shell subregion (Heimer et al., 1991).

The other major output structure of the nucleus accumbens is the ventral mesencephalon. The neurons in the medial shell project to the medial parts of the ventral tegmental area, whereas the neurons in the ventral and lateral shell project to the more lateral parts of the ventral tegmental area, as well as to the medial substantia nigra. The neurons in the core project to the substantia nigra pars compacta and pars reticulata (Nauta et al., 1978).
Taken together, the nucleus accumbens afferents originating in the frontal cortex, the midline and intralaminar thalamic nuclei, the basal amygdaloid complex and the hippocampal formation are rather strictly topographically organized. On the basis of the patterns of afferent fiber connections, the shell can be considered as the main site of convergence of visceral-limbic (ventral prefrontal cortex, and hippocampus and amygdala) and arousal stimuli (midline thalamus), while the core receives converging stimuli from limbic-cognitive and executive behavioral origins (dorsal prefrontal cortex and amygdala). The descending projections of the nucleus accumbens to the pallidum and mesencephalon likewise are organized in a topographical way. Finally, the (caudo)medial shell of the nucleus accumbens seems to be endowed with special efferent connectional characteristics.

As described in previous paragraphs, it seems also evident, however, that a further anatomical differentiation is present within these larger subregions of the nucleus accumbens. The results of the first systematic studies of the heterogeneous structure of the nucleus accumbens by Herkenham et al. (1984) revealed the existence of distinct cell clusters, characterized by dense naloxone-binding sites and, in addition, avoided by thalamostriatal fibers. Subsequent neuroanatomical tracing studies confirmed the compartmental structure of the nucleus accumbens (Voorn et al., 1989; Berendse et al., 1992b; for review, see Groenewegen et al., 1989). This will be described in the next paragraphs.

**Relationships of afferents and efferents with different compartments within shell and core**

In the rostrolateral part of the core the relatively large ‘patches’ receive input from the paraventricular thalamic nucleus and from the dorsal agranular insular area, and send outputs to the substantia nigra pars reticulata (Berendse and Groenewegen, 1990; Berendse et al., 1992b). In the same region, the ‘matrix’ is innervated by the central medial thalamic nucleus, the rostral part of the basolateral amygdaloid nucleus and the deep layers of the prelimbic area (Berendse et al., 1992a). Neurons in this compartment project to the substantia nigra pars compacta, ventral tegmental area and retrorubral field (Berendse et al., 1988; Groenewegen et al., 1989; Berendse and Groenewegen, 1990; Berendse et al., 1992b). At more caudal levels, the smaller patches receive input from the deep layers of the prelimbic area, the basolateral amygdaloid nucleus and the paraventricular thalamic nucleus, and project to the pars compacta of the substantia nigra (Berendse and Groenewegen, 1990; Kita and Kitai, 1990; Berendse et al., 1992b; Gerfen, 1992). In contrast, the matrix receives input from the superficial layers of the prelimbic area and from the central intralaminar thalamic nucleus and projects to the pars reticulata of the substantia nigra (Berendse...
and Groenewegen, 1990; Berendse et al., 1992b; Gerfen, 1992). It has not yet been established whether the patch compartments in the core project to the ventral pallidum.

In the shell, the small cell clusters receive dense input from the amygdala and the ventral prelimbic area, whereas the cone-shaped area is strongly innervated by the ventral subiculum, the infralimbic area, the amygdala and the paraventricular thalamic nucleus (Berendse et al., 1988, 1992a; Groenewegen et al., 1987; Berendse and Groenewegen, 1990). It has not yet been established whether the small compartments in the shell project to the ventral tegmental area and/or ventral pallidum.

Taken together, on the basis of the above described projection patterns of shell and core, two largely segregated limbic corticostriatal-thalamocortical routes that involve different parts of the (pre)frontal cortex-ventral striatal system can be identified. In addition, within the two larger subregions of the nucleus accumbens, i.e., shell and core, smaller compartments appear to have their unique input-output characteristics. These examples will be the subject of the following paragraphs.

Parallel organization of prefrontal corticostriatal circuits

Much in line with the parallel, functionally segregated basal ganglia-thalamocortical circuits described by Alexander et al. (1986) in primates, closed circuits between the cortex, basal ganglia and thalamus can be described for the ventral, limbic-related parts of the basal ganglia and the prefrontal cortex in rats (Groenewegen et al., 1990). In particular the shell and core of the nucleus accumbens form the striatal way station of different corticostriatal circuits (see Figs. 3). The prefrontal corticostriatal circuit that entertains the shell of the nucleus accumbens can be viewed as starting in ventral parts of the medial prefrontal cortex. Thus, the ventral prelimbic and infralimbic areas are the cortical nodal points of a circuit, which further involves the medial shell of the nucleus accumbens, the ventromedial part of the ventral pallidum, and the rostral part of the medial segment of the mediodorsal thalamic nucleus. The latter thalamic region has reciprocal connections with the ventral areas in the medial prefrontal cortex (Groenewegen, 1988; Groenewegen et al., 1990). This ventral corticostriatal circuit is paralleled by a circuit that primarily involves the core of the nucleus accumbens. This circuit is formed by the dorsal parts of the lateral prefrontal cortex, including the dorsal agranular insular area, the core of the nucleus accumbens, the dorsomedial part of the substantia nigra pars reticulata and the caudal part of the medial segment of the mediodorsal thalamic nucleus as well as the medial segment of the ventromedial thalamic nucleus. The ‘closed’ part of this circuit is completed by the reciprocal connections of the caudal medial segment of the
General introduction

mediodorsal nucleus with the dorsal agranular insular area (Groenewegen, 1988; Groenewegen et al., 1990; Deniau et al., 1994). It should be noted that this circuit, much like the cortical basal ganglia circuits involving the dorsal striatum, is composed of direct and indirect routes between the striatum and the output structures. The direct pathway can be traced from the core of the nucleus accumbens to the dorsomedial part of the substantia nigra pars reticulata and, subsequently, to the caudal part of the medial segment of the mediodorsal thalamic nucleus and medial segment of the ventromedial thalamic nucleus (Deniau et al., 1994). The indirect pathway runs from the core of the nucleus accumbens to the dorsolateral, subcommissural part of the ventral pallidum, which projects to the dorsomedial part of the subthalamic nucleus. This part of the subthalamic nucleus, in turn, projects back to the subcommissural part of the ventral pallidum as well as to the dorsomedial part of the substantia nigra pars reticulata (Groenewegen and Berendse, 1990; Maurice et al., 1998). As indicated above, the dorsomedial part of the substantia nigra pars reticulata projects to the caudal part of the medial segment of the mediodorsal thalamic nucleus and medial segment of the ventromedial thalamic nucleus that, in turn, project back to the prefrontal cortex (Deniau et al, 1994; Groenewegen et al., 1999b).

To summarize, it is clear from the above described circuits that the projections from the ventral striatum to the ventral pallidum parallel those from the dorsal striatum to the globus pallidus. Likewise, the projections from the external segment of the globus pallidus to the subthalamic nucleus, the substantia nigra and, in addition, back to the striatum, are paralleled by projections from the ventral pallidum to the same structures. For a further understanding of the (pre)frontal-basal ganglia relationships, it will be important to use this structural framework of connectivity as a basis to reveal the functional characteristics of these circuitry. This is the subject of the following paragraphs.

Current views of nucleus accumbens function: behavioral dissociation between shell and core

The nucleus accumbens became a focal point of interest among behavioral neuroscientists with the discovery that dopamine injections in the nucleus accumbens enhanced locomotor activity in rats (Pijnenburg and Van Rossum, 1973; Robbins and Everitt, 1982). This effect was mimicked by the dopamine-release enhancing agent amphetamine (Kelley et al., 1989). In more recent years, the importance of the nucleus accumbens and its dopaminergic innervation in mediating cognitive and behavioral processes associated with reward, learning, memory, strategy and response selection, feeding, behavioral sensitization, and drug addiction have become evident (for reviews, see Robbins and Everitt, 2002; Schultz, 2002; Cardinal et al., 2002; Di Chiara, 2002). This elicited a
further interest in the role of the (micro)circuitry of the nucleus accumbens in mediating behavior and more complex cognitive functions (e.g. Parkinson et al., 1999; Kelley, 1999, 2004; Corbit et al., 2001; Reynolds and Berridge, 2001, 2002, 2003; Sellings et al., 2006).

As indicated above, the nucleus accumbens is a compartmentalized structure, composed of two major subterritories, i.e., shell and core. Their specific input-output relationships suggest an involvement in different functional aspects. Indeed, the shell stands out from the core and the rest of the striatum through its involvement in the expression of certain innate, unconditioned behaviors, such as feeding or defensive behavior (Maldonado-Irizarry et al., 1995; Kelley, 1999, 2004; Reynolds and Berridge, 2001, 2002, 2003; Cardinal et al., 2002).

Both shell and core play a prominent role in various forms of Pavlovian and instrumental conditioned learning, which may be potentiated by psychostimulant drugs (Parkinson et al., 1999; Bell et al., 2000; Corbit et al., 2001; Fenu et al., 2001; Hotsenpiller et al., 2001; Cardinal et al., 2002; Di Chiara, 2002; Philips et al., 2003; Kelley, 2004; Ito et al., 2004; Sellings et al., 2006). For example, acquisition of lever pressing for food is a function of the core, whereas the acquisition of conditioned taste aversion is a function of the shell. Lesions of the core impair control over the response to conditioned reinforcers, whereas enhancement of this control by psychostimulant drugs depends on the shell. Moreover, core lesions disrupt the performance of conditioned stimulus-controlled cocaine seeking, whereas shell lesions decrease its enhancement by cocaine. Cocaine-conditioned locomotion and expression of context-specific psychomotor sensitization are also functions of the core. Moreover, the acute, unconditioned psychomotor and reinforcing effects of psychostimulant drugs are a more prominent, but not exclusive, function of the shell.

These data indicate that the nucleus accumbens is involved in behaviors as diverse as locomotor activity, and learning or conditioned-dependent behavior. The core region is preferentially involved in response-reinforcement learning, whereas the shell is not involved in motor or response learning per se, but rather integrates basic biological ‘drives’ with the viscerolimbic & motor effector system. Dopamine in the nucleus accumbens may have a role in enhancing the gain by which conditioned stimuli and contexts exert control over behavior.

As indicated above and described in previous paragraphs, the essentially parallel nature of information processing through segregated basal ganglia-thalamocortical circuits (Heimer and Wilson, 1975; Alexander et al., 1986; Groenewegen et al., 1990) allows theoretical models of function in which different functional streams integrate the information ‘flowing’ through the various circuits for the production of coherent behavior. One such model (derived from the motor circuit) focuses on the role of the basal ganglia-thalamocortical circuits in the selection and
implementation of appropriate actions while inhibiting unwanted ones (Mink, 1996). This model assumes that the behavior has been learned and the role the basal ganglia is to carry out a coordinated action. However, as described above, recent behavioral evidence demonstrates that the basal ganglia are also critical in mediating learning processes by reinforcing new behavioral-guiding rules. For this to occur, communication across parallel, functionally distinct basal ganglia-thalamocortical pathways seems critical. Indeed, behavioral studies, to be outlined below, support the role of the basal ganglia in this process (Parkinson et al., 1999; Corbit et al., 2001). While basal ganglia-thalamocortical circuits are generally topographically organized through the one-way cortical-basal ganglia circuits, as reviewed above, other parts of the neural network argue against parallel processing as the only organizational rule. As will be outlined below, the results of an emerging literature in rodents as well as in primates support the idea that there are several ways by which functionally distinct circuits can interact with each other. These observations will lead to the tentative conclusion that the cortical-basal ganglia neural networks have a role for both parallel circuits and integrative circuits (Pennartz et al., 1994; Redgrave et al., 1999).

**Interacting neural networks: from motivation to action**

Zahm and Brog (1992) were among the first authors to emphasize the existence of ‘open’ components in the loops between the cerebral cortex, the basal ganglia and the thalamus, suggesting a ‘spiral’ of connections leading from the limbic-innervated part of the basal ganglia via the thalamus and subsequently via cortical-basal ganglia-thalamocortical circuits to the premotor cortex (see Fig. 3). In a similar vein, Joel and Weiner (1994) described the so-called ‘split circuits’, that include both ‘open’ and ‘closed’ basal ganglia-thalamocortical loops, which likewise have a tendency to lead from limbic to more motor-related circuits. A split circuit contains one corticostriatal circuit and two thalamocortical circuits. One of the thalamocortical circuits re-enters the cortical area of origin, thus forming a ‘closed circuit’, and the other leads to a cortical area which is the source of a different circuit, thus forming an ‘open circuit’. Moreover, O’Donnell and coworkers (1997) showed that parallel circuits from the dorsal and ventral striatum to the mediodorsal thalamic and reticular thalamic nuclei via its projections to the ventral pallidum exhibit some interactions at the level of the thalamus (see Fig. 3). A recent behavioral study likewise provides specific suggestions for such interactions at the functional level (Corbit et al., 2001). The observation that the core is modulated by the shell is a proposed mechanism through which feedback from cues associated with reward helps the core to activate and guide actions that are instrumental to gaining access to basic supplies, such as water and food (Corbit et al., 2001). Thus,
whereas the shell and core appear to have differential functional roles, as outlined above, these two subregions and their related circuitry are thought to influence each other.

In the context of the question of interactions between different parallel, functionally segregated basal ganglia-thalamocortical circuits, the role of the ascending dopaminergic system is of importance. Already based on the results of the neuroanatomical tracing study of Nauta et al. (1978), it may be concluded that the nucleus accumbens projects to a rather broad area in the substantia nigra pars compacta, potentially influencing dopaminergic neurons projecting upon the dorsal striatum. In other words, these early observations by Nauta et al. (1978) suggest a role for dopamine in the integration of activity of functionally distinct cortico-basal ganglia circuits. In more recent years this concept has been re-examined (Groenewegen et al., 1994; Deniau et al., 1996; Maurin et al., 1999; Otake and Nakamura, 2000; Haber et al., 2000). These studies have demonstrated that the nucleus accumbens shell innervates massively the region of the ventral tegmental area and substantia nigra pars compacta that contains the dopamine neurons innervating the nucleus accumbens core in both rodents and in primates (Groenewegen et al., 1994; Maurin et al., 1999; Otake and Nakamura, 2000; Haber et al., 2000). Moreover, the nucleus accumbens core has been demonstrated to innervate a dorsomedial region of the substantia nigra pars reticulata that is overlaid by the dopamine neurons innervating medial and central portions of the dorsal striatum receiving inputs from anterior cingulate and prefrontal cortical areas (Deniau et al., 1996, Maurin et al., 1999, Groenewegen et al. 1999; Haber et al., 2000). Accordingly, each striatal domain regulates its own dopamine innervation and, in addition, that of its adjacent domain. A recent analysis of the pattern of striatonigral and nigrostriatal projections in both rodents and primates revealed that each functional territory of the dorsal striatum is innervated by two main subpopulations of dopaminergic neurons (Maurin et al., 1999; Haber et al., 2000). Additionally, Haber et al. (2000) proposed that, at least in the primate brain, the striatonigrostriatal pathways form an ascending spiral from the shell to the dorsolateral striatum, which might argue for a serial, hierarchical organization of behavior involving successively more dorsal parts of the striatum as proposed by Redgrave et al. (1999). However, it is evident that this neural network in the rat, if existent, with particular emphasis on the topography and electrophysiological properties, needs further research, in order to determine how limbic, emotional-motivational aspects might influence cognitive and motor behavior.

**Further functional and anatomical differentiation of the shell and core of the nucleus accumbens: the ensemble hypothesis**
As described in previous paragraphs, differentially characterized compartments in shell and core appear to have rather unique sets of inputs and outputs. Inputs are derived from different prefrontal cortical layers, different thalamic and amygdaloid nuclei and even from dopaminergic cell groups. Outputs are directed not only towards different parts of the ventral pallidum, substantia nigra pars compacta or pars reticulata but also to septal, hypothalamic and caudal mesencephalic areas. This suggests that a further functional and anatomical differentiation is present within these larger subregions of the nucleus accumbens. An important question arising here is whether these compartments can be identified as the functionally distinct neuronal ensembles proposed in the ‘ensemble hypothesis’ of Pennartz et al. (1994). Briefly, this hypothesis states that the nucleus accumbens consists of functional units or cell groups that are smaller in size than the nucleus accumbens core and shell subregions, and subserve distinct functions in modulating various types of behaviour, such as locomotion, approach or avoidance, defensive or aggressive behavior and consummatory behavior. The neural basis for the selective recruitment of a nucleus accumbens ensemble is proposed to be a specific pattern of neural activity in the limbic-associative areas afferent to the nucleus accumbens; different ensembles are proposed to be innervated by different subregions of the hippocampal formation, medial prefrontal and orbitoofrontal cortex, basolateral amygdala and midline thalamic nuclei. The subregions providing excitation for a common ensemble are interconnected. Their differential outputs to target structures in the basal ganglia and beyond, in the mediodorsal thalamic nucleus and brainstem, are mediating the differential effects the ensembles exert on behavior. Furthermore, a lateral inhibition between ensembles endows the nucleus accumbens with the functional element of competition.

Based on behavioral, neuroanatomical and electrophysiological studies of the nucleus accumbens, Pennartz et al. (1994) proposed in the ensemble theory a prominent role for medium-sized spiny projection neurons and local circuit neurons of the rat nucleus accumbens with a relatively ‘long-range’ complex of axonal ramifications in cross-talk and cross-regulation of activity within, as well as between, functionally distinct neuronal ensembles (Fig. 8). A possible neuroanatomical correlate of an ensemble may be a striatal compartment, due to its specific afferent and efferent connections and distinct neurochemical composition (see section “The nucleus accumbens: a highly compartmentalized structure). The term ‘ensemble’ is defined as a group of neurons characterized by similar afferent-efferent relationships and closely related functions. Through convergence of active glutamatergic, excitatory inputs from cortical, hippocampal,
thalamic and amygdaloid origin, in combination with the dopaminergic input from the ventral mesencephalon and, in addition, the intrinsic microcircuitry of the nucleus accumbens, some ensembles become activated and provide an output of the nucleus accumbens at the expense of others. Such a network of functionally competing neuronal ensembles subserves input selection functions for control over outputs via the mechanisms of lateral inhibition and output selectivity as proposed in the feedback inhibition model by Groves (1983) and in the ‘Winner-take-all’ model by Wickens (1990), respectively. Anatomical evidence in support of a relatively ‘long-range’ complex of axonal ramifications in the rat nucleus accumbens has been advanced by Heimer et al. (1991).
Heimer et al. (1991) demonstrated a fine and delicate system of ‘intrastriatal association fibers’ in the rat nucleus accumbens that is restricted in its distribution. As should be clear from the foregoing account, the circuitry at the level of the nucleus accumbens needs more research, in order to determine to what extent it would lend support for the ensemble theory. In particular, the issue of the topography of ‘long-range’ versus more local intrastriatal projections deserves more attention.

Indeed, further anatomical and behavioral evidence supports the idea of functionally distinct ensembles of neurons in the nucleus accumbens (e.g. Arts and Groenewegen, 1992; Zahm and Heimer, 1993; Reynold and Berridge, 2001, 2002, 2003). Arts and Groenewegen (1992) have demonstrated that the dendritic arborizations of medium-sized spiny projection neurons of the nucleus accumbens core that project to the ventral mesencephalon are largely segregated by compartmental boundaries, reinforcing the notion of a functional separation between groups (subsets) of medium-sized spiny projection neurons and that ensembles may be instantiated by compartments. The studies by Zahm and Heimer (1993) and Reynold and Berridge (2001, 2002, 2003) have demonstrated that there exist rostrocaudal differences within the nucleus accumbens. Zahm and Heimer (1993) designated the rostralmost part of the nucleus accumbens as the so-called rostral pole, on the basis of an efferent connectivity pattern that could be distinguished from the ‘typical’ projection patterns of both shell and core. Providing another example, using specific GABA receptor agonists and AMPA/kainate glutamate receptor antagonists, Reynolds and Berridge (2001, 2002, 2003) analyzed the differential roles of these two classes of receptors in the medial shell of the nucleus accumbens. These pharmacological studies demonstrated that the anterior medial shell is involved in feeding (appetitive) behavior (see also Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1997; Basso and Kelley, 1999; Kelley and Berridge, 2002; Kelley, 2004), while the posterior medial shell (and possibly the anterior core) is concerned with fear and defensive (aversive) behaviors. These shell-elicited affective actions depend on its projections to the lateral hypothalamus. The anterior core is involved in defensive behavior evoked by AMPA/kainate glutamate receptor antagonists, but this trend was not statistically significant (Reynolds and Berridge, 2003). These (bypass) routes to the lateral hypothalamus and the diversity of (behavioral) response types found in this target structure, after activation of specific receptor types, indicate the richness in the entire output of the medial shell of the nucleus accumbens.

In conclusion, the (rostral and caudal) shell, core and rostral pole as well as the smaller compartments therein reveal a richness of differential output projections and behavioral effects. This divergence of outputs is likely to be the main substrate for the nucleus accumbens involvement in a differential and diverse range of behavioral functions. This evidence tentatively supports the
hypothesis that different types of behavior can be sustained by spatially differentiated firing patterns and intrinsic interconnections of ensembles of medium-sized spiny projection neurons of the nucleus accumbens, as proposed by Pennartz et al. (1994).

3. AIM OF THE THESIS

In the context of the questions of what the neural basis is for the selection of functionally different outputs of the nucleus accumbens and how the limbic system influences the motor system, the specific aim of the present thesis is to investigate the interactions between cortical-basal ganglia circuits by studying direct intrastriatal connections and indirect basal ganglia loop interconnections in the rat, with particular emphasis on the nucleus accumbens. We will address two main questions:

(1) How are the intrastriatal connections within and between the nucleus accumbens shell and core subregions by medium-sized spiny projection neurons and/or interneurons organized?

(2) How are the interconnections between the medium-sized spiny projection neurons of the nucleus accumbens shell and the nigrostriatal dopamine neurons innervating the sensorimotor part of the dorsal striatum organized?

These questions have been addressed by using anterograde, retrograde and single-cell juxtacellular tracing as well as electrophysiological techniques.

4. OUTLINE OF THE THESIS

The most adequate technique for studying the intricate circuitry of the nervous system is axon tracing or ‘tract-tracing’. This method relies on a substance’s incorporation into the neuron, its transport, and its subsequent localization in other parts of the neuron. Axonal tracing of neuronal pathways can be achieved by anterograde and retrograde transport. Tracers commonly used for anterograde transport, including biotinylated dextran amine (BDA; Veenman et al., 1992; Vercelli et al., 2000; Reiner et al., 2000; Power and Mitrofanis, 2002), biocytin and neurobiotin (Pinault et al., 1996), and Phaseolus vulgaris leukoagglutinin (PHA-L; Gerfen and Sawchenko, 1984; Groenewegen and Wouterlood, 1990), are most likely incorporated by nonspecific endocytosis into the perikaryon followed by anterograde axonal transport. Two methods will be used. The first
method includes the extracellular application of BDA, PHA-L or fluor gold (retrograde tracer)(Fig. 9A). The second method includes the juxtacellular application of neurobiotin (Pinault et al., 1996)(Fig. 9B). Different techniques can be used to introduce tracer into the tissue, for example by pressure and iontophoretic injection. The term iontophoresis or electrophoresis pertains to the use of electrical current to eject substances from an electrode or micropipette. In this case, tracer molecules must carry an electrical charge and are thus driven by force of the electrical field. This technique is suited for both extracellular and juxtacellular applications. In addition, the application site can be precisely located prior to iontophoresis by conventional electrophysiological techniques with the same electrode (e.g. juxtacellular labeling using extracellular recording pipettes).

To address our first question, we initially examined in the rat nucleus accumbens the intrastriatal connections between the two major subregions of the nucleus accumbens, i.e., shell and core, and we characterized the type of neurons that give rise to these projections, using anterograde tract-tracing injections and single-cell juxtacellular tracing injections, and subsequent three-dimensional reconstructions (Chapter 2). To further clarify the anatomical organization of intrastriatal communication within the two major subregions of the rat nucleus accumbens, i.e., shell and core, in the studies described in Chapter 3 we examined the spatial orientation of the dendrites and local axon collaterals of medium-sized spiny projection neurons, as well as the spatial relationships between the dendritic and axonal arborizations of medium-sized spiny projection neurons on the one hand and the subregional (shell-core) and compartmental (patch-matrix) structure on the other hand, using single-cell juxtacellular tracing injections, and subsequent three-dimensional reconstructions and computerized visualizations (Chapter 3). To address our second question, we examined in the rat the effects of electrical stimulation of the nucleus accumbens shell on dopamine nigrostriatal neurons identified as projecting to the sensorimotor territory of the dorsal
striatum and the extent to which the shell projects onto the two main subpopulations of nigrostriatal neurons that innervate the sensorimotor region of the dorsal striatum, using electrophysiological experiments and combined anterograde tracing from the nucleus accumbens shell with retrograde tracing from the sensorimotor region of the dorsal striatum (Chapter 4).
Box.  A historical perspective on the histology of the central nervous system

The fundaments of modern neuroanatomical approaches were already laid in the late 19th century. In 1873, the Italian histologist Camillo Golgi (1843-1926) discovered a staining procedure that, by using silver impregnation, showed the fine morphology of neurons in all their exquisite details. In the decades following this great discovery, it was in particular the Spanish histologist Santiago Ramón y Cajal (1852-1934) who recognized the immense potential of the Golgi stain and studied virtually every part of the nervous system of a great variety of species. These studies culminated in 1911 in the publication of Cajal’s monumental “Histologie du Système Nerveux de l’Homme et des Vertébrés”. The German neurologist and psychiatrist Karl Wernicke (1848-1904) and Cajal introduced, at the end of the 19th century, their view of brain function called ‘cellular connectionism’. Cajal provided the histological basis for considering the neuron to be the structural unit of the brain. He also showed that neurons connect to one another in a highly precise fashion. Wernicke showed that behavior is mediated by specific brain regions and through localizable pathways connecting sensory and motor structures. He also emphasized that the same function is processed in parallel in different regions of the brain. Wernicke thereby initiated the notions of parallel and distributed processing of information that are so prominent in current thinking. Important steps forward in our understanding of which brain areas ‘talk’ to each other could be made in the past 50-60 years, first following the development of the silver impregnation techniques by Nauta and Gygax (1951, 1954) to selectively visualize degenerating fiber tracts and, in the seventies of the last century, by techniques that employ physiological axonal transport mechanisms of neurons, the so-called tract-tracing technique (Mesulam, 1982). To this very day, novel experimental approaches are still being developed, in order to gain further insight in our understanding of brain function in terms of connectional anatomy, for instance by using a MRI-detectable, neuronal tract-tracing method in living animals (Saleem et al., 2002) or a neuroimaging methodology, so-called diffusion tensor imaging (DTI), in the living human brain (Ramnani et al., 2004).
Chapter 2

Anatomical evidence for direct connections between the shell and core subregions of the rat nucleus accumbens

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ANATOMICAL EVIDENCE FOR DIRECT CONNECTIONS BETWEEN THE SHELL AND CORE SUBREGIONS OF THE RAT NUCLEUS ACCUMBENS

The nucleus accumbens is thought to subserve different aspects of adaptive and emotional behaviors. The anatomical substrates for such actions are multiple, parallel ventral striatopallidal output circuits originating in the nucleus accumbens shell and core subregions. Several indirect ways of interaction between the two subregions and their associated circuitry have been proposed, in particular through striato-pallido-thalamic and dopaminergic pathways. In this study, using anterograde neuroanatomical tracing with Phaseolus vulgaris-leucoagglutinin (PHA-L) and biotinylated dextran amine (BDA) as well as single-cell juxtacellular filling with Neurobiotin, we investigated the intra-accumbens distribution of local axon collaterals for the identification of possible direct connections between the shell and core subregions.

