Examining the difference between striatal neurons of the direct and indirect pathway of the basal ganglia that is responsible for their differential growth direction in rodents.

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Abstract

The neurons of the striatum are part of the neural network known as the basal ganglia, projecting either directly to the substantia nigra, or indirectly via the globus pallidus. Despite their different roles, direct and indirect neurons lie unsorted throughout the striatum, showing many similarities. We aim to elucidate the differences between direct and indirect striatal projection neurons by giving an overview of current knowledge of their development, axon guidance, target recognition, and synaptic plasticity. Additionally, for future research we point to genes that might also be important to striatal neurons in these various aspects.

Introduction

The basal ganglia (BG) are thought to be primarily involved in processing motor control, but also in cognitive and motivational aspects of behavior. Its main input center, the striatum, receives input from the cortex which it sends through to the major output centers, the globus pallidus pars interna (GPi) and the substantia nigra pars reticulata (SNr). This information flows via two pathways, the direct and indirect pathway. In the direct pathway the striatum inhibits the GPi and SNr by γ-aminobutyric acid (GABA)-ergic synapses. In the indirect pathway the striatum inhibits the globus pallidus externa (GPe) by GABA, which reduces GABAergic inhibition of the subthalamic nucleus (STN), which in turn innervates the GPi and SNr with glutamate. Thus, the direct and indirect pathway have an opposite effect on the GPi and SNr, which inhibit the thalamus with GABA. The thalamus stimulates the cortex via glutamate synapses, making the cycle complete. So activation of the direct pathway causes activation of the cortex through disinhibition of the thalamus, while activation of the indirect pathway causes inhibition of the cortex through disinhibition of the STN, which

stimulates GPi and SNr mediated inhibition of the thalamus (Fig 1.)(Deniau et al., 2007).

The two efferent pathways of the striatum seem to be highly segregated in two types of medium-sized densely spiny neurons (MSNs) which seem to be distributed randomly (Shuen et al., 2008). Physiologically the two types of MSNs can be distinguished by differential expression of several genes. MSNs of the direct pathway, forming the striatonigral projection pathway, is most prominently characterized by expression of dopamine receptor D1 (DRD1) and next to GABA the additional neuropeptide substance P (SP). MSNs of the indirect pathway, also known as striatopallidal neurons, express dopamine receptor D2 (DRD2), and enkephalin (Bolam et al., 2000). Interestingly, activation of DRD1 results in excitation whereas activation of DRD2 results in inhibition of action potentials (Deniau et al., 2007). Together with the opposing functions of the direct and indirect pathways, this property might be part of a 'brake' mechanism, to prevent unwanted movement (Yelnik, 2002).

Another level where striatal neurons differ is the separation into patches and matrix, where the patch compartments (a.k.a. striosomes) constitute about 15% of the striatum, and the matrix compartments constitute about 85% (Lobo et al., 2008). Striatonigral and striatopallidal neurons are both present in both patches as in the matrix, and the distinct function of patch and matrix compartments is still poorly understood.

This review will focus on what the difference is between MSNs of the direct and indirect pathway that is responsible for their differential growth direction. Notably, the model of the direct and indirect pathways of the BG has received criticism over the years (reviewed in Nambu, 2008). Nambu et al. (2003) REF show that in the monkey, cortical inputs can also influence the BG by direct innervation of the STN, which provides an input route that is faster than the direct and indirect pathways, and is currently known as the hyperdirect pathway. Also, Lévesque et al. (2003) reported that single MSNs in rats and monkeys innervate in target structures of both pathways. However, studies with BAC-transgenic mice expressing fluorescent proteins driven by DRD1 or DRD2 promoters show that in the mouse the level of segregation is very high, exhibiting low coexpression (<1%)(Shuen et al., 2008), and while some DRD1 positive neurons do give off terminals in the GPe, the MSNs projecting to the SNr expressed almost exclusively DRD1 as opposed to DRD2 (<1%) (Matamales et al., 2009). Alltogether, the classical model of BG with the direct and indirect pathway (Fig. 1) might be an oversimplification, but another possibility is that connectivity in the mouse BG is

simply different from the BG in rats and monkeys. Although this implicates that research based on mice is less comparable to humans as rat or primate research, there are still some clear benefits to using mice. Beside the fact that it is easier to genetically manipulate mice, other issues might also be resolved more easily in a more simply organized motor system.

One of those issues is how axon pathfinding is regulated. The basal ganglia are organized according to somatotopic maps, and this organization is preserved throughout both pathways, from the striatum to the GPe, STN and GPi (François et al., 2004; Romanelli et al., 2005) and converge onto the same SNr output neurons (Kolomiets et al., 2003; Deniau et al., 2007). This level of organization requires mechanisms that not only can direct an outgrowing axon to the appropriate nucleus, but very accurately to a target within that nucleus as well. The signals behind these mechanisms might be examined better in mouse brains, where the pathways are clearly separated between different populations of neurons.

