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Molecular mechanisms underlying midbrain dopamine neuron development and function

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Abstract

The mesencephalic dopaminergic system is involved in the control of multiple brain functions including movement control and emotion and is of clinical importance because it is implicated in several psychiatric disorders, of which many are considered to have a neurodevelopmental origin. Studies into the developmental pathways of these neurons have led to the identification of the transcription factors *En1*, *Pitx3*, *Nurr1* and *Lmx1b*, all shown to be important for the development of the mesencephalic dopaminergic system. In this paper, we discuss the consequences of genetic ablation of essential developmental genes. Furthermore, we discuss the consequences of changes in dopamine homeostasis for the function of the mesencephalic dopaminergic system. Finally, we analyse the potential of the mesencephalic dopaminergic system to adapt to gene dysfunction.

Keywords: Development; Mesencephalic; Dopaminergic; Pitx3; Midbrain; Dopamine

1. Introduction

Because of its implications in mental and neurological disorders, dopamine belongs to the most intensively studied neurotransmitters of the brain. Milestones in the recognition of dopamine being an important neurotransmitter were the notions that (i) dopamine was not a biosynthetic intermediate of noradrenaline and adrenaline, but a biologically active substance on its own (Von Euler and Lishajko, 1957; Bertler and Rosengrens, 1959); (ii) depletion of striatal dopamine was the cause of Parkinson's disease (Carlsson, 1959; Ehringer and Hornykiewicz, 1960); (iii) striatal and limbic dopamine originated from mesencephalic dopaminergic neurons (Andén et al., 1964, 1966; Dahlström and Fuxe, 1964), and finally, (iv) the dopamine antagonist haloperidol alleviated psychotic symptoms in schizophrenics (Janssen and Niemegeers, 1959). In view of its biological role in the control of movement and a broad array of behavioral processes like mood, reward, addiction, and stress, dopamine systems have been the main target of neuropharmacology for almost 40 years. The dramatic neurological consequences of degeneration of mesencephalic dopaminergic neurons in Parkinson's disease highlighted the functions of this dopaminergic system in the control of motor behavior at the level of the striatum, and led to rational therapeutic approaches, e.g. L-3,4 dihydroxyphenylalanine (L-DOPA) treatment (Barbeau et al., 1962). Furthermore, the effectiveness of dopaminergic antagonists as antipsychotic drugs led to the "dopamine hypothesis of schizophrenia" (Wagner et al., 1966; Meltzer and Stahl, 1976). Although the dopamine hypothesis has now been left as the sole explanation for schizophrenia, the involvement of dopamine systems in this mental disorder is nuanced, but still considered prominent.

With the advances in molecular biology, dopamine systems and their involvement in mental and neurological disorders are now subject of studies that aim to delineate the fundamental neurobiology of dopamine neurons. These are concerned with development, cell-specific gene expression and regulation, molecular pharmacology of dopamine receptors and transporters, and with genetic association of dopamine-related genes and mental disorders, e.g. dopamine receptor variants and linkage to schizophrenia. This paper concerns the genetic programs of specification of mesencephalic dopaminergic neurons, i.e. the question which genes and gene cascades determine the dopaminergic identity of these neurons, and their specific connectivity in

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mesencephalic-striatal and -limbic pathways and regulation of the dopamine synthesis and release. We review the uniqueness and origin of mesencephalic dopaminergic neurons, and the role of transcription factors in these neurons during development of mesencephalic dopaminergic neurons. As we start to understand the consequences of their inactivation, on the one hand, one may speculate on their significance as candidate genes for association with dopamine-related mental disorders, and on the other as tools to manipulate dopamine neurons in therapies aiming to rescue or replace mesencephalic dopaminergic neurons in Parkinson's disease.

2. Heterogeneity of dopamine neurons in the brain

Because of its implications in mental and neurological disorders, most attention has been given to the mesencephalic dopaminergic neurons. These neurons are anatomically and functionally heterogeneous, but distinctly different from additional groups of dopaminergic neurons that exist elsewhere in the brain. These distinctions are important to appreciate the significance of molecular findings in the mesencephalic dopaminergic system. Regarding the rodent brain, dopamine neurons are found from rostral to caudal in the following structures:

(a) In dendritic neurons of the olfactory bulb.
(b) In the hypothalamus, organized in the A11–A15 cell groups, of which the A12 cell group is the largest, providing the tuberoinfundibular and the tuberohypophysial projections involved in neuroendocrine regulation (about 1000 neurons).
(c) In the mesencephalon, organized in the A8–A10 cell groups, indicated collectively here as ''mesencephalic dopaminergic'' neurons (about 40,000 neurons in th rat). The A9 cell group is located in the substantia nigra pars compacta and has preferred projections to the striatum forming the neo-striatal pathway mediating motor control. The non-nigral cell groups A10 and A8 are located in the medial part of the ventral tegmental area and in the caudal part of the ventral tegmental area, respectively. The A8 and A10 cell groups are the main origins of the dopamine projections to cortical and limbic brain regions, i.e. the mesolimbocortical systems, serving behavioral functions.

Thus, the clusters of dopamine neurons in the central nervous system have different anatomical positions and projections and serve different functions. In principle, they should be considered as totally unrelated neurons, having in common only the synthesis of the transmitter dopamine. Consequently, one may expect that the molecular cues involved in their development and maintenance are different. As outlined below, this is certainly the case for the gene cascades in mesencephalic dopaminergic neurons in comparison to other dopaminergic neurons, although they share common events during early embryonic preparation of the neuromeres before dopaminergic neurons are born.