Our results show widespread intra-accumbens projection patterns, including reciprocal projections between specific parts of the shell and core. However, fibers originating in the core reach more distant areas of the shell, including the rostral pole (i.e., the calbindin-poor part of the shell anterior to the core) and striatal parts of the olfactory tubercle, than those arising in the shell and projecting to the core. The latter projections are more restricted to the border region between the shell and core. The density of the fiber labeling within both the shell and core was very similar. Moreover, specific intrinsic projections within shell and core were identified, including a relatively strong projection from the rostral pole to the rostral shell, reciprocal projections between the rostral and caudal shell, as well as projections within the core that have a caudal-to-rostral predominance. The results of the juxtacellular filling experiments show that medium-sized spiny projection neurons and medium-sized aspiny neurons (most likely fast-spiking) contribute to these intra-accumbens projections. While such neurons are GABAergic, the intrastriatal projection patterns indicate the existence of lateral inhibitory interactions within, as well as between, shell and core subregions of the nucleus accumbens.

Keywords: ventral striatum, intra-accumbens projections, Phaseolus vulgaris-leucoagglutinin, biotinylated dextran amine, juxtacellular labeling, limbic corticostriatal system
Shell-core interconnections

Introduction

The nucleus accumbens (Acb), located in the rostroventral part of the striatum, consists of two main subregions, a peripheral shell and a central core. These subregions can be distinguished on the basis of their specialized histological and neurochemical profiles (Zaborszky et al., 1985; Voorn et al., 1989; Zahm & Brog, 1992; Jongen-Rêlo et al., 1993; 1994) and distinctive input-output characteristics (Brog et al., 1993; Heimer et al., 1991; Zahm & Brog, 1992; Berendse et al., 1992a, b; Groenewegen et al., 1996). In line with this, the shell and core are involved in different functional processes, although their precise functions are still to be established. The Acb core is critical for mediating the ability of environmental cues with learned relevance to stimulate and guide behavior (Cardinal et al., 2002), whereas the shell is involved in modulating unconditioned behaviors, such as feeding and locomotion (Maldonado-Irizarry et al., 1995; Kelley, 1999, 2004; Cardinal et al., 2002). Notwithstanding their differential functional roles, it is important to note that the shell and core of the Acb are thought to be part of two interacting neuronal networks (Parkinson et al., 1999; Corbit et al., 2001).

Several ways in which interactions between Acb shell and core circuits might occur have been proposed. A multisynaptic pathway would lead from the shell via ventral pallido-thalamic relays to prefrontal cortical regions that project to the core (review: Zahm, 1999, 2000; cf. also Groenewegen et al., 1999). Another possible indirect pathway from shell to core, as well as to more dorsally located striatal areas, runs via dopaminergic neurons in the ventral tegmental area and the medial parts of the substantia nigra pars compacta (Nauta et al., 1978; Haber et al., 2000; Kolomiets et al., 2002). In addition to these indirect, multisynaptic pathways between Acb shell and core, direct connections have been described (Heimer et al., 1991). According to Heimer and colleagues (1991), the intrastriatal Acb fibers represent a fine and delicate system that is rather restricted in its distribution. However, these authors further noted that the ‘intrastriatal association fibers’ could cross the shell-core boundary. Direct interactions between the shell and core may be of functional significance, e.g. for the transfer of one form of learned association to another (Dayan and Balleine, 2002; Cardinal et al., 2002).

In this context, the aim of the present study was to determine in more detail the extent to which the Acb shell and core subregions are directly interconnected through intra-accumbens projections and whether a specific organisation underlies the intra-Acb projections. To this aim, small injections of the anterograde tracers Phaseolus vulgaris-leucoagglutinin (PHA-L) and biotinylated dextran amine (BDA) were placed in different parts of the Acb in order to trace the...
intrastratal distribution of anterogradely filled axons. These experiments were supplemented by single-cell juxtacelllular injections of the tracer Neurobiotin (Nb) in order to further characterize the neurons that give rise to intrastratal projections.
Experimental Procedures

Iontophoretic BDA and PHA-L injections

Animals

Twenty-six adult female Wistar rats (Harlan Centraal Proefdierbedrijf, Zeist, the Netherlands) weighing 180-250 g were injected with BDA, and nine animals with PHA-L. All animals were fed ad libitum and housed in cages with enriched food. All experimental procedures were performed according to the guidelines of the ethical committee of animal experimentation, Vrije Universiteit, Amsterdam, that are in accordance with the European Community Council Directive 86/609/EEC.

Animal surgery and anterograde tracer injections

Animals were anesthetized with a mixture of ketamine and xylazine (i.m., 4 parts of 1% solution of ketaset (ketamine; Aesco, Boxtel, the Netherlands) and 3 parts of a 2% solution of Rompun (xylazine; Bayer, Brussels, Belgium), total dose 1ml/kg body weight). Anesthesia was maintained throughout the experiment by additional doses of ketamine and xylazine (1ml/kg body weight, i.m.). Foot withdrawal reflex was checked throughout the experiment to assess the depth of anesthesia. The anesthetized animals were mounted in a stereotaxic frame. Body temperature was maintained between 36 and 37°C by the use of a homeothermic mat. In addition, 10% lidocaine (Astra Pharmaceutica BV, Zoetermeer, the Netherlands) was used as local anesthetic for the skin of the head at the site of incision. The brain was exposed through small burr holes in the skull, and the anterograde tracers biotinylated dextran amine (BDA, 10.000 MW, Molecular Probes Inc., Eugene, OR) or *Phaseolus vulgaris*-leucoagglutinin (PHA-L, Vector, Burlingame, CA) were injected in different parts of the Acb. The coordinates were derived from the atlas of Paxinos and Watson (1986).

The tracers BDA or PHA-L were delivered iontophoretically through a glass micropipette (CG-150F-15; Clark, Reading, United Kingdom) with an external tip diameter of 2-3 µm (BDA) or 10-15 µm (PHA-L). Pipettes were filled with 5% BDA or 2.5% PHA-L in 0.1 M NaH$_2$PO$_4$/Na$_2$HPO$_4$ (phosphate buffer [PB]), pH 7.4). A small positive DC current of 1.0 µA

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1 The anterograde tracer injections and the single-cell juxtacellular injections were performed in two different laboratories; minor differences in the surgical and staining procedures are the result of different traditions in these laboratories rather than that they represent essentially different steps in the protocols.
(BDA) or 7.0 µA (PHA-L) (7s on/7 s off) was delivered to the micropipette over a period of 2-10 min in order to produce small injection sites. In most cases, the BDA injections were placed bilaterally; PHA-L was injected unilaterally in all cases.

After a 7 days postoperative survival period, the animals were deeply re-anesthetized with sodium pentobarbital (Nembutal, 1 ml/kg, i.p.; Ceva, Paris, France) and rapidly perfused transcardially with 0.9% saline (NaCl), followed by a fixative containing freshly depolymerized 4% paraformaldehyde (Merck, Darmstadt, Germany) and 0.05% glutaraldehyde (Merck) in PB (0.1 M, pH 7.4) for 15 min. After fixation, the brains were removed from the skull, post-fixed for 1.5 hrs and cryoprotected by storage of the brain for 18-48 hrs at 4°C in a mixture of 20% glycerin (Merck) and 2% dimethyl sulfoxide (DMSO; Merck) in PB. The brains were then rapidly frozen with 30% sucrose onto the stage of a sliding freezing microtome and 40 µm thick sections were cut either in the horizontal plane (BDA) or coronally (PHA-L). Sections were collected sequentially in six receptacles containing either PB for direct processing, or in a mixture of DMSO/glycerin for storage at –20°C. For each brain the sections of at least three receptacles were stained for BDA or PHA-L, one receptacle without further counterstaining, the other two combined with either Nissl staining or immunohistochemistry for the calcium binding protein calbindin-D28kDA (CaB).

**Anterograde tracer histochemistry**

In the procedures described below and in the next paragraph, all intermediate steps between different incubations included three rinses of the specifically indicated buffer for 10 min each. Initially, the sections were rinsed with PB followed by 0.05 M Tris/HCl (Merck) supplemented with 0.15 M NaCl, pH 7.6 (Tris-buffered saline; TBS) and 0.5% Triton X-100 (TBS-Tx; Merck), and the sections with BDA were subsequently incubated in avidin-biotin-peroxidase complex (ABC: (1:1) mixture of reagents A (avidin) and B (biotinylated horseradish peroxidase); Vector, Burlingame, CA) in TBS-Tx for 1.5 hrs at room temperature (rt). After rinsing with Tris/HCl, the sections were stained with nickel-enhanced diaminobenzidine (DAB-Ni) substrate: 7.5 mg 3,3′-diaminobenzidine-tetrahydrochloride (DAB; Sigma, St. Louis, MO), 0.225 g nickel-ammonium sulfate (Boom B.V., Meppel, the Netherlands), 10 µl of 30% H2O2 in 50 ml Tris/HCl, pH 8.0, for 10-30 min at rt.

The sections with PHA-L were incubated for 48 hr at 4°C in goat anti-PHA-L (Vector), diluted 1:1000 in TBS-Tx. After rinsing with TBS-Tx, the sections were incubated for 18 hr at rt in
donkey anti-goat IgG (Nordic, Tilburg, The Netherlands), diluted 1:100 in TBS-Tx, followed by an incubation in rabbit peroxidase-antiperoxidase (rPAP; Nordic), diluted 1:200 in TBS-Tx for 4 hr at rt. After rinsing with Tris/HCl, the PHA-L was visualized by incubating the sections in DAB substrate: 5 mg DAB (Sigma), 3.3 µl of 30% H₂O₂ in 10 ml Tris/HCl, pH 7.6, for 10-30 min at rt.

The progress of staining was frequently monitored with the aid of a light microscope. As soon as nonspecific background staining became visible the reaction was terminated by several rinses in Tris/HCl. The sections were mounted on glass slides from a Tris/HCl solution, containing 0.2% gelatin (Oxoid, Basingstoke, United Kingdom), and air-dried. One receptacle of sections was counterstained with a 0.3% aqueous solution of Cresyl Violet for 0.5-1 min. Finally, the material was dehydrated through an ascending series of alcohol (50%, 70%, 80%, 2x 96%, followed by 2x 100%) and coverslipped from the xylene using Entellan (Merck).

**Double staining procedure for the tracer and calbindin-D$_{28kDA}$**

In order to characterize the Acb shell and core compartments, immunohistochemical staining for the calciumbinding protein calbindin-D$_{28kDA}$ (CaB) was employed in combination with staining for either tracer. The double staining procedure was as follows. Sections were rinsed with PB followed by TBS-Tx (pH 7.6), and subsequently incubated with mouse anti-CaB (Sigma; 1:2000) overnight at 4ºC. After rinsing with TBS-Tx, the sections containing BDA were incubated in ABC-kit (Vector, Burlingame, CA) and those containing PHA-L in goat anti-PHA-L, followed by donkey anti-goat IgG and rPAP as described above. For both tracers, the final staining step involved DAB-Ni as a chromogen. Following Tris-HCl rinsing, the sections were rinsed with TBS-Tx and, to complement the CaB-staining, incubated with goat anti-mouse serum (1:50; Dako, Denmark) for 60 min at rt. Next, after rinsing with TBS-Tx, the sections were incubated with mouse peroxidase-antiperoxidase complex (mPAP; 1:100; Dako) for 60 min at rt. After Tris/HCl rinse (pH 7.6), the sections were stained with DAB (1 ml DAB [5 mg/10 ml], 9 ml Tris/HCl, pH 7.6 and 3.3 µl 30% H₂O₂) for 15 min at rt. After final rinses with Tris/HCl (pH 7.6), the sections were mounted on glass slides from a Tris/HCl (pH 7.6) solution, containing 0.2% gelatin, and air-dried. Mounted sections were dehydrated and coverslipped as described above.

**Anatomical analysis**
The distribution of labeled intrastriatal axons and terminals was charted in equally spaced sections of the ventral striatum to provide an impression of the comparative distribution and density following injections located in different parts of the Acb. Drawings were performed under 2.5 - 40x objectives using a drawing tube attached to a light microscope and transferred to CorelDraw 9.0 to construct the final chartings.

Final preparation of all half-tone figures, including contrast enhancement, was done using Adobe Photoshop 5.5.

**Single-cell juxtacellular injections**

**Animals**

Juxtacellular injection experiments were performed on 23 adult male Sprague Dawley (IFFA CREDO, Les Oncins, France) weighing 250-380 g. Surgical procedures were applied in strict accordance with the European Community Council Directive 86/609/EEC.

**Animal preparation**

Animals were anesthetized by an injection of sodium pentobarbital (Nembutal, 1 ml/kg, i.p.; Sanofi, Libourne, France) and fixed in a conventional stereotaxic apparatus (Unimécanique, Epinay sur Seine, France). Anesthesia was maintained throughout the experiment by ketamine (0.5 ml/kg, i.m.; Imalgène 500, Rhone-Mérieux, France). In addition, incision and pressure points were infused with lidocaine. Throughout the experiment, heart beat and pedal withdrawal reflex were monitored to assess the depth of anesthesia and the body temperature was maintained between 37 and 38°C by the use of a homeothermic blanket. Subsequently, the brain was exposed through small burr holes in the skull.

**Electrophysiological characterization of single Acb neurons**

To label single Acb neurons, electrophysiological responses of Acb cells evoked by electrical stimulation of either the ventral hippocampus, in particular the CA1 region or the medial prefrontal cortex (PFC) were used as a guide for placements within the Acb shell or core. Stimulations (200 µsec duration, 20-100 µA intensity) were applied at a depth of 7 mm from the cortical surface for CA1 and 3 mm for the PFC, ipsilaterally or contralaterally to the recorded Acb.
Stereotaxic coordinates of recording and stimulation sites were determined using the atlas of Paxinos and Watson (1986).

Extracellular single unit recordings were made in the Acb using glass pipettes (15-20 MΩ) containing 1.5% Neurobiotin (Nb; Vector, Burlingame, CA) in 0.5 M NaCl. The hippocampus and PFC were stimulated using a coaxial stainless steel electrode (diameter 400 µm; tip-barrel distance 300 µm). Action potentials of single neurons were recorded using the active bridge mode of an Axoclamp 2 B amplifier (Axon Instruments, Foster City, CA), amplified (1000x), filtered (0.3-3 kHz) with an AC/DC amplifier (DAM 50, World Precision Instruments, Hertfordshire, UK), and viewed on a memory oscilloscope (Tektronix, Courtaboeuf, France). Medium-sized spiny projection neurons (MSN) of the Acb were clearly distinguished from the interneurons by their basic electrophysiological properties (Wilson, 1993; Mahon et al., 2001). During these experiments, the electrical activity of Acb cells was examined on-line and stored with a Digital Tape Recorder (DTR-1404, Biologic, Claix, France).

**Labeling of single Acb neurons**

Recorded neurons were labeled using juxtacellular injection of Nb (Pinault, 1994). Briefly, a positive pulsed current (1-8 nA, 200 msec duration) was applied at a frequency of 2.5 Hz through the bridge circuit of the amplifier. The current was slowly increased, and the electrode was advanced by steps of 1 µm (LSS-1000 Inchworm Motor Positioning System, Burleigh Instruments, Fishers, NY) onto the neuron until the cell discharge was driven by the injected current. Current pulses were applied for a 10-30 min period to obtain a reliable labeling of neuronal processes.

Two to five hours after the last injection, the animal received a lethal dose of sodium pentobarbital (3 ml/kg, i.p.; Sanofi, Libourne, France) and Heparin (1 ml/kg, i.p.; Choay, Paris, France) before being perfused transcardially with 200 ml of 10% Ringer solution followed by 500 ml of 0.2% glutaraldehyde (Prolabo, Fontenay s/bois, France) and 4% paraformaldehyde (CARLO ERBA, Val de Reuil, France) in 0.1 M NaH₂PO₄·H₂O/Na₂HPO₄ (phosphate buffer, pH 7.4). In all experiments, brains were post-fixed overnight at 4°C in the same fixative with or without glutaraldehyde and then immersed in 30% sucrose at 4°C until sectioned. Fifty µm thick sections were cut in the horizontal plane on a sliding freezing microtome. Sections were collected sequentially in twelve receptacles containing phosphate buffer for direct processing.
Visualization of single Acb neurons

Sections were double-stained for Nb and CaB as follows. In the procedures described below, all intermediate steps between different incubations included three rinses of the specifically indicated buffer for 10 min each. First, the sections were rinsed in phosphate buffer supplemented with 0.15 M NaCl (phosphate-buffered saline [PBS], pH 7.4), and subsequently incubated in ABC-kit (1:1 mixture of reagents A and B; Vector, Burlingame, CA) in PBS and 0.5% Triton X-100 (PBS-Tx; Merck) overnight at 4ºC. Thereafter, a rinse with phosphate buffer was followed by staining with Cobalt enhanced DAB-Ni substrate: 0.025 g DAB, 1 ml 1% nickel-ammonium sulfate, 1.25 ml 1% Cobalt chloride, 20 µl of 30% H$_2$O$_2$ in 50 ml phosphate buffer, for 10-30 min at rt. The progress of staining was frequently monitored with the aid of a light microscope. As soon as nonspecific background staining became visible, the reaction was terminated by several rinses in phosphate buffer, followed by rinsing with TBS-Tx, and incubation in mouse anti-CaB (Sigma; 1:2000) overnight at 4ºC. After TBS-Tx rinse, the sections were incubated with goat anti-mouse serum (1:50; Dako) for 60 min at rt. Following rinsing with TBS-Tx, the sections were incubated with mPAP (1:100; Dako) for 60 min at rt. Next, a Tris/HCl (pH 7.6) rinse was followed by staining with DAB (1 ml DAB [5 mg/10 ml], 9 ml Tris/HCl, pH 7.6 and 3.3 µl 30% H$_2$O$_2$) for 15 min at rt. After final rinses with Tris/HCl (pH 7.6), the sections were mounted on glass slides from a Tris/HCl (pH 7.6) solution, containing 0.2% gelatin, and air-dried. The mounted sections were dehydrated and coverslipped as described above.

Anatomical analysis

Labeled neurons and boundaries of the shell and core of the Acb were traced and reconstructed from adjacent sections. For the purpose of the present study, i.e., to establish whether recurrent axon collaterals of neurons crossed the border between the core and shell, two-dimensional (2D) reconstructions were made from drawings performed under 10-40x objectives using a drawing tube attached to a light microscope and transferred to CorelDraw 9.0 to construct the final charting.
Results

Delineation of the shell and core

For the delineation of the shell and core subregions on the basis of the pattern of CaB-immunoreactivity (CaB-IR), we refer to previous accounts (Zahm and Brog, 1992; Jongen-Relo et al., 1994; see also Fig. 1). To facilitate the descriptions of the distribution of labeled axons and terminals following anterograde tracer injections or juxtacellularly filling, the shell and core of the Acb have been subdivided on the basis of general anatomical planes that in each experimental case can be reproducibly determined. Two transverse, one sagittal and one horizontal plane divide the Acb into rostral/caudal, medial/lateral and dorsal/ventral subareas, respectively (Fig. 1). The horizontal ‘dividing’ plane is positioned at the level where the posterior limb departs from the crossing anterior commissure (Fig. 1D). All parts of shell and core dorsal to and including this level are considered as dorsal Acb subareas (Fig. 1A-D); levels shown in figure 1E-G represent the ventral subareas. A sagittal plane is positioned such that, in the dorsal-ventral dividing horizontal plane (Fig. 1D), it ‘touches’ the medial aspect of the curved anterior limb of the anterior commissure rostrally (arrow in Fig. 1D) and, in the same horizontal plane, its lateral aspect caudally (double arrow in Fig. 1D). The mediolateral division primarily concerns subareas in the core and shell of the Acb, but also applies to the caudate-putamen (CPu) (Fig. 1A-C) dorsally and the olfactory tubercle ventrally (Fig. 1H). The two transverse planes are ‘positioned’ as follows. The rostral transverse plane runs through the most rostral extension of the darkly staining core as seen in the horizontal levels shown in figure 1C and D. The caudal transverse plane ‘touches’ the most rostral extension of the major island of Calleja (Fig. 1C). All levels of the Acb shell anterior to the rostral transverse plane are defined as the rostral pole of the Acb. The rostral pole in this way exhibits mostly low CaB-IR, in continuity with the weak staining in the remaining parts of the shell. However, the most rostral, lateral and ventral aspects of the rostral pole show moderate to strong immunoreactivity for CaB (Fig. 1A-D), at some levels showing continuity with dark staining in the core (see also Zahm and Heimer, 1993; Jongen-Relo et al., 1994). The caudal transverse plane divides both the shell and core in rostral and caudal subareas.
Shell-core interconnections

Fig. 1: Overview of the location of BDA/PHA-L (indicated with different hatchings and encoded with numbers [see below]) and Nb (indicated with dots and encoded with letters [see below]) injection sites in the Acb represented in eight equally spaced horizontal sections that are ‘counterstained’ for CaB to reveal the shell and core subregions. The sections are arranged from dorsal (A) to ventral (H). Borders of shell and core are indicated with dashed lines. The transverse and sagittal planes dividing the shell and core into different subareas are also indicated (for a description of the criteria to position these planes, see the Results section). The CaB-poor area anterior to the rostral transverse plane is defined as the rostral pole in this study. The different experiment numbers (referred to in the text) are encoded in this figure as follows. BDA and PHA-L (*) injections: 1 = 87215(*); 2 = 01028R; 3 = 00102L; 4 = 00079L; 5 = 00102R; 6 = 93086(*); 7 = 00104R; 8 = 00078R; 9 = 00157; 10 = 00154R; 11 = 00109L; 12 = 00154L; 13 = 00107; 14 = 00104R; 15 = 90135(*); 16 = 00079R; 17 = 001010; 18 = 92072(*); 19 = 00111; 20 = 00105; 21 = 87358(*); 22 = 91202(*); 23 = 00106; 24 = 00102; 25 = 01201; 26 = 02018; 27 = 00154L; 28 = 00132; 29 = 00078L; 30 = 00109; 31 = 00104L; 32 = 00104L; 33 = 00107; 34 = 03091; 35 = 01174; 36 = 02016L; 37 = 88541(*); 38 = 92213(*); 39 = 93093(*); 40 = 01173; 41 = 02016L; 42 = 01172; 44 = 01171. Juxtacellularly filled neurons: a = 7R-I; b = 7L; c = 10R-I; d = 4L-I; e = 19L-II; f = 2; g = 7R-II; h = 4L-II; i = 9; j = 19L-III; k = 12; l = 10R-II; m = 5-I; n = 1; o = 19L-I; p = 14L-I; q = 14L-II; r = 18; s = 5-II.

Abbreviations: ac, anterior commissure; Acb, nucleus accumbens; CB, striatal cell bridges; ICjM, major island of Calleja; LS, lateral shell; LV, lateral ventricle; OT, olfactory tubercle; vCPu, ventral parts of the caudate-putamen. Scale bar in H = 500 µm and applies to all levels.

Anterograde tracing experiments

Initial experiments consisted of PHA-L injections, in a set of later experiments BDA was used as tracer. Injections of both tracers resulted in a dense plexus of labeled fibers and terminals in the immediate vicinity of the injection site, whether located in the Acb shell or core, but also in specific patterns of labeling in more distant Acb areas, including the adjacent subregion (shell or core) and/or the olfactory tubercle or ventral CPu. An important point of concern of most neuroanatomical tracers is their ‘selectivity’ with respect to direction of transport and/or the possibility that they might be taken up by fibers passing through the injection site. While PHA-L is generally accepted to be a tracer that is transported almost exclusively in anterograde direction and is hardly taken up by passing fibers, BDA has been described as being bi-directionally transported as well as being taken up by damaged fibers passing through the injection site (see also Discussion). To circumvent this potential problem with BDA tracing, the injection sites were kept as small as possible to avoid tissue and fiber damage at the site of injection. Furthermore, for the purpose of the present study, dealing with local projections in the Acb, it was crucial to compare the results of injections of PHA-L and BDA in the same location of the Acb. Since it turned out to be much easier to produce small BDA injections and the resulting fiber and terminal labeling could be much easier charted, we primarily rely on the results of the BDA
experiments in the remainder of this paper, referring to results of the PHA-L experiments when appropriate (see also Discussion).

**Characterization of the BDA injection sites.** BDA injection sites used in the present study varied in size from about 0.3x10^{-3} mm^3 (case 01173; Fig. 1F) to 33x10^{-3} mm^3 (case 00154L; Fig. 1C, D)(Table 1). The appearance of the injection sites also differed. In a number of cases, the center of the injection was saturated with reaction product, preventing the identification of individual cell bodies and dendrites. Only in the periphery of such injection sites could single cell bodies, dendrites and axon collaterals be observed. In other cases, a cluster of individually labeled neurons, including their cell body, dendrites and axon collaterals characterized the injection site. The shape of most of the cell bodies was round or oval, while the majority of the dendrites were densely spiny. Dendrites could extend up to 100-200 µm away from the periphery of the injection site (i.e., the most peripherally located cell bodies). Enmeshed between the neuronal cell bodies and dendrites, numerous labeled varicose axons and boutons were present. In most cases, a relatively dense plexus of axonal fiber labeling was present close to the center of the injection site (characterized by labeled cell bodies and their filled dendrites), as well as at more distance from the injection site. The density of the axonal plexus was highest in the vicinity of the injection site and diminished with distance. The distribution of the labeling showed a specific pattern within the Acb depending on the location of the injection site. Essentially two types of fibers could be observed within the Acb: 1. smooth, sparsely branching axons, often arranged in bundles, and 2. frequently branching, varicose or terminating axons. The smooth fibers could be observed to leave the Acb to the ventral pallidal, hypothalamic and mesencephalic projection areas. The second type of fibers represent a network of labeled axons and terminals of varying density that was present in specific parts of the Acb, signifying intrastriatal projections beyond the immediate vicinity of the injection site. The distribution patterns of these labeled axons and terminals will be described in the next paragraphs.
Table 1. The distribution of BDA labeled fibers and terminals in the nucleus accumbens, caudate-putamen and olfactory tubercle after injections in the shell, core and rostral pole subregions of the rat nucleus accumbens. The density of labeled fibers and terminals is represented as follows: ****** very dense labeling, *** dense labeling, ** moderate labeling, * light labeling and – sporadic labeled fibers. Case numbers followed by an ‘R’ (right hemisphere) or an ‘L’ (left hemisphere) concern rats with bilateral injections. The shading indicates the location of the injection site. Abbreviations: CPu, caudate-putamen; D, dorsal; Expnr., experiment number; L, lateral; M, medial; OT, olfactory tubercle; RP, rostral pole; Sf, subfield; V, ventral.

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<th>CPu</th>
<th>OT</th>
<th>Injection site (mm, left hemisphere)</th>
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Table 1: The density of labeled fibers and terminals is represented as follows: ***** very dense labeling, *** dense labeling, ** moderate labeling, * light labeling and ~ sporadic labeled fibers. Case numbers followed by an ‘R’ (right hemisphere) or an ‘L’ (left hemisphere) concern rats with bilateral injections. The shading indicates the location of the injection site.

Abbreviations: CPu, caudate-putamen; D, dorsal; Exp.nr., experiment number; L, lateral; M, medial; OT, olfactory tubercle; RP, rostral pole; Sf, subfield; V, ventral.
**Representation of the density of BDA labeled fibers and terminals in the Acb.** The density of labeled fibers and terminals in the Acb was scored as follows: •••• very dense labeling, ••• dense labeling, •• moderate labeling, • light labeling and ~ sporadic labeled fibers (see Fig. 2A and B). Table 1 summarizes the distribution of the labeling over the different Acb subareas as well as the maximum density of the labeling in at least one-quarter of each subarea.

