"The greatest obstacle to discovery is not ignorance — it is the illusion of knowledge."

Daniel J. Boorstin
The striatum is the major input nucleus of the basal ganglia and can be subdivided into a dorsal part and a ventral part that is also named nucleus accumbens (NAc). The dorsal part is involved in motor control and habit learning whereas the ventral part is mostly associated with reward-motivated behaviors. The neurons that populate the striatum are for 95% GABAergic medium spiny neurons. Input into the striatum comes from cortex and thalamus and is mostly glutamatergic. This glutamatergic input is the essential drive behind excitatory synaptic transmission in the striatum. Apart from glutamatergic input, the striatum also receives dopaminergic input from the midbrain.

To measure excitatory synaptic transmission and synaptic plasticity we used field potential recordings which measures the activity of a population of neurons in the striatum evoked by stimulation of glutamatergic fibers. In order to study the involvement of specific neurotransmitters and receptors involved in synaptic transmission and its modulation, we applied pharmacological tools in the perfusion solution that modify glutamatergic, dopaminergic and GABAergic synaptic transmission. We also measured the levels of the neuromodulator dopamine which plays an important part in striatal synaptic transmission. In order to study long term potentiation (LTP), we applied a high frequency stimulation-protocol. We found that glutamatergic synaptic transmission in the striatum is depressed by bath-application of N-methyl-D-aspartate (NMDA). We found that this depression is mediated by adenosine acting on A1-receptors. The NMDA-receptors that mediate this depression were shown to contain the NR1/NR2A-subunits. These NMDA-receptors are most likely located in the striatum on medium spiny neurons. Furthermore, bath-applied NMDA also depresses evoked dopamine-release in striatum via NMDA-receptors that contain NR1/NR2A-subunits.

LTP in the NAc was shown to be independent of the Mg$^{2+}$-block of NMDA receptors but rather depends on the level of NMDA-receptor activation. We also showed that LTP depends on dopamine D1- but not D2-receptors, is independent of GABA-receptor activation but requires the activation of group I mGluRs. Finally, we showed differences in neuronal excitability in the striatum and NAc between male and female mice in different stages of the estrous cycle. In addition, the excitability of striatal neurons of both male and female mice is modulated by acute administration of estrogen. Together, these results contribute to understanding the role of different neurotransmitters in the physiology of the striatum.
LIST OF PUBLICATIONS

This thesis is based on the following articles, referred to in the text by the roman numerals stated below:

I. **Sietske M. Schotanus**, Bertil B. Fredholm and Karima Chergui
NMDA depresses glutamatergic synaptic transmission in the striatum through the activation of adenosine A₁ receptors: Evidence from knockout mice. *Neuropharmacology*, 51 (2006) 272-282

II. **Sietske M. Schotanus** and Karima Chergui
NR2A containing N-methyl-D-aspartate receptors depress glutamatergic synaptic transmission and evoked dopamine-release in the mouse striatum. *Submitted to Journal of Neurochemistry*

III. **Sietske M. Schotanus** and Karima Chergui
Long-term potentiation in the nucleus accumbens requires both NR2A- and NR2B-containing NMDA receptors
*Submitted to European Journal of Neuroscience*

IV. **Sietske M. Schotanus** and Karima Chergui
Dopamine D1 receptors and group I metabotropic glutamate receptors contribute to the induction of long-term potentiation in the nucleus accumbens. *Neuropharmacology*, (2008) *in press*
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPA</td>
<td>Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid</td>
</tr>
<tr>
<td>CREB</td>
<td>cAMP response element binding protein</td>
</tr>
<tr>
<td>(a)CSF</td>
<td>(artificial) cerebrospinal fluid</td>
</tr>
<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>DAT</td>
<td>Dopamine transporter</td>
</tr>
<tr>
<td>DOPA</td>
<td>Dihydroxyphenylalanine</td>
</tr>
<tr>
<td>DYN</td>
<td>Dynorphin</td>
</tr>
<tr>
<td>EAAT</td>
<td>Excitatory amino acid transporter</td>
</tr>
<tr>
<td>ENK</td>
<td>Enkephalin</td>
</tr>
<tr>
<td>EP</td>
<td>Entopeduncular nucleus</td>
</tr>
<tr>
<td>(f)EPSP/PS</td>
<td>(field) Excitatory postsynaptic potential</td>
</tr>
<tr>
<td>EPSC</td>
<td>Excitatory postsynaptic current</td>
</tr>
<tr>
<td>ER</td>
<td>Estrogen receptor</td>
</tr>
<tr>
<td>E2</td>
<td>Estrogen (17β-estradiol)</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma (γ)-aminobutyric acid</td>
</tr>
<tr>
<td>GAD</td>
<td>Glutamic acid decarboxylase</td>
</tr>
<tr>
<td>GAT</td>
<td>GABA transporter</td>
</tr>
<tr>
<td>GP</td>
<td>Globus pallidus</td>
</tr>
<tr>
<td>HFS</td>
<td>High frequency stimulation</td>
</tr>
<tr>
<td>IPSP</td>
<td>Inhibitory postsynaptic potential</td>
</tr>
<tr>
<td>KA</td>
<td>Kainate</td>
</tr>
<tr>
<td>LTD</td>
<td>Long term depression</td>
</tr>
<tr>
<td>LTP</td>
<td>Long term potentiation</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
</tr>
<tr>
<td>mGluR</td>
<td>Metabotropic glutamate receptor</td>
</tr>
<tr>
<td>MSN</td>
<td>Medium spiny (projection) neuron</td>
</tr>
<tr>
<td>NAc</td>
<td>Nucleus accumbens</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>SNc</td>
<td>Substantia nigra pars compacta</td>
</tr>
<tr>
<td>SNr</td>
<td>Substantia nigra pars reticulata</td>
</tr>
<tr>
<td>SP</td>
<td>Substance P</td>
</tr>
<tr>
<td>STN</td>
<td>Subthalamic nucleus</td>
</tr>
<tr>
<td>VGLUT</td>
<td>Vesicular glutamate transporter</td>
</tr>
<tr>
<td>VTA</td>
<td>Ventral tegmental area</td>
</tr>
</tbody>
</table>
BACKGROUND

I. THE STRIATUM

The striatum is the major recipient of inputs into the basal ganglia. This sub-cortical nucleus participates in initiation, production and sequencing of movement and behavior and in the storage of information regarding these processes. A functional segregation can be made between the dorsal and ventral parts of the striatum, the ventral part being termed nucleus accumbens (NAc). The dorsal regions of the striatum have been closely linked to the production of task-oriented motor-sequences and habit learning (Graybiel, 1995; Barnes et al., 2005), whereas the NAc has been shown to be more involved in working-memory and reward-motivated behaviors and has been implicated in the development of addiction (Lovinger et al., 2003; Lewis et al., 2004). In the rodent brain, the striatum consists mainly of the caudate nucleus, whereas in the primate brain the striatum has differentiated into nucleus caudatus and putamen. Dorsal and ventral striatum likely have the same function within the basal ganglia-circuitry which is to translate cortical input into inhibition of downstream structures (Graybiel, 1998; Lovinger et al., 2003).

Striatal input projections

The main inputs into the striatum arise from cortex, thalamus and midbrain (Fig. 1). In addition to this, smaller projection systems provide inputs into the striatum, these include; serotonergic input from the dorsal raphe nucleus and noradrenergic input from locus coeruleus.

Cortical input into the striatum originates from most cortical areas; motor, premotor and prefrontal regions as well as limbic cortical areas. The topographic organization of these cortical areas is largely maintained in their projections to the striatum and all cortico-striatal neurons are pyramidal neurons that use glutamate as a neurotransmitter (see Fig. 4). Three different corticostriatal cell subtypes have definitely been identified, these include: neurons of the pyramidal tract; bilaterally projecting corticocortical corticostriatal neurons; and neurons that are actually corticothalamic in nature but have a collateral projection to the striatum.

Thalamic input to the striatum was long believed to consist of a single topographically organized projection, but was later established to be heterogeneous in a similar way as
the corticostriatal projection pathways. In fact, two independent thalamostriatal projections were identified; one that originates from the parafascicular/centromedian nuclei and a separate one that arises from rostral parts of the complex. These projections are excitatory and use glutamate as a neurotransmitter.

Distinct groups of neurons located in the midbrain give rise to a dense efferent projection to striatum, limbic cortex and associated subcortical structures. These neurons use the catecholamine dopamine as a neurotransmitter and their cell-bodies are located primarily in the substantia nigra pars compacta (SNC) and ventral tegmental area (VTA).

**Figure 1 – Sagittal section of rat brain showing striatal circuitry.**
Green lines represent glutamatergic pathways, red lines represent GABAergic pathways and blue lines are dopaminergic projections. GABAergic (red) medium spiny neurons in the striatum (Nst) project to globus pallidus (GP) and substantia nigra (SN). Abbreviations – STR: striatum, CTX: cortex, Th: thalamus, STN: subthalamic nucleus, SN: substantia nigra, GP: globus pallidus. (Adapted from Greengard P et al. (1999) Neuron, Vol.23; 435-447)

**Cellular composition of the striatum**
Medium spiny neurons (MSNs) or spiny projection neurons are GABAergic neurons that constitute 90-95% of the cell-population of the striatum. MSNs have a cell body of approximately 20-25 µm in diameter from which extend 7-10 moderately branched dendrites that are densely laden with spines (Fig. 2). The distribution of the dendrites is not always uniform and may be limited by compartmental boundaries within the striatum as specified in a later paragraph. MSNs project their axons to the two major
target structures of the striatum; the globus pallidus (GP) and the substantia nigra pars reticulata (SNr) (Fig. 1). Often, MSNs also sprout a local axon collateral that remains within the striatum but may extend over a very large area within this structure (Kawaguchi et al., 1990).

Three main classes of striatal interneurons have been identified based on their physiological, morphological and histochemical properties. The first type is a fast-spiking cell (FS-cell) that receives direct cortical input and is GABAergic in nature. This type of interneuron is thought to mediate feed-forward GABA-inhibition via the parvalbumin-positive terminals that make symmetrical synapses on somata and dendrites of neostriatal cells, probably including MSNs (Kita et al., 1990).

The second type of interneuron, the persistent and low-threshold spike cell (PLTS-cell) exhibits unique firing properties (Kawaguchi, 1993) and has NADPH diaphorase immunoreactivity. As NADPH diaphorase and NO-synthase are identical (Dawson et al., 1991), these neurons are considered to release NO within the striatum. They are positive for neuropeptide Y and are thought to be GABAergic. Like FS-cells, these cells regulate MSNs from the cortex through feed-forward inhibition. Thus, cortical information may affect MSNs through two types of interneurons.

The third type, the long-lasting afterhyperpolarization cell (LA-cell) is characterized by large afterhyperpolarizations which causes it to fire single spikes. They are cholinergic as they display ChAT-immunoreactivity, and they mainly receive input from the thalamus. Their terminals end on dendritic shafts and perikarya of MSNs in symmetrical synapses, and they have been proposed to stabilize the state of MSNs, whether it is depolarized or hyperpolarized (Akins et al., 1990).
**Striatal output projections**

Two parallel pathways run from the striatum to two output nuclei; globus pallidus (GP) and substantia nigra pars reticulata (SNr) (Fig. 1, 3). The first pathway, termed the indirect pathway, sends inhibitory, GABAergic projections to GP and from there to the subthalamic nucleus (STN). In the STN, the output signal is transformed into excitatory, glutamatergic projections that reach SNr. This projection from STN to SNr is the only excitatory projection in the basal ganglia. Striatal MSNs project directly to SNr in the second pathway. This inhibitory, GABAergic pathway is therefore called the direct pathway.

