

From the Department of Neuroscience
Karolinska Institutet, Stockholm, Sweden

DISSECTING THE INTRASTRIATAL NEURONAL CIRCUITRY THAT REGULATES DIRECT AND INDIRECT STRIATAL PROJECTIONS

Henrike Planert



**Karolinska
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ABSTRACT

The basal ganglia (BG) are a group of subcortical nuclei that are interconnected in multiple parallel cortico-BG-thalamocortical loops. They have been implicated in many functions, among them action control and motor learning. The striatum forms the main input nucleus of the BG. Its principal neuron type, the medium spiny neuron (MSN), projects via striatonigral (direct) and striatopallidal (indirect) BG pathways, which according to an influential model function antagonistically in motor control. D1 and D2 receptor expressing MSNs ascribed to direct and indirect pathways, respectively, are not easily discriminable based on electrophysiological properties, but are hypothesized to be oppositely affected by dopamine (DA). A small population of striatal neurons, the fast-spiking interneurons (FSNs) however show characteristic stuttering discharge *in vitro*, and have an important role in mediating feedforward inhibition onto MSNs (which are also interconnected via feedback collaterals). FSNs form electrical, as well as chemical synapses onto each other. The focus of this thesis has been to investigate the characteristic electrical properties of the mentioned striatal neuron types and their dynamic interconnectivity, as well as DAergic modulation of MSNs of the different projection systems.

In two animal models (rat and mouse), electrical properties of different MSN subtypes were similar, however, membrane excitability consistently differed with direct pathway MSNs being less excitable than their counterparts. DA had opposite effects on excitability of D1 and D2 MSNs, counteracting these initial differences. Excitability increased in D1 MSNs, across experimental conditions and parameters, and also when applying DA or D1 agonist during blockade of cholinergic, GABAergic, and glutamatergic synaptic transmission.

FSNs provided a strong and homogeneously depressing “feedforward” inhibition of both striatonigral and striatopallidal MSNs, as measured with multineuron patch-clamp recordings in the acute slice. Individual FSNs were connected to MSNs of both types. In contrast, both MSN types received sparse and variable, depressing and facilitating synaptic “feedback” transmission from other MSNs. Connection probability appeared higher for pairs with presynaptic striatopallidal MSNs; however, the type of interconnected MSNs did not determine the variability in synaptic dynamics. The differences between feedback and feedforward inhibitory pathways were clear in two species at different developmental stages.

Measurements *in vitro* and a computational FSN-model showed that FSNs that exhibit typical random stuttering discharge in response to steady depolarization do not show stuttering when they receive fluctuating input. The model predicts that electrically coupled FSNs show substantial spike synchronization only when in the stuttering regime. *In vivo* variability in FSN discharge was furthermore translated to high variability in postsynaptic amplitudes due to strong depression of the FS-MSN synapse.

Using PV-Cre mice injected with AAV virus containing ChR2 and mCherry, we selectively photostimulated FSNs. When recording from nearby MSNs, FS, low-threshold spiking (LTS), and cholinergic (ACh) interneurons while activating FSNs, most MSNs received strong and reliable synaptic input, which was mediated by GABA_A receptors, whereas ACh (and LTS) interneurons received no input at all.

In conclusion, DA induced changes in excitability of identified MSNs were consistent with an influential model of BG function, and direct pathway excitability increases were mediated by D1 receptors most probably acting on intrinsic MSN properties. Synaptic dynamics generally differed between striatal feedforward versus feedback synapses, but were similar for both output pathways. Modeling suggested that *in vivo*, neighboring FSNs are not readily in the stuttering regime simultaneously, discharge variability is rather determined by input fluctuations, and synaptic dynamics lead to highly variable postsynaptic response amplitudes in MSNs. Feed-forward inhibition mediated by FSNs is highly target selective for MSNs in contrast to other interneuron types, especially ACh interneurons.

Key words: basal ganglia, striatum, medium spiny neuron, fast-spiking interneuron, ACh interneuron, direct pathway, indirect pathway, intrinsic properties, dopamine, intrastriatal connectivity, synaptic dynamics, feedforward inhibition, feedback inhibition, short-term plasticity

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LIST OF ABBREVIATIONS

AAV – Adeno-associated virus
ACh – Acetylcholine
AP – Action potential
AP5 – 2-Amino-5-phosphopentanoic acid
BG – Basal ganglia
ChAT – Choline acetyltransferase
ChR2 – Channelrhodopsin-2
CNQX – 6-Cyano-7-nitroquinoxaline-2,3-dione
D2 R – Dopamine D2 receptor
DA – Dopamine
DA R – Dopamine receptor
dMSN – Direct pathway MSN in retrogradely labeled rat striata or striata from transgenic *Drd1a*-EGFP mice
Drd1a / D1 R – Dopamine receptor D1A
EP – Entopeduncular nucleus, primate homologue: GPi
EGFP – Enhanced green fluorescent protein
FSN – Fast-spiking striatal interneuron
GABA / GABA R – Gamma-aminobutyric acid / Gamma-aminobutyric acid receptor
GP/GPe – Globus pallidus (rodent), globus pallidus *external segment*
GPi – Globus pallidus *internal segment*, rodent homologue: entopeduncular nucleus
iMSN – putative indirect pathway MSN (nonlabeled MSN in retrogradely labeled rat striata or striata from *Drd1a*-EGFP mice)
IR-DIC – Infrared differential interference contrast
LTD – Long-term depression
LTP – Long-term potentiation
LTS/PLTS neuron – Low-threshold spiking interneuron
Mec – Mecamylamine
MLA – Methyllycaconitine citrate
MSN – Medium spiny neuron
PPR – Paired-pulse ratio
PV – Parvalbumin
SNc – Substantia nigra *pars compacta*

SNr – Substantia nigra *pars reticulata*
STDP – Spike-timing dependent plasticity
STN – Subthalamic nucleus

INTRODUCTION

THE DIRECT AND INDIRECT STRIATAL PATHWAY MODEL OF THE BASAL GANGLIA

The basal ganglia

The basal ganglia (BG) are a group of brain nuclei implicated in a multitude of functions, e.g. action control and action learning, as well as in psychiatro-neurological pathologies, among them Parkinson's Disease (PD) (Del Casale et al., 2011; Haber, 2003; Obeso et al., 2008; Raymond et al., 2011; Turner and Desmurget, 2010). The main BG structures are the striatum (caudate nucleus, putamen, and ventral striatum in primates; dorsal and ventral striatum in rodents), the subthalamic nucleus, the pallidal complex (GPe, GPi and ventral pallidum in primates; GP, ventral pallidum and entopeduncular nucleus in rodents), substantia nigra *pars reticulata* (SNr) and *pars compacta* (SNc), as well as the ventral tegmental area (VTA). Multiple parallel loops ("prefrontal", "oculomotor", "limbic", and "motor") have been described, that interconnect different regions of cortex, the BG and thalamus and are associated with different functions (Alexander and Crutcher, 1990; Alexander et al., 1986; Heimer et al., 1982; Nakano, 2000).

The direct versus indirect striatofugal pathway model

The striatum forms the main input nucleus of the BG, receiving excitatory glutamatergic input from cortex and dopaminergic (DAergic) modulation from Substantia Nigra *pars compacta* (SNc) (Calabresi et al., 1996; Joel and Weiner, 2000; Kincaid et al., 1998). Striatal neurons then project *directly* or *indirectly* (via GP/GPe in rat and primate, respectively), to BG output structures substantia nigra *pars reticulata* (SNr) and GPi/entopeduncular nucleus. The idea has been put forward that

these pathways via different effect on BG output functionally oppose each other in motor control, such that the direct pathway facilitates and the indirect pathway inhibits movements. The opposite effects of dopamine (DA) depletion on the two pathways furthermore lead to the assumption that DA affects direct and indirect pathways differentially (Albin et al., 1989). This second “parallel” (Alexander and Crutcher, 1990) direct and indirect pathway organization of striatofugal projections has since then been highly influential in models of function and dysfunction of the BG in motor control (DeLong, 1990; Mink, 1996). From gene expression experiments it was further inferred that effects of DA depletion are mediated by selective expression of D1 Rs in striatonigral, and D2 Rs in striatopallidal MSNs, respectively (Gerfen et al., 1990).

The classical functional BG model has received strong support by a recent *in vivo* study showing motor activation and inhibition by D1 and D2 MSNs, respectively (Kravitz et al., 2010). This study, however, showed inconclusive results regarding discharge rate changes after intraperitoneal injections with specific DA agonists, and this was interpreted as a result of the complexity of modulatory effects in intact circuits.