3. Midbrain development: commitment of the mesencephalic area

In order to appreciate the developmental programs of mesencephalic dopaminergic neurons, it is important to consider the events that enroll in the mesencephalic area before dopaminergic neurons can be distinguished. Events with consequences for the commitment and position of mesencephalic dopaminergic neurons can be traced back to very early stages of neural induction. Such events are related to pattern formation and organization of the midbrain for which the creation of the isthmus, the border between midbrain and hindbrain is crucial. Next, the commitment of neuronal lineages directs diversification of the region, resulting ultimately in induction of specific neuronal cell types, including mesencephalic dopaminergic neurons. These molecular events concern ''gene cascades''. Although these cascades enroll over time, they are not strictly serial but overlap in time and space.

All regulatory processes rely on ''gene cascades'', a hierarchical sequence of events leading to execution of a process. Gene cascades are best noticeable during developmental processes, in which an organism passes through irreversible stages from a single cell towards an adult organism. Therefore, gene cascades have mostly been delineated in developmental processes. However, they also underlie regulatory processes in adult physiological systems, in which they determine cellular competence and physiological set points.

Like many gene cascades, midbrain-specific cascades involve the interplay between intracellular and intercellular control mechanisms. While signaling molecules and receptors mediate intercellular communication, transcription factors are central to intracellular control by determining programs of gene expression. The interplay is bidirectional: signaling molecules secreted by one cell (type), e.g. growth factors, hormones, or transmitters, elicit changes in other responsive cells at the level of gene expression through altering activity of transcription factors. In turn, transcription factors induce or suppress specific sets of genes, thereby providing the cell with new properties.

During the development of the midbrain, one can recognize the organizing capacity of gene cascades, e.g. in the establishment of the isthmus. Looking at progressive stages of developing neuronal systems, the hierarchical role of transcription factors becomes evident in the specification of neuronal properties. Neurons not only need to adopt specific transmitters, they also need to integrate adequately in a network having specific efferents and afferents. As many inputs and decisions of the brain ultimately converge on the mesencephalic dopaminergic system, the dopaminergic neu-

rons of the midbrain must develop in a highly controlled and coordinated fashion. Based on the description of transcription factor expression in the midbrain, and the consequences of their genetic null mutation, one now starts to recognize gene cascades in mesencephalic dopaminergic neurons, and link them to specific properties of mesencephalic dopaminergic neurons.

3.1. Development of the isthmus

During brain development, molecular signaling causes the appearance of molecular borders. These borders serve to initialize specific brain regions in their local developmental program. This results in the complexity of different specialized regions of the brain. The isthmus is a very important barrier creating a region that separates anatomically and molecularly the forebrain from the hindbrain. First, the hindbrain can be defined as part of the same code that rules developing peripheral tissue. In this region, the Hox-code is still functional as for the rest of the body. Anterior to this border, no Hox-rules can be applied since these genes are not expressed in the mid- and forebrain.

Essential genes for defining the isthmus and the appearance of the mid- and forebrain are the Otx genes. Otx2 is expressed in the anterior neural tube and is limited at midbrain/hindbrain border. In mutant mice for Otx2 the mid- and forebrain are absent (Acampora et al., 1995). In line of this phenotype, the expression of Otx2 covers the midbrain and forebrain specifically: all dorsal and most ventral regions of the telencephalon, diencephalon and mesencephalon (Matsuo et al., 1995). The expression domain of the family member Otx1 overlaps that of Otx2, but it is smaller. The temporal expression is such that Otx2 is first (E5.5) and is directly followed by Otx1. In the ventral side of the forebrain, both Otx1 and Otx2 are early expressed in the mesencephalon with sharp boundaries at the anterior and posterior side. The posterior side is the early mid/hindbrain border.

The expression of Otx genes in the forebrain area is an essential component of the cascade that leads to specification and function of the forebrain area. Genes like HesX1/Rpx, Six3, Pax2, Wnt1 and En are depending, for their induction, on proper Otx expression (Rhinn et al., 1998). In Otx1 −/−,Otx2+/− animals the isthmus identified by the narrow dorsal expression of the growth factor related component, Fgf-8, is shifted to the diencephalic border. This is already notable by the loss of the sharp expression field of Fgf-8. The end result is that the mesencephalon is lost and the anatomical space is occupied by the metencephalon (Acampora and Simeone, 1999).

Recent studies have shown that the caudal limit of Otx2 expression is able to set the position of the isthmus. Ectopic expression of Otx2 by knock-in experiments using the En1 locus showed that most of the cerebellar vermis is missing and that the inferior colliculus is complementarily enlarged. Molecular analysis proved the shift of the isthmic region to a caudal position by the caudal shifted expression of the midbrain markers Wnt1 and Ephrin A5 and the isthmus markers Pax2 and Fgf8. As expected, the isthmic hindbrain marker Gbx2 was also shifted to a caudal position (Broccoli et al., 1999). In Gbx2 −/− mice, the expression border of Otx2 extended to a posterior position in the early somite stage. The downstream isthmus markers shifted similar to the new Otx2 border. Crossing experiments with Gbx2 transgenic animals showed that the Otx2 expression border could be placed at more anterior regions. So, the function of Gbx2 at the caudal border seems to be to limit the expression of Otx2, thereby creating the sharp limit of Otx2 expression at the isthmus (Millet et al., 1999). The initial isthmus boundary is apparent by the expression of the signaling molecule Fgf-8. This molecule is important for the downstream signaling in the isthmus region. If ectopically applied, Fgf-8 is capable of inducing an ectopic isthmus structure (Martinez et al., 1999). Thus, the anatomical position and the commitment of the area in which mesencephalic dopaminergic neurons will be induced are set at very early stages of brain development. Next, additional signals are required to specify different neuronal lineages, one of which will become the mesencephalic dopaminergic system.