![Diagram](https://example.com/diagram)

**Figure 3 – Striatal output projections.**

Cortex and thalamus provide excitatory input to the striatum. Striatal neurons that contain enkephalin (ENK) and the dopamine D2-receptor (D2) provide inhibitory input to globus pallidus (GP). GP sends inhibitory projections to subthalamic nucleus (STN) which in turn provides excitatory input to substantia nigra pars reticulata (SNr). Striatal neurons expressing the dopamine D1-receptor (D1), dynorphin (DYN) and substance P (SP) directly provide SNr and entopeduncular nucleus (EP) with inhibitory input. GABAergic neurons from SN and EP provide inhibition to thalamus, superior colliculus and pedunculopontine nucleus (PPN). (Adapted from Gerfen et al. (1992))

Immunohistochemical studies suggest that striatopallidal neurons (indirect pathway) express enkephalin (ENK), whereas striatonigral neurons (direct pathway) express substance P (SP) and dynorphin (DYN) (Gerfen, 1992). In addition, the two striatal output pathways are affected differently by the dopaminergic input from substantia nigra pars compacta (SNC). Striatonigral neurons containing SP and DYN also express the dopamine D1-receptor that facilitates transmission. The inhibitory dopamine D2-receptor is expressed only by striatopallidal neurons that co-express ENK (Gerfen et al., 1990; Le Moine et al., 1990). In spite of the differences in dopamine receptor expression, dopaminergic inputs into these pathways lead to the same effect in reducing inhibition in thalamocortical neurons. This reduced inhibition will facilitate movements.
initiated by the cortex. Thus, dopaminergic input into the striatum is essential for initiation of movement.

**Patch-matrix compartments**

The division of striatal neurons into two clearly identifiable output pathways that regulate movement-initiation via disinhibition of the thalamus gives a deceptive impression of the complexity of the regulatory mechanisms within the striatum itself. The considerable heterogeneity in the striatum is organized in a mosaic structure composed of two interdigitating compartments differing in cytochemical make-up and afferent and efferent targets. MSNs in the larger of these compartments, the **matrix** compartment, express calbindin and a plexus of somatostatin-immunoreactive fibers. These neurons preferentially receive inputs from sensory and motor cortices. The MSNs located in the smaller compartment, called **patch** or **striosome**, express µ-opiate receptors, display acetylcholinesterase labeling and receive input from a more restricted area of the cortex (Donoghue and Herkenham, 1986; Kawaguchi et al., 1990; Gerfen, 1992).

Retrograde axonal tracing studies show that both patch and matrix neurons project to the substantia nigra, but that patch neurons provide inputs to dopaminergic cells in both SNc and SNr whereas matrix neurons provide inputs to GABAergic neurons in SNr. Thus, the striatonigral pathway is subdivided in a patch-matrix manner with patch-neurons innervating the dopaminergic parts of SN and matrix-neurons innervating the non-dopaminergic parts of SNr. Multiple studies have shown that dendrites of subsequent patch and matrix neurons do not extend beyond their separate compartments, suggesting that inputs are confined to the separate compartments as well (Bolam et al., 1988). Indeed, dopaminergic inputs from midbrain VTA, SN and retrorubral area provide inputs into the striatum that specifically target either the patch- or the matrix-compartments (Jimenez-Castellanos and Graybiel, 1987).

**Segregation between dorsal and ventral striatum**

The cortical, thalamic and dopaminergic inputs into the striatum do not segregate along well-defined lines. The boundary between the NAc (ventral striatum) and the caudate putamen (dorsal striatum) is widely used as a demarcation line, but a clear distinction between these two striatal subregions has not been identified (Groenewegen et al., 1999; Voorn et al., 2004). In line with the dorsal-ventral striatal division, appetitive
behavior and reinforcement are generally agreed to be ventral striatal functions (Cardinal et al., 2002; Kelley, 2004b). However, some effects of psychostimulants on conditioned reinforcement (Baker et al., 1998) and feeding behavior (Kelley, 2004a) can be ascribed to parts of the dorsal striatum, slightly blurring the dorsal-ventral division. Similar functional overlap can be seen in cognitive functions (Setlow, 1997; Devan and White, 1999).

No clear boundary between dorsal and ventral striatum can be established based on cytoarchitecture or chemoarchitecture (Prensa et al., 2003). Instead, a dorsolateral to ventromedial graded density in MSN’s has been observed that resembles both the zonal organization of several neurochemical gradients and the pattern of corticostriatal inputs (Karachi et al., 2002; Haber, 2003). This dorsolateral to ventromedial distinction is also in line with regional differentiation of behavioral functions (Voorn et al., 2004). Together, these findings suggest that a dorsolateral to ventromedial functional striatal organization would provide a better framework to define striatal boundaries than would the classic dorsal-ventral divide (Fig. 4).

This divide fits well with the already established distinction of the functionally related core and shell regions of the nucleus accumbens. These two regions would represent the ventral-most sector in the dorsolateral to ventromedial functional organization with

---

**Figure 4** – Cortical and thalamic inputs to the striatum distribute in dorsomedial-to-ventrolateral zones (grayscale).

This distribution is illustrated by showing the topographical arrangement of afferents originating in the frontal cortex, midline and intralaminar thalamic nuclei, basal amygdaloïd complex and hippocampal formation. Afferent projections from these regions converge in longitudinal striatal zones with a dorsomedial-to-ventrolateral orientation. The traditional distinction between dorsal and ventral striatal areas and, more specifically, the core and shell region of the nucleus accumbens are also outlined. Abbreviations of interest – ac, anterior commissure; SMC, sensorimotor cortex; PFC, prefrontal cortex (reproduced with permission; Voorn et al. 2004).
the ventral and medial extreme being the caudomedial shell. The shell reaches areas that are unique for striatal output such as preoptic, hypothalamic and mesencephalic areas associated with locomotor functions. So, although dividing the striatum into dorsal and ventral extremes has greatly contributed to gaining understanding of striatal function, it might be more appropriate to view striatal function from a mediolateral perspective (Voorn et al., 2004).

II. SYNAPTIC TRANSMISSION AT THE CORTICOSTRIATAL SYNAPSE

Glutamatergic synaptic transmission

The major force driving synaptic transmission in the striatum is the excitatory input coming from cortex (corticostriatal pathway). These projections use the excitatory amino acid glutamate as a neurotransmitter. Glutamate is a nonessential amino acid that does not cross the blood-brain barrier and is therefore locally synthesized in neurons from precursors. The most abundant precursor for the synthesis of glutamate is glutamine, which is released into the extracellular space by glial cells. Glutamine is taken up by nerve terminals via excitatory amino acid transporters (EAATs) and converted to glutamate by the mitochondrial enzyme glutaminase in the cytoplasm. After synthesis, glutamate is packaged into synaptic vesicles by vesicular glutamate transporters (VGLUT). Once glutamate is released into the synaptic cleft, it is taken up by glial cells via EAATs and broken down to glutamine by the enzyme glutamine synthetase. Glutamine is then released back into the extracellular space and can be reused by nerve terminals to synthesize glutamate. This cooperation between nerve terminals and glial cells to maintain a sufficient store of neurotransmitter is called the glutamate-glutamine cycle (Fig. 5).

Glutamate activates postsynaptic glutamate receptors. In the striatum, both ionotropic and metabotropic glutamate receptors are present. Ionotropic glutamate receptors are ligand-gated cation channels that are subdivided into three subtypes; NMDA (N-methyl-D-aspartate), AMPA (amino-3-hydroxy-5-methyl-4-isozole propionic acid) and kainate (KA) receptors (Herrling et al., 1983; Tarazi and Baldessarini, 1999). Evidence indicates that all three subtypes are expressed in the striatum. For instance, electrical stimulation of cortical inputs evokes excitatory postsynaptic potentials (EPSPs) in striatal MSNs which are largely mediated by AMPA receptors (Calabresi et al., 1990). In addition to this, evidence indicates a significant role for the NMDA-receptor in MSNs. MSNs are susceptible to toxic doses of NMDA or over-activation of
NMDA-receptors which may result in excitotoxicity and neuronal cell-death (Gerfen, 1992).

Metabotropic glutamate receptors (mGluRs) are coupled to various intracellular signal transduction processes. To date, eight mGluR subtypes have been cloned from the mammalian brain. These subtypes are classified into three major groups based on sequence homologies, coupling to second messenger systems and pharmacological profiles. Group I mGluRs, which include mGluR1 and mGluR5, couple primarily to Gq and increase phosphoinositide hydrolysis. Groups II (mGluR2 and mGluR3) and III (mGluR 4, 6, 7 and 8) couple to Gi/Go and inhibition of adenylyl cyclase (Conn and Pin, 1997). The Group I mGluRs, mGluR1 and 5, have been identified in striatal MSNs (Kerner et al., 1997). Activation of Group I mGluRs was shown to potentiate NMDA receptor currents in striatal neurons (Pisani et al., 1997; Rouse et al., 2000).

The NMDA-receptor

NMDA receptors are glutamate-activated cation channels that are characterized by a high Ca²⁺/Na⁺ permeability ratio. The pore of the channel is blocked by Mg²⁺ in a voltage-dependent manner and activation of the receptor requires glycine as a coagonist of glutamate (Dingledine et al., 1999). The NMDA-receptor mediates synaptic transmission and neural plasticity at many sites in the mammalian central nervous system. It has slow activation and deactivation kinetics and can contribute to
epileptiform activity and excitotoxicity leading to neuronal cell death in certain experimental and pathological conditions (Zeron et al., 2002; Jarabek et al., 2004). NMDA receptors consist of an NR1 subunit and any of four NR2 subunits (NR2A-D) (Fig. 6). The NR1 subunit is expressed throughout the brain, whereas the expression of NR2 subunits is spatially and temporally regulated. Although all striatal MSNs express NR2A and NR2B subunits (Standaert et al., 1999), the relative expression of these subunits varies in different areas of the striatum. For instance, a distinct lateral to medial gradient of the NR2A mRNA distribution can be observed. In addition, NR2B was shown to be more abundantly expressed than NR2A (Buller et al., 1994). NMDA receptors are known to be located both intra- and extrasynaptically, fulfilling different roles with respect to development, synaptic plasticity and cell survival (Hardingham et al., 2002). NMDA-receptors with distinct pharmacologies appear to be involved in the release of the striatal neurotransmitters acetylcholine, dopamine and GABA (Nicolas et al., 1994; Nankai et al., 1995). Furthermore, striatal infusion of antisense oligonucleotide probes directed against NR2A and NR2B subunits produced differential effects in behavioral paradigms (Standaert et al., 1996).