In order to attempt to elucidate the mechanisms responsible for the differential growth direction of striatal neurons of the direct and indirect pathway, various levels will be studied, from development precursor cells, to axon guidance, to synapse formation and synaptic plasticity. This scope of topics reflect the possibilities of regulation on the levels of gene expression in the nucleus, growth cone interactions with intermediate and final targets, and activity dependent regulation of synaptic strength.

Development of the lateral ganglionic eminence (LGE)

In an embryo, the brain develops from the embryonic neural tube, which in early stages is not much more than a tube of cells around a cavity or ventricle. In the ventral part of the telencephalon, which is one of the neural vesicles, two lumps of inward bulging cells can be seen on both the left and the right side at embryonic day (E)11.5, in the mouse (Ghanem et al., 2008). The medial lump is called the medial ganglionic eminence (MGE) that later develops into the globus pallidus, and the lateral lump is called the lateral ganglionic emincence (LGE), that later develops into the striatum. Also, part of the more dorsal GABAergic neurons formed in the LGE migrate to other areas of the brain to become interneurons in the olfactory bulb or cortex (Waclaw et al., 2009).

In this paragraph, we will discuss which genes are thought to be involved in the development of LGE cells to MSNs. In later paragraphs we will discuss how the MSNs undergo their final differentiation to become D1R or D2R positive neurons. However, for the

development of the LGE to MSNs we do have to make a distinction between patch neurons which are born early (between E12 and E15 in rat), and matrix neurons which are born later (between E17 and E20 in rat)(Lobo et al., 2008). Since these populations of neurons are born and develop at different timepoints, these processes are at least partially governed by different genes.

One gene that is involved in early development of MSNs in the LGE is Gsx2. When specifically overexpressed in the telencephalon at early timepoints between E9 and E10, Gsx2 increases the expression of MSN markers Mash1 and Dlx1/2 in the dorsal LGE, while decreasing expression of dorsal markers Pax6 and Tbr1 was reduced. Also, expression of MGE marker Nkx2.1 was completely gone. On the other hand, when Gsx2 expression is knocked out, the striatal volume is greatly reduced and expression of MSN marker DARPP-32 is greatly decreased. However, when Gsx2 expression is progressively reduced in the LGE from E10.5, DARPP-32 levels are comparable to controls (Waclaw et al., 2009). Therefore, Gsx2 is necessary for, and capable of promoting development of LGE cells to MSNs at early stages.

Another important mechanism for development of patch neurons is Mash1-Notch signaling. Notch1 mutant mice show alterations of patch neurons but not matrix neurons (Mason et al., 2005 REF). During neurogenesis, Notch activation induces expression of transcription factor CSL, which induces Hes5 expression. Hes5 is a WRPW-bHLH transcription factor that inhibits neuronal differentiation by repressing the expression of proneural bHLH transcription factors (Yun et al., 2002). Therefore, inhibition of Notch signaling facilitates differentiation. In the Mash1 mutant, the notch ligands Dll1 and Dll3, as well as Hes5 fail to be expressed in the LGE, leading to premature expression of LGE markers (Casarosa et al., 1999). Therefore, Mash1 is an important regulator of the timing of neurogenesis in the LGE. In further support, the phenotype of reduced striatum volume of a Gsx2 mutant animal is reduced in double knock-out animals where Mash1 is also mutated (Wang et al., 2009).

Notably, although both Gsx2 and Notch1 mutant mice show defects that are limited to the patch neurons, in mice with a double knock-out for Gsx2 and Gsx1, or Notch1 and Notch3, the matrix is also affected (Toresson and Campbell, 2001; Mason et al., 2005). This suggests that Gsx2 and Notch1 are also involved in the development of matrix neurons, which can normally be compensated for by related genes.

More evidence that Notch signaling is involved in the development of striatal matrix neurons is provided by the Dlx1/2 mutant. While the production of early born neurons

appears normal, at later timepoints Notch signaling is enhanced in the Dlx1/2 mutant, as seen by the increase in Dll1, Notch1 and Hes5. Also, the phenotype of these animals resembles that of mice with constitutively Notch1 or soluble Dll1 treated animals (Yun et al., 2002). This suggests that Dlx1/2 are required to repress Notch signaling to drive later steps in LGE development. Recent efforts have been made to make an elaborate list of transcription factors with changed expression patterns in Dlx1/2 mutants, Mash1 mutants, or triple mutants (Long et al., 2009). However, since the important function of these three genes in striatal development is the correct timing of their disappearance to initiate differentiation, the approach of knocking them out altogether produces defects earlier than their critical timepoints.