**Location of the BDA and PHA-L injection sites.** In 26 rats, 43 small iontophoretic injections of BDA were placed in different parts of the shell and core of the Acb. Thirty-five (out of the 43) injection sites showed sufficient labeling and were confined to the shell or the core (see Fig. 1). The injection sites were selected on the basis of the intensity of the staining of the neurons that allowed us to visualize their dendrites and a plexus of axon collaterals. Nine BDA injections included the core, two the ventral CPu, three the core/ventral CPu, twenty-one the shell, of which seven were located in the rostral pole. The organization of the intra-accumbens projection patterns will be described on the basis of six representative BDA injection sites located in different parts of the Acb. Nine relatively small PHA-L injections, located in different subareas of the Acb, were selected for comparison with the BDA injections.

**Intrastriatal projection patterns following tracer injections in the core and adjacent caudate-putamen**

Fourteen injections were placed in the Acb core, some including the ventral CPu (see Fig. 1 and Table 1). Most of the injection sites were located in medial parts of the core. There appeared to be only slight differences in fiber and terminal labeling following injections in either the dorsal or ventral parts of the core, whereas injections at different rostrocaudal levels resulted in significantly different patterns of labeling in the Acb. Therefore, emphasis will be placed on the patterns of labeling following injections at different mediolateral or rostrocaudal locations.

**Rostral core.** Five BDA and two PHA-L (rats 87358 and 91202) injections were located in the rostromedial part of the core, while two injections of each tracer were placed in the adjacent rostrolateral part of the core/ventral CPu (see Fig. 1). The five BDA injection sites in the rostromedial core occupied slightly different positions, i.e., three cases were located close to the shell-core boundary (cases 00105 [Fig. 1C, D], 00109 [Fig. 1D], and 00107 [Fig. 1E, F]), whereas
Shell-core interconnections
those in the two other cases were located farther away from the shell-core border (cases 00106 [Fig. 1C, D] and 00104 [(Fig. 1D)]).

The results of a PHA-L injection (rat 87358 [Figs. 1C, D and 3A-C]) and a BDA injection (case 00106 [Fig. 4A-H]), both located in the rostromedial core will be described. The relatively large PHA-L injection was confined to the core region and occupies the rostral core region just medial to the anterior limb of the anterior commissure (Fig. 3B). The staining in the center of the injection site was largely amorphous, in the periphery of the injection site densely stained dendrites, fibers and terminals were present. Apart from a labeled fiber bundle travelling caudally to enter the ventral pallidum, parts of the core and shell of the Acb (Fig. 3B, C), as well the striatal elements of the rostromedial part of the olfactory tubercle (Fig. 3A) contained a fine plexus of varicose fibers and terminals. Within the core, these labeled intrastriatal fibers extended into its dorsal part as well as into the ventrally adjacent medial part of the caudate-putamen. The labeling also extended ventrally and medially into the core and labeled fibers reached the adjacent medial and ventral parts of the shell (Fig. 3B, C). In rostral direction, the rostral pole of the Acb was also reached by labeled fibers and terminals (Fig. 3A). The labeling in rostral areas of the Acb appeared to be slightly denser than in more caudal areas.

In case 00106, the BDA injection site consisted of a small cluster of labeled neurons \((4\times10^3 \text{ mm}^3)\). A dense plexus of dendrites and axons was found in the immediate vicinity of the injection site occupying most of the rostromedial core. The plexus of axon fibers and terminals extended into the ventromedial CPu where single fibers reached areas as far as 1 mm away from the injection site (Fig. 4A-C). Fewer labeled axons reached the caudal and lateral core (Fig. 4C-F). The intra-Acb fibers extended rostralward into the rostral pole and rostromedial shell (Fig. 4A-G). Ventrally beyond the rostral shell, labeled fibers and terminals reached the striatal cell bridges and layer II of the olfactory tubercle (Fig. 4H).
The injection sites that were located closer to the shell-core boundary than in case 00106 (cases 00105, 00107 and 00109), showed stronger labeling in the rostral pole, rostromedial shell, and the ventral CPu.

The two PHA-L (rats 87215 [Fig. 1A] and 93086 [Fig. 1B]) and the two BDA injections (cases 01028R [Fig. 1A, B] and 01024L [Fig. 1B, C]) that were located in the lateral core/ventral CPu differed in the density of the resulting labeling of axon collaterals and terminals, but the general pattern of labeling was rather similar. In case 01028 (Fig. 5), with a BDA injection site occupying a volume of about 30x10^-3 mm^3, a dense plexus of labeled dendrites and axons was found in the immediate vicinity of the injection site occupying most of the rostrolateral CPu (Fig. 5A-D). The plexus of axon collaterals extended into the caudolateral CPu (Fig. 5A-D), and to a lesser degree into the ventromedial CPu, the rostral pole, and both the rostral core and shell (Fig. 5A-F). A few labeled fibers and terminals were observed more ventrally in the olfactory tubercle (Fig. 5G).
Fig. 4: Chartings of the injection site and distribution of labeled fibers following a representative BDA (case 00106) injection site in the rostral core. The labeling (red) is represented in eight horizontal sections (A = dorsal, H = ventral). The smooth labeled fibers that leave the Acb to the ventral pallidal, hypothalamic and mesencephalic projection areas are omitted for clarity. The shading represents high CaB-immunoreactivity. Dashed lines indicate boundaries of shell and core as well as between different subareas. The black dots indicate individual cell bodies.

Abbreviations: ac, anterior commissure; CB, striatal cell bridges; ICjM, major island of Calleja; LV, lateral ventricle.
Fig. 5: Chartings of the injection site and distribution of labeled fibers following a BDA injection site in the rostroventral CPu (case 01028R). The labeling (red) is represented in eight horizontal sections (A = dorsal; G = ventral). The smooth labeled fibers that leave the Acb to the ventral pallidial, hypothalamic and mesencephalic projection areas are omitted for clarity. The shading represents high CaB-immunoreactivity. Dashed lines indicate boundaries of shell and core as well as between different subareas. The black dots indicate individual cell bodies.

Abbreviations: ac, anterior commissure; CB, striatal cell bridges; ICjM, major island of Calleja; LV, lateral ventricle.
**Caudal core.** Four BDA (cases 03092 [Fig. 1C], 01024R [Fig. 1C], 02016L [Fig. 1F] and 01209 [Fig. 1G]) and three PHA-L injections (rats 88541 [Fig. 1F], 92213 [Fig. 1F], and 93093 [Fig. 1F]) were located in the caudomedial core. Two BDA injections included the medial core and adjacent ventral CPu (cases 00078R [Fig. 1B-D] and 00154R [Fig. 1B, C]) and one involved only the extreme ventromedial CPu (case 00157 [Fig. 1B]). The main pattern of labeling resulting from these injections was independent of whether the injection involved exclusively the caudal core or included the adjacent ventral CPu. However, considerable differences were noted depending on the distance of the injection site from the shell-core boundary. Case 00154R, in which the BDA injection site (9x10^-3 mm^3) occupied mainly the most caudodorsal core, but also included the adjacent ventromedial CPu just lateral to the inferior tip of the lateral ventricle, will be used to illustrate the main pattern (Figs. 2 and 6). A very dense plexus of dendrites and axons was found in the immediate vicinity of the injection site occupying the caudomedial core and ventromedial CPu. The plexus of axon collaterals extended into the lateral core and ventrolateral CPu where single fibers reached areas as far as 1 mm away from the injection site. The intra-Acb fibers extended into the rostromedial core (Fig. 6A-E), as well as into the medial shell (Fig. 6A-F). In this case (00154R), as well as in rat 00078R and the three PHA-L cases, the labeling in the medial shell is densest in its ventral regions (Fig. 6D-F). In contrast, in cases 01024R, 00157 and 03092 (Fig. 1B,C) labeling predominates in the dorsomedial shell (see also Table 1). Labeling of fibers and terminals in case 00154R, as well as to a lesser extent in the other cases, extended ventrally beyond the medial shell into the olfactory tubercle (Fig. 6G). Finally, in all cases labeling in the rostral pole was less extensive than in the medial shell.

Injection sites with a more lateral position in the core (BDA cases 02016L and 01209) showed a more restricted distribution of fiber labeling. A plexus of labeled fibers was found in the immediate vicinity of the injection site occupying the caudomedial core, from which only in case 02016L a few fibers did extend into the rostral core. These two cases did not result in fiber labeling in the shell or rostral pole (see Table 1).

**Intrastriatal projection patterns following tracer injections in the shell**

Twenty-one BDA injections were placed in the Acb shell, of which seven were located in the rostral pole, five in the rostral shell and nine in the caudal shell (see Fig. 1; Table 1). All these injection sites involved the medial shell, together covering its dorsoventral extent. Two of our smaller PHA-L injections in the shell, one rostromedially and the other
Fig. 6: Chartings of the injection site and distribution of labeled fibers following a representative BDA injection site in the caudal core (case 00154R). The labeling (red) is represented in seven horizontal sections (A = dorsal; G = ventral). The smooth labeled fibers that leave the Acb to the ventral pallidal, hypothalamic and mesencephalic projection areas are omitted for clarity. The shading represents high CaB-immunoreactivity. Dashed lines indicate boundaries of shell and core as well as between different subareas. The black dots indicate individual cell bodies.

Abbreviations: ac, anterior commissure; CB, striatal cell bridges; ICJM, major island of Calleja; LV, lateral ventricle.
caudomedially located, were also selected for analysis. Main differences in the projection patterns were observed following injections that were located either in the dorsomedial or ventromedial shell, while differences following injections at different rostrocaudal levels were less apparent.

**Rostral pole.** Seven BDA injections were located in the most rostral parts of the Acb, i.e., the rostral pole (see Fig. 1). Most of the injection sites were placed in the dorsal part of the rostral pole. All injections showed a very similar distribution of fiber labeling in the Acb, independent of their position in the rostral pole. Three injections were located close to the medial border of the rostral pole (cases 01009L [Fig. 1B, C], 02018 [Fig. 1C-E], and 00154L [Fig. 1C, D]), three occupied a more intermediate position (cases 01028L, [Fig. 1B], 01009R [Fig. 1B-D], and 01004L [Fig. 1D]) and one was located more laterally (case 00102L [Fig. 1A]). Since these injections resulted in a very similar distribution of fiber and terminal labeling within the Acb, details will be described for only case 00154L (Figs. 1C, D and 7). The injection site in this case covered about $33 \times 10^{-3}$ mm$^3$. A dense plexus of axons was found in the immediate vicinity of the injection site, including most of the rostral pole. The bulk of intra-Acb fibers extended caudally into the rostromedial shell (Fig. 7A-F). A few fibers reached the caudal and lateral shell (Fig. 7A-F). From the dense plexus in the ventromedial shell, a moderate number of fibers extended via the striatal cell bridges layer II into the olfactory tubercle (Fig. 7F, G). Furthermore, the labeled fibers extended into the rostromedial core (Fig. 7B, C) and adjacent ventromedial CPu (Fig. 7A, B) where they reached as far as 1.5 mm away from the injection site. Only a few fibers reached the caudal and lateral core (Fig. 7C) and adjacent ventral CPu (Fig. 7A-C).

**Rostral shell.** Five BDA injections were located in the rostral shell, three dorsomedially and two in its ventromedial part (see Fig. 1). The three dorsally situated injection sites occupied slightly different positions in the medial shell, i.e., one was located in the vicinity of the shell-core boundary (case 01032 [Fig. 1C]), one more medially (case 00078L [Fig. 1C]) and the third injection was located in the transition between the rostral shell and rostral pole (case 01004R [Fig. 1B, C]). Since these BDA injections resulted in a very similar distribution of fiber and terminal labeling within the Acb, details will be given only for case 01032 (Figs. 1C and 8). The approximate dimensions of the injection site measured about $15 \times 10^{-3}$ mm$^3$. A dense plexus of labeled axons and terminals was found in the immediate vicinity of the injection, occupying most of the rostromedial shell. This plexus of labeled fibers extended both rostrally and caudally into the rostral pole and caudal shell, respectively (Fig 8A-F).
Fig. 7: Chartings of the injection site and distribution of labeled fibers following a representative BDA injection site in the rostral pole (case 00154L). The labeling (red) is represented in seven horizontal sections (A = dorsal; G = ventral). The smooth labeled fibers that leave the Acb to the ventral pallidal, hypothalamic and mesencephalic projection areas are omitted for clarity. The shading represents high CaB-immunoreactivity. Dashed lines indicate boundaries of shell and core as well as between different subareas. The black dots indicate individual cell bodies.

Abbreviations: ac, anterior commissure; CB, striatal cell bridges; ICJM, major island of Calleja; LV, lateral ventricle.
Fig. 8: Chartings of the injection site and distribution of labeled fibers following a representative BDA injection site in the rostral shell (case 01032). The labeling (red) is represented in eight horizontal sections (A = dorsal; H = ventral). The smooth labeled fibers that leave the Acb to the ventral pallidal, hypothalamic and mesencephalic projection areas are omitted for clarity. The shading represents high CaB-immunoreactivity. Dashed lines indicate boundaries of shell and core as well as between different subareas. The black dots indicate individual cell bodies.

Abbreviations: ac, anterior commissure; CB, striatal cell bridges; ICjM, major island of Calleja; LV, lateral ventricle.
This plexus of labeled fibers extended both rostrally and caudally into the rostral pole and caudal shell, respectively (Fig 8A-F). A very similar pattern of labeling was also observed in PHA-L case 90135 (Fig. 1B). In case 01032 only very few fibers reached the lateral shell (Fig. 8E). The rather dense plexus of labeled fibers and terminals in the ventromedial shell extended via the striatal cell bridges into layer II of the olfactory tubercle (Fig. 8G, H). Furthermore, labeling was found in the rostral and caudal core, albeit in a relatively restricted zone adjacent to the core-shell border (Fig. 8C-F). Similar patterns of labeling were observed in the above-mentioned PHA-L case 90135. Following both the PHA-L and BDA injections in the rostral shell, the labeled fiber plexus in the core extended into the ventromedial CPu (Fig. 8A, B), while only sporadically fibers extended into the lateral core (Table 1).

It is important to note that, although the general patterns of intrastriatal labeling in cases 01032, 00078L and 01004L were very similar, following an injection in the most rostral position (case 01004L), the density of fiber labeling in the rostral pole and rostral core was higher than in the more caudal cases (Table 1).

The two injections in the ventral parts of the rostral shell (cases 01173 [Fig. 1F] and 01172 [Fig. 1G]) resulted in a considerably different pattern of labeling, mostly restricted to the rostromedial and caudomedial shell. Sporadic labeling was found in the rostral pole and the olfactory tubercle, while also the medial core contained only few fibers (Table 1).

**Caudal shell.** Nine BDA injections were located in the caudal shell, five of which were situated caudally in the dorsomedial and four in the ventromedial shell (see Fig. 1). The five dorsally located injection sites occupied slightly different positions in the medial shell, i.e., one was located close to the shell-core boundary (case 01010 [Fig. 1B]), three more medially (cases 00079L [Fig. 1A, B], 00102R [Fig. 1A], and 00079R [Fig. 1B]) and the fifth injection was located in the most caudal position (case 00111 [Fig. 1B]). Four of these injections resulted in a very similar distribution of fiber labeling in the Acb, only case 00111 resulted in a considerably different projection pattern. Therefore, we will describe case 00111 separately, and use case 01010 to illustrate the overall pattern of fiber labeling in the Acb (Figs. 1B and 9). In case 01010 (15x10⁻³ mm³) a very dense plexus of axons was found in the immediate vicinity of the injection site occupying most of the caudomedial shell (Fig. 9A-F). This plexus of intra-Acb fibers and terminals extended in both rostral and caudal direction, occupying the rostral shell, the most caudal shell, and to a lesser extent the rostral pole (Fig. 9A-F). Only very few fibers reached the lateral shell (Fig. 9D, E). The rather
dense plexus of labeled fibers and terminals in the ventromedial shell extended via the striatal cell bridges into layer II of the olfactory tubercle (Fig. 9F, G). In addition, labeling was found in the rostral and caudal core, in particular in a relatively restricted zone adjacent to the core-shell boundary (Fig. 9B-F). This fiber plexus also extended into the ventromedial CPu (Fig. 9A, B). Only sporadically fibers reached more laterally into the core (Fig. 9B).

In case 00111, the BDA injection site that was located in a more caudal position resulting in a plexus of fiber labeling that preferentially occupied the caudomedial shell. The density of fiber labeling in the rostral shell and medial core was lower than in the three cases with caudal shell injections described above. Only sporadically fibers reached the rostral shell and olfactory tubercle, while also very few fibers extended into the medial core (Table 1). Fiber and terminal labeling in the Acb following a PHA-L injection in the caudomedial shell (case 92072) gave similar results.

The four ventrally located injection sites occupied slightly different positions in the medial shell, i.e., one was located close to the shell-core boundary (case 03091 [Fig. 1E]) and the other three more medially (cases 01174 [Fig. 1E], 02016R [Fig. 1F] and 01171 [Fig. 1G]). These injections resulted in a considerably different pattern of labeling that was mostly restricted to the rostromedial and caudomedial shell. Sporadic labeling was found in the rostral pole and olfactory tubercle, while also the medial core contained only few fibers (Table 1).
Fig. 9: Chartings of the injection site and distribution of labeled fibers following a representative BDA injection site in the caudal shell (case 01010). The labeling (red) is represented in seven horizontal sections (A = dorsal; G = ventral). The smooth labeled fibers that leave the Acb to the ventral pallidal, hypothalamic and mesencephalic projection areas are omitted for clarity. The shading represents high CalB-immunoreactivity. Dashed lines indicate boundaries of shell and core as well as between different subareas. The black dots indicate individual cell bodies.

Abbreviations: ac, anterior commissure; CB, striatal cell bridges; ICJM, major island of Calleja; LV, lateral ventricle.
Single-cell juxtacellular tracing experiments

In order to more specifically characterize the neurons that potentially give rise to projections within and between shell and core, in a second set of experiments we juxtacellularly filled electrophysiologically identified neurons in the rat Acb with Nb. Electrophysiological characterization was done by stimulation of either the ventral hippocampus or the medial prefrontal cortex (Table 2). In the present paper, emphasis will be placed on the identification of the morphological subtype of the Acb neurons and the relationships of their axon collaterals with the shell-core border.

Aspects of the Nb labeling. Out of a population of 85 neurons injected in the Acb, 21 cells showed sufficient staining, not only of their cell body and dendrites but also of the axon collaterals (Fig. 1 and Table 2). All but one of the neurons used in this study were identified as MSN by their electrophysiological properties and their morphological features. One neuron was identified electrophysiologically as an interneuron and morphologically characterized as medium-sized aspiny (most likely a fast-spiking GABAergic neuron). From the cell bodies of the MSN several primary dendrites radiated and strongly branched within the Acb. The orientation of the dendrites was not uniform (Van Dongen et al., in preparation). Axons of these MSN originated from the cell body forming a main stem from which several thinner collaterals with many varicosities and terminal boutons arose. In most cases, these axon collaterals arborized extensively within the Acb (up to 350 µm from the cell body). The axon collaterals of the aspiny neuron were thinner and formed a dense plexus of local axon collaterals with varicosities and terminal boutons within the Acb.

All main axons of the examined neurons were seen to turn caudally and, in most cases (66%; 14 out of 21 neurons), exit the Acb to reach the ventral pallidum (VP). In six cases, these axons could be followed caudal to the VP, in two cases reaching as far as the ventral mesencephalon. However, in a number of cases, the staining of the main axon faded before the axon reached the VP (n = 6) or within the confines of the VP (n = 8; see Table 2). All 21 examined neurons were reconstructed.
Table 2. Distribution of local axon collaterals in the nucleus accumbens and extrinsic projections after single-cell juxtacellular filling of neurons in the shell, core and rostral pole of the rat nucleus accumbens.

**Abbreviations:** Acb, nucleus accumbens; C, nucleus accumbens core; Hipp, hippocampus; L, left hemisphere; LCN, local circuit neuron; Mes, mesencephalon; MSN, medium-sized spiny projection neuron; n, number of cells; PFC, prefrontal cortex; R, right hemisphere; RP, rostral pole; Stim.Electr, stimulation electrode; S, nucleus accumbens shell; VP, ventral pallidum; VP>, ventral pallidum and beyond.

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Table 2: Abbreviations: Acb, nucleus accumbens; C, nucleus accumbens core subregion; Hipp, hippocampus; L, left hemisphere; LCN, local circuit neuron; Mes, mesencephalon; MSN, medium-sized spiny projection neuron; n, number of cells; PFC, prefrontal cortex; R, right hemisphere; RP, rostral pole; Stim.Electr, stimulation electrode; S, nucleus accumbens shell subregion; VP, ventral pallidum; VP>, ventral pallidum and beyond.
Injection sites. Nine neurons were located in the core or adjacent ventromedial CPu and twelve neurons in the shell (see Figs. 1). The majority of the analysed juxtacellularly filled cells (17 out of 21) showed a restricted axonal field observing the borders of the core and shell (see Table 2). For the purpose of the present study, only the four neurons with axonal labeling extending into the adjacent subregion will be briefly described.

Distribution of intra-accumbens axon collaterals

Core. Neurons 7R-I (Fig. 1A) and 4L-I (Fig. 1A), located in the dorsal part of the rostromedial core, showed varicose axon collaterals that extended into the adjacent subregion of the Acb, i.e., the rostral pole and rostral shell, respectively. One of these neurons was characterized as a MSN (neuron 7R-I), the other was the above-mentioned medium-sized aspiny interneuron (neuron 4L-I). Both neurons were stimulated from the ventral hippocampus (CA1). The recurrent axon collaterals of the MSN arborized away from the dendritic tree towards and into the rostral pole (Fig. 10A-E). While the extensive and fine local axon collateral network of the interneuron branched within the medial core, two of its axon collaterals arborized into the rostromedial shell close to the shell-core boundary (Fig. 11A-E). The main axon of the MSN exited the Acb and terminated within the VP (Table 2).

Rostral pole. Neuron 19L-II (Fig. 1A), located in the dorsal part of the rostral pole, stimulated from CA1, showed an extensive terminal field in the rostral pole, while from its main axon, passing through the medial core, a single branch with varicosities was observed in the rostromedial core (not illustrated). The main axon of this neuron terminated in the VP (Table 2).

Shell. Neuron 5-II (Fig. 1G), in the caudoventral shell, presented a varicose axon collateral that projected into the caudomedial core (Fig. 12A-E). This neuron was stimulated from the PFC, and the main axon terminated within the VP (Table 2).
Fig. 10: Photomicrographs and two-dimensional (2D) reconstruction of single medium-sized spiny neurons (MSN) in the rostral core (neuron 7R-I) and rostral shell (neuron 7R-II) in a horizontal section through the Acb, double-stained for Nb and CaB. A, low power photomicrograph showing the medium-sized spiny neuron in the rostral core (arrow) and some dendrites of the medium-sized spiny neuron in the rostral shell (boxed area indicated with an asterisk). The area enclosed by the small rectangle is shown at higher magnification in E; note the labeled varicose fiber in E. B, 2D-reconstruction of neuron 7R-I illustrating the distribution of the dendrites and local axon collaterals. The part of the local axon collateral enclosed by the small rectangle is located in the rostral pole (A), and is shown in E. The dashed line indicates the shell-core boundary. The arrow indicates the main axon leaving the Acb. C, high power photomicrograph of the medium-sized spiny neuron in the rostral core. Note the high density of the dendritic spines. D, photomicrograph of the medium-sized spiny neuron in the rostral shell in a section adjacent to the section shown in A. Reconstruction of the two neurons revealed that the local axon collaterals did not overlap.

Abbreviations: vCPu, ventral parts of the caudate-putamen. Scale bar in A = 125 µm, scale bar in C = 50 µm and applies to C and D, and scale bar in E = 10 µm.
Fig. 11: Photomicrographs and two-dimensional (2D) reconstruction of an interneuron (neuron 4L-I) and a medium-sized spiny neuron (MSN; neuron 4L-II) in the rostral core and rostral shell, respectively, in a horizontal section through the Acb, double stained for Nb and CaB. A, low power photomicrograph showing the interneuron in the rostral core (arrow) and some dendrites of the medium-sized spiny neuron in the rostral shell (in boxed area indicated with an asterisk). The area enclosed by the small rectangle is shown at higher magnification in E; note the labeled varicose fiber in E. B, 2D-reconstruction of neuron 4L-I illustrating the distribution of the dendrites and local axon collaterals. The part of the local axon collateral enclosed by the small rectangle is located in the rostral shell (A), and is shown in E. The dashed line indicates the shell-core boundary. C, high power photomicrograph of the interneuron in the rostral core. Note the absence of dendritic spines (inset). D, photomicrograph of the medium-sized spiny neuron in the rostral shell in a section adjacent to the section shown in A. Note the high density of the dendritic spines (inset). Reconstruction of the two neurons revealed that the local axon collaterals did not overlap.

Abbreviations: vCPu, ventral parts of the caudate-putamen. Scale bar in A = 125 μm, scale bar in C = 50 μm, scale bar in D = 100 μm, scale bar in inset = 10 μm and applies to C and D, and scale bar in E = 10 μm.
Fig. 12: Photomicrographs and two-dimensional (2D) reconstruction of a medium-sized spiny neuron (MSN) in the caudal shell (neuron 5-II) in a horizontal section through the Acb, double-stained for Nb and CaB. A, low power photomicrograph showing the neuron in the caudal shell. B, 2D-reconstruction of neuron 5-II illustrating the distribution of the dendrites and local axon collaterals. The part of the local axon collateral enclosed by the small rectangle is located in the caudal core (D), and is shown at higher magnification in E. The dashed line indicates the shell-core boundary. C, high power photomicrograph of the neuron in the caudal shell. Note the high density of the dendritic spines (inset). D, low power photomicrograph of a section adjacent to the section containing the neuron (A, C). E, high power photomicrograph of the varicose axon collateral in the caudal core. Scale bar in A = 125 µm, scale bar in C = 50 µm; scale bar in inset = 10 µm, scale bar in D = 500 µm, and scale bar in E = 10 µm.
Discussion

This study provides the first systematic analysis of the organization of intrastriatal projections in the rat Acb. Using anterograde neuroanatomical tracing and single-cell juxtacellular filling, it was demonstrated that extensive intrastriatal projections exist (Fig. 13). These include reciprocal connections between specific parts of the shell and core. However, fibers originating in the core reached more widespread areas of the shell, including the rostral pole, than those arising in the shell and reaching to the core. The latter projections are more restricted to the border region between the shell and core. The density of the fiber labeling within both the shell and core was very similar and diminishes with the distance from the injection site. Moreover, it could further be demonstrated that specific intrinsic projections exist within both the shell and core, including a relatively strong projection from the rostral pole to the rostral shell, reciprocal projections between the rostral and caudal shell, and projections within the core that have a caudal-to-rostral predominance. The results of the single-cell juxtacellular filling experiments demonstrated that axon collaterals of MSN as well as medium-sized apiny neurons significantly contribute to these intra-accumbens projections (cf. also Taverna et al., 2004). Finally, striatal elements of the medial olfactory tubercle also received projections from both the shell and, to a lesser degree, the core.

Methodological considerations

The interpretation of the results of the present study, which has the specific aim to identify intranuclear, short distance projections, requires serious consideration of the properties and limitations of the tracer substances used. Most substances characterized as anterograde tracers have been reported to be also transported in retrograde direction, some of them giving rise to subsequent collateral labeling. A further complication in the interpretation of patterns of labeled fibers may be the phenomenon of uptake of the tracer by fibers passing through the injection site. In the present study, we have used three different tracer substances. For the description of the intrastriatal projection patterns, we rely primarily on the small, iontophoretic BDA injections. In view of the potential limitations of BDA as a selective anterograde tracer, we also analysed the intrastriatal projection patterns following PHA-L injections restricted to one of the Acb subareas. Finally, we used Neurobiotin to reconstruct the intrastriatal collateral network of single neurons.