Criticism and adaptations

Even if heuristically helpful, the models of parallel functional pathways, as well as direct and indirect striatofugal pathways opposing each other in motor control are of course oversimplified. They have also been developed further and adapted to new findings in the last decades. At least a few additions need to be made at this point (see also Bolam et al. (2000); Nambu (2008); Smith et al. (1998); Turner and Desmurget (2010)):

Firstly, the architecture of the circuitry is not strictly as described in the models above: The striatum receives, apart from cortical input, important glutamatergic projections from the thalamus (Doig et al., 2010; Dubé et al., 1988; Galvan and Smith, 2011; Groenewegen and Berendse, 1994; Haber and Calzavara, 2009; Lacey et al., 2007; McFarland and Haber, 2000, 2001; Parent, 1990; Raju et al., 2008; Raju et al., 2006; Smith et al., 2009; Smith et al., 2004; Smith et al., 2011; Xu et al., 1991). Whereas specificity of D1 R (as opposed to D2 R) expression for striatonigral direct pathway MSNs seems to be established at least in the mouse (Gertler et al., 2008; Matamalas et al., 2009), in rats, direct pathway MSNs also send collaterals to GP (Fujiyama et al., 2011; Kawaguchi et al., 1990), so that the striatopallidal projection is not specific to indirect pathway projecting MSNs. Furthermore, the STN is a second important BG input structure, as it receives excitatory cortical input and relays fast excitatory signals to GP/GPi/SNr (Fujimoto and Kita, 1992; Kita, 1992; Kita, 1994; Kolomiets et al., 2003; Kolomiets et al., 2001; Maurice et al., 1999; Mink and Thach, 1993; Nambu et al., 2000; Parent, 1990; Ryan and Clark, 1991). Especially the axis cortex-STN-GPi/SNr, also termed *hyperdirect pathway*, has been seen as important in motor control (Mink and Thach, 1993; Nambu et al., 2002). Moreover, the GP is in an important position to influence all nuclei of the BG, including striatum and STN (Bevan et al., 1998; Bolam et al., 2000; Smith et al., 1998), and has been described as a dichotomous structure whose neurons, based on their spike timing *in vivo*, can be divided into two classes presenting differential neurochemistry and projection profiles (Mallet et al., 2012; Mallet et al., 2008).

Secondly, while functional theories of the direct/indirect striatofugal pathway model focus on the selection of movement by disinhibition through the direct pathway and “surrounding” inhibition of competing motor programs (Mink, 1996;

Mink and Thach, 1993), there has been substantial criticism to these views mainly based on information about timing of different neuronal activities with relation to movement execution (Turner and Desmurget, 2010). This would suggest a role of the striatum in movement monitoring rather than in the actual selection of movements (Nambu, 2008; Turner and Desmurget, 2010).

Two more considerations with respect to the functional BG models introduced above are of relevance to the work presented in this thesis: 1. While the classical model by Albin et al. clearly predicts that DA should increase excitability in direct pathway MSNs and decrease excitability in the indirect pathway, the many studies on the effect of DA and specific agonists on MSN excitability have for several reasons been very hard to interpret (Nicola et al., 2000). We are readdressing this question in the experiments that form part of paper I. We there directly tested the effect of DA on excitability of identified MSNs at different membrane potentials and with much of the intrastriatal microcircuitry intact. 2. Cortico-BG-thalamic loops are now often discussed as not strictly parallel (Alexander et al., 1986; Mink, 1996), but also integrative (Haber, 2003; Joel and Weiner, 2000; Kolomiets et al., 2003; Kolomiets et al., 2001; Nambu, 2008; Nambu, 2011; Voorn et al., 2004). For example, information was suggested to be hierarchically channeled from limbic to cognitive to motor circuits based on anatomical studies in monkeys (Haber, 2003). In rodents, the striatum is seen as one candidate structure where crosstalk between functional loops can occur (Voorn et al., 2004). An implementation of such crosstalk would be via neuronal interconnectivity within the striatum. In papers II, III and IV, we present results from experiments aiming at a better understanding of the intrastriatal circuitry.

In this thesis, we investigate the effects of DA on identified MSNs of the different pathways, specifically their excitability, in the intact slice (paper I). We also

investigated how the striatal microcircuit is organized to compute the input it receives with respect to direct and indirect output projections, i.e. whether there are similarities and differences of dynamic interconnectivity patterns in relation to these pathways (paper II). There are several different ways that striatal neurons interconnect with each other, and we will give a short introduction below. First we will however introduce the main striatal interneuron types that can be considered as candidates for synaptically communicating with MSNs and with each other.

STRIATAL MICROCIRCUITRY

It has been demonstrated in lamprey that much of the BG circuitry described above is conserved throughout vertebrate evolution (Stephenson-Jones et al., 2012; Stephenson-Jones et al., 2011). Also, subpopulations of striatal neurons show characteristic cellular properties, as well as D2 R mRNA expression (Ericsson et al., 2011; Robertson et al., 2012). In rats, MSNs comprise the vast majority of striatal neurons, which discharge at low rates *in vivo* (Berke et al., 2004; Wilson and Groves, 1981). In addition, large cholinergic (ACh) interneurons, as well as several, mainly GABAergic, interneuron types have been classified anatomically, histochemically and based on electrophysiological grounds (Kreitzer, 2009; Tepper and Bolam, 2004; Tepper et al., 2010).

Interneuron types

The striatal neuron type most easily recognizable under IR-DIC microscopy in the acute slice is the ACh interneuron. These large neurons stain positively for ChAT (Bolam et al., 1984) and discharge tonically *in vivo* (Wilson et al., 1990), a property for which they were termed tonically active neurons (TANs). Even *in vitro*, they

show irregular, but tonic endogenous discharge (Bennett et al., 2000; Bennett and Wilson, 1999; Wilson et al., 1990).

Of the remaining striatal interneurons, the most prominent in terms of electrophysiological properties are the parvalbumin-expressing GABAergic, fast-spiking interneurons (FSNs, (Gerfen et al., 1985; Kawaguchi, 1993), which are found in a dorsolateral to ventromedial gradient in the striatum (Gerfen et al., 1985; Luk and Sadikot, 2001). These cells are characterized by small action potential (AP) width and sharp afterhyperpolarization, as well as repetitive discharge in response to step current injection (Kawaguchi, 1993). As cortical FS interneurons, striatal FSNs show high-frequency repetitive “stuttering” (Gupta et al., 2000) discharge *in vitro* (Bracci et al., 2003; Golomb et al., 2007; Kawaguchi, 1993). *In vivo*, these interneurons are characterized by near continuous discharge with behavior-related changes of rate and timing (Berke, 2008, 2011; Berke et al., 2004). In this thesis, we were interested in how the type of input that the FSN receives determines its discharge behavior (paper III).

Another main striatal interneuron type is the (persistent) low-threshold spiking (PLTS) neuron, that expresses NADHP diaphorase and nitric oxide synthase and can be electrophysiologically characterized e.g. by its high input resistance. It is probably the same type as the LTS neuron described later (Kawaguchi, 1993; Koós and Tepper, 1999). LTS neurons have recently been shown to exhibit autonomous discharge *in vitro*, similar to ACh interneurons (Beatty et al., 2012).

As transgenic mice that express fluorescent protein in specific neuronal populations have become available (Gong et al., 2003), it became possible to describe other neuron subtypes electrophysiologically. With this technique, several electrophysiological phenotypes of tyrosine hydroxylase-expressing interneurons have been described (Ibáñez-Sandoval et al., 2010). Also neuropeptide Y -expressing

interneurons have been subclassified into two clearly distinguishable classes (Ibáñez-Sandoval et al., 2011). One shows the typical PLTS-characteristics (Koós and Tepper, 1999). The other GABAergic “neurogliaform” (NPY) subtype has different electrophysiological and morphological properties.

Neuronal interconnectivity

MSNs of both projection types receive strong cortical as well as thalamic innervation (Doig et al., 2010), and also FS neurons receive glutamatergic synapses from both cortex and thalamus (Wilson, 2007). Within the striatal microcircuitry, two main modes of operation have been described: “Feedforward” inhibition, mainly as the one between FSNs and MSNs (Kita et al., 1990; Koós and Tepper, 1999; Mallet et al., 2005), as well as GABAergic “feedback” inhibition between MSNs (Czubayko and Plenz, 2002; Guzmán et al., 2003; Tunstall et al., 2002). *In vitro*, FSNs can delay or inhibit MSN discharge (Koós and Tepper, 1999), and also *in vivo*, the activity of projection neurons inversely covaries with FSN activity (Mallet et al., 2005). But also LTS neurons feed forward onto MSNs (Koós and Tepper, 1999), and a disynaptic inhibitory connection between ACh interneurons and MSNs involving GABAergic NPY interneurons has been discovered (English et al., 2012).