On a pathophysiological level, NMDA receptor subunits are thought to be promising therapeutic targets for the development of new medication for treatment of several illnesses and disorders. Dysfunction of glutamatergic transmission in the striatum is believed to underlie pathologies such as Huntington’s disease, Parkinson’s disease and schizophrenia. For instance, animal models of Huntington’s disease demonstrate an increased vulnerability to NMDA-mediated cell death (Zeron et al., 2002). In animal models, NMDA receptor antagonists have proven to be effective antiparkinsonian...
agents that have the potential to reduce application of other, more complicated types of therapeutics (Hallett and Standaert, 2004; Kristiansen et al., 2007).

**Electrophysiology of striatal neurons**

Intracellular recording studies have shown that the membrane potential of MSNs shifts periodically between a hyperpolarized ‘down’-state and a depolarized ‘up’-state (Calabresi et al., 1990). In the ‘down’-state, MSNs typically display a membrane potential of around -60 to -90 mV. In this stage MSNs are at resting membrane potential. When depolarized, MSNs display a membrane potential of approximately -60 to -40 mV. The ‘up’-state, or depolarized state, is generated by simultaneous activation of a large number of corticostriatal and thalamostriatal fibers (Wilson, 1993). During the ‘up’-state, the membrane potential reaches a spike threshold which triggers a train of spikes in the MSN. Thus, the synaptic inputs to the MSNs and their membrane-properties are major determining factors of their firing-potential (Calabresi et al., 1987).

In vivo intracellular recordings of MSNs have demonstrated that MSNs fire infrequently. Periods of membrane hyperpolarization and electrical silence are briefly interrupted by periods of sustained depolarization driven by cortical inputs (Wilson, 1993).

MSNs do not fire until a cortex command arrives. When they do fire, an inhibitory intrinsic and reciprocal network controls the propagation of excitation (Flores-Hernandez et al., 1994). This network may consist of several components. Cholinergic interneurons form synapses on the dendrites of MSNs and intracellular recordings suggest that cholinergic synapses elicit both nicotinic and muscarinic responses in MSNs. In addition to this, GABAergic interneurons were shown to participate in a feedforward inhibition mechanism by forming synapses on the soma and proximal dendrites of MSNs (Kita et al.,

---

**Figure 7** – Dopaminergic and glutamatergic synaptic transmission in striatal neurons. Dopaminergic terminals synapse on necks of spines and are therefore in an excellent position to modulate intracellular signaling pathways that may affect glutamatergic transmission.
Intrastriatal stimulation was shown to evoke GABAergic inhibitory postsynaptic potentials (IPSPs) in MSNs that are mediated by GABA$_A$-receptors (Kita, 1996). Another important modulator of striatal MSNs is dopaminergic input from the midbrain. Dopaminergic terminals typically synapse on the necks of dendritic spines of MSNs. Dopamine receptors are known to regulate intracellular cAMP levels and all three subtypes of ionotrophic glutamate receptors have been shown to be functionally modulated by activation of cAMP-dependent protein kinase (PKA) (Greengard et al., 1991). Anatomic studies have localized both ionotropic glutamate receptors and dopamine receptors to dendrites of MSNs, raising the possibility that dopamine receptor activation may regulate glutamate receptor function and thereby modulate the postsynaptic responsiveness of MSNs (Hersch et al., 1995).

**Synaptic plasticity**
Changes in the efficacy of synaptic transmission at synapses can contribute to storage of information within neural circuits. These changes in synaptic strength can occur both on a short-term and long-term basis depending on synaptic activity and the type of synapse (Bliss and Collingridge, 1993). Experimentally, it is possible to induce prolonged (>1 hour) increases and decreases in synaptic strength which have been termed long-term potentiation (LTP) and long-term depression (LTD), respectively. The most extensively studied type of synaptic plasticity is NMDA-receptor dependent LTP and LTD in the CA1 region of the hippocampus (Bear and Malenka, 1994).

NMDA receptors have been shown to play an essential role in inducing long-term changes in synaptic strength (Malenka and Bear, 2004) and the subunit-composition of NMDA-receptors can be of importance for the induction of synaptic plasticity (Massey et al., 2004). Differential incorporation of NR2A or NR2B subunits within NMDA-receptor complex is thought to have profound implications for the generation of synaptic plasticity due to different interactions with proteins involved in intracellular cascades. The subunit-composition of NMDA receptors is under developmental regulation, and so is their consequential role in synaptic plasticity (Schramm et al., 2002). In fact, a developmental switch where NR2B predominates in early developmental stages and is gradually replaced by NR2A is associated with a decrease in the potential for generation of LTP due to the dramatically lower affinity for CAMKII of NR2A compared to NR2B (Barria and Malinow, 2005; Bayer et al., 2006).
The corticostriatal pathway was shown to support activity-dependent changes in synaptic plasticity (Partridge et al., 2000; Spencer and Murphy, 2000). Early studies on plasticity in striatal slices were primarily focused on LTD at corticostriatal synapses due to the relative ease with which this form of plasticity could be induced within the slice-preparation (Calabresi et al., 1992b). The induction of LTP in the dorsal striatum was generally thought to be difficult to achieve in slice-preparations until recent experiments revealed robust LTP in brain-slices using different recording techniques (Calabresi et al., 1992a). LTP-induction also seems to be dependent on the striatal subregion (Liu et al., 2004; Massey et al., 2004).

III. MODULATORS OF STRIATAL SYNAPTIC TRANSMISSION

Adenosine

The purine and neuromodulator adenosine is not considered to be a classical neurotransmitter as it is not stored in synaptic vesicles or released in a Ca^{2+}-dependent manner. Instead, it is generated from ATP by extracellular enzymes. Adenosine is thought to be involved in the regulation of important central mechanisms such as cognition, arousal, aggression and anxiety (Lang et al., 2003). In several brain regions, including brainstem, hippocampus and cortex, adenosine has been shown to be inhibitory (Greene and Haas, 1991). This inhibitory action was also demonstrated in the corticostriatal pathway (Malenka and Kocsis, 1988). In the striatum, endogenous adenosine levels are thought to be correlated with motor-activity (Huston et al., 1996). Adenosine can influence physiological processes involving NMDA receptors, and shows interactions with dopamine receptors in the basal ganglia (Manzoni et al., 1994; Fuxe et al., 1998). Adenosine has also been suggested to play a significant role in synaptic plasticity at specific synapses in the NAc and the striatum (Flagmeyer et al., 1997; d'Alcantara et al., 2001).

The adenosine A_1-receptor

Transmission via purinergic synapses is thought to be widespread in the mammalian brain which is exemplified by a wide distribution of purinergic receptors, both ionotropic and metabotropic. The adenosine A1 receptor is one of four known subtypes of adenosine receptors: A1, A2A, A2B and A3. The A1 receptor is a G-protein coupled receptor that is linked to inhibition of transmitter release. Neuroprotection through the inhibition of glutamate-release is also ascribed to activation of this receptor (Rudolphi
et al., 1992). In the striatum, endogenous adenosine acting at adenosine A1-receptors may have neuroprotective effects during traumatic or metabolic stress where NMDA-receptors are involved (Calabresi et al., 1997; Centonze et al., 2001). In general, the A1 receptor is characterized as a homeostatic receptor with protective functions in many tissues.

The expression-levels of A1-receptors in striatum were shown to be intermediate to high whereas in NAc low levels were observed (Fastbom et al., 1987). In the striatum, adenosine A1 receptors are shown to be colocalized with D1 receptors in MSNs that project to substantia nigra (SNr) and entopeduncular nucleus (EP). In this pathway, adenosine A1 receptors were found to strongly antagonize dopamine receptor D1-mediated facilitation of GABA-release into the EP suggesting the existence of an antagonistic A1/D1 interaction in the direct pathway. A1 receptors might contribute to this effect by inhibiting glutamate release (Ferre et al., 1996). A1 receptors are also present in striatopallidal MSNs as well as in glutamatergic corticostriatal neurons (Ferre et al., 1997; Ferre et al., 1999).

**Dopamine**

Dopamine (DA) is one of the three well-established catecholamine neurotransmitters that are derived from the amino acid tyrosine. DA-synthesis occurs in the cytoplasm. Tyrosine is converted into DOPA by the aid of tyrosine hydroxylase, an enzyme essential for DA-synthesis. DOPA is then converted into DA by the enzyme DOPA decarboxylase. Upon synthesis, DA is transported into vesicles by a vesicular monoamine transporter (VMAT). After release into the synaptic cleft a Na+-dependent dopamine transporter (DAT) ensures DA reuptake into nerve terminals and surrounding glial cells.

DA was first described in the central nervous system in 1964 (Dahlström and Fuxe, 1964). DA-ergic cell-bodies are shown to be primarily localized within the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). Their efferent projections terminate both in the dorsal striatum and NAc, as well as in limbic cortex and associated subcortical structures (Nauta et al., 1978; Voorn et al., 1986; Descarries et al., 1996). DA-ergic inputs importantly modulate synaptic transmission in the striatum. Postsynaptic interactions between DA and glutamate in the NAc are shown to be critical for motivation, reward and other behavioral functions that are disrupted after chronic exposure to drugs of abuse (Everitt and Wolf, 2002). Ultrastructural studies
have shown that DA-ergic terminals form both symmetrical and asymmetrical contacts on postsynaptic structures (Sesack and Pickel, 1992) and are therefore in a position to modulate synaptic transmission by releasing DA in the vicinity of glutamatergic synapses or by causing more diffuse increases in extracellular DA levels (Grace, 1991; Garris et al., 1994; Descarries et al., 1996). Evidence has shown that striatal DA-transmission occurs in two distinct temporal modes; tonic and phasic. Phasic DA-transmission is a high-amplitude transient signal that consists of DA released by burst firing of DA-terminals. The transient nature of the signal is ensured via reuptake mechanisms mediated by DAT that eliminate DA from the synaptic cleft. This type of activity is rapid and behaviorally relevant. In contrast, tonic DA-transmission represents a pool of DA that is present at steady-state concentrations in the extra-cellular space. By activating autoreceptors located extrasynaptically on the DA-terminal, tonic DA-levels act to downregulate the responsivity of the DA-system to burst-firing generated during behavioral activation (Grace, 1991; Garris et al., 1994).

**Dopamine-receptors**

Originally, two types of DA-receptors (D1 and D2) were identified on pharmacological and biochemical grounds, based on their ability to activate (D1) and inhibit (D2) adenylate cyclase. Gene cloning, however, revealed five subgroups (D1-D5) where D1 and D5 belong to the original D1-family and D2, D3 and D4 fall into the initial D2-group. All subtypes are G-protein coupled transmembrane receptors and their transduction mechanisms are linked to adenylate cyclase and phospholipid hydrolysis. Dopamine acts both pre- and postsynaptically. The impact of DA-receptor activation on MSN activity in the intact striatum is dependent on multiple factors, including the mode of DA-transmission (tonic or phasic), the relative contribution of D1 and D2 receptors and the striatal subregion involved. As was mentioned in previous chapters, dopaminergic input into the striatum is thought to modulate the response of MSNs to excitatory input by influencing voltage-gated ion channels, as well as ligand-gated ion channels such as the NMDA-receptor (Hernandez-Lopez et al., 1997). D1-receptor mediated enhancement of NMDA-receptor responses was first described in the striatum about a decade ago and later confirmed in the nucleus accumbens (Cepeda and Levine, 1998; Chergui and Lacey, 1999). Different types of NMDA-receptors were shown to be involved in the D1-receptor mediated increase in
immediate early gene expression in striatonigral neurons (Keefe and Gerfen, 1996). The interaction between NMDA-receptors and dopaminergic inputs also affects striatal synaptic plasticity as the activation of D1-receptors was shown to be required for the induction of NMDA-dependent LTP (Calabresi et al., 2000; Kerr and Wickens, 2001). DA also modulates MSNs via inhibitory pathways which involves D2 receptor-dependent mechanisms. Studies in striatal brain slices revealed that DA or D2-agonists applied in the bath decreased the amplitude of evoked corticostriatal EPSPs and locally evoked EPSCs (Levine et al., 1996; Umemiya and Raymond, 1997). This D2 receptor-mediated inhibition of corticostriatal inputs is likely to be, at least partially, mediated by presynaptic receptors (O'Donnell and Grace, 1994; Hsu et al., 1995). In addition, MSNs from D2 receptor-deficient mice exhibited increased spontaneous synaptic activity and large amplitude depolarizations that were not observed in wild-type mice (Cepeda et al., 2001). Together, these studies demonstrate that it is likely that D2-receptors play a significant role in inhibiting corticostriatal signaling in striatal MSNs.