The tracer PHA-L is one of the most selective anterogradely transported tracers (Gerfen and Sawchenko, 1984; Groenewegen and Wouterlood, 1990). However, in our hands it appeared relatively difficult to produce small enough PHA-L injections for the purpose of the present study.
Shell-core interconnections

Furthermore, the quality of the PHA-L staining and, consequently, the identifiability of the local collateral network in the Acb was less compared to the BDA staining (cf. also Veenman et al., 1992; Reiner et al., 2000; Köbbert et al., 2000). The lesser quality of the PHA-L staining may be due to penetration problems of the antibodies used in the immunohistochemical procedure (Wouterlood et al., 2002). The transport of PHA-L has also proven to be somewhat more unpredictable than that of BDA (Groenewegen and Wouterlood, 1990; Schmued and Heimer, 1990). However, BDA has been shown to be transported bi-directionally and it may be taken up by fibers of passage. Nevertheless, when BDA is delivered iontophoretically its retrograde transport component is limited (Lanciego and Wouterlood, 1994). Furthermore, uptake by fibers of passage is only rarely observed when taking specific precautions (Veenman et al., 1992; Vercelli et al., 2000; Reiner et al., 2000; Power and Mitrofanis, 2002). Therefore, in order to reduce the possibility of retrograde transport and uptake by passing fibers, we made small, iontophoretic BDA injections.
using very small tip diameters of the micropipettes, low injection currents, and brief injection periods. In line with reports in the literature, we observed minimal tissue damage at the center of the injection site, in most cases minimal or no retrograde labeling, no leakage along the injection track, and ‘healthy’ looking neuronal cell bodies, dendrites and axons. On the basis of our observations that injections of PHA-L and BDA in approximately the same location in the Acb resulted in very comparable patterns of intrastriatal labeling of fibers and terminals, we conclude that in our BDA experiments uptake by passing fibers and retrograde transport subsequently followed by anterograde collateral labeling plays a very minor or no role. However, it cannot be ruled out with absolute certainty that some of the axonal labeling is due to uptake of BDA by such extrinsic fibers from, for example, prefrontal cortical, hippocampal, thalamic, amygdaloid or ventral pallidal origin (cf. Shu and Peterson, 1988; Chen and Aston-Jones 1998; Kuo and Chang, 1992; Groenewegen et al., 1996).

The results of our BDA and PHA-L experiments were supplemented by 21 juxtacellularly filled Acb neurons. The purpose of the juxtacellular labeling of single neurons was 1) to identify the type of neuron that gives rise to these intra-Acb projections, and 2) to verify the patterns of intra-Acb projections observed following BDA injections. First, out of 21 selected juxtacellularly filled neurons, 20 neurons appeared to be MSN, while one neuron was an (electrophysiologically and anatomically identified) aspiny interneuron. Second, three MSN and the interneuron showed axonal branches projecting into the adjacent subregion, extending up to approximately 250 µm into that subregion. Although some of the MSN had long-range axon collaterals extending up to approximately 1 mm away from the parent cell body, none of the neurons in our sample that projected to the adjacent subregion showed such an extensive distribution of axon collaterals. However, in most cases the main axon could be followed over several millimeters into the ventral pallidum or beyond. Therefore, we believe that the absence in our sample of neurons with long-range collaterals crossing the border between the shell and core is not due to the limitations of the injection or staining procedure. It may rather be related to 1) the relatively small fraction of neurons that project into the adjacent subregion, or 2) the bias introduced by the selection of these neurons through the electrical stimulation of the prelimbic cortex or hippocampus.

**Connections within and between shell and core**

Our results show that axon collaterals of MSN, as well as axons of interneurons, distribute over rather extensive areas in the Acb, providing projections *within* as well as *between* the shell and
core subregions. These observations concur with the results of previous studies showing intrastriatal ‘associational’ projections in both dorsal and ventral striatum (Kawaguchi et al., 1990; Heimer et al., 1991). In the ventral striatum, Heimer et al. (1991) briefly described such intrinsic projections. The present results confirm the observations by Heimer et al. (1991) and show, in addition, the specific organization of these projections using much smaller injections in different subareas of the Acb. As to the dorsal striatum, several studies using intracellular filling have shown that MSN (Bishop et al., 1982; Kawaguchi et al., 1989, 1990), as well as interneurons (Wilson et al., 1990; Kawaguchi, 1993), have axons that project up to 1 mm away from the parent cell body. Like in our sample of single Acb neurons, dorsal striatal MSN with long-range axon collaterals form only a relatively small subgroup of MSN.

Indirect connections between the shell and core have been proposed to exist via basal ganglia-thalamocortical circuits (Zahm and Brog, 1992; Joel and Weiner, 1994; Zahm, 2000) as well as via the mesencephalic dopaminergic system (Nauta et al., 1987; Groenewegen et al., 1994; Haber et al., 2000; Kolomiets et al., 2002). Both these indirect pathways show a preference for information flowing from the shell towards the core, at the level of the core using glutamate and dopamine as the neurotransmitter inprefrontal and mesencephalic afferents, respectively. Our present results show that there are direct reciprocal connections between specific parts of the core and shell (including the rostral pole). Interestingly, the intrastriatal fibers originating in the core appear to reach more widespread areas of the shell than those arising in the shell and projecting to the core. Whereas the core-to-shell connections include virtually the entire extent of the shell, and in some cases the adjacent striatal parts of the olfactory tubercle, the shell-to-core connections remain primarily restricted to the border region of the shell and core. The single-cell juxtacellular filling experiments show that the direct reciprocal connections are, at least in part, GABAergic (cf. Taverna et al., 2004). Thus, our findings prompt a reevaluation of the relative weight of shell-to-core influences vis a vis core-to-shell influences.

Functional considerations

The results of this study provide a morphological framework for specific, possibly GABA-mediated, interactions between the Acb shell and core subregions. An important question in the context of the present results is what role the direct shell-to-core and core-to-shell interactions play in the overall circuitry. Recently, Corbit et al. (2001) have argued that through the shell cues associated with reward may affect instrumental performance via the output of the core. Thus, whereas the shell and core appear to have different functional roles (Kelley, 1999, 2004; Parkinson
et al., 1999, 2000; Reynolds and Berridge, 2001, 2002, 2003; Cardinal et al., 2002), these two subregions and their associated circuitry are thought to influence each other (cf. Dayan and Balleine, 2002; Parkinson et al., 1999). As discussed above, previous neuroanatomical studies provide evidence for indirect pathways from the shell to the core via corticostriatal and dopaminergic projections. However, the present study indicates that direct reciprocal connections between specific parts of the shell and core should also be taken into account when considering functional interactions between the shell and core. Although not much is known about the functional aspects of core-to-shell projections, the available evidence supports the hypothesis that the core may convey information to the shell to overwrite its modulation of primary, non-learned behavior (e.g. the sight of an apple may counteract the shell-mediated inhibition of feeding (cf. Kelley, 1999, 2004)).

Finally, our present findings of reciprocal connections between the rostromedial and caudomedial shell suggest a possible involvement in funneling the motivational valence of positive (appetitive) vs. negative (aversive) states. Thus, Reynolds and Berridge (2001, 2002, 2003) demonstrated that the anterior medial shell, coinciding largely with our rostromedial shell and possibly part of the rostral pole, is involved in feeding behavior (see also Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1997; Basso and Kelley, 1999; Kelley and Berridge, 2002; Kelley, 2004), while the posterior medial shell, coinciding largely with our caudomedial shell, is concerned with fear and defensive behaviors. These behavioral results are in line with the existence of functionally different neuronal ensembles in the shell (Pennartz et al., 1994) that on the basis of our present results may be interconnected via the GABAergic (and peptidergic) axon collaterals of MSN. The results of the present paper do certainly not exclude a contribution of one or more types of Acb interneurons in the intrastriatal projections. However, the GABAergic projections between the rostral and caudal shell may be of great importance in the inhibitory feedback control of 1) the rostral shell, to facilitate appetitive behavior and 2) the caudal shell, to facilitate aversive behavior. These data suggest that GABA may be a major neurotransmitter in intra-shell communication to control behavioral selection.

Reynolds and Berridge (2003) have demonstrated that the rostral core supports glutamate antagonist evoked defensive behavior, but this trend was not statistically significant. What role the intrinsic connections between the rostral and caudal core and their neurotransmitters (including GABA, glutamate and dopamine) play remains to be established.
Acknowledgements

We thank Dirk de Jong for his support in making the illustrations and Jeroen van Zanten for his assistance with the data analysis. This study was supported by a Program Grant of the Dutch Medical Research Council NWO-ZonMW (903-42-092) and a NWO-ZonMW/INSERM travel grant (910-48-029).

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Chapter 3

Three-dimensional organization of dendrites and local axon collaterals of shell and core medium-sized spiny projection neurons of the rat nucleus accumbens

Co-authors: Philippe Mailly, Anne-Marie Thierry, Henk J. Groenewegen, Jean-Michel Deniau
THREE-DIMENSIONAL ORGANIZATION OF DENDRITES AND LOCAL AXON COLLATERALS OF SHELL AND CORE MEDIUM-SIZED SPINY PROJECTION NEURONS OF THE RAT NUCLEUS ACCUMBENS

Medium-sized spiny projection neurons (MSN) in the head of the primate caudate nucleus are thought to have preferred dendritic orientations that tend to parallel the orientations of the striosomes. Moreover, recurrent axon collaterals of MSN in the rat dorsal striatum have been categorized into two types, i.e., restricted and widespread. The nucleus accumbens (Acb), on the other hand, has a highly complex compartmental organization and the spatial organization of dendritic and axonal arbors of MSN have not been systematically studied as yet. In this study, using single-cell juxtacellular labeling with neurobiotin as well as anterograde neuroanatomical tracing with biotinylated dextran amine, we investigated the three-dimensional (3D) organization of dendrites and axons of MSN of the rat Acb in relation to subregional (shell-core) and compartmental (patch-matrix) boundaries.

Our results show that dendritic arbors of MSN in both the Acb shell and core subregions are preferably oriented, i.e., that they are flattened in at least one of the 3D-planes. The preferred orientations are influenced by shell-core and patch-matrix boundaries, suggesting parallel and independent processing of information. Dendritic orientations of MSN of the Acb core are more heterogeneous, than those of the shell and dorsal striatum, suggesting a more complex distribution of striatal inputs within the core. While dendrites respect the shell-core and patch-matrix boundaries, recurrent axon collaterals may cross these boundaries. Finally, different degrees of overlap between dendritic and axonal arborizations of individual MSN were identified, suggesting various possibilities of lateral inhibitory interactions within and between, functionally distinct territories of the Acb.

Keywords: ventral striatum; juxtacellular labeling; three-dimensional models; medium-sized spiny projection neurons; dendritic orientations; local axon collaterals
Introduction

The spatial arrangement of the dendrites of a neuron is an important feature of its specific ability to process incoming information ultimately leading to an output of that neuron. For example, large pyramidal neurons in the cerebral cortex have a very specific and highly ordered three-dimensional (3D) morphology of their dendrites and intracortical axons that is related to both the horizontal cortical lamination and the vertical columnar organization and, in this way, with specific cortical input-output features (Ramón y Cajal, 1911; Gray, 1959; Feldman, 1984; DeFelipe et al., 2002). The striatum, the main input nucleus of the basal ganglia, lacks an apparent cytoarchitectonic feature like the cortical lamination and has a rather homogeneous cytoarchitectonic structure. Moreover, at first sight, the dendrites of its principal projection neurons, i.e., the medium-sized spiny projection neurons (MSN), appear to have a much less clearly ordered geometry than the cortical pyramidal neurons. However, Walker et al. (1993) described a preferred orientation of the dendritic arbors of MSN in the primate striatum along a rostral-dorsal-medial to caudal-ventral-lateral axis. These authors suggested that this orientation tends to parallel the preferred orientation of the striatal compartments, i.e., the striosomes, in the primate striatum. Thus, the geometry of striatal MSN might, at least to some extent, be related to the striatal compartmental structure.

The ventral striatum is known to differ in its compartmental organization from the dorsal striatum. On the basis of the patterns of neurohistochemical staining, the nucleus accumbens (Acb), which is the major component of the ventral striatum, can be divided into a peripheral ‘shell’ and a central ‘core’ subregion. Within these subregions different smaller compartments exist (Zaborszky et al., 1985; Voorn et al., 1989; Zahm & Brog, 1992; Jongen-Rêlo et al., 1993; 1994). In view of the differences in compartmental structure between the dorsal and ventral striatum, a question is whether the dendrites of ventral striatal MSN have preferred orientations and whether the geometry of the dendrites of these neurons is in any way related to the orientation of the shell-core border or compartmental structure of the ventral striatum.

Medium sized spiny projection neurons, in addition to innervating structures extrinsic to the striatum, have a local axon collateral network that arborizes within the striatum. Most recently, these local axon collaterals have been demonstrated to play an important role in GABA-mediated synaptic transmission between striatal spiny projection neurons (Tunstall et al., 2002; Czubayko and Plenz, 2002; Taverna et al., 2004; Venance et al., 2004). These findings challenged us to analyze and describe the spatial organization of the recurrent axon collateral network of MSN. Kawaguchi et al. (1990) described two types of MSN in the rat dorsal striatum, one with local axon collaterals
restricted to the dendritic field of the parent cell, and one with more widespread axon collaterals. The first type might be important for interactions between neurons within a particular functional striatal ‘territory’. The second type might provide a possibility for more distant interactions, i.e., between functionally different ‘territories’. The patterns of distribution of recurrent axon collaterals of MSN in relation to the parent cell body and the geometry of its dendritic arborizations, therefore, are two basic aspects of functional architecture of the striatum. Up till now, very little is known about such intrinsic relationships within the ventral striatum.

In the present study, we examined the 3D organization of the dendrites and local axon collaterals of MSN in the rat Acb shell and core subregions, by using single-cell juxtacellular filling with neurobiotin (Pinault, 1996) as well as anterograde neuroanatomical tracing with biotinylated dextran amine (Veenman et al., 1992). The geometrical aspects of the dendrites and recurrent axon collaterals of the injected neurons were studied in relation to the shell-core boundary and immunohistochemically defined compartments.
Experimental Procedures

Single-cell juxtacellular injections

Animals

Juxtacellular injection experiments were performed on 15 adult male Sprague Dawley (IFFA CREDO, Les Oncins, France) weighing 250-380 g. Surgical procedures were applied in strict accordance with the European Communities Council Directive 86/609/EEC (1986). The number of animals and their suffering was kept as low as possible.

Animal preparation

Animals were anesthetized by an injection of pentobarbital (1 ml/kg, i.p.; Sanofi, Libourne, France) and fixed in a conventional stereotaxic frame. Anesthesia was maintained throughout the experiment by ketamine (0.5 ml/kg, i.m.; Imalgène 500, Rhone-Mérieux, France). In addition, incision and pressure points were infused with lidocaine. Throughout the experiment, heart beat and pedal withdrawal reflexes were monitored to assess the depth of anesthesia and the body temperature was maintained between 37 and 38°C by the use of a homeothermic mat. Subsequently, the brain was exposed through small burr holes in the skull.

Electrophysiological characterization of Acb neurons

To label Acb neurons, electrophysiological responses of Acb cells evoked by electrical stimulation of either the ventral hippocampus (in particular the CA1 region) or the medial prefrontal cortex (PFC) were used as a guide for stereotaxic placement of recordings within the Acb shell or core, respectively. Stimulations (200 µsec duration, 20-100 µA intensity) were applied at a depth of 7 mm from the cortical surface for CA1 (ipsilaterally to the recorded Acb) and 3 mm for the medial PFC (ipsilaterally or contralaterally to the recorded Acb). Stereotaxic coordinates of recording and stimulating sites were determined using the atlas of Paxinos and Watson (1986).

Extracellular single unit recordings were made in the Acb using glass pipettes (15-20 MΩ) containing 1.5% neurobiotin (Nb; Vector, Burlingame, CA) in 0.5 M NaCl. The hippocampus and

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1 The anterograde tracer injections and the single-cell juxtacellular injections were performed in two different laboratories; minor differences in the surgical and staining procedures are the result of different traditions in these laboratories rather than that they represent essentially different steps in the protocols.
PFC were stimulated using a coaxial stainless steel electrode (diameter 400 µm; tip-barrel distance 300 µm). Action potentials of single neurons were recorded using the active bridge mode of an Axoclamp 2 B amplifier (Axon Instruments, Foster City, CA), amplified (1000x), filtered (0.3-3 kHz) with an AC / DC amplifier (DAM 50, World Precision Instruments, Hertfordshire, UK), and viewed on a memory oscilloscope (Tektronix, Courtaboeuf, France). Medium size spiny neurons of the Acb were clearly distinguished from fast spiking interneurons by their basic electrophysiological properties, i.e., a low rate of spontaneous firing, spike duration and a single spike discharge in response to stimulation of cortical inputs (Wilson, 1993; Mahon et al., 2001; Slaght et al., 2004; Mallet et al., 2005). During these experiments, the electrical activity of Acb cells was examined on-line and stored with a Digital Tape Recorder (DTR-1404, Biologic, Claix, France).

**Labeling of the Acb neurons**

Recorded neurons were labeled using juxtacellular injection of neurobiotin (Nb) (Pinault, 1996). Briefly, positive current pulses (1-8 nA, 200 msec duration) were applied at a frequency of 2.5 Hz through the bridge circuit of the amplifier. The current was slowly increased, and the electrode was advanced by steps of 1 µm (LSS-1000 Inchworm Motor Positioning System, Burleigh Instruments, Fishers, NY) until the cell discharge was driven by the injected current. Current pulses were applied for a 10-30 min period to obtain a reliable labeling of neuronal processes.

Two to five hours after the last injection, the animal received a lethal dose of pentobarbital (3 ml/kg, i.p.; Sanofi,Libourne, France) and Heparin (1 ml/kg, i.p.; Choay, Paris, France) before being perfused transcardially with 200 ml of 10% Ringer solution followed by 500 ml of 0.2% glutaraldehyde (Prolabo, Fontenay s/bois, France) and 4% paraformaldehyde (CARLO ERBA, Val de Reuil, France) in 0.1M NaH₂PO₄H₂O/Na₂HPO₄ (phosphate buffer, pH 7.4). In all experiments, brains were post-fixed overnight at 4°C in the same fixative solution with or without glutaraldehyde and then immersed in 30% sucrose at 4°C until sectioned. Fifty µm thick sections were cut in the horizontal plane on a sliding freezing microtome. Sections were collected sequentially in twelve receptacles containing phosphate buffer for direct processing.

**Visualization of the Acb neurons**
In order to visualize the relationships of labeled dendrites and axon collaterals with the compartmental structure of the Acb (i.e., the shell and core subregions, including their smaller compartments) sections were double-stained for Nb and CaB. In the procedures described below, all intermediate steps between different incubations included three rinses of the specifically indicated buffers for 10 min each. First, the sections were rinsed in phosphate buffer supplemented with 0.15 M NaCl (phosphate buffered saline [PBS], pH 7.4), and subsequently incubated in ABC-kit ((1:1) mixture of reagents A and B; Vector, Burlingame, CA) in PBS and 0.5% Triton X-100 (PBS-Tx; Merck) overnight at 4°C. Thereafter, a rinse with phosphate buffer was followed by staining with Cobalt enhanced DAB-Ni substrate: 0.025 g DAB, 1 ml 1% nickel-ammonium sulfate, 1.25 ml 1% Cobalt chloride, 20 µl of 30% H₂O₂ in 50 ml PB, for 10-30 min at rt. The progress of staining was frequently monitored with the aid of a light microscope. As soon as nonspecific background staining became visible, the reaction was terminated by several rinses in phosphate buffer, followed by rinsing with TBS-Tx, and incubation in mouse anti-CaB (Sigma; 1:2000) overnight at 4°C. After TBS-Tx rinse, the sections were incubated with goat anti-mouse serum (1:50; Dako, Denmark) for 60 min at rt. Following rinsing with TBS-Tx, the sections were incubated with mPAP (1:100; Dako, Denmark) for 60 min at rt. Next, a Tris HCl (pH 7.6) rinse was followed by staining with DAB (1 ml DAB [5 mg/10 ml], 9 ml Tris HCl, pH 7.6 and 3.3 µl 30% H₂O₂) for 15 min at rt. After final rinses with Tris HCl (pH 7.6), the sections were mounted on glass slides from a Tris HCl (pH 7.6) solution, containing 0.2% gelatin, and air-dried. The mounted sections were dehydrated and coverslipped as described above.

**Anatomical analysis**

*Neuronal reconstruction.* Labeled neurons, the boundaries of the Acb shell and core, and in a number of cases a patch compartment were traced and reconstructed from adjacent serial sections. Two-dimensional (2D) reconstructions were made from drawings performed under 10-40x objectives using a drawing tube attached to a light microscope. In total 35 neurons were reconstructed in 2D. The dendrites of each neuron could be traced from the cell body through adjacent sections. After all the dendritic segments had been drawn, each drawing was adjusted in such a way that it was aligned correctly with its neighbors, and the cut ends of the dendrites drawn were connected to make the reconstruction complete. Similar procedures were used to reconstruct the entire axonal field. A 3D computerized image-analysis system (Neurolucida, MicroBrightField, Inc.) was used to reconstruct the axonal and/or dendritic trajectory of a selection of neurons (11 out
of 35 labeled neurons) that correspond to the different geometries found within the Acb shell and core subregions. To achieve these 3D reconstructions, cell bodies, axonal and/or dendritic arborizations, boundaries of the Acb shell/core and, in relevant cases, a patch compartment were precisely drawn under 25-63x oil immersion objectives. Furthermore, 3D models of these neurons were visualized using the Lightwave software (Newtek Inc., San Antonio, TX). For this purpose a Perl script was developed. This script reads a Neurolucida data file and converts it into a Lightwave script, in which the X, Y, and Z polygon coordinates corresponding to each structure (i.e., cell body, dendrites, axons, Acb shell/core and patch compartment boundaries) are described as 3D objects. The shrinkage that occurs in the z-axis of the brain sections during dehydration was corrected for with the software. Models were then processed for solid surface rendering using the Lightwave software. The 3D-reconstructed models could be rotated. In addition, light sources and camera could be adjusted to enhance the 3D appearance of the reconstructed neurons when represented in 2D pictures.

**Polar histograms.** The orientation of the dendritic trees of the reconstructed neurons were analyzed by generating polar histograms of their somatodendritic morphology by calculating the maximum length of dendrite and axon in each 15°-wide sectors around the cell body in each plane of the section from Neurolucida data files in Neuroexplorer (MicroBrightField, Inc.).

**Geometrical parameters of labeled neurons in the Acb.** The orientation of the dendritic field of each labeled neuron (n =35) was determined using the 2D reconstructions. First, the dimension of the dendritic arborization along the dorsoventral axis was measured on the basis of the number of horizontal sections in which dendrites of the filled neuron were present multiplied by the thickness of the sections, taking into account the shrinkage factor. Second, the mediolateral and rostrocaudal extensions of the dendritic field were determined in the horizontal reconstructions by measuring the distance between the tips of the farthest medially and laterally or rostrally and caudally extending dendrites (black; Fig. 1A). A similar procedure was followed to measure the distribution of axon collaterals of the labeled neurons (gray; Fig. 1B). Third, we analyzed the maximum length of dendrites around the cell body in each plane of the section, in order to determine any orientation (or polarization) of the dendritic field.

**Statistical analysis.** The group of neurons (n=19) in which the entire neuron could be visualized, including the cell body, dendritic arborizations and local axon collaterals, was subdivided into three categories related to the degree to which dendritic arborizations and local axonal collaterals overlap with each other. The three categories include 1) a group of MSN with ‘full overlap’ of dendrites and local axon collaterals, 2) a group with ‘partial overlap’, and 3) a group with ‘no overlap’. To test
whether the extent to which the axonal field expands outside the dendritic field of MSN was significantly different between the Acb shell and core, we used the two-tailed nonparametric Mann-Whitney test (Table 1).

**Iontophoretic BDA injections**

The above-described juxtacellular injection experiments were supplemented by ‘classical’ tracer experiments with small injections of the tracer biotinylated dextran amine (BDA), as performed in the context of an earlier published study (Van Dongen et al., 2005). Animal surgery, anterograde tracer injections, perfusion, fixation, storage of the brain, sectioning of the brain, anterograde tracer histochemistry and double staining procedure for the tracer and calbindin-D28K DA were performed as described previously (Van Dongen et al., 2005).

**Data analysis**
Neuronal reconstructions were performed as described above. In short, labeled neurons, the boundaries of the Acb shell and core, as well as nearby located patch compartments were traced and reconstructed from adjacent serial sections. Two-dimensional (2D) reconstructions were made from drawings performed under 10-40x objectives using a drawing tube attached to a light microscope and transferred to CorelDraw 9.0 to construct the final chartings.

Final preparation of all half-tone figures, including contrast enhancement, was done using Adobe Photoshop 5.5.
Results

Characterization of the subregional and compartmental structure of the Acb

To differentiate between the Acb shell and core subregions, as well as between compartments therein, we employed the immunoreactivity patterns of the calcium binding protein calbindin-D\textsubscript{28kDa} (CaB) as an established maker (Fig. 2). In accordance with numerous previous studies (e.g. Zahm and Brog, 1992; Jongen-Rêlo et al., 1993, 1994), the Acb core subregion shows strong immunoreactivity for CaB while the Acb shell is only very weakly immunoreactive. Within the Acb core, smaller compartments of light immunoreactivity, i.e., the patches, stand out against the generally strongly stained matrix. Although it has been established that the weaker immunoreactive patches in the rostral and caudal core might have different neurochemical and connectional characteristics (Voorn et al., 1989; Groenewegen et al., 1996), for the purpose of the present study we consider all lightly stained compartments in the Acb core as belonging to the patch compartment. In view of the fact that the injected cells are relatively small and therefore present in only a few (three to six) adjacent sections of the Acb, we decided to stain only for CaB and not for other potential markers of compartments. As a result, it was not possible to differentiate between smaller compartments within the Acb shell subregion.

General features of the injected neurons

Thirty-five injected neurons were analyzed, 21 in the Acb shell and 14 in the Acb core (Fig. 2). In slightly more than half of these cases (n=19) the entire neuron could be visualized, including the cell body, dendritic arborizations and local axon collaterals, whereas the remaining neurons (n=16) showed sufficient filling of only the cell bodies and dendrites. All 35 injected neurons were identified as MSN by their morphological features. From the cell bodies of these MSN 3-6 primary dendrites radiated and strongly branched within the Acb. In the shell of the Acb, the dimensions of the dendritic tree of MSN ranged from 62 up to 375 μm rostrocaudally, 150-437 μm mediolaterally, and 150-400 μm dorsoventrally (see Table 1). In the core, these figures were 100-312 μm in the rostrocaudal dimension, 125-325 μm mediolaterally and 150-350 μm dorsoventrally (Table 1). All axons originated from the cell body forming a main stem from which several thinner collaterals with many varicosities and terminal boutons arose. The dimensions of the extending recurrent axon collaterals of MSN ranged from 275 up to 575 μm rostrocaudally, 200-450 μm mediolaterally, and 200-500 μm dorsoventrally in the Acb shell (Table 1). For MSN in the core these figures were 225-725 μm in the rostrocaudal direction, 125-675 μm mediolaterally, and 150-400 μm dorsoventrally (Table 1).
Fig. 2: Overview of the location of neurobiotin-injected neurons in the nucleus accumbens (Acb) represented in eight equally spaced horizontal sections that are ‘counterstained’ for the calcium-binding protein calbindin-D_{28KDA} to reveal the Acb shell and core subregions. The sections are arranged from dorsal (A) to ventral (H). Borders of Acb shell and core subregions are indicated with dashed lines. The transverse and sagittal planes dividing the Acb shell and core subregions into different subareas are also indicated (for a description of the criteria to position these planes, see Van Dongen et al., 2005). Each dot represents a single medium-sized spiny projection neuron (MSN) with a particular dendritic orientation. The blue dots stand for MSN with a rostral-medial to caudal-lateral orientation. The red dots represent MSN with a rostral-lateral to caudal-medial orientation. The black dots encompass MSN that can not be categorized into either the blue or red group, since these neurons have diverge orientations.