3.2. Factors involved in early midbrain development

The specification of the midbrain depends largely on the correct specification of its caudal bordering structure, the isthmus. Any deregulation of that structure has severe effects on the development of the midbrain. A midbrain can be subdivided initially in a dorsal part and a ventral part. Each region has its own specific developmental cascades, and removal or ectopic expression of the early signaling molecules can induce the ventral cell type in dorsal positions and the other way around. An important ventral signaling molecule is sonic hedgehog (Shh) that is expressed in the notochord. The signaling by Shh together with the signaling by Fgf8 from the isthmus will induce dopaminergic cells in the ventral midbrain (Ye et al., 1998). The inductive signal of Shh is dominant in this event, since the overexpression of Gli1, a downstream effector of Shh, can also ectopically induce dopaminergic cell-markers (Hynes et al., 1997). Ectopic expression of Shh or Gli1 in dorsal midbrain regions expressing Fgf8 will also result in ectopic dopaminergic neurons. Olfactory and diencephalic dopamine neurons also originate in overlapping expression fields of Fgf8 and Shh (Ye et al., 1998).

Although the gene cascades enrolling in the specification of the ventral midbrain are not known in detail, the following players have been implicated: the transcription factors En1, En2, Pax2, Pax5, Hes, Otx2, Hnf3α and β, Nurr1, Lmx1B and Pitx3, and the growth factors Fgf8, Shh, Wnt1, brain derived neurotrphic factor (BDNF), glia derived neurotrophic factor (GDNF) and transforming growth factor (TGF). Several of these molecules will be

discussed in relation to mesencephalic dopaminergic neuron development.

4. Induction and development of mesencephalic dopaminergic neurons

The first mesencephalic dopaminergic neurons arise at the most ventral rim of the neuroepithelium lining up along the mesencephalic flexure of the ventral mesencephalon (Fig. 1). Before the expression of dopamine-specific markers, ventral midbrain markers are present in these cells. Among the earliest markers of the region are En1, En2, Wnt1, Pax2 and Pax5. The expression is located directly anterior of the Otx2/Gbx2 border (Wassef and Joyner, 1997). The expression of En 1 and 2 is maintained by the expression of the signaling molecule Wnt1 (Danielian and McMahon, 1996). Recently another homeobox family member was implicated in the early specification of the ventral midbrain. This factor, Lmx1b, is expressed at E7.5 in the ventral mes- and diencephalon and remains expressed in the adult in brain structures derived from these areas including mesencephalic dopaminergic neurons (Smidt et al., 2000).

The first specific signs of mesencephalic dopaminergic neurons shortly follow the induction of the orphan nuclear hormone receptor Nurr1 (E10.5), although the expression pattern is not restricted to these neurons and extends in a large field in the mesencephalon and diencephalon. Immediately after Nurr1, expression of the key enzyme in dopamine synthesis tyrosine hydroxylase (TH) is induced (E11.5). This initiation is parallel to the induction of the mesencephalic dopaminergic-specific homeobox gene Pitx3. In short, one can abstract the course of developmental

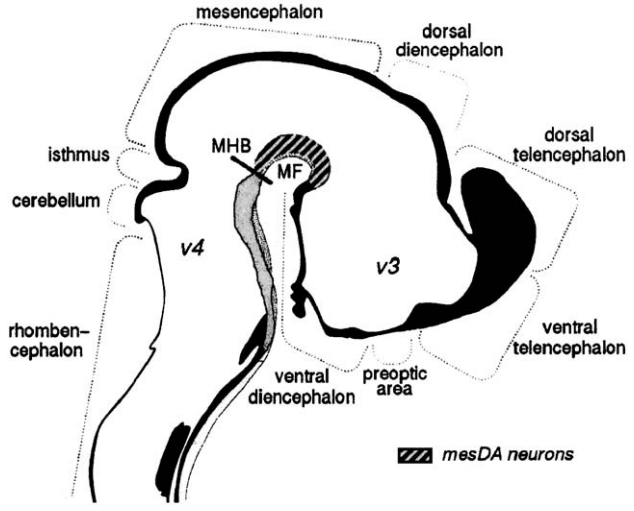

Fig. 1. Schematic representation of the developing murine central nervous system at stage E12.5. The region where the first appearance of mesencephalic dopaminergic neurons is detected is indicated by a shaded area (v3: third ventricle; v4: fourth ventricle; MHB: midbrain/hindbrain border (isthmus); MF: mesencephalic flexure).

decisions leading to mesencephalic dopaminergic neurons in the following stages:

(I) Dopaminergic progenitor cells are defined by the intersection actions of Fgf8 and Shh.
(II) The genes En1, En2, Wnt1, Pax2 and Pax5 are expressed in the region and are probably necessary in the differentiation program of the mesencephalic dopaminergic progenitor cells together with the recently identified player Lmx1b.
(III) Just before the dopaminergic phenotype emerges Nurr1 is activated and,
(IV) With the induction of TH, which requires Nurr1, and of Pitx3, the mesencephalic dopaminergic neurons are born. At present, no later emerging regulatory proteins have been identified.
(V) Finally, mesencephalic dopaminergic neurons need to acquire the complete molecular make-up involved in all aspects of dopamine neurotransmission, and to establish the appropriate connectivity with efferent and afferent neurons.

Around stage II, dopaminergic progenitor cells stop proliferating and enter into a differentiation program. This transition is a milestone in the birth of mesencephalic dopaminergic neurons. Therefore, recent observations on factors potentially involved in these processes are highlighted below.