**Estrogen**

Traditionally, a distinction is made between hormones (chemicals that are released from glands into the blood-stream), and neurotransmitters (chemicals that are synthesized by neurons, stored in vesicles and exert their actions on post-synaptic receptors). This distinction, however, was challenged in the 1950s by the discovery of neurohypophysal hormones such as oxytocin and vasopressin that are synthesized by neurons, but are released into the blood-stream (Harris, 1951). From that moment onwards, the concept of a neurotransmitter was liberalized which allowed for a more integrative perspective on the interaction between neuronal and endocrinological compartments. Recent work on brain steroids, particularly locally synthesized estrogen (E2), reveals that E2 in the brain displays many features that are traditionally associated with neurotransmitters. Indeed, estrogens are synthesized in the brain by aromatization (aromatase) of androgens such as testosterone. Rapid changes in aromatase-activity can be induced by phosphorylation and changes in intracellular Ca^{2+}-levels. E2 was found to be present in pre-synaptic boutons, displays rapid effects at a cellular level and is subject to rapid inactivation mechanisms (Balthazart et al., 2001).
Estrogen-receptors

Two nuclear estrogen-receptors, ERα and ERβ, modulate gene-transcription when activated by estrogen-agonists. These receptors are transcription factors that dimerize upon ligand-binding. This dimer then gathers other factors and forms a receptor complex that binds to hormone response elements of target genes. Many of the cellular effects of estrogens are mediated by these nuclear or ‘genomic’ actions (Sanchez et al., 2002).

Accumulating evidence indicates ‘non-genomic’ membrane-effects of estrogens in the brain that might either be mediated by membrane-bound ERα and ERβ (Toran-Allerand, 2004) or by a novel, plasma membrane-associated, putative ER with unique properties (Toran-Allerand et al., 2002). In fact, nuclear and membrane ERs can be derived from a single gene transcript and display near-identical affinities for E2 (Razandi et al., 1999). Membrane-bound ERs can trigger activation of second messenger systems such as cAMP and IP3 production and CREB activation (Levin, 2002) as well as activation of MAP kinase (Wade et al., 2001).

The expression of mRNA for ERα and ERβ has been reported in the striatum (Kuppers and Beyer, 1999), and striatal expression of these two ERs is thought to be low to moderate (Shughrue et al., 1997; Zhang et al., 2002; Morissette et al., 2008). Several studies have demonstrated rapid effects of E2 in the striatum. For instance, in vitro administration of E2 rapidly stimulates striatal DA-release (Becker, 1990), and pretreatment with E2 was shown to increase the firing rate of MSNs in response to DA (Arnauld et al., 1981). Indeed, such effects of E2 in the striatum have been associated with the activation of second messenger pathways via interaction of E2 with membrane-associated ERs (Mermelstein et al., 1996).

GABA

The inhibitory neurotransmitter γ-aminobutyric acid (GABA) was identified in brain tissue during the 1950s (Roberts and Frankel, 1950; Udenfriend, 1950). It is now known that as many as a third of the synapses in the brain use GABA as their neurotransmitter. GABA is commonly found in local circuit interneurons as can be identified in the striatum. Importantly, striatal MSNs are GABAergic projection-neurons that exert an inhibitory influence on their target-structures.

The predominant precursor for GABA synthesis is glucose, but pyruvate and glutamine can also act as precursors. These precursors are metabolized into glutamate and
converted into GABA by the catalyzing enzyme glutamic acid decarboxylase (GAD). This enzyme is almost exclusively localized in GABAergic neurons and is therefore commonly used as a specific marker for these neurons. Once GABA is synthesized, it is transported into synaptic vesicles. Removal upon release is carried out by high-affinity transporters for GABA (GATs) that transport GABA back into neurons or glial cells.

**GABA-receptors**

Three types of postsynaptic GABA-receptors (GABA<sub>A-C</sub>) have been characterized, two of which are ionotropic receptors (GABA<sub>A</sub> and C) and one of which is metabotropic (GABA<sub>B</sub>). Ionotropic GABA-receptors are permeable to Cl<sup>-</sup> and are therefore mostly inhibitory, since in most neurons the reversal potential for Cl<sup>-</sup> is more negative than the threshold for neuronal firing. These receptors are pentamers assembled from five types of subunits (α, β, γ, δ and ρ) giving rise to a diverse range of combinations. As a consequence, the function of ionotropic GABA-receptors varies widely among neuronal types.

GABA<sub>A</sub>-receptors are essential for the function of the basal ganglia providing fast synaptic inhibition between basal ganglia nuclei (Smith et al., 1998). Most of the GABA-effects in the striatum are mediated by this receptor. Tonic GABA-inhibition due to low concentrations of GABA diffusing from the synapse and activating extrasynaptic GABA<sub>A</sub>-receptors also occurs in basal ganglia circuits (Goetz et al., 2007). In the striatum, GABA<sub>A</sub>-receptors mediate fast transmission at symmetrical synapses on dendrites and spines of MSNs and GABA interneurons. They are also present on GABAergic as well as non-GABAergic pre-synaptic terminals (Fujiyama et al., 2000).

Like ionotropic GABA-receptors, metabotropic GABA-receptors (GABA<sub>B</sub>) are inhibitory and widely distributed in the brain. GABA<sub>B</sub>-mediated inhibition is mostly due to the activation of K<sup>+</sup>-channels, but GABA<sub>B</sub>-mediated inhibition can also be due to blockade of Ca<sup>2+</sup>-channels leading to inhibition of transmitter-release if GABA<sub>B</sub>-receptors are located pre-synaptically. GABA<sub>B</sub>-receptors are heterodimers of two subunits GABA<sub>B1</sub> and GABA<sub>B2</sub> (Kaupmann et al., 1998). In the striatum, GABA<sub>B</sub>-receptors are located both pre- and postsynaptically. Presynaptic GABA<sub>B</sub>-receptors can modulate input-projections from cortex and thalamus and are present on terminals of
MSNs and GABAergic interneurons. Furthermore, postsynaptic GABA\textsubscript{B}
receptors may affect excitability of striatal neurons (Lacey et al., 2005).
AIMS

The general aim of the studies brought together in this thesis was to identify the role of several neurotransmitters; glutamate, dopamine and GABA as well as the neuromodulator adenosine in synaptic transmission and plasticity in the striatum of the mouse. Specific focus was placed upon the NMDA-receptor; its activation, its subunits and its role in synaptic plasticity.

The specific aims of this thesis are:

- To investigate the effect of NMDA on synaptic transmission and long-term synaptic plasticity and the interaction between NMDA-receptors and the neuromodulator adenosine in the striatum

- To investigate if the NR2A and NR2B subunits of the NMDA-receptor contribute to NMDA-induced synaptic depression of glutamatergic transmission and evoked dopamine-release in the striatum

- To examine the contribution of NMDA-receptor subunits NR2A and NR2B to high frequency stimulation (HFS)-induced long term potentiation (LTP) in the nucleus accumbens (NAc)

- To identify the contribution of the neurotransmitters dopamine, glutamate and GABA to high frequency stimulation (HFS)-induced long term potentiation (LTP) in the nucleus accumbens (NAc)

- To identify gender-related differences in synaptic transmission in the striatum and NAc and to identify the acute effect of estrogen (E2)
MATERIALS AND METHODS

Animals and brain slice preparation

Experiments using mice were approved of by our local ethical committee (Stockholms norra djurförsöksetiska nämnd). Male and female C57Bl6 mice aged 4-7 weeks (females 5-7 weeks) were used in all experiments on wild-type mice. Knockout mice with a deletion in the A₁ receptor gene were back-bred against C57Bl6 in the speed congenic protocol of Jackson laboratories and judged to be congenic by 140 gene markers (Johansson et al., 2001). A₁ receptor knockout mice used in the experiments were 4-7 week old males.

Mice were anaesthetized by inhalation of fluothane and subsequently underwent cervical dislocation and decapitation. The brain was swiftly removed and coronal striatal slices (400 µm thick) were prepared in ice cold aCSF on a microslicer (Leica, VT1000S). In the experiments using decorticated striatal slices, the cortex was removed from each slice immediately after sectioning. The slices were then equilibrated during at least 1 hour in carbogen rich (95:5) artificial cerebrospinal fluid (aCSF) (NaCl 126 mM, KCl 2.5 mM, NaH₂PO₄ 1.2 mM, MgCl₂ 1.3 mM, CaCl₂ 2.4 mM, glucose 10 mM and NaHCO₃ 26 mM, pH 7.4) at a constant temperature of 32°C. Using a glass Pasteur's pipette filled with oxygenated aCSF, the slices were transferred to a recording chamber (Warner Instruments, Hamden, CT) mounted on an upright microscope (Olympus, Solna, Sweden) and were continuously perfused with oxygenated aCSF.

Identification of the estrous cycle of female mice

Since the first characterization of the estrous cycle in rats by Long and Evans (Evans and Long, 1922) the evaluation of changes in epithelial cell structure (vaginal smear) in spontaneously ovulating laboratory animals has been used as a principal approach to measure reproductive cyclicity. In juvenile mice a fully cornified smear indicates the onset of the first ovulation (around 5 weeks). The following cycles will tend to average 5 days, although irregularities may occur due to environmental conditions. For instance, female mice housed in the same room as male mice exhibit more regular cycles than females housed in an all-female environment. In addition, continuous illumination can induce a persistent estrus (Goldman et al., 2007).
In a standard cycle, proestrus is identified by the presence of round, nucleated epithelial cells, which often have a granular appearance under the microscope (Fig. 8A). Proestrus lasts for one day and is followed by vaginal estrus, which is routinely identifiable by large numbers of cornified (or keratinized) cells which have either a needle-like or a more rounded shape (Fig. 8B). The predominance of cornified cells can last one or two days, depending on the length of the cycle (4-5 days). Metestrus reflects the transitional period during the early part of diestrus, and its smear is characterized by a combination of leukocytes and cornified and rounded epithelial cells (Fig. 8C, D). During diestrus, the smear can often be almost exclusively leukocytic (Fig. 8E), but may also show a few small clumps of nucleated epithelial cells that indicate initiation of proestrus the next day.