Abbreviations: ac, anterior commissure; Acb, nucleus accumbens; CB, striatal cell bridges; IC,M, major island of Calleja; LS, lateral shell; LV, lateral ventricle; OT, olfactory tubercle; vCPu, ventral caudate-putamen. Scale bar in H = 500 µm and applies to all levels.

To analyze a possible difference of the extent to which the axonal field expands outside the dendritic field of MSN between the Acb shell and core, we used the two-tailed nonparametric Mann-Whitney test, but we found no significant difference (Table 1).

Nucleus accumbens core: location and geometrical aspects of injected neurons

Location. Fourteen MSN were injected in the core. These MSN were located in the rostromedial part of the nucleus (Figs. 2 and 3). Twelve MSN were located in the matrix compartment, while two MSN were located in a patch compartment. Among the MSN located in the matrix, two MSN were lying close to a patch compartment (neurons 4L-III and 8) and four MSN were situated along the shell-core boundary (neurons 9-I, 10R-I, 10L and 5-IV). One neuron was located close to the anterior commissure (neuron 1), while the remaining MSN were distributed across the matrix of the medial core (Figs. 2 and 3; Table 1).

Geometrical aspects of injected neurons. The analyses of the 3D aspects of the injected neurons showed that the dimensions of the dendritic arborizations are not equal in all three dimensions. In other words, the geometry of the dendrites of all individual neurons showed a flattened shape and, therefore, showed a preferred orientation. Considering the orientation in the coronal plane, it appeared that five MSN showed a dendritic orientation in dorsal-medial to ventral-lateral direction, while four MSN showed a preference for the dorsal-lateral to ventral-medial direction. The remaining five MSN showed a variety of dendritic orientations. An example of a MSN located in the matrix compartment with a dorsal-medial to ventral-lateral dendritic orientation is illustrated in Figure 4.

The specific geometry of the dendritic arborizations of MSN appears to be profoundly influenced by the proximity of the shell-core boundary, a patch compartment or the anterior commissure, as illustrated in Figures 5-10. An example of a MSN close to the shell-core boundary is illustrated in Figure 5. The geometry of the dendritic arborizations of this neuron (neuron 5-IV) is...
Table 1. Distance from the shell-core boundary, extension of the dendritic and axonal fields, extent to which the axonal field expands outside the dendritic field, and orientation of the dendritic field of medium-sized spiny projection neurons in the rat nucleus accumbens shell and core.

<table>
<thead>
<tr>
<th>Neuron</th>
<th>Dist. from shell core boundary</th>
<th>Extension of dendritic field (μm)</th>
<th>Extension of axonal field (μm)</th>
<th>Axonal field expands dendritic field (μm)</th>
<th>Orientation of dendritic field</th>
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| Core   |       |       |       |       |       |       |       |       |       |       |       |       |
|--------|--------------------------------|-----------------------------------|-------------------------------|------------------------------------------|-------------------------------|
| 7R-I   | 187   | 200   | 125   | 275   | 250   | 300   | 225   | 50    | 175   | 100   | RM-CL | v     |
| 7L-I   | 187   | 150   | 250   | 200   | 550   | 300   | 200   | 550   | 300   | 200   | v     | DL    |
| 14L-I  | 125   | 300   | 275   | 225   | 350   | 255   | 125   | 50    | -     | -     | RL-CM | DM-VM |
| 5-I    | 125   | 150   | 100   | 125   | 150   | 725   | 675   | 150   | 725   | 675   | RM    | v     |
| 1      | 375   | 250   | 300   | 325   | 250   | 300   | 300   | -     | -     | -     | v     | v     |
| 9-I    | 62    | 200   | 200   | 225   | 350   | 375   | 300   | 150   | 175   | 25    | v     | v     |
| 10R-I  | 62    | 350   | 250   | 275   | 400   | 425   | 400   | 50    | 175   | 125   | RL-CM | DM-VM |
| 5-LV   | 62    | 250   | 325   | 187   | -     | -     | -     | -     | -     | -     | v     | VL    |
| 8      | 125   | 150   | 250   | 125   | -     | -     | -     | -     | -     | -     | v     | DL    |
| 10L    | 62    | 250   | 250   | 125   | -     | -     | -     | -     | -     | -     | RL-CM | DL-VM |
| 9-I    | 125   | 250   | 312   | 187   | -     | -     | -     | -     | -     | -     | RM-CL | DM-VM |
| 4L-III | 375   | 250   | 250   | 125   | -     | -     | -     | -     | -     | -     | v     | v     |
| 18L    | 250   | 200   | 250   | 125   | -     | -     | -     | -     | -     | -     | RL-CM | DM-VM |

* n = 2
v = variety of dendritic orientations
Geometry of accumbens medium spiny neurons

Fig. 3: Overview of the location of neurobiotin-injected neurons in the nucleus accumbens (Acb) represented in a coronal section that is ‘counterstained’ for the calcium-binding protein calbindin-D<sub>28KDA</sub> to reveal the Acb shell and core subregions. The border between the Acb shell and core subregions is indicated with a dashed line. The horizontal planes that correspond to eight equally spaced horizontal sections (see Figure 2) are also indicated. Each dot represents a single medium-sized spiny projection neuron (MSN) with a particular dendritic orientation. The blue dots stand for MSN with a dorsal-lateral to ventral-medial orientation. The red dots represent MSN with a dorsal-medial to ventral-lateral orientation. The black dots encompass MSN that can not be categorized into either the blue or red group, since these neurons have diverge orientations.

Abbreviations: ac, anterior commissure; AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell; LS, lateral shell; LV, lateral ventricle; OT, olfactory tubercle; vCPu, ventral caudate-putamen. Scale bar is 500 µm.

strongly asymmetric, such that the dendrites are mainly oriented parallel to and away from the boundary of shell and core, without dendrites crossing this boundary. Such features can also be observed in experiments with small BDA injections that label multiple MSN in a small cluster close to the shell-core boundary (Fig. 6A, B). Likewise, dendrites of neurons lying close to a patch compartment (Figs. 7) or to the anterior commissure (not illustrated) appear to approach, but not cross the boundaries, the main dendritic orientation diverted away from the patch or the fiber bundle. Examples of the influence exerted by the compartmental boundaries on the dendritic arborizations are illustrated with a single BDA-labeled neuron (neuron 03091) and neurons 7L-I and 4L-III (Figs. 7-9). The cell body of neuron 4L-III was situated within one of the curvatures of a patch compartment and its dendritic field curved nicely along the border of the compartment conforming to the geometry of this compartment resulting in a strongly asymmetric orientation of the dendrites related to the cell body (Fig. 8).
Fig. 4: Three-dimensional (3D) reconstruction of a single medium-sized spiny projection neuron (MSN), neuron 9-II (A-C). A, The neuron is located in the rostromedial part of the core subregion of the nucleus accumbens (Acb) and examined from a dorsal view in A, B and a lateral view in C. In blue is indicated the contour of the Acb shell. B, A zoomed view of the 3D-reconstruction of the neuron shown in A. C, The 3D reconstruction shows that the dendritic arbors of the neuron are extended from dorsal-medial-rostral to ventral-lateral-caudal.

Abbreviations: AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell. Scale bar in A= 100 µm.

Fig. 5: Three-dimensional (3D) reconstruction of a single medium-sized spiny projection neuron (MSN), neuron 5-IV (A-C). A, The neuron is located in the rostromedial part of the core subregion of the nucleus accumbens (Acb), along the shell-core boundary at a range of approximately 60µm from the shell-core border. In blue is indicated the contour of the Acb shell. B, Polar histogram of the dendritic orientation (in gray). The polar histogram shows that the dendrites extend in rostral-lateral and caudal-lateral direction. C, A zoomed view of the 3D-reconstruction of the neuron shown in A. Note that the dendritic arbors extend parallel to and away from the shell-core boundary.

Abbreviations: AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell. Scale bar is 100µm.
Fig. 6: Photomicrographs of two BDA injection sites in the nucleus accumbens (Acb) shell and core subregions in close vicinity to the shell-core boundary. A, Low power photomicrograph showing the location of the BDA injection site in the Acb core. Note that the BDA-injected neurons are oriented along the shell-core border in a slightly dorsal-medial to ventral-lateral direction. B, High power photomicrograph illustrating the high density of dendritic spines. Note that the BDA-injected neurons respect the shell-core border. C, Low power photomicrograph showing the location of the BDA injection site in the Acb shell. D, High power photomicrograph illustrating the high density of dendritic spines.

Abbreviations: ac, anterior commissure; AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell; LS, lateral shell. Scale bar in C = 250 µm and applies also to A, scale bar in D = 30 µm and applies also to B.
Nucleus accumbens shell: location and geometrical aspects of injected neurons

**Location.** Twenty-one MSN were injected in the shell. The majority of MSN were located in the medial part of the nucleus, two of these MSN being located along the shell-core boundary (Figs. 2 and 3; Table 1).

**Geometrical aspects of injected neurons.** Like in the Acb core, the dimensions of the dendritic arborizations of the MSN are not equal in all three dimensions, such that the geometry of the dendrites of all individual neurons showed a flattened shape and, therefore, showed a preferred orientation. Unlike the Acb core, the dendritic orientations of MSN in the Acb shell are more homogeneous. Considering the orientation in coronal plane, it appeared that twelve MSN showed a dendritic orientation in dorsal-medial to ventral-lateral direction, while four MSN showed a preference for the dorsal-lateral to ventral-medial direction. The remaining five MSN do not fit into these two larger categories and showed different dendritic orientations. Figure 10 illustrates an individual MSN located in the medial shell showing a dorsal-medial to ventral-lateral dendritic orientation.

Like in the Acb core, the specific geometry of the dendritic arborizations of MSN is profoundly influenced by the proximity of the shell-core boundary (Figs. 6 and 11). Thus, as illustrated in Figure 11, the geometry of the dendritic arborizations of neuron 5-V is strongly asymmetric, such that the dendrites are mainly oriented parallel to and away from the boundary of shell and core, without dendrites crossing this boundary. Such features can also be observed following small BDA injections (case 03091) that labeled a cluster of MSN in close vicinity of the shell-core border (Fig. 6C and D).
Fig. 8: Three-dimensional (3D) reconstruction of a single medium-sized spiny projection neuron (MSN), neuron 4L-III (A, B). A and B, The neuron is located adjacent to a patch compartment (gray) in the rostromedial part of the core subregion of the nucleus accumbens (Acb). Note that its dendrites arborize along the curvatures of the patch compartment and that the dendritic arbors do not cross the border between the patch and matrix. Scale bar in A = 100 µm.

Fig. 9: Photomicrographs and three-dimensional (3D) reconstruction of two medium-sized spiny projection neurons (MSN; neurons 7L-I) in a patch compartment of the rostromedial part of the core subregion of the nucleus accumbens (Acb) in a horizontal section through the Acb, double-stained for the tracer Nb and the calcium-binding protein calbindin-D$_{28KDA}$ to reveal the shell and core subregions and its compartments. A, Low power photomicrograph showing the location of the neurons in a patch compartment. B, High power photomicrograph of the two neurons, note the high density of dendritic spines. C, 3D-reconstruction of one of the neurons illustrating the distribution of its dendrites (yellow) and local axon collaterals (orange), as well as its relationship with the patch compartment. The arrow indicates the main axon leaving the Acb. Note that the dendritic arbors of the neuron extend parallel to and away from the border of the matrix compartment.

Abbreviations: AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell. Scale bar in A = 150 µm, scale bar in B = 50 µm and scale bar in C = 100 µm.
Fig. 10: Three-dimensional (3D) reconstruction of a single medium-sized spiny projection neuron (MSN), neuron 6 (A-C). A, The neuron is located in the caudomedial part of the shell subregion of the nucleus accumbens (Acb) and examined from a dorsal view in A, B and a rostral view in C. In blue is indicated the contour of the Acb shell. B, A zoomed view of the 3D-reconstruction of the neuron shown in A. C, The 3D-reconstruction illustrates that the dendrites of the neuron are oriented from dorsal-medial-rostral to ventral-lateral-caudal.

Abbreviations: AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell. Scale bar in A = 100 µm.

Fig. 11: Three-dimensional (3D) reconstruction of a single medium-sized spiny projection neuron (MSN), neuron 5-V (A-C). A, The neuron is located in the rostromedial part of the shell subregion of the nucleus accumbens (Acb), along the shell-core boundary at a range of approximately 60µm from the shell-core border. In blue is indicated the contour of the Acb shell. B, Polar histogram of the dendritic orientation (in gray). The polar histogram shows that the dendrites extend in rostral-medial and caudal-medial direction. C, A zoomed view of the 3D-reconstruction of the neuron shown in A. Note that the dendritic arbors extend parallel to and away from the shell-core boundary.
The distribution of the local axon collaterals of medium-sized spiny projection neurons in relation to the geometry of the dendrites

The recurrent axon collaterals of MSN in both the Acb shell and core subregions terminate within and outside the dendritic field of their parent neuron. Among the population of MSN examined, a considerable variability was observed in the extension of the axon collaterals. Most likely, such variability was not due to the limitations of the injection or staining procedure, since in all the selected cases the axon collaterals were strongly stained suggesting adequate filling. In addition, in most cases the main axon could be followed over several millimeters into the ventral pallidum and beyond (see Table 1 in Van Dongen et al., 2005), indicating that the animal survival time was long enough to label axons over distances greater than the length of intra-accumbens collaterals. Therefore, the observed differences in the extension of the axon collaterals most likely reflect an existing structural variability in the local axonal network of Acb MSN.

**Nucleus accumbens core.** Eight MSN located in the medial Acb core were sufficiently labeled to allow full reconstruction of their axon. Several patterns of the distribution of the local axon collaterals in relation to the dendrites were observed. The dendrites and recurrent axon collaterals showed either full (neuron 1), partial (neurons 9-I, 7R-I, 14L-I and 10R-I) or no overlap (neurons 5-I and 7L-I). The axon collaterals of neuron 5-I showed a widely distributed network extending away from the cell body up to 1 mm away from the cell of origin (Fig. 12). Two MSN with a largely overlapping distribution of dendrites and local axon collaterals, but with a difference in the extension of their axon collaterals, are illustrated in Figures 7 and 13. Neuron 7R-I shows an example of a local axonal network that extended into the adjacent subregion, i.e., the rostral pole. As can be seen in Figure 13, three axonal branches traversed the border between the shell and core (cf also Van Dongen et al., 2005). Additionally, a single BDA-injected MSN (neuron 03091) forms an example of a local axonal network that extended to an adjacent compartment (Figure 7). This neuron was closely related to a nearby located patch compartment (Fig. 7A and B). Interestingly, two axonal branches of this matrix neuron traversed the border with the patch to enter this compartment (Fig. 7C).

**Nucleus accumbens shell.** In the shell, the dendrites and recurrent axon collaterals of all 11 reconstructed MSN showed a partial overlap of dendrites and axonal collaterals. Interestingly, in most cases the axon collaterals tended to extend rostrally beyond the reach of the dendrites. A representative neuron (neuron 4L-II) is illustrated in Figure 14.
Fig. 12: Three-dimensional (3D) reconstruction of a single medium-sized spiny projection neuron (MSN), neuron 5-I (A, B). A, The neuron is located in the rostromedial part of the core subregion of the nucleus accumbens (Acb). In blue is indicated the contour of the Acb shell. Note the lack of overlap between dendrites and local axon collaterals and the widespread distribution of its axonal network. B, A zoomed view of the 3D-reconstruction of the neuron shown in A. The arrow indicates the main axon leaving the Acb.

Abbreviations: AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell. Scale bar is 100 µm.

Fig. 13: Three-dimensional (3D) reconstruction of a single medium-sized spiny projection neuron (MSN), neuron 7R-I (A, B). A, The neuron is located in the rostromedial part of the core subregion of the nucleus accumbens (Acb). In blue is indicated the contour of the Acb shell. Note that some of the local axon collaterals cross the shell-core boundary and extend into the rostral pole. B, A zoomed view of the 3D-reconstruction of the neuron shown in A. The arrow indicates the main axon leaving the Acb.

Abbreviations: AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell. Scale bar is 100 µm.
Fig. 14: Three-dimensional (3D) reconstruction of a single medium-sized spiny projection neuron (MSN), neuron 4L-II (A, B). A, The neuron is located in the rostromedial part of the shell subregion of the nucleus accumbens (Acb). In blue is indicated the contour of the Acb shell. Note that the axodendritic morphology (in yellow the dendrites and in orange the local axon collaterals) shows a partial overlap between the dendritic and axonal field and in addition a rostralward extension of some of its local axon collaterals. B, A zoomed view of the 3D-reconstruction of the neuron shown in A. The arrow indicates the main axon leaving the Acb.

Abbreviations: AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell. Scale bar is 100 µm.
Discussion

This study provides the first analysis of the spatial organization of both the dendritic and axonal arborizations of MSN in the shell and core subregions of the rat Acb. Our principal finding is that the dendritic arborizations of MSN in both the Acb shell and core subregions are preferentially oriented, i.e., that they are flattened in at least one of the 3D-planes. Moreover, the data show that the orientations of dendritic arborizations of MSN in the Acb are influenced by the shell-core boundary and they confirm earlier observations that the dendritic arborizations conform to compartmental (patch-matrix) boundaries (e.g., Gerfen et al., 1985; Bolam et al., 1988; Kawaguchi et al., 1989; Arts and Groenewegen, 1992). Thus, in the Acb shell, the dendrites of MSN situated close to the shell-core boundary are oriented parallel to or away from the border but they do not cross the border. The majority of MSN in the shell have dendritic arborizations that have a rostral-medial-dorsal to caudal-lateral-ventral orientation. In the Acb core, the dendritic arborizations of MSN likewise respect the shell-core border as well as patch-matrix boundaries. The preferred orientations of the dendrites of MSN in the core are more varied when compared to the Acb shell and the rest of the striatum. Unlike the dendrites, recurrent axon collaterals of MSN were observed to cross subregional (shell-core) and compartmental (patch-matrix) boundaries. Finally, our data also show that different degrees of overlap exist between dendritic and axonal arborizations of individual MSN, including full, partial or no overlap. This suggests various ways of communication between MSN in the ventral striatum.

Spatial organization and orientation of dendritic arborizations of MSN

It has been shown previously, both in the dorsal (Gerfen et al., 1985; Penny et al., 1988; Bolam et al., 1988; Kawaguchi et al., 1989; Yelnik et al., 1994; Onn et al., 1994) and ventral striatum (Arts and Groenewegen, 1992), that the geometry of the dendritic arborizations of MSN is influenced or limited by the borders between striatal compartments, i.e., the boundaries between patch and matrix. Our present observations in the core of the Acb confirm these findings, indicating that the dendrites of MSN remain within the compartment in which their parent cell body is located. This neuronal architecture supports the generally held view, primarily based on immunohistochemical and connectional data, that the patch and matrix form two largely segregated information processing compartments within the striatum (for reviews see Graybiel, 1990; Gerfen, 2004). However, the present study extends these observations in showing that MSN in either of the two larger subregions of the Acb, i.e., the shell and core, likewise retain their dendrites within the subregion in which the cell body resides. Like the dendrites of neuronal cell bodies that lie close to
the border between patch and matrix, the dendrites of MSN in close proximity of the shell-core boundary tend to run in parallel to or are oriented away from this boundary. For neurons situated close to the boundary, this results in a very asymmetric and in some cases strongly flattened shape of the dendritic arborizations. The fact that the dendrites of MSN in shell or core do not cross the border between these two subregions of the Acb supports the notion that the shell and core constitute two morphologically and functionally largely segregated ‘compartments’ of the Acb. The distinction between shell and core on the basis of (immuno)histochemical and connectional criteria has been described extensively since its recognition by Zaborszky and colleagues (1985; for reviews, see Groenewegen et al., 1991; Zahm, 1999). Likewise, the functional differentiation between shell and core has received extensive coverage in the literature (for reviews, see Cardinal et al., 2002; Di Chiara, 2002; Kelley, 2004). Our present findings suggest that it is unlikely that MSN in either Acb subregion are able to pick up information from incoming fibers in the adjacent subregion. This supports the idea of parallel and independent processing of information in both the shell and core to be transmitted to their respective segregated target areas in the basal forebrain, diencephalon and mesencephalon (Groenewegen et al., 1990). As will be discussed below, recurrent axon collaterals of MSN in either core or shell might cross the border between the two subregions or the compartments therein and yet in this way providing a way of mutual influences at the striatal level (see also Van Dongen et al., 2005).

Apart from the direct influence of compartmental or subregional boundaries on the orientation and geometry of the dendritic arborizations of MSN that are situated in close proximity to such boundaries, the 3D reconstructions of most of the injected MSN in the present study show that the dendritic fields of MSN are flattened and that they show a preferred orientation. In the shell subregion more than half of the neurons have a dendritic field that is preferentially oriented from rostral-dorsal-medial to caudal-ventral-lateral. Another, less numerous population of MSN shows a preferred orientation from dorsolateral to ventromedial. In the core the preferred orientations appear to be more heterogeneous with about one-third having an orientation from dorsomedial to ventrolateral, one-third from dorsolateral to ventromedial and the remaining neurons with a variety of orientations. It must be emphasized that the majority of neurons in the shell are located in the medial part. In this part of the shell the afferent fibers from the ventral hippocampus enter, through the lateral septum, from a dorsal and medial position to run in ventral, lateral and rostral direction (Groenewegen et al., 1987). The amygdaloid afferents from the magnocellular basal amygdaloid nucleus enter the medial shell, through the bed nucleus of the stria terminalis, from caudal, medial and dorsal and run in rostral, lateral and ventral direction (Wright et al., 1996). In other words, the
preferred orientation of the dendritic arborizations is more or less in parallel with the main afferent inputs of the medial shell of the nucleus accumbens. This might be interpreted such that the dendrites of these neurons pick up information from a relatively restricted set of afferent fibers that make a maximal traverse through the dendritic arbors of individual neurons. Such an arrangement may lead to more contacts from a restricted afferent source, i.e., a form of convergence, as compared to the situation for the smaller population of MSN with a preferred dendritic arborization from dorsolateral to ventromedial. These dendritic fields tend to be oriented more or less perpendicular to the main stream of incoming fibers and such an arrangement could result in fewer contacts with individual neurons but also with a larger number of neurons, i.e., a form of divergence. Whether these differences in arrangement of incoming fibers versus orientations of dendritic arborizations make a real difference with respect to the number of contacts being made by single axons from hippocampal or amygdaloid origin with individual MSN in the medial shell remains to be established.

Within the core of the Acb the preferred orientations of dendritic arborizations of MSN show more variations. This might well be related to the less ordered input into this part of the ventral striatum and the complex 3D curvature of the shell-core boundary. Thus, although more dorsal parts of the core merge with the caudate-putamen complex and contains the typical patch-matrix structure and orientation of the dorsal striatum, the more ventral, lateral and rostral parts of the core are closely associated with the ventral and lateral shell, and the rostral shell (rostral pole) that curve around the core subregion. Whereas the orientation of afferent fiber systems in the caudate-putamen complex and the dorsal core is from dorsomedial to ventrolateral (cf. Berendse and Groenewegen, 1990; Berendse et al., 1992; Wright et al., 1996; Voorn et al., 2004), the orientation of afferents to the ventral and rostral parts of the core is less well ordered.

In the head of the primate caudate nucleus, Walker et al. (1993) have shown that there exists a predominant dorsomedial to ventrolateral orientation of the dendritic arborizations of MSN, an orientation that parallels the main orientation of the striosomes in this part of the striatum. Desban et al. (1993) have demonstrated that the architecture of the patch network in the rat caudate-putamen likewise exhibits an overall dorsomedial to ventrolateral orientation. However, our results reveal that the preferred dendritic orientation of only a minority of the MSN in the Acb core conforms to this orientation. This might well be related to a somewhat more complex spatial orientation of the compartments and, as discussed above, a less ordered organization of afferents in this part of the striatum.
**Relationships of MSN recurrent axon collaterals with subregional and compartmental boundaries and with the dendritic arborizations**

While there are quite some studies describing the morphology of the dendrites and the 3D-geometry of the dendritic arborizations of MSN in the striatum, it has clearly been more difficult to fill the very delicate recurrent collateral, intrastriatal axonal ramifications of these neurons. In the present study, in slightly more than half of the neurons that were juxtacellularly injected, both dendrites and axons were sufficiently filled to allow adequate visualization of these fine intrastriatal axons. For the majority of these neurons the main axon exiting the striatum and terminating in the ventral pallidum or ventral mesencephalon could be identified (see also Van Dongen et al., 2005), indicating the adequacy of the labeling procedure. The present study reveals two interesting aspects with respect to the recurrent axon collateral network of MSN in the ventral striatum. First, in contrast to the dendrites of MSN, recurrent axon collaterals of these neurons may cross subregional (shell-core) or compartmental (patch-matrix) boundaries in the ventral striatum and in this way interconnect these functionally different subregions or compartments. Indications for patch-matrix communication on the basis of axon collaterals of the MSN projection neurons has been very sparse up till now. Kawaguchi et al. (1989), on the basis of intracellular injections of biocytin in *in vitro* slices of the dorsal striatum, concluded that both dendrites and axons of MSN respect the boundaries between patch and matrix. While Onn et al. (1994), using *in vivo* intracellular labeling, describe main projection axons of MSN located in the matrix of the dorsal striatum that cross patches on their way to the pallidum, these authors explicitly state that these axons do not show terminations in these patches. In a previous study from this laboratory, using intracellular injections of Lucifer Yellow in lightly fixed slices of the ventral striatum, a single MSN in the matrix could be identified that had extensive axon collaterals in the adjacent patch compartment (Arts and Groenewegen, 1992). The results of the present study confirm these observations in showing that in the core of the Acb axons of labeled MSN may enter and terminate in a nearby patch. It must be emphasized, however, that intercompartmental connections on the basis of MSN projections are scarce and intercompartmental connections might be more prominent for interneurons with long axon collaterals, such as cholinergic (Meredith et al., 1989; Kawaguchi, 1993) and somatostatin interneurons (Chesselet and Graybiel, 1986). The axons of the large aspiny cholinergic interneurons, however, tend to respect compartmental boundaries (Meredith et al., 1989; Kawaguchi, 1993). This has been established most directly with intracellular labeling of individual neurons in the dorsal striatum (Kawaguchi, 1993). With immunohistochemical studies, Chesselet and Graybiel (1986) have demonstrated that somatostatin interneurons might cross compartmental
boundaries. The parvalbumin and calretinin interneurons may also be involved in patch-matrix intercommunication, as demonstrated by immunohistochemical studies (Cowan et al., 1990; Fortin and Parent, 1994). As discussed previously (Van Dongen et al., 2005), MSN recurrent axon collaterals might also be the substrate for communication between shell and core of the Acb. Although these projections are bidirectional, as also shown in the present study, there appears to be dominance for core-to-shell projections when the results of larger tracer injections are taken into account.

The second interesting aspect revealed in the present study concerns the distribution of the recurrent axonal network in relationship to the dendritic arborizations of the same striatal projection neuron. Principally, three patterns were distinguished: 1. MSN with a distribution of axon collaterals largely falling within the space of the dendritic distribution (‘full overlap’); 2. MSN with axonal collaterals extending beyond the reaches of the dendritic arborizations, but with a considerable degree of overlap (‘partial overlap’); 3. MSN with recurrent axon collaterals occupying a 3D-space that showed no or only minimal overlap with the dendrites of the same neuron (no overlap). In the shell subregion, all injected neurons belonged to the second category, indicating that the axon collaterals of MSN in this part of the Acb in principle reach other shell neurons primarily within but also beyond the ‘receptive’ sphere of their dendrites. In the core subregion, neurons of all three categories were identified. One neuron with no overlap between dendrites and axon collaterals exhibited a widely distributed axonal network extending up to 1 mm away from the parent cell body. In particular the findings in the core are largely in agreement with those of earlier studies in the dorsal striatum. Both Bishop et al. (1982) and Kawaguchi et al. (1990) distinguished two categories of striatal projection neurons. One category consists of neurons with axons that are largely overlapping the dendritic field of the same neuron. Within this category several subtypes were described on the basis of their extrinsic projections to pallidal and mesencephalic targets. A second category consists of neurons with axons that have a wide distribution of their axon collaterals, up to 1 – 2 mm within the striatum, and that do not project beyond the pallidum (Kawaguchi et al., 1990). Like in our sample, neurons in the latter category were far less numerous than MSN with short range recurrent axonal fields. Whether MSN with longer ranging axon collaterals exist within the Acb shell remains uncertain; the present sample of juxtacellulary filled neurons does not include neurons of that category.