4.1. Pitx3 and development of the mesencephalic dopaminergic system

Mesencephalic dopaminergic neurons are the only neurons that express the homeobox gene Pitx3. Such exclusivity is unique in the brain and suggests that Pitx3 serves a function unique to mesencephalic dopaminergic neurons (see below). From a reverse transcriptase polymerase chain reaction (RT-PCR) experiment using rat brain mRNA and degenerated primers based on OTP and other paired-like homeobox genes (Simeone et al., 1994), a homeobox-DNA fragment of approximately 180 bp was cloned (Smidt et al., 1997). This fragment was used to screen a rat brain cDNA library, which resulted in the characterization of a cDNA encoding a protein of 302 AA. Comparison of its amino acid sequence to the database revealed that it was related to Pitx1 and Rieg (hereafter named Pitx2). These two related proteins were implicated in pituitary-specific gene expression and in development of stomodeal structures (Lamonerie et al., 1996; Drouin et al., 1998) and in the Rieger syndrome, an autosomal-dominant human disorder characterized by craniofacial malformation (Semina et al., 1996), respectively. Based on homology to Pitx1 and Pitx2, the novel protein was called Pitx3. At the same time, Pitx3 was independently cloned from a mouse embryo library by homology screening to Pitx2 (Semina et al., 1997). The homeodomains of the Pitx proteins are of the paired-like type and are highly

conserved, only differing by one or two amino acids, displaying a lysine residue at position 50 of the homeodomain, which is typical for bicoid-type homeodomains. The homology also extends into the proximal and extreme C terminus (67% homology, Pitx3 vs. Pitx1, Pitx3 vs. Pitx2). Thus, these three genes constitute a distinct and closely related subfamily within the paired-like class of homeoproteins. Strikingly, the invertebrate species *C. elegans* and *Drosophila melanogaster* also contain homeobox genes with an almost identical homeodomain to the mammalian Pitx family, termed Unc30 and dPitx, respectively, but homologies beyond the homeodomain are insignificant (Jin et al., 1994; Vorbruggen et al., 1997).

At the macroscopic level, hybridization of Pitx3 was confined to the substantia nigra pars compacta (SNc; A9) and the ventral tegmental area (VTA; A8, A10), together harboring the mesencephalic dopaminergic system, but was not seen in rostral regions having dopaminergic neurons, nor in the adrenal gland. In order to determine if Pitx3 is indeed expressed in mesencephalic dopaminergic neurons, we performed double in-situ hybridization with Pitx3 and tyrosine hydroxylase (TH) cRNA probes (Fig. 2). Pitx3 expression completely overlapped with TH-positive cells, demonstrating that Pitx3 is expressed exclusively in dopaminergic neurons of the mesencephalic dopaminergic system (Smidt et al., 1997).

Ablation of mesencephalic dopaminergic neurons by microinjection of the neurotoxin 6-hydroxy-dopamine has provided an animal model of motor dysfunction as in Parkinson's disease (Ungerstedt et al., 1974). Unilateral 6-hydroxy-dopamine lesions of the mesencephalic dopaminergic neurons in adult rats caused the characteristic rotational behavior and resulted in the total disappearance of TH expression on the lesioned side. Notably, Pitx3 expression was no longer detectable in the 6-hydroxy-dopamine injected side, but was normally expressed contralaterally. This shows that Pitx3 expression is confined to dopamine neurons. In situ hybridization experiments on the human substantia nigra showed strict co-localization of the human counterpart of Pitx3 with pigmented cells, which represent the dopaminergic neurons. Furthermore, in situ analysis of the substantia nigra of Parkinson patients revealed a reduced density of Pitx3-expressing neurons as compared to normal controls. Thus, the loss of Pitx3 expression correlated with loss of the mesencephalic dopaminergic neurons, both in animal models and human disease. Taken together, these results demonstrated that only mesencephalic dopaminergic neurons express Pitx3 both in the rodent and human ventral midbrain.

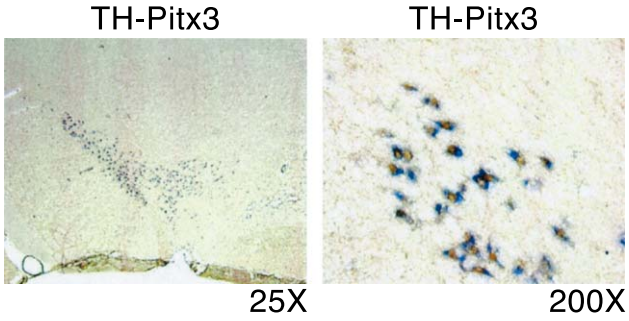

Fig. 2. Combined in situ hybridization for TH and immunohistochemistry for Pitx3 on a rat coronal brain section containing the substantia nigra. The TH signal is shown in purple and the positive nuclei staining for Pitx3 are shown in brown. The right panel represents a higher magnification of the most lateral/dorsal position of the substantia nigra.

As homeodomain proteins are usually involved in pattern formation, the close association of Pitx3 expression with an intact mesencephalic dopaminergic system suggested that Pitx3 might be involved in development and/or maintenance of mesencephalic dopaminergic neurons. In situ hybridization on mouse embryos from E8.5 to E16.5 showed the correlation of Pitx3 expression with development of mesencephalic dopaminergic neurons. At E11.5, a small layer at the ventral surface of the mesencephalic flexure expressed Pitx3. At E12.5, a complete field of TH-positive, Pitx3-positive neurons has been obtained. Higher magnifications show that Pitx3-positive cells are restricted to the marginal layer of the mesencephalic tegmentum. This group of about 50 cells corresponds to the first TH-expressing cells in the developing rodent brain. At later stages, the expression remains restricted to the mesencephalic dopaminergic system and this association is conserved in adult rat brain. Apart from the mesencephalic dopaminergic system, Pitx3 expression in the developing skeletal muscle, in the tongue and in the developing eye lens was detected at this stage (Smidt, unpublished data; Smidt et al., 1997; Semina et al., 1997).