The appearance of different cell-types in the vaginal smear is a reflection of the hormonal changes that are taking place in the rodent. The two main hormones that regulate the cyclicity in the female reproductive system are estrogen and progesterone. Overall, estrogen promotes proliferation and growth of endometrial cells and increases

Figure 8 – Stages of the mouse estrous-cycle identified by means of vaginal smear samples.

A – Pro-estrus; characterized by a large amount of nucleated cells (white arrows), B – Estrus; characterized by cornified cells (black arrows), C, D – Met-estrus; a combination of nucleated cells and leukocytes (C, black arrows) and cornified cells (D, white arrow), E – Di-estrus; predominating leukocytes (black arrows).
vascular permeability. Estrogen also stimulates the proliferation and differentiation (keratinization) of the squamous epithelium of the vagina (Li and Davis, 2007). Progesterone exerts an opposite effect of estrogen and leads to a decrease in the number of keratinized cells (Goldman et al., 2007; Li and Davis, 2007). Different studies report slight differences as to the exact moment when the fluctuations in hormone-concentrations take place. However, it can generally be stated that the levels of estrogen are high during proestrus, low during estrus and metestrus and intermediate during the diestrus-phase of the cycle. The differences observed in vaginal cytology are a relevant reflection of the circulating estrogen-levels, and therefore also of the hormone-status in the brain.

We collected vaginal smear samples from the female mice used in the electrophysiology-experiments by means of a micro-spatula (Fisher Scientific). The samples were fixed in 100% ethanol and were stained according to the Nissl-staining protocol (Cresyl violet). The samples were then identified on a regular light-microscope (Motic) by at least two separate, unbiased researchers.

**Electrophysiology**

Experiments were performed in a submerged-type chamber perfused with carbogen rich (95:5) aCSF. Extracellular field potentials were recorded using a borosilicate glass micropipette (low resistance; G120F-3; Warner Instrument Corp.) filled with aCSF positioned on the slice surface. A concentric bipolar stimulating electrode (FHC, Bowdoinham, ME) was placed in close proximity to stimulate fibers. Signals were amplified 500 times via an Axopatch 200B amplifier (Axon Instruments, Foster City, CA), acquired at 10 kHz, filtered at 2 kHz and recorded on a Dell computer using acquisition and data analysis software from Axon Instruments (pClamp9).

To evoke synaptic responses single stimuli (0.1 ms duration) were applied every 15 s at an intensity of 50-70% of the maximal response as established by a stimulus/response curve, for each slice, at the beginning of a recording session. The maximal stimulation intensity was determined measuring the amplitude of the field potential evoked by gradually increasing stimulation intensities. Baseline responses were recorded for at least 15-20 minutes. If the fEPSP slope was observed to drift by more than 25% during baseline recording the pulse intensity was re-adjusted and another baseline was recorded. If the fEPSP slope continued to drift the slice was abandoned.
**Amperometry**

Amperometric detection of dopamine release was performed with methods described earlier (Schmitz et al., 2001; Chergui et al., 2004). A carbon fiber electrode (WPI, 10µm diameter) with an active part of 100 µm was positioned within the striatum in the brain slice. A constant voltage of +500 mV was applied to the carbon fiber via an Axopatch 200B amplifier (Axon Instruments) and currents were recorded with the same amplifier. A stimulating electrode (patch micropipette filled with aCSF) was placed on the slice surface in close proximity to the carbon fiber electrode. Stimulation consisted of a single pulse (0.1 ms, 8-14 µA) applied every minute, which evoked a response corresponding to oxidation of dopamine at the surface of the electrode. When the carbon fiber electrode was held at 0 mV, stimulation of the slice did not produce any current.

**Drugs and chemicals**

In the experiments performed in this thesis, acute bath-administration of different kinds of drugs is an important tool. Drugs were dissolved according to the solubility-protocol. Stock solutions of most drugs were stored at -20°C and fresh solutions were prepared by diluting with aCSF before each experiment. Drug-application to the slices was controlled by switching a three-way tap. The perfusion flow rate was maintained at 1-1.5 ml/min.

**Drugs targeting the glutamatergic system**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMDA</td>
<td>prototypic NMDA-receptor agonist (Study I &amp; II) (Carter et al., 1989)</td>
</tr>
<tr>
<td></td>
<td>(N-methyl-D-aspartate, Sigma)</td>
</tr>
<tr>
<td>DL-APV</td>
<td>unselective, potent NMDA antagonist (Study III)</td>
</tr>
<tr>
<td></td>
<td>(DL-2-amino-5-phosphonopentanoic acid, Tocris)</td>
</tr>
<tr>
<td>Ifenprodil*</td>
<td>selective antagonist for NR1/NR2B-diheteromeric receptors (Study II &amp; III)</td>
</tr>
<tr>
<td></td>
<td>(2-(4-benzylpiperidino)-1-(4-hydroxy-phenyl)-1-propanol (hemi) tartrate, Tocris &amp; Sigma)</td>
</tr>
<tr>
<td>Ro 25-6981*</td>
<td>selective antagonist for NR1/NR2B-diheteromeric</td>
</tr>
</tbody>
</table>
receptors, derivative of ifenprodil  
([R-(R*,S*)] - α - (4-hydroxyphenyl) - β - methyl 
- 4 - (phenylmethyl) - 1 - piperidinepropanol – hydrochloride, Sigma)

**NVP-AAM 077** specific antagonist of NR1/NR2A-diheteromeric receptors  
([R)-(S)-1-(4-bromo - phenyl) – ethyl-amino - (2,3-dioxo-1,2,3,4-tetrahydro-quinoxalin-5-yl) - methyl] - phosphonic acid, Novartis Pharma)

**CNQX** potent, competitive AMPA/kainate receptor antagonist  
(6-cyano-7-nitroquinoxaline-2,3-dione disodium salt, Sigma)

**MPEP hydrochloride** potent and highly selective non-competitive antagonist of the mGlu5 receptor subtype  
(2-methyl-6-(phenylethynyl) pyridine hydrochloride, Tocris)

**LY 367385** selective mGlu1a receptor antagonist  
((S)-(+)a-amino-4-carboxy-2- methylbenzeneacetic acid, Tocris)

**DL-TBOA** potent, competitive, non-transportable blocker of excitatory amino acid transporters with high selectivity for EAATs  
(DL-threo-b-benzyloxyaspartic acid, Tocris)

* Ifenprodil and Ro 25-6981 are structurally closely related and are suggested to exert their antagonistic action on the LIVBP-like domain (leucine/isoleucine/valine-binding protein-like domain) of the NR2B subunit (Perin-Dureau et al., 2002; Malherbe et al., 2003). The specific affinity of these drugs in blocking NR2B (Ro 25-6981; IC50 ± 0.02 µM and Ifenprodil; IC50 ± 0.4 µM) exceeds that for NR2A (Ro 25-6981; IC50 ± 52 µM and Ifenprodil; IC50 ± 49 µM) (Fischer et al., 1997).

** NVP AAM077 was a gift from Dr. Y.P. Auberson, Novartis Pharma, Basel, Switzerland. The affinity of NVP-AAM 077 for NR2A (IC50 = 14nM) clearly exceeds that for NR2B (IC50 = 1800nM) (Auberson et al., 2002).
### Drugs targeting the dopaminergic system

<table>
<thead>
<tr>
<th>Drug</th>
<th>Description</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SCH 23390</strong></td>
<td>D$_1$ dopamine receptor antagonist</td>
<td>(Study IV)</td>
</tr>
<tr>
<td>(R(+)-SCH-23390 hydrochloride, Sigma)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sulpiride</strong></td>
<td>D$_2$ dopamine receptor antagonist</td>
<td>(Study IV)</td>
</tr>
<tr>
<td>(S)-(−)-Sulpiride, Sigma)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nomifensine</strong></td>
<td>selective dopamine uptake inhibitor, interacts with the dopamine transporter at a site different from that of cocaine</td>
<td>(Study IV) (Wieczorek and Kruk, 1994)</td>
</tr>
<tr>
<td>(1,2,3,4-tetrahydro-2-methyl-4-phenyl-8-isouquinolinamine maleate salt, Sigma)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Drugs targeting purinergic neurotransmission

<table>
<thead>
<tr>
<th>Drug</th>
<th>Description</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adenosine</strong></td>
<td>endogenous neurotransmitter, purine</td>
<td>(Study I)</td>
</tr>
<tr>
<td>(adenosine, Calbiochem)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DPCPX</strong></td>
<td>potent and selective A$_1$ adenosine receptor antagonist</td>
<td>(Study I)</td>
</tr>
<tr>
<td>(8-cyclopentyl-1,3-dipropylxanthine, Tocris)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Drugs targeting the GABAergic system

<table>
<thead>
<tr>
<th>Drug</th>
<th>Description</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bicuculline</strong></td>
<td>GABA$_A$ receptor antagonist</td>
<td>(Study II &amp; IV)</td>
</tr>
<tr>
<td>(−)-Bicuculline methiodide, Sigma)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CGP 55845</strong></td>
<td>potent, selective GABA$_B$ receptor antagonist</td>
<td>(Study IV)</td>
</tr>
<tr>
<td>(2S) – 3 - [(1S) – 1 - (3,4 – dichlorophenyl) ethyl ] amino – 2 – hydroxypropyl ] (phenylmethyl) phosphinic acid, Tocris)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Effects of NMDA-receptor activation on glutamatergic synaptic transmission and evoked-dopamine release in the striatum

In order to examine glutamatergic synaptic transmission in the striatum we performed recordings of extracellular field potentials in acute corticostriatal brain slices. In earlier studies a brief electrical stimulation of corticostriatal slices was shown to elicit a characteristic biphasic response (Fig. 9) with two negative components (Calabresi et al., 1997). The first negative component, termed N1, is a non-synaptic compound action potential which is independent of glutamate release as it is unaffected by the AMPA-receptor antagonist CNQX (Study I). The second negative component, termed “field excitatory postsynaptic potential/population spike” (fEPSP/PS), is mediated by endogenous glutamate released upon electrical stimulation of glutamate-containing fibers present in the slice. In the striatum, this component is mostly mediated by glutamate receptors of the AMPA type as blocking these receptors with CNQX resulted in a full blockade of the fEPSP/PS (Study I).

NMDA depresses striatal glutamatergic synaptic transmission

Apart from AMPA-receptors, glutamate also activates NMDA-receptors to mediate fast synaptic transmission. NMDA-receptors are known to play a role in synapse development and neurotoxicity and are critically involved in certain forms of synaptic plasticity (Malenka and Bear, 2004). In the hippocampus, exogenous application of NMDA was shown to cause an initial inhibition of glutamatergic synaptic transmission followed by LTD (Lee et al., 1998). The ability of NMDA to induce long-term changes in glutamatergic transmission in the striatum has not yet been extensively studied. We
therefore wanted to examine the ability of NMDA to regulate glutamatergic synaptic transmission and to induce long-term changes in synaptic strength in the striatum. As we set out to evaluate the effect of NMDA-receptor activation on glutamatergic synaptic transmission we applied NMDA in the perfusion solution at various concentrations while measuring fEPSP/PS amplitude. We observed a depression of the fEPSP/PS in response to NMDA-application which was concentration-dependent and culminated in a full blockade at 40\(\mu\)M which lasted for 1 hour. At 32°C, this depression of fEPSP/PS was not a form of LTD as the fEPSP/PS returned to baseline levels after 2 hours. However, at room temperature (RT) the depression was longer lasting and was therefore identified as a form of LTD (Study I). These results show that NMDA profoundly disrupts glutamatergic synaptic transmission in a concentration- and temperature-dependent manner. Indeed, previous studies have indicated that NMDA-application at lower concentrations than we used in our study is able to induce a chemical form of LTD in hippocampus and dentate gyrus (Lee et al., 1998; Rush et al., 2001). However, such LTD is not induced in the striatum at a physiologically relevant temperature but only at RT suggesting that different expression mechanisms are activated at different temperatures.