Intrastriatal communication: interactions between MSN?
Medium size spiny projection neurons (MSN) are the most prevalent neuronal cell type in both the dorsal and ventral striatum, by far outnumbering the smaller and heterogeneous population of striatal interneurons. MSN with their extensive ‘receptive’ surface in the form of dendrites and spines, represent the neuronal substrate *par excellence* for the integration of information from functionally different cortical areas, limbic structures, and midline and intralaminar thalamic nuclei in the striatum. These inputs arrive mainly on the heads of the dendritic spines of MSN while the transfer of this information, that is excitatory in nature, can be modulated by dopaminergic inputs and contacts from striatal interneurons. Dopaminergic fibers make contact on the neck of the spines or on the dendritic shafts while the terminals of cholinergic and GABAergic interneurons are positioned more proximally on dendritic shafts and the perikaryon of the MSN (reviews, e.g. Smith and Bolam, 1990; Gerfen, 2004; Tepper et al., 2004). As discussed above, it has long been known that MSN have an extensive local recurrent collateral axonal network (Ramón y Cajal, 1911; Preston et al., 1980; Bishop et al., 1982; Chang and Kitai, 1985) and on the basis of ultrastructural studies, it has been demonstrated that these axons make inhibitory synaptic contacts on the dendritic shafts of neighbouring MSN (Meredith et al., 1993). Thus, the inhibitory inputs from spatially closely related MSN appear to be in a strategic position on the dendrites of a MSN to influence the transfer of information from the dendrites to the cell body. While it has been notoriously difficult to show functional synaptic contacts between MSN with electrophysiological methods (e.g., Jaeger et al., 1994; see also Tepper et al., 2004), several recent studies have confirmed their functional existence in both the dorsal (Tunstall et al., 2002; Czubayko and Plenz, 2002; Venance et al., 2004) and ventral striatum (Taverna et al., 2004). Taverna et al. (2004), using dual whole cell patch-clamp recordings in acute slices of the Acb, showed that no less than 34% of the patched pairs of MSN had synaptic connections, most of them uni-directionally. It could further be shown that the connections between MSN exhibit various forms of short-term synaptic plasticity and that the functional connectivity can be modulated by dopamine (Taverna et al., 2004, 2005; also Czubayko and Plenz, 2002).

In theories of striatal functioning, synaptic connections between MSN have been interpreted as being important for striatal learning and selection mechanisms through lateral inhibitory processes. Lateral inhibition between MSN is thought to play a role in the interactions between collections or ‘ensembles’ of neurons that are influenced by different sets of cortical inputs and code for competing outputs of the striatum (e.g., Pennartz et al., 1994; Plenz, 2003; Tepper et al., 2004). In this way, lateral inhibition between MSN would support a ‘winner take all’ mechanism in the selection of striatal outputs. Recent insights in the role of an important class of inhibitory striatal
interneurons, i.e., the fast-spiking GABAergic interneurons that are driven by excitatory cortical inputs and have a much more powerful inhibitory influence on MSN than recurrent collaterals of MSN themselves, have again questioned the significance of MSN-MSN interactions (Tepper et al., 2004). However, as argued by Plenz (2003), the inhibitory mechanisms of fast-spiking interneurons and collateral connections between MSN could both play a distinctive role in the striatal network dynamics that underlie timing and selection of cortical information that is processed through the basal ganglia circuitry. Thus, even though the direct electrophysiological effect of MSN inputs on adjacent neurons might not be as strong, the consistent findings that these MSN-MSN connections exhibit several forms of short-term plasticity and are influenced by dopamine suggests a role for such interactions at the striatal microcircuit level (Taverna et al., 2004, 2005; also Czubayko and Plenz, 2002), a role that has still to be further explored. In further considering the functional role of connections between MSN, the results of the present study with respect to the distribution of recurrent axon collaterals of MSN in the ventral striatum should be taken into account. First, most theoretical models depart from the assumption that the geometry of the axonal arborizations of MSN largely concurs with the dendritic geometry of the parent cell body. Our study, in agreement with Kawaguchi et al. (1990), shows that such congruence is not always the case and that there exist MSN with long-range axonal terminations and several forms of non-overlapping axonal and dendritic arborizations. This could imply that such recurrent axon collaterals connect functionally different sets MSN and provide a way of interaction or competition between such populations of neurons. Important in this context is the demonstration of directionality in the intrastriatal connections as described in our previous study (Van Dongen et al., 2005) and confirmed in the present study at the single cell level. Second, our findings of recurrent axon collaterals of MSN that cross the patch-matrix and the shell-core borders emphasize and strengthen the idea that such collaterals play a role in the interaction between functionally different compartments or ‘ensembles’ of neurons (cf also Pennartz et al., 1994 and Van Dongen et al., 2005).

References


Geometry of accumbens medium spiny neurons


Geometry of accumbens medium spiny neurons


Chapter 4

A subpopulation of mesencephalic dopamine neurons interface the shell of the nucleus accumbens and the dorsolateral striatum in rats

Co-authors:

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A SUBPOPULATION OF MESENCEPHALIC DOPAMINE NEURONS INTERFACE THE SHELL OF THE NUCLEUS ACCUMBENS AND THE DORSOLATERAL STRIATUM IN RATS

Nigro-striatal dopaminergic neurons are usually considered to interface the ventral limbic and dorsal sensorimotor striatum, since the shell of the nucleus accumbens (Acb shell) projects to the ventral tegmental area/substantia nigra pars compacta (VTA/SNC) complex. However, both the organization of Acb shell projections to the nigro-striatal neurons innervating the sensorimotor striatum and the synaptic influence exerted by the Acb shell on these neurons remain to be determined. These questions were addressed in the rat using neuroanatomical and electrophysiological approaches.

Combined anterograde tracing from the Acb shell with retrograde tracing from the sensorimotor region of the dorsal striatum revealed that labeled fibers from the Acb shell overlap retrogradely labeled nigro-striatal neurons located in the medial SNC and the lateral VTA but avoid the nigro-striatal neurons located laterally. In addition, stimulation of the Acb-shell induced an inhibition of dopaminergic nigro-striatal neurons projecting to the sensorimotor striatal territory. In agreement with the anatomical observations, these responses were observed in nigro-striatal neurons located in the medial SNC and the lateral VTA but not in nigro-striatal neurons located laterally.

These data further establish the existence of a functional link between the Acb shell and the sensorimotor striatum via dopaminergic nigro-striatal neurons. The present study also reveals that among the dopaminergic nigro-striatal neurons innervating the sensorimotor striatal territory, only the subpopulation located in the medial SNC and lateral VTA receives an inhibitory input from the Acb shell. This indicates a functional heterogeneity within the population of dopaminergic neurons innervating a given striatal territory.

Keywords: substantia nigra, electrophysiology, ventral tegmental area, ventral striatum, neuroanatomical tracing
DA neurons interface limbic and motor striatum

Introduction

The striatum transmits cortical signals to the basal ganglia output nuclei, i.e., the substantia nigra pars reticulata (SNR) and the internal segment of globus pallidus. Growing evidence indicates that signals originating from functionally distinct cortical areas are processed in separate striatal territories and remain segregated in the striato-nigral and striato-pallidal pathways, supporting the concept of parallel cortico-basal ganglia circuits (Alexander & Crutcher, 1990; Groenewegen et al., 1999; Deniau & Thierry, 1997; Mailly et al., 2001). Classically, the dorsal striatum is associated with sensorimotor and cognitive processes, the ventral striatum with motivation and reward.

Dopaminergic (DA) fibers from the ventral tegmental area/substantia nigra pars compacta complex (VTA/SNC) innervate both dorsal and ventral striatum. Based on observations that the ventral striatum innervates the VTA/SNC complex, Nauta et al. (1978) first proposed that DA neurons constitute an interface between the limbic and extrapyramidal motor systems. Corroborating this hypothesis, fibers from the ventral striatum were shown to establish synaptic contacts on dendrites of nigral neurons projecting to the dorsal striatum (Somogyi et al., 1981). However, the functional-anatomical properties of interactions between the limbic and sensorimotor domains of the striatum via DA nigro-striatal neurons remain to be determined.

Histochemical and anatomical studies have now established that the major component of the ventral striatum, the nucleus accumbens (Acb), comprises two main subdivisions, the core and shell, that present distinctive connectional characteristics (Heimer et al., 1997). Like the dorsal striatum, the Acb core receives major inputs from the cerebral cortex, i.e., the medial and lateral prefrontal areas, and projects to the subcommissural ventral pallidum and the SNR. The Acb shell receives major inputs from hippocampus and amygdala and innervates predominantly the VTA/SNC complex in addition to the ventral pallidum (Heimer et al., 1997; Groenewegen et al., 1999; Wright et al., 1996; Montaron et al., 1996; Maurice et al., 1997, 1998). On the other hand, a recent comparative 3D analysis of the striato-nigral and nigro-striatal projections in the rat revealed that each functional territory of the dorsal striatum is innervated by two main subpopulations of VTA/SNC neurons (Maurin et al., 1999). The first population, located in the SNC and denominated "proximal", occupies a position in register with the striato-nigral projections in the subjacent SNR. Such a close spatial relationship suggests that this subpopulation may be involved in a reciprocal striato-nigro-striatal feedback circuit. The second population, denominated "distal", is located more medially and dorsally in the SNC and VTA, and is likely involved in non-reciprocal connections with the dorsal striatum.
Chapter 4

The aim of the present study was to investigate to which extent the shell exerts a synaptic influence on these two subpopulations of nigro-striatal neurons which innervate the sensorimotor dorsal striatum. For this purpose injections of an anterograde tracer into the Acb shell were combined with injections of a retrograde tracer into the dorsolateral striatal territory innervated by the sensorimotor cortical areas. In addition, the effects of electrical stimulation of the Acb shell on DA nigro-striatal neurons identified as projecting to the sensorimotor territory of the dorsolateral striatum were determined.
Experimental procedures

Anatomical tracing studies

Animals

All experimental procedures were in accordance with the European Community Council Directive 86/609/EEC.

Surgical procedures

A total of 15 female Wistar rats weighing 180-240 gm (Harlan/CPB, Zeist, Netherlands) were used. Animals were anaesthetized with a 4:3 parts mixture of a 1% solution of ketamine (Aesco, Boxtel, Netherlands) and a 2% solution of xylazine (Bayer, Brussels, Belgium) by intramuscular injections (1ml/kg). During surgery, anesthesia was maintained by additional doses of the same solution, while body temperature (36-37 °C) was maintained by a homeothermic mat. Anaesthetized animals were mounted in a stereotaxic frame. Local anesthetics were injected under the skin of the head at the site of incision. Tracers were injected using coordinates from the atlas of Paxinos & Watson (1986).

Tracer injections

The anterograde tracer biotinylated dextran amine (BDA, 10.000 MW, Molecular Probes, Eugene OR) and the retrograde tracer Fluorogold (FG, Fluorochrome, Denver CD) were iontophoretically delivered through glass micropipettes (external diameter 3 µm for BDA and 15 µm for FG) using a positive pulsed current of 1 µA (7 sec on/off) for BDA and 2.5 µA (7 sec on/off) for FG, employing a constant current source (Midgard CCS-3, USA). Pipettes were filled with either 5% BDA in 0.1 M NaH₂PO₄/Na₂HPO₄ (phosphate buffer [PB], pH 7.4) or 2% FG in 0.1 M cacodylate buffer (pH 7.3). Tracer delivery lasted 2 min for BDA and 5 min for FG.

Histological procedures

Following a 7 days postoperative survival period, animals were deeply re-anaesthetized with sodium pentobarbital (60 mg/kg body weight, i.p.; Ceva, Paris, France) and perfused transcardially with 0.9% saline, followed by a fixative containing 4% paraformaldehyde (Merck, Darmstadt, Germany) and 0.05% glutaraldehyde (Merck-Schuchardt, Hohenbrunn, Germany) in PB (0.1 M, pH
7.4) for 15 min. Brains were post-fixed for 1.5 h and cryoprotected by storage for 18-48 h at 4°C in a mixture of 20% glycerin (Merck) and 2% dimethyl sulfoxide (DMSO; Merck) in PB (0.1M, pH 7.4). Horizontal or transverse 40 µm sections were cut on a sliding microtome. Sections were collected sequentially in six vials containing either PB (0.1 M, pH 7.4) for direct processing, or a mixture of glycerin/DMSO for storage at –20°C.

**Double staining of BDA and FG**

Sections to be double-stained for BDA and FG were rinsed with PB (0.1 M, pH 7.4) followed by a rinse in 0.05 M Tris/HCl (Merck) supplemented with 0.15 M NaCl, pH 7.6 (TBS) and 0.5% Triton X-100 (TBS-Tx; Merck). They were incubated with rabbit anti-FG (Chemicon, Temecula CA; 1:5000) overnight at 4°C. Subsequently, the sections were stained for BDA with nickel-enhanced dianaminobenzidine substrate: 7.5 mg diaminobenzidine-tetrahydrochloride (DAB; Sigma, St. Louis, MO), 0.225 g nickel-ammonium sulfate (Boom, Meppel, Netherlands), 10 µl 30% H$_2$O$_2$ in 50 ml TrisHCl (pH 8.0) for 10-30 min.

After rinsing with TBS-Tx, sections were incubated with swine anti-rabbit serum (Dako, Glostrup, Denmark; 1:100) for 60 min at room temperature. Following a rinse with TBS-Tx, the sections were incubated with rabbit peroxidase-antiperoxidase (Dako, Denmark; 1:800) for 60 min at room temperature. Finally, a TrisHCl rinse (pH 7.6) was followed by staining with DAB: 1 ml DAB [5 mg/10 ml], 9 ml Tris HCl, pH 7.6 and 3.3 µl 30% H$_2$O$_2$, for 15 min.

**Combination of BDA and calcium binding protein calbindin-D$_{28kDA}$**

In order to determine the site of the BDA injections in relationship to either Acb shell or core, we performed a double-staining for BDA and the calcium binding protein calbindin-D$_{28kDA}$ (CaB). The immunohistochemical staining followed exactly the procedures described by Wright et al. (1996).

**Mounting and coverslipping**

Sections were mounted on glass slides from a Tris-HCl (pH 7.6) solution, containing 0.2% gelatin (Oxoid LTD, Hampshire, UK). The mounted sections were dehydrated through an alcohol
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gradient (50%, 70%, 80%, 2x 96%, followed by 2x 100%) and coverslipped from the xylene using Entellan (Merck).

Electrophysiological studies

Experiments were performed on 18 adult male Sprague Dawley rats (275-300 gm) anesthetized with ketamine (100 mg/kg, i.p., supplemented every hour by 50 mg/kg i.m. injections; Imalgène 500, Rhone-Mérieux, Courbevoie, France) and fixed in a stereotaxic frame (Horsley-Clark; Unimécanique, Epinay-sur-Seine, France). Body temperature was monitored rectally and maintained at 36-38°C with a homeothermic warming blanket (Harvard Apparatus, Kent, UK).

Electrical stimulation of the Acb shell (A: 10.4; L: 0.8; H: 7.4 mm from the cortical surface) and of the sensorimotor territory of the dorsal striatum (A: 9.2; L: 3.8; H: 7.4 mm from the cortical surface) was conducted with coaxial stainless steel electrodes (diameter 400 μm; tip-barrel distance 300 μm) positioned stereotaxically (Paxinos & Watson, 1986). Electrical stimuli consisted of pulses of 0.6 ms width and 200-600 μA intensity delivered at a frequency of 1 Hz.

Single-unit activity of VTA/SNC cells ipsilateral to the stimulation sites was recorded using glass micropipettes (6-10 MΩ) filled with a 0.6M NaCl solution containing 4% Pontamine Sky Blue. Action potentials were amplified and displayed on a memory oscilloscope. Spikes were separated from noise using a window discriminator and sampled on-line using a CED 1401 interface (Cambridge Electronics Design, Cambridge, UK) connected to a computer. Peristimulus time histograms were generated from 100 to 200 stimulation trials using 1 ms bins. The duration of the inhibitory response corresponds to the time during which no spike was observed. DA cells projecting to the dorsal striatum were identified using their classically defined electrophysiological characteristics: large duration spikes > 2 ms, low discharge frequency < 8 Hz and latency of the antidromic spike evoked from stimulation of the dorsal striatum (Deniau et al., 1978; Guyenet & Aghajanian, 1978). The antidromic spikes were characterized by their fixed latency and their collision with spontaneous discharges within an appropriate time interval. In four rats, the conduction time of the shell-VTA/SNC pathway was determined from the latency of the antidromic spikes evoked in shell cells following stimulation of the VTA/SNC complex (A: 3.8; L: 1.3; H: 7.9 from the cortical surface).
At the end of each recording session, the tip of the stimulating electrode was marked by a deposit of iron (15 μA anodal, 20 sec) and localized in histological sections after a ferri-ferrocyanide reaction. The tip of the recording electrode was marked with Pontamine Sky Blue (8 μA cathodal, 20 min) allowing the determination of the recorded cells. Brains were removed and fixed in a 12% formalin solution, and the positions of electrodes were identified on serial frozen sections (100 μm) stained with safranin.
Results

Anatomical tracing studies

Following FG injections centered in the orofacial sensorimotor part of the dorsal striatum (Deniau et al. 1996, Deniau & Thierry, 1997), verified by the presence of retrograde labeling in the sensorimotor cortex (Figure 1A-D), retrogradely labeled neurons in the ventral mesencephalon were observed virtually over the entire mediolateral extent of the SNC as well as more medially in the dorsolateral part of the VTA. However, there was a clear difference in the strength of the labeling of neurons in different parts of the VTA/SNC complex. In the dorsal tier of the SNC densely labeled neurons were intermingled with more weakly labeled cells, while in the most medial aspects of the SNC and in the VTA retrogradely labeled neurons were in general only lightly labeled. The relationships between the distribution of anterogradely labeled fibers and terminals originating in the medial Acb shell and retrogradely labeled neurons following a FG injection in the dorsal striatum are shown in Figure 1G for a representative case. As illustrated, an extensive overlap between anterograde and retrograde labeling was observed in the dorsal parts of the medial SNC and the lateral VTA. In this region, frequent close appositions between retrogradely labeled, FG-containing neuronal cell bodies or dendrites and varicosities or boutons of anterogradely filled, BDA-containing fibers could be observed (Fig. 1E, F). By contrast in more lateral aspects of SNC, the anterogradely labeled fibers were mostly segregated from the retrogradely labeled nigro-striatal neurons, these cells occupying a ventral position with respect to the labeled fibers.

Effect of shell stimulation on the activity of VTA/SNC dopaminergic neurons projecting to the sensorimotor territory of the dorsal striatum

In 14 rats, the responses evoked by electrical stimulation of the shell of the Acb were investigated in 152 cells antidromically driven from the orofacial sensorimotor territory of the dorsal striatum (Deniau et al. 1996, Deniau & Thierry, 1997). These cells were characterized as DA on the basis of their long duration action potential (> 2 ms), a relatively low spontaneous activity (< 8 Hz) and a mean latency of antidromic activation of 11.7 ± 0.2ms. The cells antidromically driven from the dorsal striatum were located throughout most of the medio-lateral extent of the SNC and the lateral part of the VTA. In addition, 15 other cells were antidromically driven from the shell; these cells were located more medially in the VTA.
Electrical stimulation of the shell induced an inhibitory response in 69 of the 152 cells antidromically driven from the dorsal striatum. The evoked response had a mean latency of $17.8 \pm 0.9$ ms and mean duration of $38.8 \pm 3.0$ ms (Fig. 3A, B). As shown in figure 3C, these cells were located in the medial SNC and the lateral part of the VTA mainly dorsally.

In order to determine the conduction time of the shell-VTA/SNC pathway, the latency of the antidromic responses evoked in the shell (41 cells) by stimulation of the VTA/SNC complex was determined. The mean latency of the antidromic responses was $16.7 \pm 0.2$ ms (range: 13 – 20 ms).
Fig. 2: Inhibitory effect evoked by stimulation of the Acb shell in electrophysiologically identified DA nigrostriatal neurons projecting to the sensorimotor striatal region. 

A. Electrophysiological identification of a nigrostriatal neuron by antidromic activation following an electrical stimulation applied in the orofacial sensorimotor territory of the dorsolateral striatum. Upper trace: fixed latency (17 ms) of the antidromic response. Arrow indicates the time of application of the stimulation. Middle and lower trace: collision test. Note the lack of the antidromic response (star) when the striatal stimulation is preceded by a spontaneous discharge of the neuron upon an appropriate time interval. 

B. Inhibitory response of this nigrostriatal neuron to stimulation of the Acb shell. Arrow indicates the time of application of the stimulation. 

C. Distribution within the VTA/SNC complex of the neurons antidromically activated from the dorsolateral striatum and either inhibited by stimulation of the Acb shell (filled circles) or uninfluenced by the Acb shell stimulation (open circles). Open triangles indicate the location of neurons antidromically activated by stimulation of the Acb shell.

Abbreviations: A, anterior; cp, cerebral peduncle; SNR, substantia nigra pars reticulata.
Discussion

The present anatomical and electrophysiological data show that 1) the Acb shell, a major component of the limbic striatum, exerts an inhibitory influence on DA nigro-striatal neurons that project to the sensorimotor territory of the dorsolateral striatum and 2) this inhibitory influence is addressed to the subpopulation of nigro-striatal neurons located in the medial SNC and lateral VTA and not to the nigro-striatal neurons located more laterally. These data provide the first evidence for a direct functional link between the limbic ventral striatum and the sensorimotor dorsolateral striatum and stress the functional heterogeneity of the population of DA nigro-striatal neurons innervating the sensorimotor domain of the striatum.

Mesencephalic dopamine neurons as a link between the shell and the dorsolateral striatum

Various earlier studies indicate that mesencephalic projections from the Acb shell innervate the VTA/SNC complex (Nauta et al., 1978; Heimer et al., 1991). Accordingly, following injections of the anterograde tracer BDA within discrete regions of the Acb shell, labeled fibers and terminals distributed primarily among DA neurons of both the VTA and dorsomedial SNC (Groenewegen et al., 1994). The organization of the VTA/SNC neurons that innervate distinct functional territories of the rat dorsal striatum has now been clarified (Maurin et al., 1999). This organization obeys to complex three dimensional rules that cannot be systematized on a simple point to point topographical basis. As revealed by combined anterograde and retrograde tracing, each functional territory of the dorsal striatum receives an innervation from a neuronal population largely distributed along the mediolateral axis of the VTA/SNC complex. As a rule the most dense and largest neuronal contingent lies in proximal position with respect to the corresponding striato-nigral projection field, in register with the labeled striato-nigral fibers. The lateral extension of this contingent of “proximal” neurons never transcends the lateral edge of the corresponding striato-nigral projections. A less numerous contingent of neurons extends medially in the SNC and the adjoining VTA. This “distal” subpopulation of neurons does not follow topographical rules. Consequently, the “distal” contingent of nigro-striatal cells innervating different functional territories of the dorsal striatum intermingle within the medial SNC and lateral VTA. Interestingly, following combined injections of a retrograde tracer centered in the orofacial territory of the sensorimotor striatum and of an anterograde tracer in the Acb shell, labeled fibers were observed among retrogradely labeled neurons located in the medial SNC and the adjoining VTA corresponding primarily to the location of the "distal" population of nigro-striatal neurons. By
contrast, the retrogradely labeled neurons localized in the lateral SNC corresponding to the “proximal” subpopulation of nigro-striatal neurons was devoid of anterogradely labeled Acb shell fibers.

A further confirmation of a functional link between the Acb shell and the VTA/SNC neurons that project to the dorsal striatal sensorimotor territory is derived from the findings that an important proportion of electrophysiologically identified DA neurons antidromically activated from the orofacial striatal territory presented an inhibitory response to shell stimulation. In agreement with the anatomical data, these cells were mainly located in the medial SNC and lateral VTA suggesting that the Acb shell exerts an inhibitory influence preferentially on the "distal" subpopulation of nigro-striatal neurons. Whether indeed the shell targets this subgroup as identified by Maurin et al. (1999) awaits further investigation. The inhibitory responses evoked by Acb shell stimulation are likely monosynaptic since their latency is consistent with the conduction time of the Acb shell-VTA/SNC pathway (present study). In addition, it is known that efferent neurons from the Acb shell are GABAergic (Ferraguti et al., 1990). Finally, VTA/SNC cells antidromically driven from the dorsal striatum and inhibited by Acb shell stimulation presented the electrophysiological characteristics of dopaminergic neurons (Deniau et al., 1978, Guyenet & Aghajanian, 1978). A further, rigorous test of their dopaminergic nature will be to apply apomorphine or D2 receptor agonists, known to inhibit these neurons. Taken together, these data suggest that the "distal" subpopulation of dopaminergic neurons and not the “proximal” one provides a functional link between the Acb shell and the dorsolateral striatum. These observations also support the notion that the “proximal” and “distal” subpopulations of nigro-striatal neurons constitute functionally distinct sets of neurons involved in different regulatory processes. Although the present anatomical and electrophysiological study was focused on the neurons of the VTA/SNC innervating the orofacial sensorimotor territory of the dorsal striatum, it is likely that a similar link exists between the Acb shell and the distal subpopulations of neurons innervating other sensorimotor districts of the dorsal striatum since these subpopulations share the same spatial distribution in the medial part of the VTA/SNC complex (Maurin et al., 1999).

**Functional considerations**

The parallel organization of the corticostriatal circuits allows for the simultaneous processing of various kinds of sensorimotor, cognitive and motivational information in the basal
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ganglia. However, for producing a coherent behavior, a communication between these specific circuits is required. In monkeys, anatomical studies have revealed that striato-nigro-striatal relationships comprise both reciprocal and non-reciprocal components suggesting that the DA system is involved in both feedback and feedforward loops with the striatum. On the basis of the arrangement of feedforward connections, Haber et al. (2000) have proposed that "information from the limbic system reaches the motor system through a series of connections" that form an ascending spiral from the Acb shell to the dorsolateral striatum. In this functional scheme, the limbic and motor circuits of the basal ganglia would be linked through an ordered sequential chain of events proceeding successively from the Acb shell through the cognitive processing circuits involving the Acb core and the central striatum to finally reach the motor circuits of the dorsolateral striatum. In rats, the striato-nigro-striatal relationships also comprise reciprocal and non-reciprocal components. As in the monkey brain, the organization of the non-reciprocal components of the striato-nigro-striatal circuits suggests that the different functional regions of the rodent striatum might also be interconnected by DA neurons through an ascending spiral. Indeed, as shown from the present anterograde labeling experiments and confirming earlier reports, the Acb shell innervates massively the region of VTA/SNC containing DA neurons projecting to the Acb core (Heimer et al., 1991; Maurin et al., 1999; Otake & Nakamura, 2000). The Acb core innervates a dorso-medial region of the SNR that is overlaid by DA neurons innervating medial and central portions of the rostral striatum receiving inputs from anterior cingulate and prefrontal cortical areas (Deniau et al., 1996, Maurin et al., 1999, Groenewegen et al. 1999). However, in contrast to monkeys in which direct limbic-motor connections seem rather limited, the present study indicates that in rats, a relatively large subpopulation of DA cells projecting to the sensorimotor territory of the dorsal striatum lies in the medial SNC and lateral VTA, a region innervated massively by the Acb shell, and receives a direct synaptic input from the Acb shell. In that respect, the Acb shell in rats appears to be in a unique position.