In comparison to FSN-MSN synaptic inhibition, the effect of collateral inhibition between MSNs is rather small as measured at the soma (Gustafson et al., 2006; Koos et al., 2004), arguing against a competitive winner-take all mechanism (Koos et al., 2004; Wilson, 2007). Also the convergence of FSNs onto MSNs is large (Wilson, 2007). Connectivity as measured in the slice is consequently robust and widespread (Gittis et al., 2011; Gittis et al., 2010; Koós and Tepper, 1999), whereas interconnectivity for MSNs is sparse (Czubayko and Plenz, 2002; Taverna et al., 2008; Tunstall et al., 2002). We describe both of these connectivity principles of the

striatal microcircuit in paper II of this thesis (Planert et al., 2010). This pattern somewhat resembles the organization of cortical neuronal microcircuits. Here, similar fast-spiking interneurons feed forward onto pyramidal cells seemingly unspecifically and with high connection probabilities (Packer and Yuste, 2011), while projection neurons themselves are only sparsely connected (Brown and Hestrin, 2009; Markram et al., 1997).

A fast mode of communication by which signals can be directly transmitted between neurons are the connections via electrical synapses (Pereda et al., 2012). In the cortex, FSNs are electrically coupled by these so-called gap junctions, which can synchronize them (Galarreta and Hestrin, 1999; Gibson et al., 1999; Kita et al., 1990). Also in the striatum, FSNs interconnect via electrical synapses (Kita et al., 1990; Koós and Tepper, 1999) and they are also connected through chemical synapses, just as neocortical FSNs (Galarreta and Hestrin, 1999; Gibson et al., 1999; Gittis et al., 2010). They, however, do not appear to be broadly synchronized *in vivo* and in a network model (Berke, 2008; Hjorth et al., 2009).

The relationships between intrastriatal connectivity and the projections via different output pathways are a major focus of this thesis work (paper II). In this paper, we were furthermore interested in how synaptic dynamics of feedback and feedforward inhibition relate to these functionally important pathways. A second focus was on the effect that naturally occurring FSN discharge patterns can induce in their postsynaptic targets, taking into account the dynamic nature of synaptic connections (paper III), and on the interconnectivity of striatal FSNs with other neuron types in the striatum, specifically ACh-expressing interneurons (paper IV). Before we discuss analysis and function of synaptic dynamics in detail, we will therefore shortly introduce the ACh modulation of the striatum, and at the same time review some of the literature on DAergic modulation.

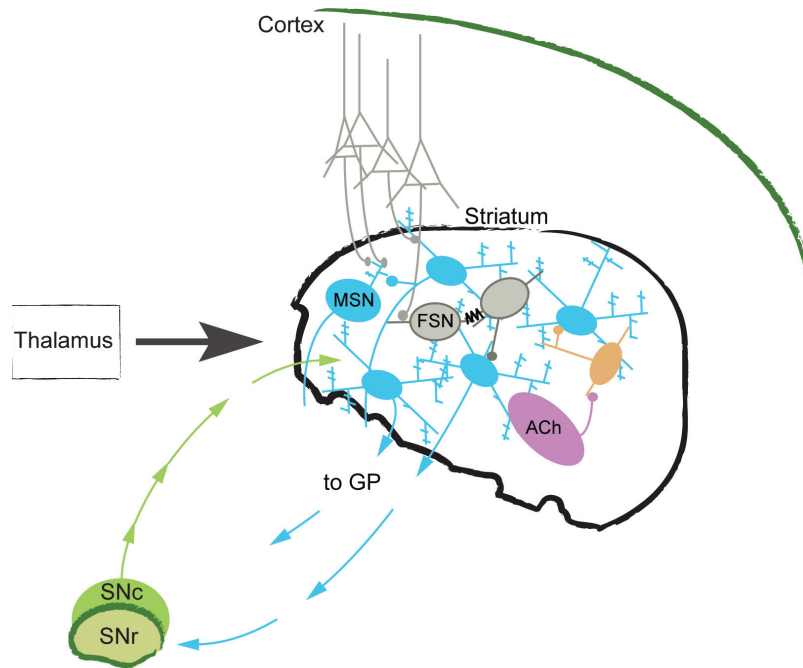


Fig. 1. Features of intrastriatal neuronal circuitry with afferent and efferent connections: Striatal neurons receive excitatory input from cortex and thalamus, and cholinergic modulation from ACh interneurons, as well as DAergic projections from the midbrain SNc (green). Feedforward inhibition has been described from FSNs (gray) to MSNs (blue), feedback inhibition between MSNs, and electrical synapses between FSNs. ACh neurons (pink) feed forward onto MSNs via a disynaptic pathway involving NPY interneurons (orange). See text for details.

STRIATAL NEUROMODULATION BY DA AND ACh

As mentioned above, striatal neurons receive DAergic modulation from the midbrain (Joel and Weiner, 2000; Moss and Bolam, 2008), as well as ACh neuromodulation from within. The traditional view that ACh and DA functionally oppose each other in the striatum has recently been challenged (Surmeier and Graybiel, 2012), based on influences of ACh neuron activity on DA release or DA-release related function (Cachope et al., 2012; Threlfell et al., 2012; Witten et al., 2010).

Tonic and phasic DA changes in the striatum have been implicated in multiple

functions such as action selection or action motivation, as well as action learning (Costa, 2007; Goto et al., 2007; Schultz, 2007; Turner and Desmurget, 2010; Wickens et al., 2007). DA modulation studies have focused on MSNs, yielding complex results (Nicola et al., 2000; Surmeier et al., 2007). However, apart from MSNs also ACh and GABAergic striatal interneurons express DA Rs (Kreitzer, 2009), suggesting that network mechanisms may play an important role in the overall effect of DA on striatal output. Furthermore, investigations of DAergic or agonist-related effects on identified neurons of direct and indirect output pathways are sparse (André et al., 2010; Day et al., 2008; Janssen et al., 2009; Tecuapetla et al., 2009). In paper I, we therefore addressed the question whether DA has the effects on excitability of identified MSNs assumed by the classical functional model of the BG. We investigated MSNs of the different projection systems in the slice preparation, in which much of the microcircuitry remains intact.

In vivo, TANs acquire characteristic pauses in relation to reward-predicting stimuli, and have long been associated with sensorimotor learning (Aosaki et al., 1994a; Aosaki et al., 1994b; Graybiel et al., 1994). Thalamostriatal synapses onto ACh interneurons have been implicated in the gating of corticostriatal signals onto MSNs (Ding et al., 2010). As described above, both ACh interneurons, as well as FSNs are involved in feedforward inhibition of MSNs via GABAergic interneurons. It is therefore interesting to investigate, whether the ACh-to-MSN feedforward pathway may communicate with the FSN-MSN feedforward system, a question that was addressed by the experiments conducted for paper IV. Here, FSNs were selectively activated by light stimulation, while recording from multiple possible postsynaptic targets, mainly ACh interneurons.

INVESTIGATING SHORT-TERM SYNAPTIC DYNAMICS

Synaptic dynamics in neuronal networks

Synaptic transmission in the nervous system is dynamic, such that synaptic response amplitudes change depending on previous activity. Such changes can occur as long-term effects, for example in response to sustained stimulation as long-term potentiation (LTP) of the synaptic response (Bliss and Lomo, 1973). Furthermore, depending on the stimulation protocol, or as effects of the timing of pre- and postsynaptic activity, long-term depression (LTD), or spike-timing dependent plasticity (STDP) can occur, respectively. In a multitude of studies, these plastic phenomena have been described in the striatum, and here mainly at glutamatergic (corticostriatal) synapses (Calabresi et al., 2007; Fino and Venance, 2011; Kreitzer and Malenka, 2008; Lovinger, 2010). Such long-term effects of synaptic activity are often used to model learning in synaptic networks.

At synapses between principal cortical neurons, response amplitude decreases for consecutive APs, and other cortical synapses show facilitating dynamics (Thomson and Deuchars, 1997; Tsodyks and Markram, 1997). Thus, also when receiving multiple presynaptic inputs within tens or hundreds of milliseconds, postsynaptic response amplitudes do not remain static. This may allow for dynamic change of the weight of specific synapses within small networks.