**NMDA-induced depression is mediated by adenosine via A1-receptor activation**

Activation of NMDA-receptors has been shown to cause the release of adenosine in cortex and hippocampus (Craig and White, 1993; Manzoni et al., 1994). We now wanted to test the possibility that the observed actions of NMDA might be mediated at least in part by the release of this purine. Of the four adenosine-receptors identified (Fredholm et al., 2001), the A1-receptor is the most likely candidate to mediate such an effect as it was shown to be involved in NMDA-mediated downregulation of glutamatergic synaptic transmission in hippocampus (Dunwiddie and Fredholm, 1989). It is also highly expressed in the cortex (Mahan et al., 1991) and might therefore mediate adenosine-effects at corticostrial terminals. We investigated whether the NMDA-induced decrease in fEPSP/PS was mediated by the adenosine A1-receptor by examining the effect of NMDA in the presence of the selective A1-receptor antagonist DPCPX and by studying this effect in A1-receptor knockout mice (Study I). We found that the A1-receptor antagonist DPCPX dramatically reduces NMDA-induced synaptic depression both at 32°C and RT.
Moreover, the LTD we observed when applying NMDA (40µM) at RT was abolished by this intervention. However, the initial depression induced by NMDA at 40µM remained largely unaffected. It is possible that incomplete blockade of A1-receptors was responsible for this remaining inhibition and we therefore repeated the experiments in brain slices from A1-receptor knockout mice. We found that the effect of NMDA at 40µM was significantly reduced and that the initial inhibition that we saw in wild-type slices with DPCPX was also dramatically attenuated, although not completely abolished. This observation suggests that post-synaptic membrane depolarization might account for a small component of NMDA-induced short term depression.

Finally, we wanted to find out whether adenosine itself would be capable of producing a similar effect to that induced by NMDA. We applied adenosine in the perfusion-solution at different concentrations and found a depression of the amplitude of the fEPSP/PS both at 32°C and RT. Similar to the NMDA-effect, the depressant effect of adenosine was larger at RT than at 32°C. The depressant effect of adenosine was absent in slices from A1-receptor knockout animals at both temperatures examined. These results clearly demonstrate that NMDA-receptors and adenosine A1-receptors cooperate to downregulate glutamate release in the striatum (Study I).

**NMermanns depression is mediated by NMDA-receptors containing the NR2A-subunit**

The precise molecular composition of functional NMDA-receptors in the striatum is currently unknown. As we now identified a specific mechanism involving NMDA-receptors which leads to down-regulation of striatal glutamate release (Study I), we wanted to identify the subunit-composition of these NMDA-receptors. Several studies have identified the subunit composition of striatal NMDA-receptors using different biochemical approaches and have found that the NR2B-subunit is most abundantly expressed (Standaert et al., 1994; Dunah and Standaert, 2003). The NR2A-subunit is also expressed in the striatum, but at more moderate levels while NR2C and NR2D-subunits are virtually absent in this brain region (Standaert et al., 1994; Landwehrmeyer et al., 1995; Ghasemzadeh et al., 1996). We therefore wanted to determine whether NR2B and/or NR2A subunits contribute to NMDA-induced synaptic depression in the striatum.
Having established a concentration-response curve (Study I) we chose a concentration of NMDA (25µM) that induced a reproducible and reversible depression of the fEPSP/PS and evaluated the involvement of NR2B-subunits in this depression by examining the effect of two NR2B-antagonists, ifenprodil and Ro 25-6981. We found that NR2B-selective antagonists did not affect the depressant action of NMDA on fEPSP/PS in the striatum, demonstrating that NR2B does not play a significant role in NMDA-mediated synaptic depression (Study II). We then proceeded to examine the effect of the NR2A-selective antagonist NVP-AAM077 and found that slices perfused with this drug did not show a significantly decreased fEPSP/PS in response to NMDA. Thus, an NR2A-antagonist, but not NR2B-antagonists, was able to block NMDA-induced depression indicating that NMDA-induced depression of glutamatergic synaptic transmission in the striatum is mediated by NR2A-containing NMDA receptors (Study II).

NR2A-containing NMDA-receptors are located in the striatum on medium spiny neurons

In the corticostriatal brain slices we used in these experiments, functional connections between the cortex and the striatum are preserved. Evidence has indicated that the expression of NR2A is higher in the cortex than in the striatum (Kawaguchi et al., 1989; Standaert et al., 1994; Li et al., 2003). Since we applied NMDA in the perfusion-solution, all NMDA-receptors present in the slice would be activated including those present in the cortex. We wanted to examine the possibility that the observed NMDA-induced depression could be due to activation of NR2A-containing NMDA-receptors located on cortical neurons that project to the striatum. To test this hypothesis, we repeated the experiments described in acutely decorticated striatal slices (see Materials and Methods).

In decorticated striatal slices application of NMDA depressed the amplitude of the fEPSP/PS similar to intact slices. Perfusion with the NR2B-antagonist Ro 25-6981 did not block this NMDA-induced decrease, but NMDA failed to produce a significant decrease in the amplitude of the fEPSP/PS in the presence of NR2A-antagonist NVP AAM077. So, in decorticated striatal slices, an NR2A-antagonist, but not an NR2B-antagonist, blocks the depressant action of NMDA. This clearly demonstrates that NMDA-induced decrease is mediated by NR2A-containing NMDA-receptors present in the striatum and not in the cortex (Study II).
Striatal GABAergic interneurons have been shown to express the NR2A-subunit in moderate to high levels and display a higher NR2A/NR2B ratio than MSNs (Standaert et al., 1999; Kuppenbender et al., 2000). Furthermore, NMDA induces the release of GABA in the striatum, most likely as a result of NMDA-induced firing activity in GABAergic interneurons as well as MSNs (Fantin et al., 2007). We examined the possibility that the release of GABA could contribute to NMDA-induced inhibition of the fEPSP/PS by applying NMDA in the presence of the GABA <sub>A</sub>-receptor antagonist bicuculline. We found that there was no significant change compared to NMDA-application under control conditions, although we did observe a tendency for bicuculline to reduce the depressant effect of NMDA, but this difference was not statistically significant (Study II). These results show that the NMDA-induced inhibition of glutamatergic synaptic transmission in the striatum is independent of cortical inputs and of GABA-release. This strongly indicates that NMDA-receptors involved in NMDA-induced decrease of the fEPSP/PS might be located on MSNs.

**NM<sub>A</sub> depresses evoked DA-release via NM<sub>D</sub>-receptors containing NR2A-subunits**

It is possible that bath-applied NMDA could act on presynaptic NMDA-receptors located on dopamine terminals and modulate dopamine release in the striatum (Ohta et al., 1994; Cheramy et al., 1998). Midbrain dopaminergic neurons have been shown to express low levels of NR2A and NR2B subunits in the soma and dendrites (Albers et al., 1999) and these receptors could therefore possibly also be present on dopaminergic terminals in the striatum. We therefore wanted to evaluate the possible effect of NMDA on stimulation-evoked release of dopamine from dopamine-terminals in corticostriatal slices (Study II).

We applied NMDA in the bath as described earlier and found that this produced a profound decrease in the peak amplitude of the evoked release of dopamine. When we applied NMDA in the presence of the NR2B-antagonist Ro 25-6981 this depression still occurred. However, the depressant action of NMDA on evoked dopamine-release was significantly reduced in the presence of NR2A-receptor antagonist NVP AAM077, but was not completely blocked. These results show that NMDA depresses evoked dopamine-release in the striatum and that this effect is largely mediated by NMDA-receptors containing NR2A-subunits.
This finding identifies an important role for NR2A-containing NMDA-receptors in the regulation of the release of dopamine in the striatum. The mechanism by which NMDA exerts its action could be through direct activation of NR2A-containing NMDA-receptors present on dopaminergic terminals (Albers et al., 1999). Further involvement of other subunits, particularly that of NR2D (Dunah et al., 1996), is hereby not excluded as dopaminergic neurons also express functional NMDA-receptors containing this subunit. Alternatively, adenosine, or other diffusible retrograde messengers (Avshalumov et al., 2003) could be released following post-synaptic NR2A-containing NMDA-receptor activation and contribute to NMDA-induced inhibition of evoked dopamine-release by a mechanism similar to NMDA-induced depression of glutamatergic synaptic transmission (Study I).

Long term potentiation (LTP) in the core region of the nucleus accumbens (NAc)

In order to examine the properties of glutamatergic synaptic plasticity in the core region of the nucleus accumbens (NAc), we measured the amplitude of the fEPSP/PS evoked by brief electrical stimulation of glutamatergic fibers present in the brain slice. Previous studies demonstrated that 100 Hz trains of stimuli applied up to 4 times were able to induce LTP in the NAc (Kombian and Malenka, 1994; Schramm et al., 2002; Li and Kauer, 2004). We recorded a 20 min baseline of the fEPSP/PS evoked by single pulse stimulation before inducing LTP by HFS (HFS-protocol; 3 trains at 100 Hz, 1 sec duration, 10 sec inter-train intervals, same stimulation intensity as baseline) (Fig. 10). We found that the amplitude of the fEPSP/PS measured 1 hour following HFS was significantly increased compared to baseline values (Study III & IV).
Lack of subunit-specificity in LTP induction in NAc

Studies in cortex and hippocampus have shown that NMDA-receptors contributing to LTP-induction contained NR2A- but not NR2B-subunits (Liu et al., 2004; Massey et al., 2004). However, another study in NR2A knock-out mice showed intact LTP in dorsolateral bed nucleus of the stria terminalis (Weitlauf et al., 2005) and a report on transgenic mice overexpressing NR2B showed that enhanced NR2B-expression in the forebrain facilitated synaptic potentiation (Tang et al., 1999). Furthermore, still other studies show a lack of subunit selectivity in hippocampal LTP, but rather a combined requirement of both NR2A- and NR2B-subunits (Berberich et al., 2005; Bartlett et al., 2007; Berberich et al., 2007). In short, the involvement of NMDA-receptor subunits in LTP-induction has been a source of controversy in the scientific community.

LTP induction in the NAc by means of HFS has been demonstrated to be dependent on NMDA-receptors (Pennartz et al., 1993; Schramm et al., 2002) but to date, the subunit-composition of these receptors has not been studied in detail in this brain region. We therefore examined the subunit composition of NMDA-receptors involved in LTP-induction in the NAc by bath-applying antagonists for the NR2B-subunit (ifenprodil and Ro25-6981) and the NR2A-subunit (NVP AAM077). We found that LTP-induction is fully blocked by two different NR2B-antagonists. This suggests that LTP could be selectively mediated by the predominating NR2-subunit in the striatum, NR2B. We also showed that antagonism of NR2A-subunits results in a full blockade of LTP-induction, even though NR2A is known to be only moderately expressed in the striatum (Study III). Together, these results indicate a lack of subunit-specificity in the induction of LTP in the NAc.