Recently, another role for LGE cells has been reported. LGE cells tangentially migrate into the MGE in a narrow line, forming a corridor of cells expressing LGE markers Isl1, Ebf1 and Meis2 (López-Bendito et al., 2006). In later stages, the internal capsule sends its axons from the cortex through this structure. Another group reported that OL-protocadherin, a cell adhesion molecule, is necessary for corridor formation (Uemura et al., 2007). Similarly, corridor formation is absent or severely impaired in Mash1 mutants (López-Bendito et al., 2006), and in conditional Rac1 mutants (Chen et al., 2007). Less severely, specific deletion of Pax6 resulted in a broader, less well defined corridor (Simpson et al., 2009b). Also, a conserved reporter element present in the promoter regions of Dlx5 and Dlx6 was specifically activated in corridor cells, and both Meis2 and Isl1 had an activating effect on transcription of a reporter gene containing the conserved containing the conserved sequence (Ghanem et al., 2008). This suggests that Meis2 and Isl1 are potential upstream regulators of Dlx genes, orchestrating development of the LGE corridor.

In summary, there are various genes implicated to be involved in the development of the LGE to become either patch or matrix striatal projection neurons. However, which transcription factor is responsible for which aspect of cell specialization via which genes remains unclear. This point is illustrated in Table 1, listing transcription factors with specific expression patterns regarding the basal ganglia and cortex at E15.5 as identified by microarray and visualized by In Situ Hybridization (Long et al., 2009). The genes are sorted in three categories; 1) Expression largely restricted to basal ganglia. These genes are likely responsible for regulating regional identity or phenotypes specific to basal ganglia neurons, such as gene programs responsible for making GABAergic medium spiny neurons of

BG restricted	BG > cortex	Cortex > BG
ATBF1	Arx	BF1
Brn4	Asb4	Brn2
Dlx1/2/5/6	Brn5	COUP-TF1
Ebf1	COUP-TFII	Ctip1
ESRG	Egr3	Ctip2
Gbx1/2	ER81	Cux2
Gsx1/2	Evi3	Dlx6os1
Ikaros	FoxP1	Emx1
Islet1	FoxP2	Emx2
Lhx6/7	Lmo4	Erm
Lim1	MafB	FoxG1
Med6	Mash1	FoxP4
Meis1	Ost6	Gli1
Nkx2.1	Olig1/2	Hes1
Nkx2.2	Sox1	Hes4
Nkx5.1	Sox10	Hes5
Nkx6.2	Sp8	Id4
Nolz1	Sp9	Lhx2
Npas1	TLE4	Lin7
Otx2		Lmo3
Pbx3		Mef2c
Peg3		Meis2
Pbx1		Nex1
RARβ		NHLH2
RXRγ		Nur77
Six3		Otx1
Vax1		Pax6
Zic1		Pbx1
		RORβ
		Sall3
		Sox4
		Sox11
		Tlx
		TLE4

restricted: expression largely restricted to basal ganglia; BG > cortex: at least a 2-fold bias toward basal ganglia expression; Cortex > BG: Expression in cortex roughly equal to, or higher than in basal ganglia (Long et al., 2009).

the striatum or GABAergic local circuit neurons of the cortex and olfactory bulb. 2) at least a 2-fold bias toward basal ganglia expression. These genes may share similar functions within the cortical and subcortical telencephalon, but can also influence processes specific to the basal ganglia. 3) Expression in cortex roughly equal to, or higher than in basal ganglia. These TFs likely have general roles in regulating developmental processes common to both parts of the telencephalon. The amount of genes involved shows us that there still is much work to be done before we fully understand which set of transcription factors is responsible, and which effector genes are required for LGE development.

Differentiation into medium spiny neurons (MSNs)

After an MSN is born, either as a patch or a matrix neuron, it can become either a direct or an indirect projection neuron. This means that there are possibly four different groups of neurons with four different groups of genes required and responsible for their full maturation. Various groups have investigated which genes are specific to which type of neuron. With the use of mice that express a fluorescent reporter gene under the control of Drd1 or Drd2, Lobo et al., (2006) analyzed expression by sorting the striatal cells based on fluorescence. However, their results lack various well known positive controls (e.g., Chrm4, Dyn, Drd1, and Drd2). Another study used a fluorescent tagged ribosomal protein under the control of the Drd1 or Drd2 promoter to separate transcripts by immunoaffinity, producing a long list of genes that includes well known positive controls (Heiman et al., 2008). Moreover, the researchers also combined direct and indirect expression, which they compared to the cortex, which produced a list of striatum enriched genes such as DARPP-32 and FoxP1 (Heiman et al., 2008). Alternatively,