One common way of quantifying short-term plasticity is by calculating the ratio of two consecutive synaptic responses (the paired-pulse ratio, PPR). A PPR that is larger than one is an indication for a presynaptic mechanism of vesicle depletion, leading to diminished presynaptic release probability. Tsodyks and Markram developed a model in order to describe the dynamical properties of cortical synapses quantitatively, extracting parameters for both recovery from depression and facilitation (Markram et al., 1998; Tsodyks and Markram, 1997), and we used this

model to describe synaptic dynamics of striatal microcircuitry in papers II, III and IV.

A quantitative model for the description of synaptic dynamics

The phenomenological model of Tsodyks and Markram estimates several parameters to fit the average postsynaptic responses to a train of APs (Tsodyks and Markram, 1997). These include the absolute synaptic efficacy of the synapse (i.e. the response if release probability is maximal, this could depend on the postsynaptic receptor density and the number of release sites). The initial PSP amplitude, as well as the amplitudes in response to consecutive APs will depend on the release probability of the synapse. The available fraction of resources is reduced with each AP according to the release probability. A time constant describes the time course of recovery of these resources. In a later version of this model, a facilitatory mechanism is added, where the release probability is dynamic as well (Markram et al., 1998).

The model shall be described in the form that it was used for the studies of this thesis (papers II, III, and IV): Synaptic amplitudes are calculated from postsynaptic responses to presynaptic depolarizing pulses. In order to extract correct amplitudes of postsynaptic responses lying on the decay phase of previous responses, the synaptic decay is fitted by an exponential curve and subtracted. The amplitude of the postsynaptic response, PSP_n is a product of the fraction of available resources, R_n , and a facilitating utilization factor, u_n , scaled by the absolute synaptic efficacy, A_{se} :

$$PSP_n = A_{se} R_n u_n.$$

The utilization factor is increased by each AP and decays back towards U (which is equivalent to the baseline release probability) in the time between APs, t_{ISI} . This process is described by utilization of the synaptic efficiency in the first AP ($u_0 = U$),

and the recovery time constant from facilitation, F :

$$U_{n+1} = u_n \exp\left(-\frac{t_{ISI}}{F}\right) + U\left(1 - u_n \exp\left(-\frac{t_{ISI}}{F}\right)\right).$$

Each AP utilizes the fraction u_n from the synaptic resources, R_n , which then recovers to a value of 1 at rate of D :

$$R_{n+1} = R_n(1 - u_n) \exp\left(-\frac{t_{ISI}}{D}\right) + 1 - \exp\left(-\frac{t_{ISI}}{D}\right).$$

In the cortex, the same presynaptic cell can lead to different dynamics of postsynaptic response, depending on the target neuron (Markram et al., 1998). This may be important in shifting the weights between different interconnected subnetworks, depending on the input pattern the presynaptic neuron receives. Short-term plasticity of synaptic contacts within the striatum has begun to be investigated (Czubayko and Plenz, 2002; Gustafson et al., 2006; Guzmán et al., 2003; Koos et al., 2004; Taverna et al., 2004; Venance et al., 2004), but the relationship of dynamical parameters with projection via direct and indirect pathways had not been addressed. In paper II, we used the model for synaptic dynamics to better understand dynamics of different (feedforward versus feedback) connections, especially in their relationship to the output via direct and indirect striatal pathways. We used experimental data in order to model the postsynaptic effects of depressing feedforward inhibition that result from naturally occurring FSN discharge patterns in paper III.

AIMS

The two main foci of this thesis work were:

- The investigation of **intrinsic and microcircuit properties of the two striatofugal projection pathways** and their modulation.
- The characterization of the **striatal feedforward inhibitory system involving FSNs**

Specifically, we relate intrinsic properties of MSNs and their neuromodulation by DA to output target and D1 vs D2 R expression (paper I). We then describe and quantify MSN-MSN, as well as FSN-MSN connectivity and dynamics in relation to the different output projections (paper II). Paper III investigates the determinants of FSN discharge patterns *in vitro* and in a computational model, relates the results to *in vivo* patterns, and uses parameters describing the dynamics of FSN-to-MSN synapses recorded *in vitro* to model possible effects of *in vivo* FSN discharge patterns on postsynaptic MSNs. Finally, in paper IV, we shed light on the postsynaptic connectome of FSNs by selectively activating FSNs in the slice and recording postsynaptic responses from different cell types, mainly ACh interneurons.

METHODS

The main technique used for this thesis work was patch clamp electrophysiology in the acute slice. In the following sections, some of the specific preparations and techniques will be described. Please refer to the methods section of each paper for methodological details.

IDENTIFICATION OF STRIATAL DIRECT PATHWAY AND FAST-SPIKING NEURONS

In striatal slices, ACh interneurons are easily discriminable from the remaining neuron types based on their size, but it is not readily possible to differentiate between projection neurons and other interneuron types using in vitro optics. However, even if FSNs, once patched, are easily discriminable from MSNs based on their characteristic membrane response to current injections (see Introduction), MSNs themselves are electrophysiologically relatively homogeneous, which has made it difficult in the past to discriminate between MSNs of the different output pathways.

In the first two papers, two methods were therefore used to identify direct pathway MSNs in two different species: Striatonigral MSNs were retrogradely labeled by stereotactic injection of fluorescent latex microspheres (Katz et al., 1984) into the SNr of juvenile rats. In electrophysiological experiments, striatal areas with high percentage of labeled MSNs were chosen for recordings. Assuming randomly intermingled distribution of direct and indirect pathway MSNs, nearby nonlabeled MSNs will be mostly striato-GPe projecting, and therefore indirect pathway MSNs. To identify direct pathway projecting MSNs in mice, we used transgenic bacterial artificial chromosome (BAC) *Drd1a*-EGFP mice. These animals express EGFP under control of the promoter for the D1 R (*Drd1a*), and were originally generated by the

Gene Expression Nervous System Atlas program at the Rockefeller University (Gong et al., 2003). The mice used in our experiments had been crossed on a C57/BL/6 background (Santini et al., 2009). Identifying the direct pathway by fluorescence and comparing to nonfluorescent MSNs makes our experimental approach comparable across species. As studies in BAC mice have shown complete colocalization of D1 R and retrogradely labeled SNr-projecting MSNs, as well as no or extremely low (0.7%) colocalization of retrograde labeling and D2 MSNs (Gertler et al., 2008; Matamales et al., 2009), direct pathway neurons can be easily identified with this approach. Apart from this, it can also be assumed that most *Drd1a*-EGFP negative MSNs in our model are D2 R expressing: D1 and D2 R expression was mutually exclusive in double transgenic lines (Shuen et al., 2008), and Matamales et al. (2009) saw full coverage of DARPP positive neurons by either *Drd1a* or *Drd2*-EGFP labeling. In our experiments, only clearly nonfluorescent cells were taken as EGFP negative and for simplicity, we refer to these as D2 MSNs.

For the experiments in paper IV, we were interested in investigating the postsynaptic connectome of striatal FSNs. The adapted algal protein channelrhodopsin-2 (ChR2), a cation channel activated by blue light, can be used for optical control of neuronal activity (Boyden et al., 2005; Deisseroth et al., 2006). In Cre- knockin mice, viral-mediated gene transfer furthermore enables targeted labeling and manipulation of specific neuronal populations in a Cre-dependent manner (Kuhlman and Huang, 2008). To identify as well as selectively stimulate the parvalbumin expressing striatal FSNs, we used PV-Cre mice (Hippenmeyer et al., 2005) that had been stereotactically injected with adenoassociated viral vector (AAV) double-floxed inverted open reading frame ChR22mCherry into the striatum (see also Cardin et al. (2009)).

Mice were injected with virus 10-16 days before experiments. We targeted

injections to the dorsolateral striatum, and found virally transduced cells within a diameter of $300 \pm 100 \mu\text{m}$. Neurons expressing mCherry-ChR2 were PV positive, confirming the genetic restriction of ChR2 to PV+ cells. *In vitro*, mCherry expressing striatal FSNs were identified by switching from infrared to epifluorescence mode, and, in order to activate FSNs, photostimulation was generated through a 1 Watt blue LED (wavelength 465 nm) and was controlled by a LED driver (Mightex systems, CA, USA) connected to the ITC-18 acquisition board.