LTP induction in NAc is independent of the Mg\(^{2+}\)-block but rather depends on the level of NMDA-receptor activation

Earlier studies in NAc and striatum demonstrated that LTP-induction can be facilitated by removing Mg\(^{2+}\)-ions from the perfusion solution (Calabresi et al., 1992a; Schramm et al., 2002). Removal of extracellular Mg\(^{2+}\) relieves the NMDA-receptor from its voltage-dependent blockade and can therefore result in a more efficient activation. Although this condition is not physiologically relevant and can potentially lead to excitotoxicity due to NMDA-receptor overactivation, we examined the effect of low Mg\(^{2+}\) on LTP induction in the NAc. We found no significant difference in LTP-magnitude between control conditions and low Mg\(^{2+}\). Application of the NR2B-
antagonist Ro 25-6981 under low Mg$^{2+}$-conditions did not produce significant alterations in its ability to block LTP. Similarly, low Mg$^{2+}$ did not change the ability of the NR2A-antagonist NVP AAM077 to block LTP (Study III). These results indicate a lack of NMDA-receptor subunit-specificity in LTP-induction in the NAc which is independent of the voltage-dependent blockade.

In previous studies, the threshold for LTP-induction was shown to depend on the degree of NMDA-receptor activation (Malenka, 1991; Malenka and Bear, 2004). In agreement with this suggestion, recent findings showed that the amount of charge transfer over the post-synaptic membrane is more critical to LTP-induction than the contribution of a particular NMDA-receptor subtype (Berberich et al., 2007). It is therefore possible that, in the presence of NR2-antagonists, NMDA-receptor activation is insufficient to trigger the intracellular cascade necessary for LTP-induction. This could be an underlying explanation for the complete blockade of LTP by either of these antagonists. We bath-applied the non-subunit selective NMDA receptor antagonist APV at a low concentration to partially block NMDA-receptors and found that this abolished LTP-induction both under control conditions and in low Mg$^{2+}$ (Study III). This strongly suggests that the degree of NMDA receptor-activation, rather than the NMDA-receptor subunit involved, determines whether LTP is induced.

**Dopamine D1-receptors, but not D2-receptors are necessary for LTP induction in NAc**

Apart from glutamatergic innervation, the NAc also receives dopaminergic inputs from SNc and VTA. Modulation by dopamine of synaptic plasticity at glutamatergic synapses in this brain region could have important consequences for reward-directed behaviors and might underlie the mechanisms leading to addiction (Wolf et al., 2004; Hyman et al., 2006). High frequency stimulation (HFS) of the dorsal striatum has been shown to elicit a massive release of dopamine (Partridge et al., 2002). In the dorsal striatum, LTD induction was shown to depend on dopamine D1 and D2 receptors, whereas LTP induction requires the activation of only D1-receptors (Calabresi et al., 1992b; Kerr and Wickens, 2001). We wanted to investigate the contribution of dopamine D1 and D2 receptors to the induction of LTP in the NAc (Study IV).

We found that HFS did not induce LTP in the presence of the D1-receptor antagonist SCH 23390 (10µM), although an earlier study demonstrated that this drug failed to inhibit LTP in NAc at lower concentrations (1µM) (Pennartz et al., 1993). Apparently
the blockade of D1-receptors at a low dose of the D1-receptor antagonist is incomplete as a tenfold increase of the concentration was able to fully block LTP induction (Study IV). We also found that HFS still induced LTP at similar levels as in control slices in the presence of D2-receptor antagonist sulpiride. This indicates that HFS-induced LTP-induction is independent of D2-receptor activation.

In the dorsal striatum, dopamine transport blockers such as nomifensine or cocaine do not affect the induction of LTD (Partridge et al., 2002). In contrast, observations in the NAc showed that LTP is impaired by the psychomotor stimulant amphetamine, and by D1 and D2 receptor agonists (Li and Kauer, 2004). We wanted to investigate the ability of excess dopamine to modulate HFS-induced LTP induction in our slice model. We found that the dopamine reuptake blocker nomifensine prevented the induction of LTP (Study IV).

The possibility existed that the effect of nomifensine was mediated by D2-receptors activated by the excess dopamine. However, co-application of the D2-receptor blocker sulpiride failed to rescue LTP-induction. The inhibitory effect of excess dopamine is therefore more likely due to a decreased excitability of MSNs during HFS (Li and Kauer, 2004).

**LTP induction in NAc does not require GABA-receptor activation**

The main neurotransmitter used by MSNs and several types of striatal interneuron is the neurotransmitter GABA. High frequency stimulation (HFS) has been shown to produce the release of GABA in the striatum (Sung et al., 2001; Gubellini et al., 2004). Electrophysiological studies that investigate the properties of glutamatergic synaptic plasticity often use GABA_A-receptor antagonists to pharmacologically isolate glutamate-mediated responses. We investigated whether treatment with the GABA_A-receptor antagonist bicuculline affected the properties of LTP in the NAc, and whether this modified LTP-induction. We found that LTP was induced after HFS, but with a slight alteration in the initiation phase. The amplitude measured 1 hour after HFS was not significantly different from control slices. We also examined the effect of the GABA_B-receptor antagonist CGP55845 on LTP-induction and found that LTP-induction was not altered compared to control slices. These results suggest that neither GABA_A- nor GABA_B-receptors contribute to HFS-induced LTP in the mouse NAc (Study IV). This observation can be explained by the finding that striatal GABA-
release following HFS is lower and shorter lasting than the release of glutamate (Calabresi et al., 1995).

LTP induction in NAc requires the activation of group I mGluRs

The group I metabotropic glutamate receptors mGluR1 and mGluR5 are present at post-synaptic sites in MSNs of the dorsal striatum. These receptors contribute to the induction of LTD and LTP in this brain region as antagonists targeting these receptors have been shown to block HFS-induction of both LTD and LTP (Gubellini et al., 2004). To investigate if these two receptors also play a role in the induction of LTP in the NAc we applied the mGluR1-antagonist LY 367385 and the mGluR5-antagonist MPEP in the perfusion solution. We found that both the mGluR1-antagonist and the mGluR5-antagonist blocked the induction of LTP by HFS (Study IV). This indicates that LTP-induction in the NAc requires activation of both mGluR1 and mGluR5. As Group I mGluRs have been associated with the regulation of NMDA-receptors and intracellular signaling pathways, antagonists against these receptors may interfere with these mechanisms.

We also examined the possibility that excess glutamate could modulate LTP induction by applying the glutamate reuptake blocker DL-TBOA in the bath in order to promote spillover of glutamate outside the synaptic cleft. The application of this glutamate reuptake blocker blocked HFS-induced LTP. However, application of this drug without applying HFS increased the fEPSP/PS amplitude to a degree similar to that observed following HFS in control slices suggesting that drug-induced LTP may already have occurred before HFS was applied, thus excluding the possibility of generating more LTP by HFS (Study IV). Another possibility would be that excess glutamate impairs LTP via a mechanism involving dopamine release, as previous studies showed that glutamate reuptake blockers depress evoked dopamine-release in the dorsal striatum through the activation of mGluRs located on dopaminergic presynaptic terminals (Zhang and Sulzer, 2003).
Gender-related differences in synaptic transmission and the effect of estrogen (preliminary data)

The properties of synaptic transmission might be different in male and female brain. For instance, estradiol has been shown to increase NMDA-receptor binding in hippocampus in female rats, whereas in males this is not the case. It is suggested that this is due to a failure of estradiol to upregulate hippocampal NMDA-receptors in males (Romeo et al., 2005). In striatum, treatment with estradiol was shown to decrease AMPA-receptor specific binding (Cyr et al., 2001). Thus, glutamatergic transmission seems to be directly affected by estrogens. We therefore wanted to examine possible gender-related differences in synaptic transmission in the striatum. As estrogen-levels

![Graphs showing evoked fEPSP/PS responses to increasing stimulation intensities in proestrus female.](image)

**Figure 11** – Evoked fEPSP/PS responses to increasing stimulation intensities (input-output curves) in presence and absence of exogenous E2.

Responses were recorded in striatum (A and C) or NAc (B and D). **A, B** – The response to increasing stimulation-intensities in proestrus female. Control slices and slices perfused with E2 (1nM) in striatum (A) and NAc (B). **C** - Average value of the fEPSP/PS amplitude at 70-90 µAmp intensities in the striatum of male and female mice in different cycle stages. **D** - Average value of the fEPSP/PS amplitude at 70-90 µAmp intensities in the NAc of male and female mice in different cycle stages. *Abbreviations* – MC, male control; ME, male +E2; DC, diestrus control; DE, diestrus +E2; PC, proestrus control; PE, proestrus +E2; EC, estrus control; EE, estrus +E2; MtC, metestrus control; MtE, metestrus +E2. *p<0.05 two-tailed t-test unpaired obs.*
vary according to the phase of the estrous cycle, we also wanted to investigate possible
differences in synaptic transmission between female mice in different stages of the
cycle.

First, we examined neuronal excitability in striatum and NAc by measuring the
fEPSP/PS response to increasing stimulation intensities (input-output curves). In this
paradigm, an increase of the amplitude of the evoked fEPSP/PS will indicate increased
excitability of the neurons. We did this in male and female mice in different stages of
the estrous cycle. To examine the effect of estrogen (E2) we applied this hormone in
the bath at pro-estrus (1nM) concentrations.

In striatum, our preliminary data shows no significant differences in input-output

![Graphs showing fEPSP/PS responses to trains of eight stimuli at 25 Hz.](image)

**Figure 12 – Evoked fEPSP/PS responses to trains of eight stimuli at 25 Hz.**

Responses were recorded in the striatum (A and C) or the NAc (B and D). **A, B** – Example in proestrus female of the progression of fEPSP/PS responses to the different pulses of the train in striatum (A) and NAc (B). **C** – Average value of the fEPSP/PS amplitude in response to pulse 2-4 in the striatum of male mice and female mice in different cycle stages. **D** – Average value of the fEPSP/PS amplitude in response to pulse 2-4 in the NAc of male and female mice in different cycle-stages.

* $p<0.05$ two-tailed t-test unpaired obs.

*** $p<0.001$ two-tailed t-test unpaired obs.
curves between males and females in the different stages of the cycle under control conditions (Fig. 11C, open bars). However, acute administration of E2 elevated the plateau levels of the curves in male mice and in female mice in proestrus phase (Fig. 11A, C closed bars). In the NAc, we observed no significant differences in input-output curves between males and females, but females in diestrus phase displayed higher plateau-levels than did females in proestrus and estrus phase (Fig. 11D, open bars). Acute administration of E2 did not have a significant effect in any of the groups investigated (Fig. 11B, D closed bars). As this data is preliminary, metestrus data in NAc is still incomplete and therefore not represented in the graphs.