Patch		Matrix	
Direct	Indirect	Direct	Indirect
DARPP-32	DARPP-32	DARPP-32	DARPP-32
FOXP1	FOXP1	FOXP1	FOXP1
Pde10a	Pde10a	Pde10a	Pde10a
Zfp503	Zfp503	Zfp503	Zfp503
Rarb	Rarb	Rarb	Rarb
Rasgrp2	Rasgrp2	Rasgrp2	Rasgrp2
Gpr88	Gpr88	Gpr88	Gpr88
MuOR	MuOR	Calb1	Calb1
Kcnip2	Kcnip2		
Pcp4L1	Pcp4L1		
Neto1	Neto1		
Drd1	Drd2	Drd1	Drd2
Chrm4	Gpr6	Chrm4	Gpr6
SP	ADORA2A	SP	ADORA2A
Slc35d3	Enk	Slc35d3	Enk
Zfp521	Adk	Zfp521	Adk
Stmn2	Plxdc1	Stmn2	Plxdc1
Gnb4	BC4004044	Gnb4	BC4004044
Nrxn1	Hist1h2bc	Nrxn1	Hist1h2bc
Dyn		Ebf1	

Table 2. Expression patterns of striatal genes at ages P20 to adult. Genes enriched in MSNs vs cortex are depicted in orange. The rest of the genes are enriched in comparison to other striatal MSNs: Patch enriched genes are depicted in red, Matrix enriched genes in yellow, direct enriched in green, indirect enriched in blue (Lobo et al., 2006; Arlotta et al., 2008; Heiman et al., 2008; Lobo et al., 2008)

fluorescent labeling has not been utilized to compare patch and matrix expression patterns, but using other methods, some genes have been identified such as the matrix specific gene Ebf1 (Lobo et al., 2008) or patch specific genes Kcnip2, Pcp4L1 and Neto1 (Arlotta et al., 2008). Table 2 summarizes some well known genes specific to a certain group of MSN neurons. Note that genes that are enriched in two groups of MSNs might actually be specific to a single group, such as Ebf1, which was first thought to be matrix specific, but was later shown to be specific to striatonigral matrix neurons (Lobo et al., 2006).

It should also be noted that all experiments mentioned in this chapter thus far, are performed at postnatal day (P)20 at the earliest, to adult stages, and secondly that expression does not automatically mean that these genes are also causally related to MSN development. But also in that aspect progress is made. Lobo et al., (2008) report that Ebf1 is crucial for the development of striatonigral matrix MSNs, because Ebf1 mutant mice show a loss of Drd1 neurons in the matrix, but not in the patch compartment. Additionally, this shows that at least one of the factors that is important for MSN development is still expressed at later stages, when the expression screens were performed, because Ebf1 is still overexpressed in striatonigral cells of adult mice (Heiman et al.,2008). In a similar manner, Arlotta et al., (2008) report that Ctip2 mutant mice show severe defects in striatal development. Since Ctip2 expression is first expressed in migrating MSNs, it likely acts downstream of genes such as Dlx1/2 and Mash1 to organize MSN development at a later stage. However, these developmental defects could also be explained by defects in the cortex and abnormal dopaminergic innervation of the striatum in Ctip2 mutants, because the maturation of striatal projection neurons and interneurons is influenced by the development and integrity of their connectivity (Pezzi et al., 2005). In later paragraphs we will discuss connectivity in more detail.

Axon Pathfinding

Over the years, researchers have discovered several families of guidance cues and receptors, and the four main systems are (*a*) semaphorins and their plexin and neuropilin receptors (Pasterkamp and Kolodkin, 2003), (*b*) netrins and their DCC and UNC5 receptors (Kennedy, 2000), (*c*) Slits and their Robo receptors (Brose and Tessier-Lavigne, 2000), and (*d*) ephrins and their Eph receptors (Kullander and Klein, 2002). A common view of axon guidance mechanisms is that it is the type of receptor, or receptor complex, expressed on the

growth cone's surface, rather than a given guidance cue, that determines the direction of axon growth (O'Donnell et al., 2009). The actual effectors of growth descisions are Rho GTPases, which are regulated by Rho family guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). The number of GEFs and GAPs encoded in most genomes far exceeds the number of Rho GTPases, suggesting that upstream regulation is likely to provide tissue-specific, as well as temporal control of Rho GTPase signaling during growth cone guidance (O'Donnell et al., 2009).

Since the MSNs of the direct and indirect pathway have different targets, they require different guidance mechanisms for various points along the route that is to be traveled. Although many factors remain unknown, some mechanisms have been identified for both pathways. In striatonigral neurons, the expression of PlexinD1, but not Neuropilin1, results in repulsion from Sema3E signals, while other neurons that express both these receptors are attracted to Sema3E (Chauvet et al., 2007). Interestingly, Sema3E was also found to be enriched in striatopallidal MSNs (Heiman et al., 2008), suggesting that via this mechanism, the MSNs of the two pathways are stimulated to part ways. In striatopallidal neurons, activation of Adora2a, an adenosine receptor, results in increased differentiation and neurite outgrowth in striatal primary culture neurons. In SH-SY5Y cells, this effect is mediated by PKA via adenylyl cyclase, by PKC, and by Erk1/2 via the Ras pathway (Canals et al., 2005). However, this mechanism is probably not involved in regulation of the direction of axon growth.