SLICE PREPARATION AND ELECTROPHYSIOLOGICAL RECORDINGS

For electrophysiological experiments, brain slices were cut in ice-cold extracellular solution, kept at 35°C for 30 minutes, and moved to room temperature before recordings. Patch pipettes were pulled with Flaming/Brown micropipette pullers. Whole-cell patch clamp recordings were obtained at a temperature of 35°C. Neurons were visualized using IR-DIC microscopy and up to four neurons were recorded simultaneously (see cover picture). The extracellular solution for cutting and recording contained (in mM) 125 NaCl, 25 glucose, 25 NaHCO₃, 2.5 KCl, 2 CaCl₂, 1.25 NaH₂PO₄, 1 MgCl₂. Recordings were amplified using multiclamp 700B amplifiers.

STIMULATION PROTOCOLS, DATA ANALYSIS AND NEURON MODEL

Recorded neurons were subject to various stimulation protocols to determine synaptic and intrinsic electrical properties. The main striatal neuron types (MSNs, FSNs, LTS and ACh interneurons) were generally identified by their passive and active membrane responses to step and ramp current injections in current clamp mode. For the detailed characterization of MSN membrane properties of the different output

systems (paper I), a series of somatic current injection protocols was applied, scaled to an initial near-threshold step pulse designed to capture key active and passive properties (Wang et al., 2004). Membrane properties were extracted in IGOR Pro (Wavemetrics, USA).

Intrastriatal synaptic connections (papers II, III and IV) were characterized by recording up to four neurons simultaneously and stimulating presynaptically with a train of APs (blue light pulses for virus-injected PV-Cre mice), followed by a recovery test pulse. Postsynaptic neurons were held at hyperpolarized potentials in order to ensure depolarizing responses to GABAergic synapses. Averaged postsynaptic responses were analyzed using the model of synaptic dynamics (see Introduction) running in Matlab (MathWorks Inc., USA).

For paper III, the effect of *in vivo* discharge patterns on the distribution of synaptic responses was investigated, using results from the analysis of FSN-MSN synaptic dynamics. Samples of awake *in vivo* rat FSN spike trains were taken from a previously described data set (Berke et al., 2004; Berke, 2008). For the investigation of the effect of fluctuating versus step current injection on the discharge pattern of FSNs (first part of paper III), we used an adaptation of the one-compartment model of a cortical FSN by Golomb et al. (2007). The FSN model was implemented in Parallel GENESIS (Bower and Beeman, 1998) when modeling a pair of electrically coupled FSNs.

PHARMACOLOGY AND STAININGS

In a subset of electrophysiological experiments of papers I and IV, pharmacological agents were added to the bath solution to either test their effect on MSN-intrinsic properties, or in order to prevent specific (muscarinic and nicotinic cholinergic, as well as GABA_A-, and glutamatergic AMPA- and NMDA- receptor mediated)

synaptic transmission. These were: Dopamine (DA) and the specific D1 agonist SKF 81297; furthermore Atropine, Methylycaconitine citrate (MLA) and Mecamylamine hydrochloride (Mec) as well as SR-95531 (GABazine), CNQX and AP5. All drugs were purchased from either Tocris Bioscience or SIGMA-Aldrich.

Also, some striatal neurons from electrophysiological experiments were filled with biocytin during patch clamp recordings and subsequently fixed and incubated with either DAB or streptavidin-Cy2 (Jackson ImmunoResearch Laboratories) for morphological reconstruction. For paper IV, in order to investigate labeling efficiency in virus-injected PV-Cre mice, immunohistochemistry for parvalbumin was performed on striatal sections and co-labeling of ChR2-mCherry and parvalbumin examined using a confocal microscope (Zeiss LSM 510, Germany).

RESULTS AND DISCUSSION

MEMBRANE PROPERTIES OF STRIATAL “DIRECT” AND “INDIRECT” PATHWAY NEURONS AND THEIR MODULATION BY DOPAMINE IN MOUSE AND RAT SLICES (PAPER I)

The direct and indirect striatofugal BG pathways have been important in functional models of the BG in health and disease (see INTRODUCTION), but the MSNs giving rise to these pathways appeared similar based on anatomical and electrophysiological grounds, and were investigated as one electrophysiological subtype in slice studies (Kawaguchi et al., 1990; Nisenbaum and Wilson, 1995; Nisenbaum et al., 1996; Nisenbaum et al., 1994). Only relatively recently, transgenic mice have become available, allowing the investigation of the two pathways on the grounds of their differential D1 and D2 R expression (Gong et al., 2003). During the course of this thesis work, a few studies have addressed intrinsic differences between electrophysiological properties of the neuron types in the transgenic mouse model (Cepeda et al., 2008; Gertler et al., 2008). Considering however that many of the slice studies of the striatum have been conducted in rats, and that the question of D1 and D2 R specificity for direct and indirect pathways has been a matter of debate (Bertran-Gonzalez et al., 2010), it was important to determine how intrinsic membrane properties vary between identified MSNs in rats and how they correspond to differences in transgenic mice.

In rat retrogradely labeled direct pathway and nonlabeled (putative indirect pathway) MSNs, the resting membrane potentials were similar, and so was the AP threshold. However, striatonigral MSNs were less excitable than their counterparts, as seen in the ramp current leading to the first AP, as well as the

average minimal step current needed to reach threshold discharge. These differences were paralleled by different input resistances at membrane potentials depolarized from rest. Also, the membrane time constant was significantly shorter in striatonigral MSNs. In mice, D1 and D2 MSNs were remarkably similar in their membrane properties. D1 MSNs were however less excitable than D2 MSNs in terms of current needed to obtain discharge. A difference in excitability between the two MSN subtypes was the common feature we observed in both mice and rats, such that direct pathway MSNs were less excitable than their counterparts. This finding is in agreement with results from other studies in the mouse model (Cepeda et al., 2008; Gertler et al., 2008; Kravitz et al., 2010; Kreitzer and Malenka, 2007), and it allows the generalization of differential excitability of MSNs in the slice preparation across species.

The second main question addressed in this paper was whether MSNs identified by their receptor expression in transgenic mice (see METHODS) would show the DA-mediated changes in electrical excitability consistent with the classical model of BG function. Only few studies have addressed DA or agonist-related effects directly in the two MSN populations (André et al., 2010; Day et al., 2008; Janssen et al., 2009; Kravitz et al., 2010; Tecuapetla et al., 2009). Also, DA-mediated effects should differ depending on the membrane potential, as many conductances with different voltage-dependencies are modulated (Surmeier et al., 2007). When we bath-applied DA at depolarized membrane potentials near -60 mV, MSNs showed an overall depolarizing response. However, post-hoc tests revealed a significant change for D1 MSNs only. Moreover, and as predicted (see Surmeier et al. (2007)), neuronal excitability increased in D1 MSNs and decreased in D2 MSNs near -60 mV. More surprisingly however, in D1 MSNs, the discharge in response to a current step strongly increased also at -80 mV (Fig. 2B, C).

These excitability increases in D1 MSNs could be mediated directly by the action of DA on intrinsic conductances, or indirectly by affecting the activity of synaptic transmission of connected neurons. In order to rule out such network effects, we investigated the effect of DA and a D1 agonist on MSN excitability while blocking muscarinic and nicotinic cholinergic, as well as GABA_A and glutamatergic AMPA and NMDA receptor mediated signaling. After addition of Atropine, MLA and Mec, as well as GABAzine, CNQX and AP5 to the bath, AP discharge still increased in D1 MSNs, strongly suggesting that the increase in excitability was caused by direct action of DA on D1 MSN-intrinsic conductances, and not by synaptic modulations (Fig. 2 D).

In D2 MSNs, application of D2 agonist reduces excitability (Day et al., 2008; Janssen et al., 2009). We extend these findings by demonstrating opposite effects of DA on excitability of the two MSN types. Our results suggest that, while the indirect pathway appears more excitable under baseline or low DA conditions, application of DA counteracts this tendency by increasing the intrinsic excitability of direct pathway MSNs. The DA induced membrane depolarization of D1 MSNs acts synergistically with increased excitability, and both serve to enhance the activity of the direct pathway. These results support the classical model of striatal function (Albin et al., 1989). Within this scheme, multiple cellular and synaptic mechanisms may interact synergistically to shift the balance between the direct and indirect pathways following DA input.