4.2. Pitx3 and the dopaminergic phenotype in Pitx3 null mutants

The Pitx3 gene is mutated in the *Aphakia* (*ak*) mouse by deletions in the upstream promoter area and parts of exon 1, as was published recently (Semina et al., 2000; Rieger et al., 2001). This leads to absence of expression of the Pitx3 gene in the eye lens, which leads to blindness of these mice (Semina et al., 2000). To analyze the expression of Pitx3 in mesencephalic dopaminergic neurons, in situ hybridization experiments on the brains of adult *ak* mice compared to controls were done. The results indicated that the expression of *Pitx3* was not detectable in the mesencephalic dopaminergic neurons (Smidt et al., unpublished data). In addition, at E12.5, the stage when the mesencephalic dopaminergic neurons have been born and normally express Pitx3 and TH, no expression of Pitx3 was detected at the ventral side of the mesencephalon in *ak* embryos (Smidt et al., unpublished data). Since the expression of TH was not affected in the neurons that were located at the ventral midbrain, it was concluded that Pitx3 is not essential to induce the dopaminergic neurotransmitter phenotype. The initial data did hint on the fact that the anatomical organization of the midbrain dopaminergic

neuronal field was affected. Studies on the neuro-anatomical distribution on mesencephalic dopaminergic neurons revealed that the substantia nigra pars compacta was diminished in these animals (Van den Munckhof et al., 2003; Smidt et al., unpublished data). This neuronal loss was accompanied by loss of innervation in the caudate putamen. These data were confirmed by use of retrograde tracing experiment showing that the actual axons and terminals are lost (Van den Munckhof et al., 2003; Smidt et al., unpublished data).

The dopaminergic phenotype of remaining mesencephalic dopaminergic neurons was not affected as was indicated by the presence of all enzymes involved in dopamine synthesis. As yet, experiments did not answer the question as through which target gene Pitx3 exerts this phenotype. Control experiments on known genes involved in development and function of the mesencephalic dopaminergic system like Nurr1, Lmx1b, En1 and 2, cRET, and NLI/CLIM2 did not show any loss of expression of these genes. Until now, targets of Pitx3 in this neuronal population have not been described and therefore the mechanism behind the failing substantia nigra pars compacta development remains unclear (Smidt et al., unpublished data).

To determine if functional consequences of the anatomical and molecular alterations in the *ak* brain were apparent, the behavior of the *ak* mice was analyzed with respect to dopaminergic activity. For all behavioral analysis the *ak*-mutants were matched with wild-type mice on the same genetic background (C57/Bl.6) and, in addition, mice were enucleated at postnatal day 1 to induce blindness. Despite the lost dopaminergic innervation of the caudate putamen, no abnormal posture or tremor could be detected. In a set-up for automated quantitative gait analysis (Hamers et al., 2001), no abnormal walking patterns were detected either (Smidt et al., unpublished data), indicating that the motor control itself was not affected in Aphakia mice. However, in a ‘‘climbing test’’ the animals showed a strikingly aberrant behavior. This test is developed as a measuring tool to analyze the dopamine release in the striatum (Costall et al., 1978). Upon stimulation by, for example, psychostimulants as amphetamine, mice show increased climbing. In this test, both blind-wild-type mice and *ak*-mutants have increased climbing behavior compared to controls. The *ak*-mutants display higher activity compared to blind mice, but the difference was not significant by non-parametric statistical analysis (Smidt et al., submitted). During these experiment we noticed that the overall activity of the blind mice appeared to be elevated especially compared to the *ak*-mutants. This hyperactivity can be the cause for the relative high scores in the climbing paradigm for the blind-wild-type mice. To control for the overall activity and to asses whether the altered striatal innervation in the *ak*-mutants is mirrored by the activity, the animals were analyzed in an open field and scored for their horizontal movement by an automated observation. The data confirmed that blind-wild-type mice are hyperactive. Second, the *ak*-mutants performed strikingly low in overall activity. These data, combined with the initial data from the climbing test, indicate that the altered organization and connectivity in the *ak* mesencephalic dopaminergic system causes hyperactivation of the nucleus accumbens area, resulting in elevated climbing behavior, and it causes hypo-activation of the caudate putamen resulting in lower overall activity levels. Moreover, it is striking that only the motor output, and not the motor skills, is affected. These studies show clearly that the neuroanatomical aberrations in the *ak*-mutant are reflected by the behavioral output of the animal (Smidt et al., unpublished data). Notably, mutations in the Pitx3 gene have been described (Semina et al., 1998). These cause autosomal dominant eye defects, i.e. cataract and anterior segment mesenchymal dysgenesis (ASMDS). Human heterozygous for these Pitx3 mutations do not have apparent neurological abnormalities (personal communication).

4.3. Nurr1 and the dopaminergic phenotype in Nurr1 null mutants

Nurr1 is an orphan member of the nuclear hormone receptor superfamily of transcription factors (Law et al., 1992). It is expressed in several unrelated regions of the central nervous system including limbic areas, and the ventral mesencephalon including the mesencephalic dopaminergic neurons. Thus, its brain expression is not uniquely linked to mesencephalic dopaminergic neurons as in the case of Pitx3 (Smidt et al., 1997). The onset of expression in mesencephalic dopaminergic neurons is at E10.5 in the mouse, just before the onset of the dopaminergic markers TH and Pitx3 (E11.5) (Saucedo-Cardenas et al., 1998; Smidt et al., 1997), and expression continues in the adult stage. The protein has therefore functions during development and in the adult functional dopamine neuron. The function of Nurr1 in the mesencephalon has been studied by the creation of knock-out models. Mice with a targeted deletion of the Nurr1 gene develop until gestation, but die soon after birth. Analysis of the brain of these animals showed that in the newborn animals no mesencephalic dopaminergic neurons could be detected by using markers as Adh2, cRet and TH (Zetterström et al., 1997). From these studies it was concluded that these mice fail to induce Adh2, cRet and TH genes and that the initial mesencephalic dopaminergic neurons do not survive. The main conclusion was that Nurr1 is essential for induction of several mesencephalic dopaminergic markers and that it is essential for the survival and/or maintenance these neurons. Another independent study showed that the mesencephalic dopaminergic neurons are initially generated and that the only marker that is initially missing is TH (Saucedo-Cardenas et al., 1998). The developmental program of mesencephalic dopaminergic neurons does proceed in the Nurr1 −/− animals as illustrated by the proper induction of the mesencephalic dopaminergic spe-

cific homeobox gene Pitx3 (Saucedo-Cardenas et al., 1998) and the maintenance of Lmx1b expression (see below). The specificity of the phenotype is remarkable since other neurons that express Nurr1 are not affected by the ablation of Nurr1. It has been tentatively speculated that the specificity of the phenotype to the mesencephalic dopaminergic neurons lies in the fact that there might be a cross-talk between Nurr1 and the mesencephalic dopaminergic specific homeodomain gene Pitx3. Such interactions have been described for the nuclear hormone receptor Ftz-F1 and the homeodomain gene Ftz (Yu et al., 1997). Recent studies in cultured neuronal stem cells showed that Nurr1 can enhance the number of TH^+ cells, but there was no effect of Pitx3 in the absence or presence of Nurr1 (Sakurada et al., 1999).