Because there are no other mechanisms known to be involved in axon guidance of MSNs, an alternative approach is needed. Information on other neighboring tracts might be informative, because it might be that MSN axons grow alongside these tracts during development. One obvious candidate is the nigrostriatal tract, which grows in the opposite direction of the striatonigral tract. Developing dopaminergic neurons are attracted to the striatum through striatal Sema3A expression, because E14.5 midbrain explant cultures grow in the direction of Sema3A transfected HEK-293 cell aggregates, but not toward control HEK-293 cells. This phenomenon persists in the presence of antineuropilin1 antibodies, and is therefore independent of the receptor neuropilin1 (Hernández-Montiel et al., 2008). In a similar manner, Netrin-1 was found to have an attracting effect on midbrain explant cultures in a DCC dependant manner, while slit-2 had a repulsive effect in a Robo dependent manner (Lin et al., 2005). Also, EphA5 is expressed in dopaminergic cells of the midbrain, which might be responsible for the repulsive effect of the ligand EphrinA5 on midbrain dopaminergic

neurons in stripe assays. Since EphrinA5 is expressed in a rostro-caudal and ventro-dorsal gradients, it might be an important agent for axon guidance in the midbrain together with the receptor EphA5 (Deschamps et al., 2009). It is possible that striatonigral axons use the same guidance cues as nigrostriatal axons, by expressing different compositions of receptors so that they respond in opposite manner to these cues.

Another structure that could be important for axon guidance of MSNs is the LGE corridor discussed earlier. The formation of this corridor is known to be crucial for connections between the thalamus and the cortex (López-Bendito et al., 2006), and might also be important for striatonigral axons because their routes partially overlap. If this is the case, then it would likely be mediated by a direct cell-cell interaction signaling mechanism. OL-protocadherin poses a candidate for this interaction, since it is essential for corridor formation, and is a cell adhesion molecule known to show homophilic binding (Uemura et al., 2007). Another candidate is CRD-Nrg1, another guidance molecule expressed by the cells forming the corridor. Thalamocortical neurons preferentially grow in contact with cells expressing CRD-Nrg1 in slice cultures, and mutant embryos in which CRD-Nrg1 expression is disrupted show a disorganized arrangement. CRD-Nrg1 directly binds to ErbB receptors, which are expressed by thalamic neurons, and thalamocortical axons also show defects in ErbB4 mutants (López-Bendito et al., 2006).

	M-value	
Gene	MGE vs. LGE	Reason of interest
Epha3	-1,23	Eph-Ephrin guidance molecule
*Ephb3**	1,75	Eph-Ephrin guidance molecule
Epha5	-0,42	Eph-Ephrin guidance molecule
*Efnb3**	0,64	Eph-Ephrin family guidance molecules / receptors
*Punc**	1,11	Putative Unc, guidance molecule
*Sema3f**	-0,45	Semaphorin family guidance molecule
*Sema6d**	-1,40	Semaphorin family guidance molecule
Nrp1	0,89	Neuropilin, receptor to semaphorins
Pcdh21	-2,03	Protocadherin family cell adhesion molecule
Nrg1	-1,07	Neuregulin family cell adhesion molecule
*Arhgap1**	0,66	GAP family Rho GTPase regulator
*Arhgap18**	0,93	GAP family Rho GTPase regulator
*Iqgap1**	0,35	GAP family Rho GTPase regulator

Table 3. Expression patterns of axon guidance related genes in rat MGE vs. LGE at E16. Predicted genes are marked with an asterisk (*). (Willi-Monnerat et al., 2008)

While much remains unclear, steady progress is made in the area of MSN axon guidance. Until the picture is complete, exploratory expression screens identify many genes known to be involved in axon guidance and are

expressed in the region, posing new candidate genes to be investigated (Table 3; Willi-Monnerrat et al., 2008).

Target identification and synapse formation

Similar to axon guidance, not much is known about how MSNs identify which specific neuron they should connect with. It would be impossible to have a special gene or combination of genes responsible for a single synapse, so there must be more general mechanisms for neural circuit formation. One possible mechanism is the expression of one or several gradients of guidance molecules to determine a location within a structure (Simpson et al., 2009a). In the striatum, such patterning signals are known. While Netrin1 is expressed at a steady level in MSNs, Netrin1 expression in interneurons shows a decreasing dorsal-ventral and caudal-rostral gradient (Shatzmiller et al., 2008). Also, EphA4 and ligands EphrinA5 and EphrinB2 show specific temporal expression patterns that are required for sorting of patch and matrix neurons (Passante et al., 2008).