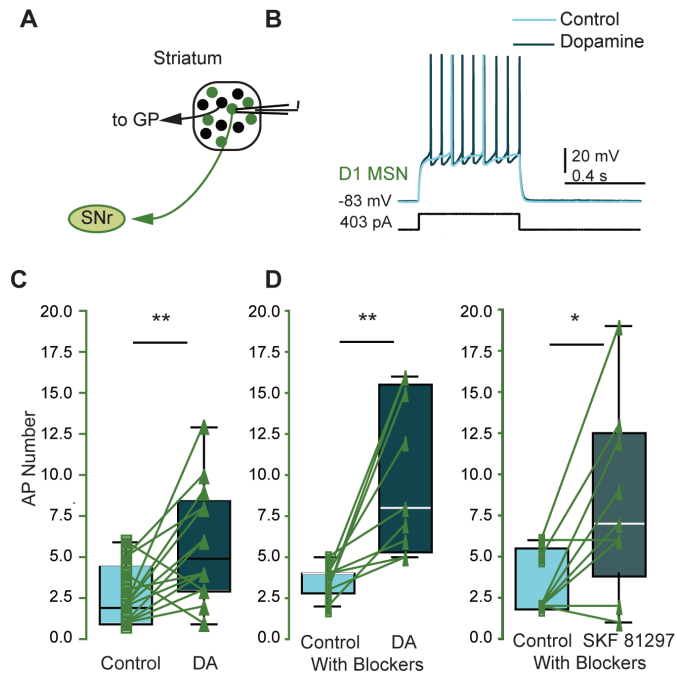


Fig. 2. Excitability increases in direct pathway MSNs after application of DA or D1 agonist. A, Schematic of patching of EGFP labeled D1 R expressing direct pathway neurons. B, Response of a direct pathway neuron to a current step before and after application of DA. C, Discharge of D1 R expressing direct pathway MSNs increases after application of DA. D, The effect is also seen when applying DA or D1 agonist SKF 81297 (left and right panel, respectively) in presence of blockers of synaptic transmission Atropine, MLA, Mec, GABAazine, CNQX and AP5, suggesting that the increase in excitability is direct and D1 R mediated.

DYNAMICS OF SYNAPTIC TRANSMISSION BETWEEN FAST-SPIKING INTERNEURONS AND STRIATAL PROJECTION NEURONS OF THE DIRECT AND INDIRECT PATHWAYS (PAPER II)

MSNs receive feedforward inhibition from FSNs, as well as collateral connections from nearby MSNs (see INTRODUCTION). How these computational principles relate to projections via direct and indirect pathways, and whether their synaptic dynamics differ, were questions addressed in paper II. In order to investigate synaptic connectivity between FSNs and MSNs of the different output pathways, as well as

between MSNs of different types, we used the same animal models as for the investigation of intrinsic properties. We thereby acquired data from both transgenic *Drd1*-EGFP mouse and retrogradely labeled rat striata. Up to four neighboring neurons were recorded simultaneously (see cover), stimulating one neuron with trains of (presynaptic) APs and at the same time recording from the remaining (postsynaptic) neurons. For simplicity, we refer to retrogradely labeled rat MSNs as well as EGFP positive MSNs in mice as dMSNs, and to the nonlabeled (putative indirect pathway) MSNs as iMSNs.

Initial experiments with unselected MSNs in rat slices showed that MSN–MSN connections were not only smaller, but also more diverse than connections from FSN to MSNs: Both pathways displayed on average paired-pulse depression; however, paired-pulse facilitation was observed in some MSN–MSN connections, but never in FSN–MSN connections. When using the phenomenological model for synaptic dynamics (Markram et al., 1998; Tsodyks et al., 1998), the recovery test pulse used for this analysis revealed a large difference between the two pathways, in which connections between MSNs displayed strong facilitation compared with the depression observed in FSN–MSN synapses. Also when using the measured synaptic amplitudes to extract the model parameters quantifying the synaptic dynamics, in particular the time constants for recovery from facilitation (F) and depression (D), FSN–MSN synapses were purely depressing. MSN–MSN synapses on the other hand had a clear facilitatory component alongside depression, as evident from their respective time constants and the high F/D ratio.

In retrogradely labeled rat slices, we recorded from a total of 83 pairs, 8 of which had synaptic connections (6 connections between MSNs and 2 from FSN to MSNs). Both MSN types also received synaptic connections from presynaptic iMSNs, and 1 connection was found between dMSNs. From the six MSN–MSN

connections, four had facilitation of the test response, and two were depressing connections. Labeled and nonlabeled MSNs received synapses from FSNs, both of which were depressing connections with clear decrease in the amplitude of the recovery test response.

In labeled and nonlabeled nearby neurons in slices of D1-EGFP mice we found synaptic interactions in 23 of 294 MSN–MSN tested connections. From these connections, a majority (74%) was from presynaptic iMSNs and 6 connections (26%) had a presynaptic dMSN. The connections from dMSNs did not have significantly different amplitudes than those from iMSNs. These connections displayed recovery test facilitation, with two (of three) facilitating dMSN-iMSN connections and one facilitating dMSN-dMSN connection (the three others were depressing synapses).

iMSNs formed connections onto dMSNs (13%) and iMSNs (23%), both of which had depressing and facilitating synapses. The connections from iMSNs onto the two types of target cells were not significantly different in their amplitude, paired-pulse ratio, recovery test facilitation, as well as the utilization factor (U). In these connections, with presynaptic iMSNs, the type of postsynaptic MSNs did not determine the dynamics of the synaptic connection, as we saw in two cases in which a dMSN received both a depressing and a facilitating connection from two different iMSNs (paper II, figure 5A therein). In two separate cases, the same presynaptic iMSN contacted a dMSN and an iMSN with facilitating synapses, showing that the individual presynaptic MSN, but not the MSN type, may determine the synaptic dynamics of the connection.

As in rat striatum, FSN-MSN connectivity recorded in mice had higher prevalence than MSN-MSN connectivity, larger amplitude, and displayed only depressing dynamics. Both iMSNs and dMSNs received inhibitory connections from FSNs with very similar dynamic properties. Moreover, we found individual FSNs

forming divergent connections on both MSN types, suggesting unspecific inhibition mediated by FSNs. The synaptic properties of FSN-dMSN and FSN-iMSN were not significantly different in terms of their amplitudes, paired-pulse depression, recovery test response, and dynamic model parameters. In summary, these experiments showed that both MSN subpopulations receive ubiquitous and homogeneous inhibition from FSNs, which is, moreover, at least partly mediated by the same pool of presynaptic interneurons (see Figure 3).

Our results are in line with an early immunohistochemical and electron microscopic report of interconnectivity between the different projection neuron types (Yung et al., 1996), as well as the finding of rather differential synaptic coupling of D1 and D2 MSNs *in vitro* by Taverna et al. (2008). The targeting of both MSN subtypes by FSNs were corroborated by similar results from Gittis et al. (2010).

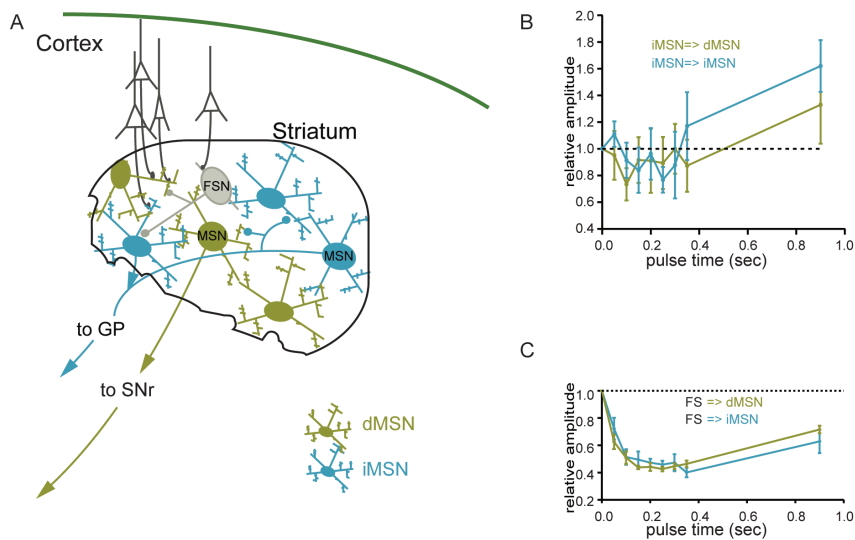


Fig. 3. Striatal feedforward and feedback inhibition with relation to the output projections via direct and indirect striatofugal pathways. A, FSNs target MSNs of both types. iMSNs (blue) are often seen as presynaptic neuron and one MSN can at the same time target a dMSN as well as an iMSNs via local feedback axon collaterals. B, C: Synaptic dynamics for feedback and feedforward inhibition are similar for the two MSN types (see also Figures 5 and 6 in paper II).