Another method for measuring synaptic transmission is to apply a train of stimuli at a certain frequency and subsequently measure the progression of the amplitude of the fEPSP/PS. The amplitude of the fEPSP/PS tends to show a frequency-dependent short term depression. This phenomenon is generally attributed to a temporary depletion of releasable vesicles or can arise from presynaptic feedback mechanisms or postsynaptic receptor desensitization (Zucker and Regehr, 2002). The progression of the train, however, may vary considerably and can show both short-term depression and potentiation which reflects the properties of presynaptic terminals (Hjelmstad, 2004). We evoked fEPSP/PS trains by applying eight pulses at 25 Hz in striatum and NAc in slices from males and females in different stages of the cycle. We averaged the responses to pulse 2, 3 and 4 in the train and compared these between the different stages of the cycle.

![Figure 13 – Evoked fEPSP/PS responses to trains of eight stimuli at 25 Hz in presence and absence of E2.](image)

Responses were recorded in the striatum (A) or the NAc (B).

A – Average value of the fEPSP/PS amplitude in response to pulse 2-4 in the striatum of male mice and female mice in different cycle stages. B – Average value of the fEPSP/PS amplitude in response to pulse 2-4 in the NAc of male and female mice in different cycle stages.

**Abbreviations** – MC, male control; ME, male +E2; DC, diestrus control; DE, diestrus +E2; PC, proestrus control; PE, proestrus +E2; EC, estrus control; EE, estrus +E2; MtC, metestrus control; MtE, metestrus +E2.

* p<0.05 two-tailed t-test unpaired obs.
** p<0.01 two-tailed t-test unpaired obs.
groups (Fig. 12). We found significant differences between males and females and also between females in different stages of the estrous-cycle. In the striatum, neurotransmission seems to be elevated in females in pro-estrus and estrus phases of the cycle as compared to males and females in metestrus and diestrus-phase. This finding would suggest that endogenous E2 enhances synaptic transmission of neurons in the striatum since E2-levels are especially high during proestrus (Goldman et al., 2007). This enhanced transmission seems to persist into estrus and thereafter declines back to levels that are comparable to males (Fig. 12 C). In the NAc, significant differences can be found only between females in different phases of the cycle. Here, neurotransmission is lowest during the diestrus phase and increases step-wise through proestrus and estrus phases and reaches its highest levels during met-estrus phase (Fig. 12 D). As E2-levels are highest in proestrus, synaptic transmission in the NAc does not seem to show a direct correlation with the cyclicity of only E2, however, interaction with other female hormones might contribute to the step-wise increase observed.

We also investigated the effect of acute application of E2 in the perfusion solution (Fig. 13). In the striatum, we found that bath-applied E2 acutely diminishes synaptic transmission in male mice and in pro-estrus females (Fig. 13A). This finding, together with the finding that E2 elevates plateau-levels input-output curves (Fig. 11C) in these two experimental groups strongly indicates a responsivity to acute E2-administration in both males and females. In the NAc, the effect of bath-applied E2 seems to be opposite since neurotransmission is enhanced in response to E2-administration in males and proestrus and estrus females (Fig. 13B).
CONCLUDING REMARKS

The obtained results have led to the following conclusions:

1. Activation of NMDA-receptors profoundly disrupts glutamatergic transmission in the striatum in a concentration- and temperature-dependent manner. This depression is mediated by adenosine acting on A1-receptors. Furthermore, we showed that adenosine A1 receptors mediate the inhibitory action of adenosine on glutamatergic synaptic transmission in striatum.

2. In addition to this, we showed that activation of NMDA-receptors also depresses evoked-dopamine release in the striatum. This NMDA-induced depression of glutamatergic synaptic transmission and evoked dopamine-release is mediated by NMDA receptors containing the NR2A subunit. Thus, these findings demonstrate a crucial role for NR1/NR2A receptors in the physiology of the striatum.

3. We induced long term potentiation (LTP) in the nucleus accumbens (NAc) and demonstrated that the activation of both NR2A- and NR2B-containing NMDA-receptors is required for this induction to take place. We also showed that this requirement is independent of Mg\(^{2+}\) ions. Partial inhibition of NMDA-receptors prevented LTP from being induced, which demonstrates the requirement of high levels of NMDA-receptor activation.

4. We evaluated the role of GABA, dopamine and glutamate in the induction of LTP in the NAc. We demonstrated that GABA does not play a role in LTP induction. We also showed that activation of dopamine D1-, but not D2-receptors, and of group I mGluRs is required for HFS-induced LTP in NAc.

5. Preliminary data shows differences in neuronal excitability and synaptic transmission in the striatum and NAc between male and female mice in different stages of the estrous cycle. In addition, the excitability of striatal neurons and neurotransmission in both male and female mice is modulated by acute administration of estradiol.
SVENSK SAMMANFATTNING

Striatum är den största inmatningskärnan i de basala ganglierna. Striatum är uppdelad i en dorsal och en ventral del, som även kallas för nucleus accumbens (Nac). Den dorsala delen är involverad i motorisk kontroll och inlämning av vanor medan den ventrala delen är mest associerad med belöningsmotiverat beteende. 95% av neuronen i striatum är GABAerga medium spiny neurons. Striatum tar emot signaler från cortex och thalamus som är mestadels glutamaterga. Dessa signaler driver excitatorisk synaptisk transmission i striatum. Förutom de inkommande glutamaterga signalerna mottar striatum också dopaminerg innervering från medelhjärnan.

I denna studie har vi använt inspelningar av fältpotentialer framkallade genom stimulation av glutamaterga fibrer för att mäta effektiviteten av excitatorisk synaptisk transmission och synaptisk plasticitet i en population av neuron i striatum. För att studera specifika neurotransmittorer och receptorer som är involverade i synaptisk transmission och dess modulation har vi applicerat farmakologiska ämnen i perfusionsvätskan, som modifierar glutamaterg, dopaminerg och GABAerg signalering. Vi har också mätt nivån av regleringssubstansen dopamin som har en viktig roll i striatal synaptisk transmission. För att studera långtidspotentiering (LTP) applicerade vi ett högfrekvent stimuleringsprotokoll.

Vi upptäckte att glutamaterg synaptisk transmission i striatum hämmades av N-methyl-D-aspartate (NMDA). Vi upptäckte också att denna inhibition medieras av adenosin via påverkan på A1-receptorer. NMDA-receptoreerna som medierar hämningen visade sig innehålla NR1/NR2A subenheter. Dessa NMDA-receptorer tycks vara lokalisera på MSNs i striatum. Dessutom hämmade NMDA också frisättningen av dopamin i striatum genom aktivering av NMDA-receptorer som innehåller NR1/NR2A subenheter.

LTP i Nac visade sig vara oberoende av Mg²⁺-blockad av NMDA-receptorn, och berodde istället på graden av NMDA-receptorkoncentration. Vi har också visat att LTP beror på dopamin D1- men inte D2-receptorer och är oberoende av GABA-receptorkoncentration. Däremot krävs aktivering av grupp I mGluRs. Slutligen har vi upptäckt skillnader i neuronal excitabilitet i striatum och Nac mellan hanar och honor i olika faser av östrogencykeln. Sammantaget bidrar dessa resultat till ökad förståelse kring den fysiologiska betydelsen av olika neurotransmittorer i striatum.
Het striatum is de voornaamste invoer-kern van de basale ganglia. Dit hersengebied is ondervoorverteld in een dorsaal deel en een ventraal deel, dat ook wel nucleus accumbens (NAc) wordt genoemd. Het dorsale deel is betrokken bij het regelen van motorische functies en het aanleren van gewoontes terwijl het ventrale deel vooral geassocieerd wordt met beloningsgemotiveerd gedrag. De neuronale cellen in het striatum zijn voor 95% GABAerge medium spiny neurons. Invoer van informatie in het striatum komt vooral vanuit de cortex en de thalamus en is glutamaterg en daarom excitatorisch. Deze glutamaterge invoer drijft de excitatorische synaptische transmissie in het striatum aan. Behalve glutamaterge invoer, ontvangt het striatum ook dopaminerge invoer vanuit de middenhersen.

Om de efficiëntie van excitatoire synaptische transmissie en plasticiteit te meten gebruikten we veld-potentiaal metingen die door stimulatie van glutamaterge vezels werden opgeroepen. Deze metingen geven de activiteit van een populatie neuronen in het striatum weer. Om de bijdrage van specifieke neurotransmitters en receptoren aan synaptische transmissie en de modulatie daarvan te bestuderen voegden we pharmaca toe aan de perfusie-vloeistof. Deze middelen modificeren glutamaterge, dopaminerge en GABAerge synaptische transmissie. We hebben ook de dopamine-uitscheiding gemeten, wat een belangrijke rol speelt in synaptische transmissie in het striatum. Om het niveau van lange-termijn potentiaitie (LTP) te bestuderen pasten we hoogfrequente stimulatie toe.


LTP in de Nac is onafhankelijk van de Mg2+-blokkade van NMDA receptoren maar is wel afhankelijk van het niveau van NMDA-receptor activatie. We hebben ook aangetoond dat LTP afhangt van dopamine D1- maar niet D2-receptor activatie en onafhankelijk is van GABA- receptor activatie maar wel activatie van groep I mGluRs behoeft. Ten slotte hebben we verschillen aangetoond in neuronale excitabiliteit in het striatum en de NAc tussen mannetjes en vrouwtjes-muizen in verschillende stadia van de voortplantings-cyclus. Daar komt bij dat de excitabiliteit van striatale neuronen van zowel mannetjes als vrouwtjes wordt gemanipuleerd door acute toediening van oestrogeen. Tezamen dragen deze resultaten bij aan begrip van de rol van verschillende neurotransmitters in de fysiologie van het striatum.
ACKNOWLEDGEMENTS

The four years that I spent here in Stockholm have been fruitful in many ways; I have learned about science, life, culture, music and traveling. I met new people and made new friends. Now, I am very grateful to be able to bring this adventure to a conclusion with this thesis.

This adventure would not have been possible without the help and dedication of a number of people who I would like to thank very much for all they have done for me. I would like to express my special gratitude to:

My supervisor, Dr. Karima Chergui; thank you for sharing your extensive experience, knowledge and know-how in the world of science. I am very lucky to have worked with you and I have learned a lot from you.

The students that have joined our group; Kalle Svennersten and Shuo Huang.

Dr. Per Svenningsson; thank you for lending a helping hand wherever this was necessary.

My fellow PhD- students; thank you for your friendship and for all those funny and memorable moments we shared. I am happy that our paths crossed so we could learn from each other during this challenging period of our lives.

Prof. Bertil Fredholm, co-author.

My music-friends, my heartbeat during these years; you may not have been aware of your contribution to this thesis, but you have been invaluable.
Tack så mycket!!

My family in Holland: I think you were sometimes wondering; ‘What on earth is she doing up there in the cold?’ Well, the answer is: THIS
Bedankt voor jullie geduld, steun en vertrouwen;
Jaap, Joke, Berend, Eva, Jakob en Dora.

The work that has led to this thesis was carried out at the Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden. This was made possible with means of support from the Swedish Research Council, the Center for Gender-medicine at Karolinska Institutet, other funding from Karolinska Institutet, Stiftelsen Lars Hierta Minne and the Swedish Society of Medicine.


Sietske Schotanus


Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. Annu Rev Neurosci 15:285-320.