We speculate that the degeneration of the mesencephalic dopaminergic neurons in the Nurr1 −/− animals is not only due to the lack of dopamine synthesis, but that other unknown functions of Nurr1 in specific survival of these neurons are involved (see below, TH knock-out). Possibly the Nurr1 −/− mesencephalic dopaminergic neurons cannot form or maintain the connections to their targets and therefore the neurons degenerate as occurs after transection of the projecting axons. The absence of TH alone is probably not sufficient for the severe neuronal loss that is found in Nurr1 −/− mice since TH −/− mice still form projections to the striatum (Zhou and Palmiter, 1995).

Alternatively, Nurr1 may not directly control the induction of TH, but rather function in parallel in a switch from proliferating stem cells to non-proliferating differentiating cells, and be more fundamental in the control of the cell cycle. In mesencephalic dopaminergic neurons, TH seems to be expressed only in differentiating neurons, and marks the transition in the cell cycle. In any case, it is intriguing to know why Nurr1 displays this marked function in the development of mesencephalic dopaminergic neurons, and not in the many other neuronal populations in which it is expressed. Vice versa, olfactory, diencephalic and adrenal dopamine cells do not involve Nurr1 at all. This unique link of Nurr1 to mesencephalic dopaminergic neurons will relate to other specific aspects of the developmental cascades in mesencephalic dopaminergic neurons. Recently, several lines of evidence emerged, which show that Nurr1 regulates more genes in a direct or indirect way. Castillo et al. (1998) showed that at P0, Nurr1 −/− mice do not express the vesicular monoamine transporter 2 (VMAT2), aminoacid-decarboxylase and the dopamine transporter (DAT) in the ventral midbrain. These experiments do not rule out that expression is lost as a consequence of neuronal loss during development. Therefore, these data are not sufficient to conclude that these genes are downstream targets. Data generated in our own lab showed that aminoacid-decarboxylase is induced normally at E12.5. The VMAT2 and DAT gene were not induced at their respective induction time points and can therefore be considered as targets of Nurr1, but not aminoacid-decarboxylase (Smits et al., unpublished data).

4.4. Lmx1b and the dopamine phenotype of Lmx1b null mutants

Lmx1b is a member of the LIM homeodomain family that is an essential regulator of dorso-ventral patterning of the developing limbs. Mutations in Lmx1b evoke the nail patella syndrome (Dreyer et al., 1998; Chen et al., 1998). We cloned Lmx1b by RT-PCR in the same screen that resulted in the cloning and characterization of Pitx3 (Smidt et al., 1997, 2000). In situ hybridization on the adult rat brain using a cloned fragment as well as the full-length mouse Lmx1b cDNA revealed a restricted expression pattern characterized by high expression in the substantia nigra pars compacta and ventral tegmental area. Neural Lmx1b expression starts at E7.5 (Johnson and Chen, personal communication). Early developmental brain expression extends anterior of the mesencephalic dopaminergic region into the ventral hypothalamic area. More posterior, Lmx1b expression shifts to dorsal in the hindbrain and extends to the dorsal part of the spinal cord. This expression shift to dorsal seems to coincide with the position of the mid/hindbrain border.

The expression of Lmx1b in mesencephalic dopaminergic neurons was demonstrated by combined immunohistochemical and in situ hybridization analysis (Smidt et al., 1997). The expression of Lmx1b and TH co-localized with Pitx3-positive nuclei in the adult brain and in the ventral tegmentum at day E16 of development. This mesencephalic dopaminergic localization is also found in the human melanin-containing substantia nigra dopaminergic neurons. The intrinsic potential of the homeobox gene Lmx1b as a developmental regulator, together with the fact that Lmx1b is expressed earlier than the first appearance of Nurr1-(E10.5), TH-(E11.5) and Pitx3-(E11.5) expression, suggested that Lmx1b may act genetically as an upstream activator of these genes, and may be involved in preparing the region for genesis and differentiation of the mesencephalic dopaminergic system. To validate this hypothesis, we analyzed the expression of TH, Pitx3 and Nurr1 in brain sections of homozygous Lmx1b knock-out mice (Chen et al., 1998) of stage E12.5, when the field of expression of TH and Pitx3 is first complete. In sections of E12.5 Lmx1b −/− embryos, TH-positive cells in the ventral tegmentum were observed. Interestingly, these cells did not express Pitx3, indicating that Pitx3 is not necessary for TH expression (Smidt et al., 2000) as was also found in the Pitx3 null mutant (see above). A few Pitx3-positive cells were identified, posterior and dorsal to the TH positive cells. At further stages up to E16, TH-positive cells could be detected in the ventral mesencephalon.

In line with the conclusion that TH-positive cells develop independent of Pitx3 are gain-of-function experiments in vitro and in vivo. It has been shown that Pitx3 does not alter the number of TH-positive cells in neural stem cell cultures, but Nurr1 does (Sakurada et al., 1999). Furthermore, ectopic expression of Pitx3 in the dorsal mesencephalon driven by the En2 promoter did not result in the induction of dorsalized TH- or Nurr1-positive neurons.