STRIATAL FAST-SPIKING INTERNEURONS: FROM FIRING PATTERNS TO POSTSYNAPTIC IMPACT (PAPER III)

This study comprised the modeling of the effects of FSN discharge as found *in vivo* on the distribution of postsynaptic potentials in MSNs, taking into account the strongly depressing nature of the FSN-MSN connection. Before doing this, we investigated the characteristic discharge pattern of FSNs to step current injections as opposed to more physiological fluctuating input, and this was done *in vitro*, as well as in a computational model (see Figures 4 and 5).

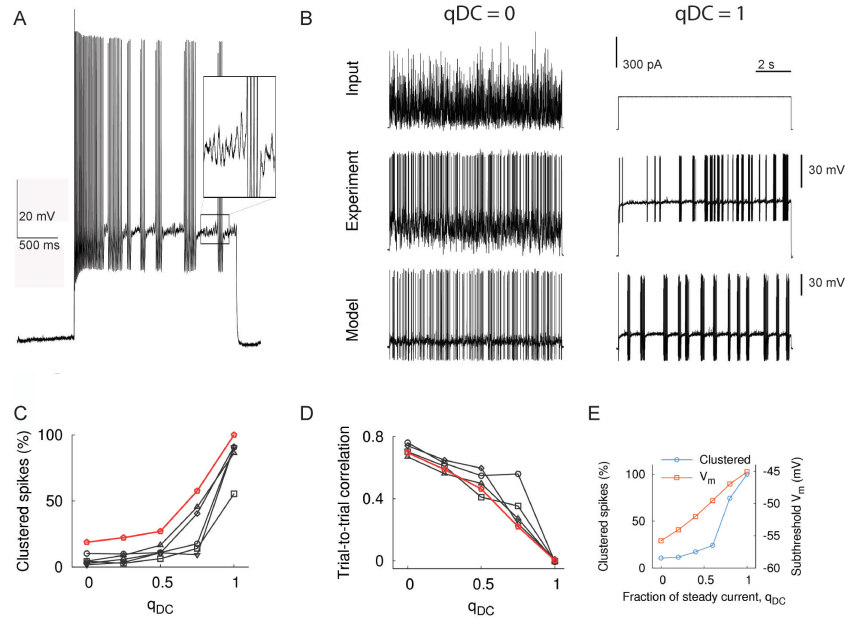


Fig. 4. Discharge pattern of FSNs in vitro and in a model in response to different input patterns showing stuttering discharge for step, but not fluctuating current input. A, Typical stuttering response of FSN to step current injection. B, Example traces for the response of striatal FSN and the model to input with varying levels of steady current, q_{DC} . C, D, Percentage of clustered APs and trial to-trial correlation in the model (red) and *in vitro*. For increasing fraction of steady current, the percentage of clustered APs increases and the trial-to-trial correlation decreases. E, In the FSN model, the subthreshold voltage increases linearly with the amount of steady current. AP clustering increases in a step-like fashion.

To briefly describe some results of the first part here, our experiments indicate that the typical random stuttering of FSNs observed *in vitro* (Gupta et al., 2000) is a result of the rather artificial step current stimulation, as FSNs both *in vitro* and in our model did not respond with clustered APs when receiving fluctuating “synaptic” input (see Figure 4). Also, the trial-to-trial correlation of the spike signature was increased, implying that these responses were driven by the input that the neuron received. This has been similarly shown in cortical neurons by Mainen and Sejnowski (1995). Furthermore, the model cells seemed to be more depolarized when entering the stuttering regime than they were for comparable discharge rates in response to fluctuating input: the subthreshold membrane potential increased linearly while replacing synaptic by steady current input. This was furthermore accompanied by an abrupt change towards clustered discharge (Fig. 4E). When connecting two modeled FSNs by electrical coupling, they did not synchronize their discharge, unless when both in the stuttering regime. Similarly, *in vivo*, in awake, behaving animals, neighboring FSNs in the striatum do not show signs of broadly synchronized discharge (Berke, 2008). This further suggests that FSNs are not readily in the stuttering regime simultaneously. Together with the results stated above, this would imply that their discharge pattern is mainly determined by the fluctuations of synaptic input that they receive.

An interesting hint that the ionic mechanisms that lead to stuttering discharge in response to step current injection may also be important in determining the precise response of FSNs to cortical input gives a recent study by Sciamanna and Wilson (2011). The authors addressed the mechanisms underlying striatal FSN stuttering and gamma resonance, which manifests itself as minimum *in vitro* firing rate and locking to gamma frequency input *in vivo*. The results stress the importance of Kv1 currents for both phenomena, and suggest that the Kv1-mediated resonant properties may

phase-lock FSN activity to the gamma component of the input it receives.

Next, we investigated the effect of the depressing nature of FSN-MSN synapses (paper II) for realistic FSN discharge patterns. We used the discharge patterns of three FSNs from prior *in vivo* studies by Berke et al. (2004) and Berke (2008), and simulated resulting trains of synaptic potentials in postsynaptic MSNs, making use of the parameters for synaptic dynamics extracted from *in vitro* recordings (see Figure 5). The model synapse was able to replicate the PSP amplitudes for different FSN discharge frequencies. Differences in the PSP amplitudes and their steady-state values became evident for longer AP trains. Lower discharge frequencies allowed more synaptic recovery between APs, resulting in larger steady state amplitudes. Full recovery of the synaptic resources required longer discharge pauses. The time course for the synaptic recovery (τ_F) ranged from 230 ms to almost 5 s. Thus, depressing FSN-MSN synapses particularly emphasize the onset of FSN discharge after prolonged pauses. These results suggest that differences in the FSN discharge pattern have an influence on the amount of synaptic depression and/or recovery and therefore on PSP amplitudes.

The discharge of striatal FSNs in awake, behaving animals is characterized by large fluctuations of the instantaneous discharge rate with numerous high-frequency bursts, single spikes, and periods of silence (Berke et al., 2004; Berke, 2008). We determined the distribution of PSP amplitudes in the FSN-to-MSN synapse model in response to FSN discharge patterns that were recorded *in vivo*, and compared it to PSP distributions that resulted from random, shuffled APs. In the *in vivo* AP train, more than 10% of the APs were organized in groups of at least nine. For Poisson and uniformly shuffled AP trains, the percentage of APs that were members of large spike clusters was comparatively smaller. High-frequency clusters and intermittent discharge pauses in the *in vivo* AP train furthermore resulted in a wide range of PSP

amplitudes (see Figure 5B). The relatively large number of extended spike clusters in the *in vivo* data resulted in a reduction of the smallest PSP amplitudes for all three FSNs ($n = 11$ synapses). Importantly, pauses in the *in vivo* FSN activity allowed also for the recovery of synaptic amplitudes. Consequently, the ratio PSP_{large}/PSP_{small} was largest for the *in vivo* AP train, and shuffling of the data destroyed the structure of the *in vivo* discharge and resulted in a smaller range of observed PSP amplitudes. These results suggest that FSN-to-MS synapses utilize a large fraction of their possible amplitude spectrum in response to *in vivo*-like input, i.e., from strongly depressed to entirely recovered.

Our modeling results indicate that *in vivo*, FSN-MSN synapses operate over a wide dynamic range. This will lead to enhancement of inhibition when the cell discharges APs after a period of silence. Mechanisms such as summation of several of the highly convergent depressing FSN inputs (even if there is not much evidence for AP synchronization *in vivo*) may further strengthen this mechanism of onset-enhancement.

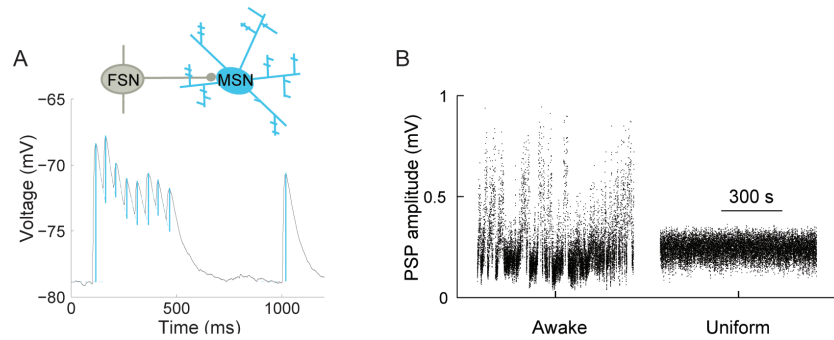


Fig. 5. Large variability in PSP amplitudes to discharge patterns as recorded *in vivo*. A, Example of a FSN-MSN feedforward synaptic response to presynaptic train of APs and recovery pulse, and the extracted amplitudes (light blue) used for the computation of parameters describing the synaptic dynamics. B, Modeled PSP amplitudes resulting from discharge patterns as recorded *in vivo* (awake, left) and when using a uniformly distributed pattern (right), demonstrating high variability in response to the *in vivo* pattern. See text and paper III for details.