Gene	M-value E12.5 vs E16 MGE	Reason of interest
*Sema6d**	-1,33	Semaphorin family guidance molecule
*RGD1566269**	-1,90	Similar to Neuropilin, receptor to semaphorins
*Plxna2**	-1,09	Plexin family guidance molecule
*Plxnc1**	-1,21	Plexin family guidance molecule
*Punc**	1,92	Putative Unc, guidance molecule
Efna3	-1,23	Eph-Ephrin guidance molecule
*RGD1560587**	-1,26	Similar to EphA4, Eph-Ephrin guidance molecule
Slit1	-1,03	Slit-Robo guidance molecule
Slit2	1,33	Slit-Robo guidance molecule
Robo1	-1,03	Slit-Robo guidance molecule
Robo2	-2,37	Slit-Robo guidance molecule
*Robo3**	1,96	Slit-Robo guidance molecule
*Cdc42ep3**	-1,37	Cdc42, family of Rho GTPase, -effector protein
Arhgef7	-1,27	GEF family Rho GTPase regulator
LOC301334	-1,47	Similar to GEF4a, Rho GTPase regulator
Rapgef4	-1,31	GEF family Rho GTPase regulator
Rap1ga1	-1,97	GAP family Rho GTPase regulator

Table 4. Temporal expression patterns of axon guidance related genes in rat MGE at E12.5 vs. E16. Predicted genes are marked with an asterisk (*). (Willi-Monnerat et al., 2008)

For the globus pallidus and substantia nigra, patterning is less well charted. Nkx2.1 is known to be enriched in the globus pallidus (Uemura et al., 2007), and similarly, Cxcl12 has

been shown to be enriched in the globus pallidus and substantia nigra (Banisadr et al., 2003). However, whether these markers are expressed in a specific pattern within the neuronal centers is not known. Future research should reveal which of the guidance genes known to be expressed in the globus pallidus (Table 4) and substantia nigra are required for target identification by MSNs.

At the level of the SNr a distinction can be made between incoming synapses from the striatum or the globus pallidus. Striatonigral axons innervate distal dendrites, whereas pallidonigral axons innervate proximally with large boutons, sometimes forming a pericellular basket around the somata (Brashnik et al., 2008). Therefore, future research should focus on proteins expressed at distal sites in SNr neurons to discover which genes are necessary for target recognition by striatal projection neurons.

Synaptic plasticity

Another way of finding the appropriate target is by a sort of trial and error, where correct synapses get strengthened, and incorrect synapses get weakened and pruned (Simpson et al., 2009a). If this is the case, then the different types of MSNs might have different mechanisms of synaptic strength regulation. One manner of regulation could be that the amount of incoming excitatory and inhibitory post synaptic potentials (EPSPs/IPSPs), and the success of outgoing IPSPs, causes a change in the electrophysiological characteristics of the synapse such as long term potentiation or depression (LTP/LTD). Secondly, another mechanism could be that synaptic strength is regulated by specific ligand-receptor pairs.

Regarding the first hypothesis, Drd1 and Drd2 MSNs might not even have similar electrophysiological characteristics to start out with, because Drd2 cells display increased excitability and reflect ongoing cortical activity more faithfully than Drd1 cells (Cepeda et al., 2008). The authors suggest that the effect could result from stronger synaptic coupling, but it could also be explained by a recent report that the total dendritic length of Drd1 MSNs is significantly greater than that of Drd2 MSNs in adult mice (Gertler et al., 2008). Puzzlingly however, excitatory post synaptic current (EPSC) amplitudes do not appear to differ between Drd1 and Drd2 MSNs, suggesting the possibility that compensatory synaptic scaling mechanisms exist (Gertler et al., 2008).

The theory that input signals are important is supported by the finding that lesion of