Yet, an important question remains: what is the functional role of this disynaptic, DA link from the Acb shell to more dorsal striatal regions? DA afferents constitute slow, modulatory inputs to a striatal network that processes information propagated by fast glutamatergic inputs from the cerebral cortex, thalamus and amygdala. In line with this, electrophysiological data support a DA modulation of glutamatergic transmission in striatum (West et al., 2003). To date there are no behavioral data to support a role for the Acb shell in orchestrating functions ascribed to dorsal striatal regions but behavioral observations do indeed support a dopaminergic modulation of processes in other striatal regions, viz. Acb core, invigorated by the Acb shell (Parkinson et al.,
1999). Therefore, it is likely that through the DA system, the Acb shell may also influence the striatal motor functions by regulating at the level of the dorsal striatum the transmission of information originating from sensorimotor cortical areas.

In conclusion, the Acb shell receives predominant afferents from limbic structures such as the hippocampus and amygdala, and is implicated in motivation and emotional behavior. By contrast, the dorsolateral striatum, via its afferents from the sensorimotor cortical areas and its connections with the motor output pathways of the basal ganglia, is involved in executive behavior and procedural learning. Therefore, it can be proposed that the subpopulation of DA neurons that innervates the sensorimotor dorsal striatum and receives inputs from the Acb shell, by funneling information from the limbic system to the extrapyramidal motor system, provides a link through which motivational and emotional states can influence motor outcomes.

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References


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Chapter 5

Summary and General Discussion
SUMMARY AND GENERAL DISCUSSION

The main aim of the present study was to determine how integration of information may take place within and between cortical-basal ganglia-thalamocortical systems in order to better understand how the complex neuronal forebrain circuits contribute to the production of goal-directed and complex behavior. As introduced in the first Chapter of this thesis, the basic principle of the cortical-basal ganglia relationships is essentially based on the parallel processing of information in functionally distinct basal ganglia-thalamocortical circuits (Alexander et al., 1986, 1990; Groenewegen et al., 1987; Wiesendanger et al., 2004). Theoretical and experimental approaches to explain and predict how these basal ganglia circuits function and interact, have been taken at different levels of analysis. In very general terms, it has been suggested that the basal ganglia may play an important role, in close association with the (pre)frontal cortex, in selecting an appropriate motor or behavioral output in a particular context (Pennartz et al., 1994; Mink, 1996; Redgrave et al., 1999). For instance, Pennartz et al. (1994) focused on the microcircuit level in proposing in the ‘ensemble hypothesis’ of striatal function a prominent role for medium-sized spiny projection neurons (MSN) of the rat nucleus accumbens (Acb) with a relatively ‘long-range’ complex of axonal ramifications from MSN for cross-talk and cross-regulation of activity within, as well as between, compartments or functionally distinct neuronal ensembles. At the system level, Haber et al. (2000) proposed that the striato-nigrostriatal pathways in primates form an ascending spiral from the Acb shell to the dorsolateral striatum, which might argue for a serial, hierarchical organization of behavior involving successively more dorsal parts of the striatum as proposed by Redgrave et al. (1999). These striato-nigrostriatal relationships comprise both reciprocal and non-reciprocal components suggesting that the DA system is involved in both feedback and feedforward loops with the striatum. We sought to determine to what extent the functional-anatomical organization of the microcircuitry of the Acb would lend support for the ‘ensemble hypothesis’ (Chapter 2 and Chapter 3) and, in addition, to what extent the Acb shell would exert a synaptic influence on the two subpopulations of nigrostriatal neurons that innervate the sensorimotor dorsolateral striatum in rodents (Chapter 4).

The following section will provide a summary of the findings by chapter. This will be followed by a discussion of the results of the present thesis in light of a prominent idea in basal ganglia literature - that parallel circuits and integrative circuits must work together, so that the

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behaviors associated with these circuits are coordinated, and focused (via parallel networks), but also can be modified and changed according the appropriate context (via integrative networks).

1. SUMMARY OF THE FINDINGS BY CHAPTER

In Chapter 2, the anatomical organization of intrastriatal communication between the two major subregions of the rat Acb, i.e., shell and core, was investigated. As highlighted in the first Chapter of the present thesis, the Acb is thought to subserve different aspects of adaptive and emotional behaviors. The anatomical substrates for such actions are multiple, parallel ventral striatopallidal output circuits originating in the Acb shell and core subregions. Several indirect ways of interaction between these two subregions and their associated circuitry have been proposed, in particular through striato-pallido-thalamic (Zahm and Brog, 1992; Joel and Weiner, 1994; Groenewegen et al., 1994; O’Donnell et al., 1997) and dopaminergic pathways (Otake and Nakamura, 2000; Haber et al., 2000). In addition to these indirect, multisynaptic pathways between the Acb shell and core, Heimer et al. (1991) described intrastriatal ‘associational’ projections in the ventral striatum. According to Heimer and colleagues (1991), these intrastriatal Acb fibers represent a fine and delicate system that are rather restricted in its distribution. These authors further noted that the ‘intrastriatal association fibers’ could cross the shell-core boundary. In order to investigate to which extent the Acb shell and core are directly interconnected and whether a specific organization underlies the intra-accumbens distribution of axon collaterals, we placed small injections of the anterograde neuroanatomical tracers Phaseolus vulgaris-leucoagglutinin (PHA-L) and biotinylated dextran amine (BDA) in different parts of the Acb. These experiments were supplemented by single-cell juxtacellular injections with the tracer neurobiotin, in order to characterize the neurons that give rise to intrastriatal projections.

Our results confirm the observations by Heimer et al. (1991) of intrastriatal ‘associational’ projections and show, in addition, the specific organization of these projections using much smaller injections in different subareas of the Acb. For instance, we demonstrate for the first time widespread intra-accumbens projection patterns, including reciprocal projections between specific parts of the shell and core. However, fibers originating in the core reach more distant areas of the shell, including the rostral pole (i.e., the predominant calbindin-poor part of the shell anterior to the core) and striatal parts of the olfactory tubercle, than those arising in the shell and projecting to the core. The latter projections are more restricted to the border region between the shell and core. The
density of the fiber labeling within both the shell and core was quite similar. Moreover, specific intrinsic projections within shell and core were identified, including a relatively strong projection from the rostral pole to the rostral shell, reciprocal projections between the rostral and caudal shell, as well as projections within the core that have a caudal-to-rostral predominance. The results of the single-cell juxtacellular tracing experiments show that MSNs and medium-sized aspiny neurons (most likely fast-spiking) contribute to these intra-accumbens projections. As to the dorsal striatum, several studies using intracellular filling have shown that MSNs (Bishop et al., 1982; Kawaguchi et al., 1989, 1990), as well as interneurons (Wilson et al., 1990; Kawaguchi, 1993), have axons that project up to 1 mm away from the parent cell body. While such neurons are GABAergic, the intrastriatal projection patterns indicate the existence of lateral inhibitory interactions within, as well as between, shell and core subregions of the Acb. Although not much is known about the functional aspects of shell-to-core and core-to-shell projections, the available evidence supports the hypothesis that the core may convey information to the shell to overrule its modulation of primary, non-learned behavior (e.g. the sight of an apple may counteract the shell-mediated inhibition of feeding)(Parkinson et al., 1999). Finally, our present findings of reciprocal connections between the rostromedial and caudomedial shell suggest a possible involvement in funneling the motivational valence of positive (appetitive) vs. negative (aversive) states (Kelley, 1999, 2004).

To further clarify the anatomical organization of intrastriatal communication within the two major subregions of the rat Acb, i.e., shell and core, in the studies described in Chapter 3 we investigated the three-dimensional (3D) organization of dendrites and axons of MSNs of the Acb in relation to subregional (shell-core) and compartmental (patch-matrix) boundaries. Previous studies in the primate caudate nucleus have demonstrated that MSNs have preferred dendritic orientations that tend to parallel the orientations of the striosomes (striatal compartments; Walker et al., 1993). Moreover, recurrent axon collaterals of MSNs in the rat dorsal striatum have been categorized into two types, i.e., restricted and widespread (Kawaguchi et al., 1990), which tend to respect the boundaries between patch and matrix (Kawaguchi et al., 1989). As highlighted in the general introduction of this thesis, the Acb has a highly complex compartmental organization. Arts and Groenewegen (1992) described the relationships of the dendrites of MSNs with the compartmental structure of the Acb using in vitro intracellular tracing. However, the spatial orientation of dendritic and axonal arborizations of MSNs, as well as the spatial relationships between the dendrites and local axon collaterals of MSNs on the one hand and the subregional (shell-core) and compartmental (patch-matrix) structure on the other hand, have not been systematically studied as yet. These issues
were addressed by using single-cell juxtacellular tracing with neurobiotin and anterograde neuroanatomical tract-tracing with biotinylated dextran amine.

Our results show for the first time that dendritic arbors of MSN in both the Acb shell and core subregions are preferably oriented, i.e., that they are flattened in at least one of the 3D-planes. The preferred orientations are influenced by the shell-core and patch-matrix boundaries, they conform to the boundaries, suggesting a particular interaction between the MSN and their afferents. This supports the idea of parallel and independent processing of information in both the shell and core to be transmitted to their respective segregated target areas in the basal forebrain, diencephalon and mesencephalon. Dendritic orientations of MSN of the Acb core are more heterogeneous, than those of the Acb shell and dorsal striatum, suggesting a more complex distribution of striatal inputs within the Acb core. While dendrites respect the shell-core and patch-matrix boundaries in shell and core, we demonstrate for the first time that local axon collaterals may cross these boundaries. Finally, different degrees of overlap between dendritic and axonal arborizations of individual MSN were identified, suggesting various possibilities of lateral inhibitory interactions within, as well as between, functionally distinct territories of the Acb. In this way, lateral inhibition between MSN would support a ‘Winner-take-all’ mechanism in the selection of striatal outputs.

In Chapter 4, we investigated the indirect dopaminergic pathway of interaction between the rat Acb shell and the nigrostriatal neurons innervating the sensorimotor region of the dorsal striatum. A recent analysis of the pattern of striatonigral and nigrostriatal projections in both rats and primates revealed that each functional territory of the dorsal striatum is innervated by two main subpopulations of nigrostriatal dopaminergic neurons (Maurin et al., 1999; Haber et al., 2000). The first population, denominated “proximal”, occupies a position in register with the striatonigral projections, and may be involved in reciprocal striato-nigrostriatal connections. The second population, denominated “distal”, is likely involved in non-reciprocal connections with the dorsal striatum. However, both the anatomical organization of Acb shell projections to the nigrostriatal dopaminergic neurons innervating the sensorimotor striatum and the synaptic influence exerted by the Acb shell on these neurons, remain to be determined. These issues were addressed in the rat using neuroanatomical and electrophysiological approaches.

Combined anterograde tracing from the Acb shell with retrograde tracing from the sensorimotor region of the dorsal striatum revealed that labeled fibers from the Acb shell overlap retrogradely labeled nigrostriatal neurons located in the medial (substantia nigra pars compacta (SNC) and the lateral ventral tegmental area (VTA) but avoid the nigrostriatal neurons located
laterally. In addition, stimulation of the Acb-shell induced an inhibition, as noted by a decrease of firing rate, of dopaminergic nigrostriatal neurons projecting to the sensorimotor striatal territory. In agreement with the anatomical observations, these responses were observed in nigrostriatal neurons located in the medial SNC and the lateral VTA but not in nigrostriatal neurons located laterally. These data further establish the existence of a functional-anatomical link between the Acb shell and the sensorimotor striatum via dopaminergic nigrostriatal neurons. The present study also reveals that among the dopaminergic nigrostriatal neurons innervating the sensorimotor striatal territory, only the subpopulation located in the medial SNC and lateral VTA receives an inhibitory input from the Acb shell. This indicates a functional heterogeneity within the population of dopaminergic neurons innervating a given striatal territory. This suggests that not all dopaminergic neurons act in the same way, i.e., in reacting to unexpected rewards (Schultz, 1997). Rather, some dopaminergic neurons may get different information than others, which should enable them to react differently to different stimuli or context.

To summarize, our present studies in rats (Chapter 2-4) outline several nodal points at which information from separate cortical-basal ganglia-thalamocortical loops can influence each other. Chapter 2 and Chapter 3 describe the interacting neural networks of shell and core at the level of the Acb. The directionality in these intranuclear projections indicates that in addition to a ‘limbic-to-motor’ flow, ‘motor-to-limbic’ transfer of information may also occur. These data argue against a strict spiralling hierarchy, in which the shell would primarily influence the core. Rather, the core may also influence the shell directly. Chapter 4 describes an open component of the striato-nigrostriatal pathway in the rat that allows direct interactions between segregated corticostriatal channels through non-reciprocal connections to the substantia nigra. The directionality in these projections indicate a ‘ventral-to-dorsal’ transfer of information.

2. DIRECT AND INDIRECT COMMUNICATION: PERSPECTIVES FROM MODELS OF CORTICAL-BASAL GANGLIA CIRCUITRY AND BASAL GANGLIA FUNCTION

Several theories have been proposed to explain and predict how parallel cortical-basal ganglia circuits interact and function in forming behavioral responses. In the following paragraphs, this integrative circuitry and their role in basal ganglia function will be highlighted, and the results of the present thesis will be related to these theories.
The ensemble hypothesis

The first question that we addressed in the first Chapter was - what is the neural basis for the selection of functionally different outputs of the Acb? The ‘ensemble hypothesis’ by Pennartz et al. (1994) states that the functions of the Acb are based on the organization of collections or ‘ensembles’ of striatal neurons that may be variably active in different behavioral connotations and are driven by selection processes. The postulate of functionally distinct neuronal ensembles in the hypothesis of Pennartz et al. (1994) (Fig. 1) emphasizes the existence of lateral inhibition between these functionally different groups of striatal neurons, in order to lead to an appropriate behavioral output. At the striatal microcircuit level, the MSN were in the 1980s already known to be important to striatal function, because of their preponderance within the striatum and the density of their associated local axon collaterals (Wilson and Groves, 1980). Anatomical evidence in support of a relatively ‘long-range’ complex of axonal ramifications in the rat Acb has been advanced by Heimer et al. (1991), who demonstrated using a combination of anterograde and retrograde tract-tracing methods, including Phaseolus vulgaris-leucoagglutinin, horseradish peroxidase and fluorescent tracers, a fine and delicate system of ‘intrastriatal association fibers’ in the rat Acb that is restricted in its distribution.

The results in Chapter 2 and Chapter 3 provide further anatomical evidence for the existence of a relatively ‘long-range’ complex of axonal ramifications in the rat Acb. Our small anterograde tracer injections in different subareas of the rat Acb resulted in a dense plexus of labeled fibers and terminals in the immediate vicinity of the injection site, whether located in the Acb shell or core, but also in specific patterns of labeling in more distant areas, including the adjacent subregion (shell or core) and/or the olfactory tubercle and ventral caudate-putamen complex. This enabled us to identify the intrinsic connections between the different subareas of the Acb. Our single-cell juxtacellular tracing experiments revealed, like in the dorsal striatum (Bishop et al., 1982; Kawaguchi et al., 1990), a relatively small subgroup of MSN with a ‘long-range’ system of axon collateralization that were preferentially found within the Acb core. Whether MSN with ‘long-range’ axon collaterals exist within the Acb shell remains uncertain, because our sample of juxtacellularly labelled neurons did not include MSN of that category. Nevertheless, regardless of the precise cell types involved, the present results provide firm evidence for neuronal cross-talk between the shell and core of Acb.
Based on electrophysiological studies, the mechanism of lateral inhibition proposed through the ‘ensemble hypothesis’ of Pennartz et al. (1994) have been advanced by Taverna et al. (2004). Taverna et al. (2004), using dual whole cell patch-clamp recordings in acute slices of the Aeb, demonstrated that no less than 34% of the patched pairs of MSN provide synaptic GABA_{A} receptor-mediated lateral inhibition, most of them uni-directionally. The results in Chapter 2 and Chapter 3 provide further indications for a role of MSN in GABA-mediated lateral inhibitory interactions between populations of MSN and, in addition, in output selectivity of the Aeb. In our studies described in Chapter 2, the Aeb core projects primarily to more rostral parts of the Aeb, while the Aeb shell projects diffusely throughout the rostrocaudal extent of the Aeb. Using specific GABA
receptor agonists and AMPA/kainate glutamate receptor antagonists, Reynolds and Berridge (2001, 2002, 2003) analyzed the differential roles of these two classes of receptors in the Acb shell and core subregions. These pharmacological studies demonstrated that the anterior medial shell is involved in feeding (or appetitive) behavior (see also Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1997; Basso and Kelley, 1999; Kelley and Berridge, 2002; Kelley, 1999, 2004), while the posterior medial shell (and possibly the anterior core) is concerned with fear and defensive (or aversive) behaviors. The shell-elicited affective actions depend on its projections to the lateral hypothalamus. The anterior core is involved in defensive behavior evoked by AMPA/kainate glutamate receptor antagonists, but this trend was not statistically significant (Reynolds and Berridge, 2003). Therefore, interactions between the anterior and posterior medial shell through MSN may be of great importance in the GABA-mediated inhibitory feedback control of 1) the anterior medial shell, to facilitate appetitive behavior and 2) the posterior medial shell, to facilitate fear or aversive behavior.

The patch-matrix connections demonstrated in Chapter 3 on the basis of MSN projections seem to exhibit compartmental specificity, i.e., matrix-to-patch connections (uni-directional). These findings suggest a role for MSN at the compartmental level, a role that has still to be further explored. For instance, by in vitro or in vivo electrophysiological studies, using dual whole cell patch-clamp, intracellular or single-unit recordings, in combination with immunohistochemical staining for Acb compartments. However, the intercompartmental interactions on the basis of MSN projections are rather scarce and, therefore, might be more prominent for interneurons with widespread axon collaterals, such as cholinergic and somatostatin interneurons. The axons of the large aspiny cholinergic interneurons, however, tend to respect compartmental boundaries (Meredith et al., 1989; Kawaguchi, 1993). This has been established directly with intracellular labeling of individual neurons in the dorsal striatum (Kawaguchi, 1993). Indeed, immunohistochemical studies by Chesselet and Graybiel (1986), Cowan et al. (1990) and Fortin and Parent (1994) in the dorsal striatum have demonstrated that axonal processes of somatostatin, parvalbumin and calretinin interneurons crossed compartmental boundaries. Despite these observations, the question of what the neuronal substrate is for the intercompartmental communication in the Acb has still not been fully elucidated. This issue deserves further exploration, for instance by single-cell labelling studies combined with patch-matrix immunohistochemical staining.
Taken together, lateral inhibition mediated by GABAergic axon collaterals of MSN is thought to play a role in the interactions between collections or ‘ensembles’ of neurons that are influenced by different sets of cortical inputs and code for competing outputs of the striatum (e.g. Pennartz et al., 1994; Plenz, 2003; Tepper et al., 2004). In this way, lateral inhibition between MSN would support a ‘Winner-take-all’ mechanism in the selection of striatal outputs (Fig. 2). Our present results, in agreement with Kawaguchi et al. (1990), show that MSN with ‘long-range’ axonal terminations and several forms of non-overlapping axonal and dendritic arborizations exist. This could imply that such recurrent axon collaterals connect functionally to different sets of MSN and provide a way of interaction or competition between such populations of neurons. This implication especially holds in case spatial segregation of MSN is coupled to segregation of function, which is a likely assumption on account of the different afferent and efferent connections of Acb compartments. For instance, the findings of recurrent axon collaterals of MSN that cross patch-matrix borders emphasize and strengthen the idea that such collaterals play a role in the interaction between functionally different ‘ensembles’ of neurons (Pennartz et al., 1994), at least when it is assumed that compartments contain such functionally different groups of cells. Finally, these findings support the idea that the information flow from separate cortical-basal ganglia loops is less segregated than previously assumed, allowing interactions across functional regions.

Shell-core interconnections

The idea that the Acb shell and core influence each other, at least indirectly, has been supported by several authors on account of anatomical tracing of corticostriatal-thalamic loops (Zahm and Brog, 1992; Joel and Weiner, 1994; Groenewegen et al., 1994; O’Donnell et al., 1997). These neuroanatomical tracing studies have emphasized the existence of ‘open’ components in the larger circuits between the cerebral cortex, the basal ganglia and the thalamus, suggesting a spiral of connections leading from the limbic-innervated part of the basal ganglia via the thalamus and subsequently cortical-basal ganglia-thalamocortical loops to the premotor cortex. These mechanisms of indirect shell-core interactions show a preference for information flowing from the Acb shell towards the core, at the level of the core using glutamate and dopamine as the neurotransmitter in prefrontal and mesencephalic afferents, respectively. The observation that the core is modulated by the shell is a proposed mechanism through which feedback from cues
associated with reward helps the core to activate and guide actions that are instrumental to gaining access to basic supplies, such as water and food (Corbit et al., 2001).

The mechanism of direct shell-core interactions as described in Chapter 2 shows a preference for information flowing from the core towards the shell, at the level of the shell using GABA as the neurotransmitter in the intrinsic microcircuitry of the Acb. The observation that the shell is modulated by the core is a proposed mechanism by which the core may convey information to the shell to overrule its modulation of primary non-learned behaviors (Parkinson et al., 1999). For
instance, the sight of an apple may counteract the shell-mediated inhibition of feeding (Kelley, 1999, 2004). On the basis of the large area of the shell that receives input from the core, the direct core-to-shell interconnections are likely to be a significant connection.

Taken together, the demonstration of directionality in our findings prompts a reevaluation of the relative weight of shell-to-core influences vis a vis core-to-shell influences. For instance, ongoing efforts in physiological and behavioral research are likely to supplement our existing knowledge on the role of the two subregions of the Acb, i.e., shell and core, as well as on their competitive and cooperative abilities at the level of the Acb.

**The spiral theory: from motivation to action**

The second question that we addressed in the first Chapter was - how does the limbic system influence the motor system? The observation that the dorsal striatum is modulated by the ventral striatum was a proposed mechanism by which limbic circuitry affects motor outcome directly (Nauta et al., 1978; Somogyi et al., 1981; Groenewegen et al., 1994). For instance, following injections of the anterograde tracer BDA within discrete regions of the Acb shell, labeled fibers and terminals distributed extensively within the dopaminergic system spanning from the VTA to lateral regions of the SNC (Groenewegen et al., 1994). In turn, the dopaminergic neurons of the VTA/SNC complex collectively innervate the entire striatum where they regulate the integration of cortical signals. Rather than a direct limbic-motor connection, most recently Haber et al. (2000) proposed that through several midbrain components, information from the limbic system reaches the motor system through a series of connections. Haber et al. (2000) examined on the basis of a collection of tracing studies the basic principles of the nigrostriatal relationships in non-human primates and described the organization of striato-nigrostriatal projections. Haber et al. (2000) have demonstrated that each striatal domain has both reciprocal and non-reciprocal connections with the midbrain (Fig. 3). Based on this pattern of striatonigral and nigrostriatal projections, Haber et al. (2000) proposed that the striato-nigrostriatal pathways form an ascending spiral from the shell to the dorsolateral striatum, which might argue for a serial, hierarchical organization of behavior, involving successively more dorsal parts of the striatum as proposed by Redgrave et al. (1999).
However, in contrast to primates in which direct limbic-motor connections seem rather limited, the studies described in Chapter 4 revealed in rats that a relatively large subpopulation of dopaminergic neurons projecting to the sensorimotor territory of the dorsal striatum lies in the medial SNC and lateral VTA, a region innervated massively by the Acb shell and receiving a direct synaptic GABA-mediated inhibitory input from the Acb shell. These findings emphasize that the position of the Acb shell in rats in the interactions between functionally different parts of the striatum is unique and the disynaptic limbic-to-motor interface is likely to be a significant connection.

Despite these observations, there are no behavioral data yet that support a role for the Acb shell in ‘orchestrating’ functions specifically ascribed to more dorsal striatal regions. Indeed, behavioral observations support a dopaminergic modulation of processes in other striatal regions, namely the core, invigorated by the shell (Parkinson et al., 1999). Therefore, it is likely that through
the dopaminergic system, the Acb shell may also influence the striatal motor functions by regulating at the level of the dorsal striatum the transmission of information originating from the sensorimotor cortical areas, a role that has still to be further explored. Conceivably, the disynaptic pathway from the shell to the dorsal striatum may allow a relatively powerful control of habit execution (linked to the dorsal striatum) by reinforcers and proximal reward-predicting cues (mediated by the Acb shell).

3. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The functional-anatomical data from the experimental studies described in this thesis emphasize various kinds of crosstalk within and between cortical-basal ganglia circuits in the midst of an overall parallel loop-like organization. Therefore, in stead of a hierarchy, the present data are much more in line with a ‘heterarchical’ arrangement, in which interactions might be mediated not only by striatonigral circuits, but also by corticostriatothalamic loops and by intrastriatal interactions. In this scenario, interactions between individual parallel circuits, which could grow stronger in time and with learning, might well be essential.

Our present findings are essential to the better understanding of normal brain function and several mental health disorders. These results open new perspectives to augment our knowledge of the basic functional-anatomical organization of the ventral, limbic-related corticostriatal system. For instance, the direct (non-)reciprocal connections, as presented in Chapter 2 and Chapter 4, should also be taken into account when considering functional interactions between different parts of the striatum. As should be clear from Chapter 3, our anatomical and behavioral knowledge does not yet permit to give an exact answer to the question of how large the hypothesized ensembles in the Acb are, since many connectional and behavior-related differences have not yet been worked out at the detailed (e.g. single cell) level of the striatal compartments. Future research may be able to reveal more fine-grained subdivisions corresponding to functionally distinct areas. Finally, with the support of novel and impressive techniques in neuroanatomical research, such as three-dimensional reconstruction and computer-graphic visualization techniques, the complex circuitry along which brain areas function are likely to be further explored and revealed.
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Nederlandse Samenvatting – Dutch Summary
De menselijke hersenen zijn opgebouwd uit ongeveer 10-100 miljard zenuwcellen (of neuronen), waarbij elk neuron met 100-1000 andere neuronen contact maakt. Op deze manier worden in onze hersenen neurale netwerken gevormd. Deze neuronale netwerken stellen ons in staat informatie uit het interne en externe milieu te verwerken, motorische handelingen en gedragingen te initiëren en coördineren, te denken, nieuwe dingen aan te leren en ons gebeurtenissen te herinneren.

Dit proefschrift richt zich op de neuronale netwerken tussen de cerebrale cortex (de hersenschors) en de grootste groep subcorticale (onder de hersenschors gelegen) kernen in de voor- en middenhersenen, de zogenaamde basale ganglia. De basale ganglia ontvangen ‘externe’ informatie van vrijwel de gehele cerebrale cortex, terwijl de output van de basale ganglia hun invloed voornamelijk uitoefenen, weliswaar indirect via de thalamus, op de cortex van de zogeheten voorhoofdskwab (of frontale kwab). In functionele termen geeft dit de betrokkenheid van de basale ganglia aan bij de motorische, cognitieve en emotioneel-motivationele gedragsprocessen waarbij de frontale kwab als geheel een rol speelt.

Tot de basale ganglia behoren een viertal hersenstructuren, te weten het striatum, het pallidum, de subthalamische kern en de substantia nigra. Deze hersenstructuren (op de subthalamische kern na) zijn alle onder te verdelen in macroscopisch-anatomisch te onderscheiden kerngebieden. Het striatum omvat de nucleus caudatus en het putamen (tesamen aangeduid als het dorsale striatum) en de meer ventraal gelegen nucleus accumbens (ook wel aangeduid als het ventrale striatum). Het striatum wordt wel beschouwd als de inputstructuur van de basale ganglia en ontvangt vezels van de gehele cerebrale cortex, de thalamus en, met name het ventrale striatum, van een aantal hersenstructuren die deel uitmaken van het limbische systeem, zoals de hippocampus en de amygdala. Het pallidum bestaat uit een drietal te onderscheiden kerngebieden, te weten het externe (of laterale) segment en het interne (of mediale) segment van de globus pallidus, en het ventraal gelegen ventrale pallidum. Het interne segment van de globus pallidus en het ventrale pallidum verzorgen een belangrijk deel van de output van de basale ganglia. De substantia nigra is onderaan de middenhersenen, in het ventrale mesencephalon, gelegen en bestaat uit twee delen, namelijk de pars compacta en de pars reticulata. De substantia nigra pars reticulata bevat neuronen die naar de thalamus projecteren en zorgt dus mede voor de output van de basale ganglia. De pars
compacta van de substantia nigra bevat neuronen die het dorsale striatum van dopamine voorzien. Het gebied mediaal van de eigenlijke substantia nigra, het zogenoemde ventrale tegmentale gebied (VTA), dat eveneens dopaminerge neuronen bevat, voorziet het ventrale striatum en de prefrontale cortex (het voorste gedeelte van de frontale kwab) van dopaminerge vezels.