It is concluded that Lmx1b is essential for the proper specification of the mesencephalic dopaminergic neurons. Lmx1b −/− mice lack the necessary molecular signals to differentiate and maintain mesencephalic dopaminergic neurons in the ventral midbrain, which leads to early loss of mesencephalic dopaminergic neurons during developmental maturation of the system. This loss of mesencephalic dopaminergic neurons is much more severe and earlier compared to the Nurr1 −/− mice.

4.5. Engrailed and the dopamine phenotype of EN mutants

In the mouse two engrailed genes are expressed in the midbrain. Their expression patterns partly overlaps but the timing is different between the two genes. En1 expression starts at the one-somite stage in the anterior neuroepithelium, which later forms the midbrain–hind brain junction region. En2 expression starts later in a similar manner at the five-somite stage. Mutation analyses of En1 and En2 were performed to investigate the role of these genes in mouse development (Wurst et al., 1994; Simon et al., 2001). Here, we consider only those aspects that are relevant for the development of the mesencephalic dopaminergic neurons.

Mice defective in the En1 gene die at birth. Analysis of the brains of these mice showed that the cerebellum was absent and the colliculi were truncated. The pons and the substantia nigra, both derived from En expressing cells in the mes/metencephalon, were not clearly affected. The lack of En1 had an influence on the expression of En2, which was up-regulated at the mid–hindbrain region. The data indicate that one of the engrailed genes is enough to ensure the functional phenotype. This works only if a copy of En1 is present; the other way around, only one copy of En2 does not restore the complete phenotype. Interestingly, in En1 and En2 double mutants the substantia nigra and ventral tegmental area are completely absent, showing that at least one of the engrailed genes is essential for this neuronal structure to develop. Comparison of the En1 and En2 mutants shows that in both animals the substantia nigra and ventral tegmental area present; in the En1 mutant, only the cytoachitecture is slightly altered in such a way that the dopaminergic neurons in the ventral tegmental area appear more loosely arranged compared to wild-type mice. Notably, after lowering the doses of En1 or En2 by applying the expression of one allele of either gene (En2 −/−, En1+/−), mutants with one functional allele of En1 show a similar phenotype as the mutant containing both alleles. Whether dosage has any consequences in the adult animal is at present unknown. It is interesting, however, that the terminal field in the En1+/− mice is not complete. Immunohistochemical staining for TH shows abundant TH protein in the nucleus accumbens but fails to identify TH in the caudate putamen (Simon et al., 2001). So, although the neuronal field is close to normal in these mice the projections might be compromised. Finally, an interesting link between Engrailed genes and Parkinson's disease was discussed since the expression of α-synuclein, which is genetically linked to some cases of Parkinson's disease, was shown to be regulated by En1 and En2. This indicates that genetic variation in engrailed genes may be a risk factor for developing Parkinson's disease.

5. Mice defective in the dopamine synthesis/release pathway

5.1. Dopamine transporter (DAT) knock-out and the mutant phenotype

Dopamine is an important regulator of many central nervous system functions. Hyperactivity of the dopaminergic system is believed to be related to several neuropsychiatric disorders. Genetic deletion of the DAT gene in mice (DAT −/−) results in a persistent extracellular hyperdopaminergic tone that is functionally evident as hyperactivity (Giros et al., 1996; Jaber et al., 1997; Jones et al., 1998a,b). The lack of a re-uptake mechanism produces a marked increase in functional extracellular dopamine (300%), which results in profound changes in pre- and postsynaptic parameters of dopamine homeostasis. In order to cope with the loss of the DAT, adaptive changes occur in the dopaminergic nerve terminal. In the caudate putamen and nucleus accumbens of DAT −/− mice, constitutive hyperdopaminergia resulted in differential regulation in gene expression for the dopaminergic receptors. D3 receptor mRNA levels increased as mRNA levels for both D1 and D2 receptors decreased (Fauchey et al., 2000). This decrease of D2 receptors also resulted in nearly complete loss of autoreceptor function (Jones et al., 1999). Levels of TH and dopamine stores were dramatically decreased, vesicular transport was slightly decreased, and DOPA decarboxylase (aminoacid-decarboxylase) levels were not modified in striata of DAT −/−; mice. Interestingly, the rate of dopamine synthesis was doubled (Jaber et al., 1999). Indeed, the reduced number of TH molecules present in DAT −/− mice seems to function at a much higher rate than those present in normal animals. Thus, the DAT not only regulates the lifetime of extracellular dopamine, but also is critically involved in maintaining the delicate balance between dopamine synthesis, release, and degradation (Jones et al., 1998a,b).

The neurotoxin 1-methyl-4-fenyl-1,2,3,6-tetrahydropyridine (MPTP) has been shown to induce parkinsonism both in human and in nonhuman primates. MPP+, which is the oxidation product of MPTP, is actively transported into presynaptic dopaminergic nerve terminals through the DAT. DAT −/− mice do not show degeneration of dopaminergic cells after MPTP treatment (Gainetdinov et al., 1997; Fumagalli et al., 1998), indicating that neuronal cell loss is related to levels of the DAT (Bezard et al., 1999). Thus, mice lacking the DAT gene may represent an appropriate model to elucidate the molecular adaptive changes accompanying pathological states associated with hyperdopami-

nergic function. They have provided key elements leading to possible clinical and behavioral implications for Parkinson's disease. In a conditioned place preference test, DAT −/− mice exhibited a stronger rewarding response to morphine (5 mg/kg, s.c.) compared with control littermates. However, the same dose of morphine failed to increase locomotor activity in DAT −/− mice, while enhancing locomotion in DAT+/− and DAT+/+ animals. Morphine-induced analgesia was unaffected in mutant mice, but the behavioral expression of naloxone-induced withdrawal signs was blunted. Morphine-induced rewarding responses are thus firmly established in DAT mutant mice, despite dopamine transmission that is already tonically activated, independent of any effect on locomotion (Spielewoy et al., 2000).