the dopaminergic input from the subtstantia nigra, or of the glutamatergic input from the cortex, affects the maturation of MSNs. Interestingly, lesion of glutamatergic input specifically affects Drd1 MSNs, while lesion of dopaminergic input specifically affects Drd2 MSNs (Pezzi et al., 2005). In the case of Drd2 MSNs, this decreased maturation in response to a loss of dopamine signaling might be mediated by an increase in Cav1.3 L-type calcium channel activity, which is usually inhibited by Drd2 activity, leading to a profound loss of spines (Day et al., 2006). For Drd1 MSNs, the differential response to a loss of glutamate signaling suggests that their maturation depends on enforcement by glutamate. This theory is illustrated by a recent report that high frequency stimulation (HFS) of MSNs induces LTP in striatonigral neurons but LTD in striatopallidal neurons (Rueda-Orozco et al., 2009). This would explain why a reduction in glutamatergic input would specifically affect Drd1 neurons, because unstimulated Drd1 neurons would not be enforced to be maintained. Notably, the observed LTP was accompanied by a large decrease in the paired pulse ratio (PPR), suggesting that the LTP is mediated presynaptically, while the observed LTP was not accompanied by any change in PPR, suggesting that the LTD is mediated postsynaptically. In a related manner, the release of dopamine in the striatum promotes LTP of glutamatergic signaling in striatonigral neurons in a Drd1 dependent manner, but in striatopallidal neurons it promotes LTD of glutamatergic synaptic transmission in a Drd2 dependent manner (Surmeier et al., 2007). Also, PTEN mutant mice, which exhibit an increase in dopamine signaling by increasing growth, proliferation, and survival in the substantia nigra, display a specific increase in Drd1 and Prodynorphin, but not Drd2 and enkephalin expression (Diaz-Ruiz et al., 2009) This differential regulation of MSNs of the direct and indirect pathway by glutamate and dopamine makes sense if we consider their opposing functions in motor control, offering a potential mechanism for selection of an appropriate motor plan, while inhibiting inappropriate ones.

Changes in synaptic plasticity are often associated with the NMDA receptor. Recent research into expression of NMDA receptor subunits using pharmacological antagonists revealed that Drd1 positive MSNs are mainly modulated by NMDA receptor subunit 2A, while Drd2 positive MSNs are mainly modulated by NMDA receptor subunit 2B (Fantin et al., 2007). Another difference in expression of receptors between striatal MSNs is the expression of the AdenosineA2A receptor (Adora2a) by Drd2 positive MSNs, forming heterodimers with Drd2. Activation of Adora2a leads, among other things, to activation of the cAMP-PKA cascade,

resulting in DARPP32 phosphorylation (Schiffmann et al., 2007). The cAMP pathway is also hypothesized to be specifically important to the indirect pathway in PDE10A mutant mice. PDE10A is involved in the degradation of cAMP, and mutant mice show behavioral defects implicating an affected indirect pathway, however, this remains to be confirmed (Siuciak et al., 2006).

Altogether, the differences in electrophysiological responses to stimuli of MSNs of the direct and indirect pathway to glutamate and dopamine is probably caused by differences in expression of receptors and other signal transducing genes. Possibly, those same mechanisms are also involved in selecting which synaptic connections should be maintained, thereby also aiding the MSNs in the formation of a functional neural network.

Discussion

Altogether, we know quite a bit about MSNs, but a larger part is still unknown. As shown by Long et al., (2009), a whole range of transcription factors are expressed in the basal ganglia, both specifically, or non specifically expressed, and both of these categories contain genes that have been shown to play important roles in MSN development (e.g. Dlx1/2; Yun et al., 2002; and Ctip2; Arlotta et al., 2008). There is more to be learned about the workings of both the genes that have been investigated and those that have not. For instance, in the case of Dlx1/2 and Mash1, it was discovered that their crucial function is executed by the exact timing of their disappearance (Yun et al., 2002), therefore it would be interesting to examine a mutant where Dlx1/2 or Mash1 expression fails to drop. Ultimately, we would like to know which genes are responsible for each of the four subtypes of MSNs. While much research is being done on the differential gene expression of direct vs. indirect MSNs, some of the reported genes might actually also be specific to patch or matrix MSNs as has been shown for Ebf1 (Lobo et al., 2008). Future

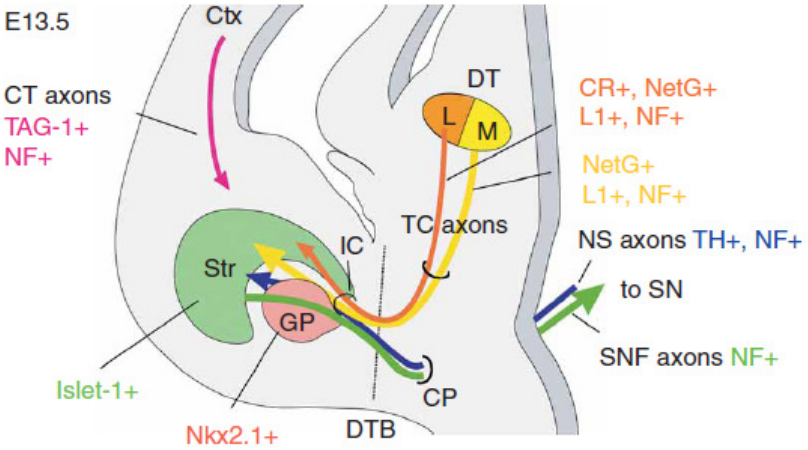

Fig 1. Schematical representation of Axonal connections between developing brain structures. Ctx, cortex; CT, corticothalamic; TC, thalamocortical; CP, cerebral peduncle; L, lateral part of dorsal thalamus; M, medial part of dorsal thalamus; Str, striatum; GP, globus pallidus; IC, internal capsule; NS, nigrostriatal fibers; SN, substantia nigra; SNF, striatonigral fibers; TH, tyrosine hydrolase; DTB, ditelencephalic border (Uemura et al., 2007).