De input- en outputstructuren van de basale ganglia zijn direct en indirect met elkaar verbonden en vormen met de thalamus en de cerebrale cortex een reeks parallel geschakelde, functioneel gescheiden basale ganglia-thalamocorticale circuits. Functioneel verschillende delen van de cerebrale cortex, waaronder sensorisch-motorisch, associatief-cognitief en limbisch, projecteren op een topografische wijze naar het striatum en leggen op die manier een functionele indeling op aan het striatum: motorisch-gerelateerde delen van het striatum zijn dorsolateraal gelegen, cognitief-gerelateerde delen omvatten het centrale deel van het striatum en het limbisch, motivationeel-emotioneel gerelateerde deel van het striatum wordt gevormd door het ventrale striatum. Aangezien de verbindingen van het striatum naar de outputstructuren van de basale ganglia, en vandaaruit naar de thalamus en de frontale cortex eveneens topografisch zijn georganiseerd, vormt de organisatie van deze serie van projecties van de cerebrale cortex via de basale ganglia en de thalamus terug naar de cortex de basis voor een functionele, en deels ook anatomische, scheiding tussen de verschillende basale ganglia-thalamocorticale circuits en het principe van een parallele organisatie van deze circuits. Dit principe werd voor het eerst in globale termen beschreven door Heimer & Wilson (1975) in de rat voor dorsale versus ventrale circuits. Later zijn deze organisatorische principes als zodanig benoemd en verder uitgewerkt door Alexander en medewerkers (1986) in primaten.

In zijn algemeenheid participeren de basale ganglia in het uitvoeren van onbewust verlopende, aangeleerde bewegingspatronen. De basale ganglia ‘ordenen’ het werkplan voor een aantal gelijktijdige of juist sequentiële bewegingselementen die de basis vormen voor complexe handelingen en deze hersenstructuren zijn essentieel voor de soepele overgang van het ene bewegingspatroon in het andere. Deze aaneenschakeling van verschillende motorische of cognitieve componenten tot adequaat gedrag geschiedt op basis van de integratie van verschillende parallelle informatiestromen om zo tot een voor de context en het moment adequate, geïntegreerde en snelle gedragsresponse te leiden.

De algemene vraagstelling van het onderzoek dat in dit proefschrift wordt beschreven betreft de manier waarop integratie van informatie plaats vindt binnen en tussen basale ganglia-thalamocorticale circuits. Dit zou inzicht kunnen verschaffen over de manier waarop deze voorhersencircuits een bijdrage leveren aan complexe gedragsfuncties. Onze interesse ging hierbij
met name uit naar de circuits die via het ventrale striatum, in het bijzonder de nucleus accumbens, naar de prefrontale cortex verlopen. Zoals hierboven reeds aangegeven is de nucleus accumbens een onderdeel van het ventrale, limbisch-gerelateerde deel van het striatum en vervult belangrijke functies in het aansturen van motivationeel en emotioneel gedrag. De nucleus accumbens valt uiteen in een tweetal structureel en functioneel verschillende subregionen, de ventraal en mediaal gelegen shell en de meer dorsaal en lateraal gelegen core die bij de rat zonder duidelijke grens overgaat in het dorsaal daarvan gelegen caudatus-putamen complex. De nucleus accumbens als geheel speelt een belangrijke rol in het integreren en verwerken van signalen die in limbische hersenstructuren zoals de amygdala, hippocampus, en prefrontale cortex worden gegenereerd. Verschillende parallel georganiseerde ventrale basale ganglia-thalamocorticale circuits, die verlopen via de shell en core van de nucleus accumbens, en die via het ventrale pallidum, de substantia nigra pars reticulata en verschillende kernen van de thalamus terug projecteren naar de prefrontale cortexgebieden die corticostriatale projecties zenden naar de shell en core, vormen de basis voor de rol van de nucleus accumbens in het aansturen van complex, motivationeel en emotioneel gestuurd gedrag. Hoewel deze circuits via de shell en de core van de nucleus accumbens deels parallel verlopen, zijn er duidelijke aanwijzingen dat een strikte anatomische en ook functionele scheiding tussen de verschillende circuits onwaarschijnlijk is. In de literatuur zijn verschillende mogelijkheden gesuggereerd voor interacties tussen deze functioneel verschillende basale ganglia-thalamocorticale circuits. Eén van deze mogelijkheden is dat een gebied in de nucleus accumbens dat door een bepaald deel van de prefrontale cortex wordt geïnnerveerd, via het pallidum naar een deel van de thalamus projecteert dat voornamelijk verbonden is met een aangrenzend deel van de prefrontale cortex. Als voorbeeld kunnen worden genoemd de projecties van de shell naar het ventrale pallidum en vervolgens naar een deel van de mediodorsale thalamuskern dat is verbonden met een gebied in de prefrontale cortex dat voornamelijk naar de core projecteert. Een andere mogelijkheid voor interacties tussen verschillende circuits berust op de organisatie van de projecties van het dopaminerg systeem vanuit het ventrale mesencephalon. Reeds in 1976 is door Nauta en medewerkers gewezen op het feit dat vezels vanuit de nucleus accumbens dopaminerg neuronen in de substantia nigra pars compacta kunnen bereiken die projecteren naar het dorsale striatum. Dit zou betekenen dat, via het dopaminerg systeem het limbisch-gerelateerde deel van het striatum het dorsale, motorisch-gerelateerde deel kan beïnvloeden. Een laatste mogelijkheid die belangrijk is om in het kader van dit proefschrift te noemen, betreft verbindingen tussen functioneel verschillende gebieden binnen het striatum op basis van intrastriatale projecties. Met behulp van de anterograde en retrograde tracing techniek (zie volgende paragraaf voor een uitgebreide beschrijving van deze

Onze meer specifieke vragen hebben zich in de eerste plaats gericht op de mogelijke verbindingen tussen de shell en de core van de nucleus accumbens, de organisatie van de verbindingen binnen de shell en de core, en de morfologische kenmerken van de ventrale striatale neuronen die deze verbindingen tot stand brengen. Om deze vragen te kunnen beantwoorden hebben we gebruik gemaakt van zogenoemde anterograde tracing techniek. Hierbij worden kleine hoeveelheden van een tracerstof ingespoten in een hersengebied. Ter plaatse van de injectie wordt de tracer opgenomen in de cellichamen van neuronen en vandaaruit langs de dendrieten en het axon in anterograde (dat wil zeggen van het cellichaam naar de uiteinden van de axonen) getransporteerd. Deze tracerstoffen kunnen dan na enkele dagen in weefsectoorns van de hersenen met behulp van (immuno)histochemische technieken worden aangetoond. In de huidige studie zijn het plantenlectine *Phaseolus vulgaris*-leucoagglutinine (PHA-L) en het gebiotinyleerde dextranamine (BDA) gebruikt als anterograde tracerstoffen. Bij injecties van deze twee stoffen wordt in de injectieplaats altijd een cluster neuronen gelabeld en het is moeilijk individuele neuronen te onderscheiden, hoe klein de injecties ook zijn. Aangezien we ook geïnteresseerd waren in de morfologie van individuele neuronen, inclusief hun uitlopers, hebben we tevens een andere neuroanatomische tracing techniek gebruikt, namelijk de zogenoemde single-cell juxtacellulaire tracing techniek. Hierbij worden neuronen geïdentificeerd op basis van electrofysiologische stimulatie in een hersengebied dat projecteert naar het gebied waarin neuronen gelabeld moeten worden. In de huidige studie werd electrisch gestimuleerd in de hippocampus of prefrontale cortex en afgeleid in de nucleus accumbens. Indien een neuron in de nucleus accumbens reageerde op stimulatie in de hippocampus of de prefrontale cortex dan werd dit neuron via een juxtacellulaire injectie ‘gevuld’ met de tracerstof neurobiotine. Op die manier konden het cellichaam, de dendrieten en, in een groot aantal gevallen, ook de axonen en hun locale vertakkingen (recurrente collateralen) zichtbaar worden gemaakt (zie Hoofdstuk 2 en Hoofdstuk 3).

Met behulp van neuroanatomische tracing technieken kan ook een serieuze schakeling van een tweetal neuronen zichtbaar worden gemaakt. Hiertoe wordt dan van een combinatie van anterograde en retrograde tracerstoffen gebruik gemaakt. Een retrograde tracerstof wordt bij voorkeur opgenomen door de uiteinden van axonen en in de richting van het cellichaam (=
Dutch summary

retrograad) getransporteerd. In de huidige studie is de retrograde tracer Fluorogold geïnjecteerd in het dorsale striatum in combinatie met een anterograde tracer (gebiotinyleerd dextranamine) in het ventrale striatum. Met behulp van (immuno)histochemische technieken kunnen dan beide tracers, met verschillende kleuren, worden zichtbaar gemaakt in een en dezelfde weefels coupe (in ons geval in de substantia nigra in het ventrale mesencephalon). Op die manier kan worden bestudeerd of de anterograad gelabelde vezels in de nabijheid komen van het cellichaam of de dendrieten van retrograad gelabelde neuronen en of deze mogelijk synaptisch kontakt maken. Om met zekerheid synaptische contacten aan te tonen zijn electronenmicroscopische technieken nodig; die zijn echter niet toegepast in de huidige studie. Wel hebben we electrofysiologische technieken gebruikt om te bestuderen of bepaalde vezels daadwerkelijk contact maken met geïdentificeerde neuronen. Neuronen in een bepaalde kern kunnen worden geactiveerd door electrische stimulatie in een gebied waar deze kern naartoe projecteert. Actiepotentialen, opgewekt door de stimulatie, worden dan voortgeleid van het zenuwuiteinde naar het cellichaam (= antidroom, want eigenlijk tegengesteld aan de normale geleidingsrichting van actiepotentialen van cellichaam naar zenuwuiteinde). Aldus geïdentificeerde neuronen kunnen worden afgeleid en de reactie van deze neuronen bestudeerd na orthodrome stimulatie (actiepotentialen verlopen van cellichaam naar zenuwuiteinde) in een hersengebied dat projecties stuurt naar deze geïdentificeerde neuronen. Deze techniek is in de huidige studie toegepast om de invloed te bepalen van de projecties van het ventrale striatum op de dopaminerge neuronen die naar het dorsale striatum projecteren (zie Hoofdstuk 4).

1. SAMENVATTING VAN DE RESULTATEN PER HOOFDSTUK

In Hoofdstuk 2 hebben we met behulp van kleine injecties van anterograde tracers en door middel van juxtacellulaire injecties de organisatie van de intrastriatale vezelverbindingen binnen de nucleus accumbens en tussen de hierboven genoemde twee subregionen van deze kern, de shell en de core, bestudeerd.

Nederlandse samenvatting

Over het algemeen bestrijken de projecties van de core een uitgebreider gebied in de shell dan andersom. Binnen de shell en de core zijn de intrinsieke projecties evenmin willekeurig georganiseerd maar zijn bepaalde patronen herkenbaar. Dit komt tot uiting in wederzijdse verbindingen tussen de rostrale en caudale shell en een dominante vezelverbinding van de caudale core naar de rostrale core. In het kader van de single-cell juxtacellulaire tracing experimenten zijn voornamelijk medium-sized spiny projectieneuronen gelabeld en een enkel interneuron (een neuron waarvan de axonen binnen het striatum blijven). De resultaten van deze experimenten laten zien dat de medium-sized spiny projectieneuronen door middel van een uitgebreid netwerk van recurrente collateralen een bijdrage leveren, niet alleen aan deze intrinsieke vezelverbindingen van de nucleus accumbens in het algemeen, maar ook aan de wederzijdse verbindingen tussen shell en core. Aangezien deze neuronen de inhibitoire neurotransmitter γ-aminoboterzuur (GABA) bevatten, suggereren deze vezelverbindingen een rol in het proces van wederzijdse inhibitie (of remming) binnen en tussen de shell en core subregionen van de nucleus accumbens.

In Hoofdstuk 3 hebben we getracht de anatomische organisatie van de intrastriatale communicatie binnen de twee subregionen van de nucleus accumbens, de shell en core, verder te ontrafelen. Hiertoe hebben we gebruik gemaakt van de populatie juxtacellulair geinjiceerde neuronen uit Hoofdstuk 2. Met behulp van 3-D reconstructietechnieken is de ruimtelijke oriëntatie van de dendrieten en de recurrente axon collateralen van de met neurobiotine gevulde medium-sized spiny projectieneuronen bestudeerd in relatie tot de subregionale (shell-core) en compartimentale (patch-matrix) grenzen van de nucleus accumbens. De resultaten van deze 3-D gereconstrueerde neuronen laten zien dat de dendrieten van medium-sized spiny projectieneuronen in de shell en core van de nucleus accumbens een uitgesproken oriëntatie hebben, te weten afgeplaat in tenminste één van de richtingen in het driedimensionale vlak. De dendrieten houden zich aan de grenzen van de patch en matrix compartimenten en eveneens aan de shell-core grens. De dendrietbomen van de neuronen in de shell vertonen een specifieke diagonale oriëntatie, te weten van rostraal, dorsaal en mediaal naar caudaal, ventraal en lateraal. De orientaties van de dendrietbomen van de neuronen van de core hebben een meer heterogeen patroon. Het is interessant om te zien dat de orientatie van de dendrietbomen van medium-sized spiny projectieneuronen in verschillende delen van de nucleus accumbens zich lijken te oriënteren in min of meer dezelfde richting als de binnenkomende vezelprojecties, bijvoorbeeld vanuit de hippocampus en de amygdala naar de shell, of van de prefrontale cortexgebieden naar de core. Dit suggereert dat individuele
vezels vanuit een bepaald gebied een relatief beperkt aantal neuronen in de nucleus accumbens kan bereiken. Dit pleit voor een zekere mate van parallele organisatie van inputs en outputs.

Terwijl de dendrieten van medium-sized spiny projectie neuronen van de nucleus accumbens niet over shell-core en patch-matrix grenzen heen gaan, geldt dit principe niet voor de locale axon collateralen. Bovendien geven onze resultaten een diversiteit in de mate van overlap aan tussen de dendrieten en locale axon collateralen van individuele medium-sized spiny projectie neuronen. De overlap tussen de vertakkingen van de dendrieten en recurrente axon collateralen kan vrijwel volledig zijn, maar ook gedeeltelijk en kan, in een enkel geval, volledig afwezig zijn. De locale axon collateralen van medium-sized spiny projectie neuronen spelen een rol in het proces van wederzijdse inhibitie (of remming) binnen en tussen verschillende delen van de core en de shell van de nucleus accumbens. Dergelijke inhibiterende acties zijn belangrijk in het selectieproces van de functioneel verschillende outputs van de nucleus accumbens.

Hoofdstuk 4 richt zich op de vraag of projecties vanuit het limbisch-gerelateerde deel van het striatum, de shell van de nucleus accumbens, via de substantia nigra pars compacta in het ventrale mesencephalon de dorsolaterale, motorisch-gerelateerde delen van het striatum kan bereiken. Hiertoe hebben we gebruik gemaakt van neurofysiologische en neuroanatomische tracing technieken. In het neuroanatomische deel van de studie zijn injecties van de anterograde tracer BDA in de shell van de nucleus accumbens in hetzelfde proefdier gecontinueerd met injecties van de retrograde tacer Fluorogold in het dorsolaterale deel van het caudatus-putamen complex. De relaties tussen anterograad gelabelde vezels en retrograad gelabelde cellen in de substantia nigra werden bestudeerd. In het electrofysiologische deel van de studie werd de reactie gemeten van antidroom (vanuit het dorsale striatum) gekarakteriseerde neuronen in de substantia nigra op stimulatie in de shell van de nucleus accumbens. De neuroanatomische experimenten laten een duidelijke overlap zien in het mediale deel van de substantia nigra pars compacta en het laterale deel van het ventrale tegmentale gebied (VTA) tussen anterograad gelabelde vezels afkomstig van de shell en retrograad gelabelde neuronen, die de dorsolaterale sensorimotorische delen van het striatum innerveren. De electrofysiologische experimenten laten een inhibiterende (remmende) werking zien vanuit neuronen in de shell op de antidroom geidentificeerde neuronen die de dorsolaterale somatomotorische delen van het striatum innerveren. Op basis van hun electrofysiologische karakteristieken zijn dit zeer waarschijnlijk dopaminerge neuronen. Deze resultaten bevestigen dat er een functionele schakel bestaat tussen de shell van de nucleus accumbens en het dorsolaterale somatomotorische deel van het striatum via dopamine-bevattende
neuronen in het mediale deel van de substantia nigra pars compacta en laterale deel van het ventrale tegmentale gebied. Op basis hiervan kan worden geconcludeerd dat er via het dopaminerge systeem interacties bestaan tussen ventrale en dorsale basale ganglia-thalamocorticale circuits.

2. DIRECTE EN INDIRECTE COMMUNICATIE TUSSEN BASALE GANGLIA-THALAMOCORTICALE CIRCUITS EN BASALE GANGLIA FUNCTIE

De resultaten die zijn beschreven in de experimentele hoofdstukken van dit proefschrift laten verschillende mogelijkheden zien van integratie en selectie van informatie tussen basal ganglia-thalamocorticale circuits. In Hoofdstuk 2 en Hoofdstuk 3 worden de intrastriatale vezelverbindingen binnen en tussen de nucleus accumbens shell en core subregionen beschreven. De richting waarin de vezelverbindingen verlopen geeft aan dat zowel een ‘limbisch-naar-motorische’ alsook een ‘motorisch-naar-limbische’ overdracht van informatie mogelijk is. Deze resultaten vormen een aanvulling op het heersende idee van een spiraliserende, hiërarchische organisatie, waarbij de shell voornamelijk de core beïnvloedt en er stapsgewijs, via een ‘ascenderende spiraal’, meer dorsale delen van het striatum betrokken raken. Echter, zoals uit onze studies blijkt, is de core ook in staat de shell te beïnvloeden, dus in een richting tegengesteld aan de ‘ascenderende spiraal’ van ventraal naar dorsaal. Wel in overeenstemming met de ‘ascenderende’ beïnvloeding van ventraal naar dorsaal zijn de bevindingen in hoofdstuk 4 waarin de verbindingen van het ventrale naar het dorsale striatum via het ventrale mesencephalon worden beschreven. Deze vezelverbindingen verzorgen directe interacties tussen verschillende corticostriatale systemen door middel van specifieke verbindingen via het dopaminerge systeem.

In het laatste deel van het proefschrift (Hoofdstuk 5) worden deze bevindingen geplaatst in het kader van de literatuur over de organisatie en functies van de basale ganglia, in het bijzonder betrekkend hebbend op de functionele anatomie van de nucleus accumbens en de interacties tussen basale ganglia-thalamocorticale circuits.

**Interacties tussen medium-sized spiny projectieneuronen**

In de eerste plaats wordt ingegaan op de ‘ensemble hypothese’ van Pennartz et al. (1994). Deze auteurs hebben de vraag gesteld hoe medium-sized spiny projectieneuronen in de nucleus
accumbens met elkaar communiceren om de gedragsoutput van emotionele leersystemen te reguleren. Hierbij wordt uit gegaan van de hypothese dat er in dit proces sprake moet zijn van selectie door middel van wederzijdse inhibitie (remming) en dat striatale projectieneuronen met axon collateralen die een aanzienlijk bereik hebben binnen het striatum, daarin een rol spelen. Tevens wordt in deze hypothese aangenomen dat verschillende ‘neuronale ensembles’ verspreid over de nucleus accumbens coderen voor verschillende gedragscomponenten. Onze huidige resultaten bevestigen dat er medium-sized spiny projectieneuronen zijn in de nucleus accumbens met een “lange-afstand” axon collateraal netwerk. Evenals in het dorsale striatum zijn deze neuronen met een “lange-afstand” axon collateraal netwerk in het ventrale striatum niet talrijk. We hebben dit type neuron uitsluitend kunnen aantonen in de core van de nucleus accumbens. Of dergelijke neuronen ook voorkomen in de shell van de accumbens zal verder moeten worden onderzocht, aangezien onze single-cell juxtacellular tracing experimenten daarover geen uitsluitsel hebben gegeven.


De resultaten van onze experimenten laten een relatief specifiek patroon van verbindingen tussen de shell en core en tussen verschillende onderdelen binnen deze subregionen van de nucleus accumbens zien (zie Hoofdstuk 2 en Hoofdstuk 3). Deze bevindingen sluiten mogelijk goed aan op de resultaten van gedragsexperimenten van Berridge en collega’s. Met behulp van specifieke GABA receptoragonisten en AMPA/kainate glutamaat receptorantagonisten, hebben Reynolds & Berridge (2001, 2002, 2003) aangetoond dat de anteriore (of rostrale) shell betrokken is bij voedingsgedrag, terwijl de posteriore (of caudale) shell een belangrijke rol speelt bij angst en defensief gedrag. Deze gedragingen, die elkaar in zekere zin uitsluiten, komen tot stand via projecties van de nucleus accumbens naar de laterale hypothalamus. In hoeverre de projecties tussen rostrale en caudale shell, zoals aangetoond in de huidige studie bij het over en weer beïnvloeden van deze gedragspatronen een rol spelen valt op dit moment niet op grond van de experimenten van Berridge en collega’s te concluderen, maar is zeker de moeite van het testen waard.
Nederlandse samenvatting

Interacties tussen shell en core van de nucleus accumbens

In de literatuur zijn verschillende aanwijzingen te vinden dat de nucleus accumbens shell en core subregionen elkaar, in ieder geval indirect, kunnen beinvloeden. Zoals eerder in dit hoofdstuk aangegeven, is het mogelijk dat de shell via het ventrale pallidum en de mediadorsale thalamuskern projecteert naar een gebied in de prefrontale cortex dat voornamelijk corticostriatale projecties zendt naar de core, waarbij glutamaat als neurotransmitter wordt gebruikt op het niveau van de core. De resultaten van functionele onderzoeken ondersteunen deze anatomische bevinding van een indirecte shell-naar-core verbinding. De resultaten van dit proefschrift (Hoofdstuk 2) laten zien dat er eveneens directe verbindingen bestaan tussen shell en core, waarbij zeer waarschijnlijk GABA als neurotransmitter wordt gebruikt. Zoals in Hoofdstuk 5 wordt besproken, geven de resultaten van functionele onderzoekingen eveneens aanleiding te veronderstellen dat er een rechtstreekse, onderlinge beinvloeding kan plaatsvinden tussen shell en core van de nucleus accumbens.

Een belangrijk resultaat in de huidige studie is de richting waarin de vezels verlopen tussen de shell en core: op basis van onze anatomische bevindingen kan de conclusie worden getrokken dat de core een groter deel van de shell bereikt dan omgekeerd. Met behulp van elektrofysiologische- en gedragsonderzoekingen zullen de interacties tussen de shell en core verder moeten worden bestudeerd.

De ‘spiraal hypothese’: van motivatie tot actie

Zoals hierboven al is aangegeven, spelen de dopaminerge projecties vanuit het ventrale mesencephalon eveneens een rol bij de interacties tussen de verschillende basale ganglia-thalamocorticale circuits. Reeds in 1978 is dit door Nauta en collega’s aangegeven als een mogelijkheid waarlangs limbisch-gerelateerde delen van het striatum een invloed kunnen uitoefenen op motoriek en gedrag. Terwijl de oorspronkelijke bevindingen van Nauta et al. (1978) in de rat pleiten voor een rechtstreekse beinvloeding van het dorsale, motorisch-gerelateerde deel van het striatum door projecties vanuit de nucleus accumbens, suggereren de meer recente bevindingen van Haber en collega’s (2000) in primaten een meer indirecte, spiraalgewijze beinvloeding van ventrale naar dorsale delen van het striatum via verschillende celgroepen in de substantia nigra. Met andere woorden, de nucleus accumbens shell beïnvloedt via mediale delen van de substantia nigra pars compacta de core, de core beïnvloedt via meer lateraal gelegen celgroepen in de substantia nigra het centrale deel van het striatum dat op zijn beurt via nog meer lateraal gelegen dopaminerge
celgroepen het dorsolaterale deel van het striatum beïnvloedt. De resultaten van onze experimenten in ratten (beschreven in Hoofdstuk 4) laten zien, in overeenstemming met de bevindingen van Nauta et al. (1978), dat er een directe functionele schakel bestaat tussen de nucleus accumbens shell en het dorsolaterale, sensorimotorische deel van het striatum via een nigrostriatale, dopamine-bevattende neuronen in het mediale deel van de substantia nigra pars compacta en het laterale deel van het ventrale tegmentale gebied. Deze resultaten suggereren dat de directe limbisch-motorische beïnvloeding tussen het ventrale en dorsale striatum via het dopaminerge systeem een belangrijke rol speelt in het proces van integratie van informatie tussen verschillende basale ganglia-thalamocorticale circuits. De resultaten van functionele onderzoeken geven aan dat er mogelijk een ‘modulerende’ rol is vanuit de shell via het dopaminerge systeem op ‘dorsaal gelegen striatale gebieden, zoals de core en het caudatus-putamen complex. Het lijkt voor de hand te liggen dat de rol van de shell gezocht moet worden in het ‘versterken’ van gedragingen die door andere striatale gebieden worden gereguleerd, een rol die verder zal moeten worden onderzocht.

3. ALGEMENE CONCLUSIES EN TOEKOMSTIG ONDERZOEK

De functionele-anatomische onderzoekingen en bevindingen die in dit proefschrift zijn beschreven benadrukken verschillende nieuwe mogelijkheden voor integratie en selectie van informatie binnen en tussen de in parallel georganiseerde basale ganglia-thalamocorticale circuits. In plaats van een ‘hiërarchische’ organisatie van deze interacties, pleiten de huidige bevindingen voor een ‘heterarchische’ organisatie, waarbij verschillende mogelijkheden van interacties tussen de functioneel verschillende, in parallel georganiseerde circuits bestaan, te weten via striato-nigrale circuits, cortico-striato-thalamische circuits en intrastriatale interacties. In dit scenario zijn interacties tussen de functioneel verschillende, in parallel georganiseerde circuits, die in de loop van leerprocessen kunnen worden versterkt, van essentieel belang.

De resultaten die in dit proefschrift zijn beschreven geven nieuwe inzichten in de functionele-anatomische organisatie van het ventrale limbisch-gerelateerde corticostriatale systeem. Hoewel onze anatomische en functionele resultaten nog geen definitief antwoord geven op de vraag hoe de neuronale ensembles in de nucleus accumbens precies functioneren, vormen ze wellicht wel de basis voor nieuwe experimenten. Wellicht kunnen in de toekomst met behulp van nieuwere neuroanatomische en neurofysiologische technieken de complexe neuronale netwerken van de basale ganglia verder worden ontrafeld om uiteindelijk te leiden tot een beter begrip van de rol van deze hersenstructuren bij onze motoriek en gedrag.
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DANKWOORD - ACKNOWLEDGEMENTS

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Full articles


Van Dongen Y.C., Kolomiets B.P., Groenewegen H.J., Thierry A.M., Deniau J.M. A subpopulation of mesencephalic dopamine neurons interfaces the shell of nucleus accumbens and the dorsolateral striatum in rats (*manuscript in preparation*).


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