TARGET SELECTIVITY OF FEEDFORWARD INHIBITION BY STRIATAL FAST-SPIKING INTERNEURONS (PAPER IV)

In this study, we characterized the postsynaptic connectome of striatal FSNs with respect to the different striatal neuron types, especially ACh neurons.

In order to selectively activate striatal FSNs, we genetically targeted expression of the light-sensitive opsin ChR2 to PV+ cells by stereotactic injection of AAV vector that expressed a ChR2-mCherry fusion protein in a Cre-dependent manner into the striatum of PV-Cre mice (see METHODS). We targeted whole-cell recordings to mCherry-expressing neurons using combined fluorescent and IR-DIC microscopy and characterized the optogenetic activation of these neurons by delivering short pulses of blue light. All neurons identified by both fluorescence and response to light stimulation were FSNs, as determined by their characteristic electrophysiological properties. Interneurons responded to light pulses with immediate depolarization, leading in most cases to AP discharge. Light pulses of 2 ms duration were usually sufficient to induce APs, enabling the stimulation of FSNs with trains of APs.

To determine the precise connectivity between FSNs and other striatal neuron types, we characterized the synaptic connectivity of ChR2-expressing FSNs onto nearby striatal neurons. When recorded in proximity to the ChR2-expressing FSNs, most MSNs responded to brief 2 ms light pulses with a strong and reliable synaptic response. Recorded light-induced synaptic responses were GABA(A)-R dependent. Light-induced synaptic responses were threefold larger than unitary direct connections in FSN-MSN pairs, suggesting that photostimulation could stimulate several labeled FSNs. Stimulation with 10 Hz trains of light pulses induced depressing synaptic responses. The dynamic properties of light-evoked synaptic responses were quantified using the model for synaptic dynamics, and compared to

direct FSN-MSNs connections obtained from paired recordings. Time constants for depression and facilitation in optical vs. direct synaptic connections were similar, however, the release probability (utilization factor, U) was higher in the light induced responses, apparent also in a smaller paired-pulse ratio in light induced responses compared with direct connections. This apparent difference in synaptic release is likely to be caused by the doublets of APs observed in some cases, also described previously for the activation of Chr2 (Gunaydin et al., 2010).

To study the synaptic connectivity from FSNs to the other main striatal interneurons (see INTRODUCTION), we recorded from ACh and LTS interneurons during photostimulation of Chr2-expressing FSNs (Figure 6). To ensure the proper optogenetic light activation of FSNs and their synaptic transmission at the recording area, we simultaneously recorded from pairs or triplets of neurons, in which at least one MSN or FSN exhibited light-induced synaptic responses or APs, respectively. ACh interneurons were identified by their typical morphological and electrophysiological properties, including large soma, depolarized membrane potential, voltage sag response, and pronounced discharge accommodation.

Interestingly, ACh interneurons did not show any synaptic events following light activation of Chr2-expressing FSNs, in contrast to the simultaneous strong and reliable synaptic response recorded at nearby MSNs ($n = 12$). We also recorded from 3 LTS interneurons, characterized by their high input resistance, low AP discharge threshold, depolarized membrane potential and long membrane time constant. These did not receive synaptic inputs following photostimulation of FSNs while neighboring neurons recorded simultaneously responded to the stimulation.

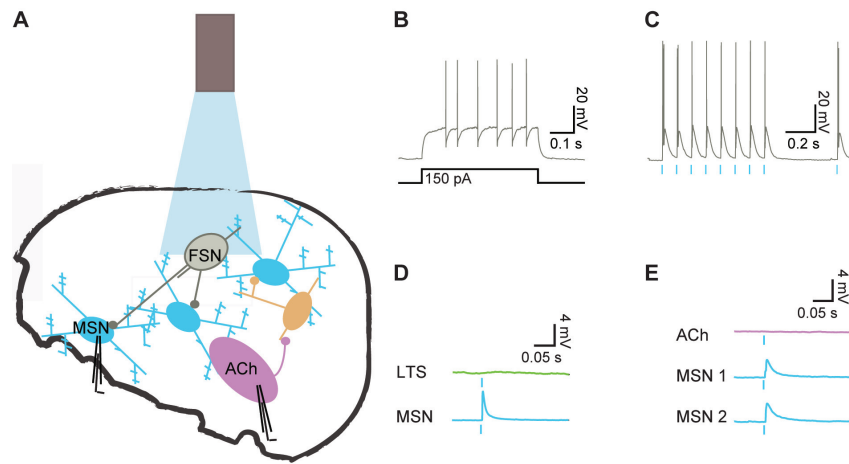


Fig. 6. Optogenetic activation of FSNs reveals feedforward inhibition onto MSNs, but not ACh interneurons. A, Schematic showing light-activation of FSN (grey) and patching of nearby MSN (blue) and ACh interneuron (purple). MSNs received input upon light stimulation, but ACh neurons remained silent. B, Example of a patched FSN response to step current injection. C, FSN responding to light stimulation (light blue bars) with a train of APs. D, A LTS interneurons that does not receive synaptic input, while a nearby MSN responds to the stimulation. E, Two MSNs showing synaptic responses to light stimulation, while a nearby ACh interneuron remains silent.

In summary, this work shows that feed-forward inhibition from striatal FSNs is highly selective, reliably targeting MSNs, but not ACh interneurons, which indicates that the two neuron types may engage in differential parallel feedforward systems within the striatal circuitry (see INTRODUCTION).

CONCLUSIONS AND FUTURE PERSPECTIVES

With the studies comprised in this thesis, we have shown opposite effects of DA on excitability of identified MSNs of the different projection systems, counteracting differences seen at baseline. Evidence for direct, D1 R-mediated increased excitability of the direct pathway is especially strong. When describing and quantifying MSN-MSN as well as FSN-MSN connectivity and dynamics in relation to the different output projections, differences in synaptic dynamics were apparent between feedforward and feedback inhibition. Neither feedforward nor feedback inhibition of single neurons was specific to one of the two pathways. Also synaptic dynamics did not differ markedly between projection systems for either of the general inhibition types. FSN discharge patterns *in vivo* are probably largely determined by the input the neuron receives. Based on depressing synaptic dynamics of FSN-MSN synapses, it can be expected that *in vivo* discharge patterns result in highly variable response amplitudes on postsynaptic MSNs. Also, while FSNs are readily connected to nearby MSNs, they do not target nearby ACh interneurons, and may engage in differential computations within the striatal circuitry.

Further studies should investigate the *in vivo* effects of DAergic stimulation on discharge of MSNs of the different output projections. This could be done with *in vivo* patch clamp recordings in either Drd1-EGFP mice (with post-hoc identification of the respective neuron type), or via optogenetic activation of the specific pathways (Kravitz et al., 2010). Striatal neurons have a wide distribution of membrane potentials during sleep and anesthesia, but also in the awake animal (Mahon et al., 2001; Mahon et al., 2006; Wilson, 1993; Wilson and Kawaguchi, 1996). It will therefore be especially interesting to see whether effects of transient DA increases on MSN excitability vary with the membrane potential.

Variability in membrane potential may also have an effect on the strength of intrastriatal connectivity. Synaptic connectivity in the cortex can be modulated in a graded fashion depending on presynaptic membrane potential (Alle and Geiger, 2006, 2008; Shu et al., 2006; Zhu et al., 2011), and this modulation may differ depending on the synapse type. In light of the very consistent differences between feedforward inhibitory dynamics and feedback dynamics within the striatal microcircuit (paper II), it would be interesting to investigate whether in striatal GABAergic circuits a similar mechanism exists at the level of graded connectivity modulation by presynaptic voltage. One way by which such a mechanism could potentially discriminate between different connection types would be via Ca^{2+} buffering in parvalbumin expressing interneurons (see also Alle and Geiger (2008)).

“True wisdom comes to each of us when we realize how little we understand about life, ourselves, and the world around us.” – Socrates

“One step inside doesn’t mean you understand” – The Notwist

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