research should try to identify subtype-specific genes, possibly by double fluorescent tagging of both a direct/indirect and a patch/matrix specific marker. For RNA isolation methods, pulldown of ribosomal tags is preferred over FACS sorting, giving more, and more reliable results than FACS sorting, measured by the presence of known positive controls in the results

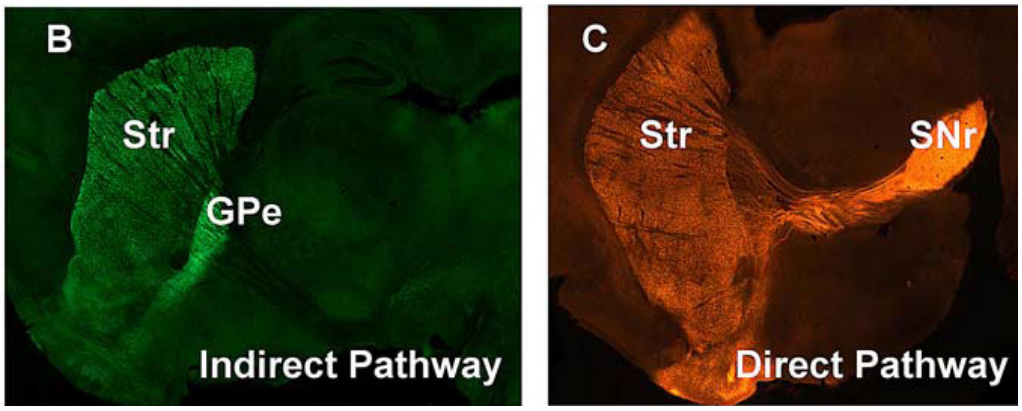

Fig 2. Sagittal view of direct and indirect pathway marked by fluorescent marker driven by the Drd1 (**B**) or Drd2 (**C**) promoter. Str, striatum; GPe, globus pallidus; SNr, substantia nigra pasr reticulata. Scale bar: 500 mm. (Valjent et al., 2009).

(Heiman et al., 2008; Lobo et al., 2006).

As with the aspect of MSN development, there is still much to be discovered on MSN axon guidance. Although there are similarities with other tracts in the brain, Uemura et al., (2007) point out that the routes of striatal and thalamocortical axons are adjacent and partially overlap in the globus pallidus region, and that the amount of overlap is very small (Fig. 1, Uemura et al., 2007). While thalamocortical axons grow along the side of the MGE, the striatal axons grow within it. There, in the later GPe, striatopallidal axons terminate, while striatonigral axons grow on to the GPi and SNr (Fig 2, Valjent et al., 2009). It would be interesting to see which signals are responsible for the decision to terminate, or to keep growing. The answer might lie in Table 3 or 4, which contain temporal and spatial expression patterns of axon guidance molecules in the MGE and LGE (Willi-Monnerat et al., 2008). The striatonigral axons do grow on, and might be helped in their path by nigrostriatal axons, which follow the same route in the opposite direction (Fig 1, Uemura et al., 2007). This theory is supported by the finding that the nigrostriatal tract is largely intact in OL-protocadherin mutants (Uemura et al., 2007), because if that tract is not dependent on successful striatonigral axon formation, the other way around might be the case. However, we are not certain if the nigrostriatal tract is formed before the striatonigral tract, which is a

prerequisite for this theory. Moreover, it cannot be the only way striatonigral axons navigate, since the tract is not formed in OL-protocadherin mutants (Uemura et al., 2007). Altogether, the task of revealing the mechanisms behind axon guidance and target recognition remains a formidable one, requiring that we discover which factors, both soluble and membrane bound, are involved in the steering decisions for every miniscule part of the route. Expression arrays can offer help to identify candidate genes, but many remain to be examined.

Astoundingly, the basal ganglia exhibit a somatotopy that is retained throughout multiple nuclei, which means that parts of the direct and indirect pathway representing the same part of the body are connected to the same neurons in the output nuclei (François et al., 2004; Romanelli et al., 2005). Since the amount of genes available to orchestrate this is limited, a complex mechanism is required. Mathematical models reveal that the solution might lie in combinations of biochemical signals, activity based regulation, and other factors such as competition for space (Simpson et al., 2009a), therefore it requires the efforts of bioinformatics, electrophysiology and cell biology to discover which biologically plausible mechanisms are necessary and sufficient for the formation of neural networks in the basal ganglia.

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