5.2. Vesicular mono amine transporter 2 (VMAT2) knock-out and the mutant phenotype

Vesicular monoamine transporter is expressed in the mesencephalic dopaminergic system and functions to load vesicles with monoamines as dopamine to generate a ready releasable pool of neurotransmitter. Also, mesencephalic dopaminergic neurotoxins like MPTP are transported from the cytosol into these secretory vesicles. Newborn VMAT2 −/− mice move little, feed poorly and die within a few days after birth, manifesting severely impaired monoamine storage and vesicular release. VMAT2 −/− mice can therefore not be studied extensively, but VMAT2 function can be studied by its disruption through application of amphetamine or by studying VMAT2+/− mice. VMAT2+/− mice are viable into adult life and display VMAT2 levels one-half of wild-type values (Takahashi et al., 1997).). The lower level of VMAT2 in VMAT2+/− mice results in drastic decrease in the levels of dopamine, despite a near doubling in the rates of synthesis in the brain (Wang et al., 1997). Striatal DAT protein levels were reduced in the VMAT2+/− mice compared to wild-type mice, while no significant differences in DAT binding between VMAT2+/− and wild-type controls were reported (Gainetdinov et al., 1998). In terms of vulnerability to the neurotoxin MPTP, it was apparent that after MPTP administration more than twice the dopamine cell loss was detected if compared to wild-type mice. Disruption of VMAT2 apparently leaves the neurotoxin in the cytoplasm of the neuron, where it can cause extensive degeneration. In vitro overexpression of VMAT2 reduces MPP+ toxicity, whereas overexpression of DAT enhances this toxicity (Kitayama et al., 1992; Liu et al., 1994).

5.3. Dopamine D1 receptor knock-out and the mutant phenotype

Dopamine receptors play a pivotal role in the pathophysiology and pharmacological treatment of Parkinson's disease and schizophrenia (Albin et al., 1989; Starr, 1995). To study the functions of each receptor, mice lacking the dopamine D1 receptor (Drago et al., 1994; Xu et al., 1994), dopamine D2 receptor (Baik et al., 1995; Kelly et al., 1997; Jung et al., 1999), dopamine D3 receptor (Accili et al., 1996; Xu et al., 1997a,b; Jung et al., 1999), dopamine D4 receptor (Rubinstein et al., 1997) and dopamine D5 receptor have been generated (Holmes et al., 2001; Hollon et al., 2002). Among the five subtypes of dopamine receptors, two, the Dopamine D2 receptor and the dopamine D3 receptor, are of special interest, as they have been postulated to be involved in the pharmacological activity and therapeutic efficacy of antipsychotic drugs (Seeman, 1992; Malmberg et al., 1993). The exact role of the Dopamine D1 receptor is still not clear, but the expression pattern (hippocampus and prefrontal cortex) suggests a role in cognition and behavior (Lidow et al., 1991). Dopamine D1 receptor −/− mice are growth-retarded and die shortly after weaning unless their diet is supplemented with hydrated food (Drago et al., 1994). Dopamine D1 receptor −/− mice display several behavioral changes. They exhibit normal coordination and mild hyperlocomotion (Drago et al., 1994; Xu et al., 1994), but they have a deficit in spatial learning without visual or motor impairment (El Ghundi et al., 1999) and display a drastic reduction of rearing behavior (Drago et al., 1994). These animals also fail to show cocaine-induced stimulation of locomotor activity (Drago et al., 1996). Drago et al. (1999) showed that novelty-induced grooming is less expressed in dopamine D1 receptor −/− mice compared to dopamine D2 receptor −/− mice and their littermate controls. A decrease in spontaneous grooming of dopamine D1 receptor −/− mice was already described by Xu et al. (1994). However, some controversy exists about this since Clifford et al. (1998) reported that Dopamine D1 receptor −/− mice show unaltered grooming activity. Dopamine D1 receptor −/− mice show also attenuated alcohol-seeking behavior, suggesting that the Dopamine D1 receptor has an important role in the motivation for alcohol consumption (El Ghundi et al., 1998). In agreement with their conclusion, Ng and George (1994) showed that hypodopaminergic function in the mesolimbic system promotes alcohol intake, which is attenuated by increasing synaptic dopamine (George et al., 1995).

5.4. Dopamine D2 receptor knock-out and the mutant phenotype

Dopamine D2 receptors are of crucial importance in the striatal processing of motor information received from the cortex. Disruption of the dopamine D2 receptor gene function in mice results in hypoactivity and insensitivity to the hypolocomotor and hypothermic effects of D2/D3 receptor agonists (Boulay et al., 1999), indicating that the presence of the dopamine D2 receptor is necessary for the expression of the locomotor- and core temperature-decreasing effects of D2/D3 receptor agonists (7-OH-DPAT and

PD 128907). The expression levels of dopamine D3 receptor proteins during later stages of their postnatal development are higher in dopamine D2 receptor mutants, and dopamine D2 receptor/dopamine D3 receptor double mutants show more severe impairment of locomotor activity than single dopamine D2 receptor mutants, suggesting that dopamine D3 receptor can compensate for some of the missing dopamine D2 receptor functions (Jung et al., 1999). Presynaptic D2 autoreceptors, which are well known to modulate dopamine release, have recently been shown to regulate DAT activity. DAT function is decreased in dopamine D2 receptor −/− mice (i.e. clearance of locally applied dopamine is decreased by 50%), but no changes in dopamine release in dorsal striatum are observed (Dickinson et al., 1999). The absence of differences in either density or affinity of DAT binding sites in the striatum indicates that the differences seen in DAT activity are not a result of decreased DAT expression (Dickinson et al., 1999). Absence of the dopamine D2 receptor generates animals that are akinetic and bradykinetic in behavioral tests and show significantly reduced spontaneous movements. This phenotype presents analogies with symptoms characteristic of Parkinson's disease (Baik et al., 1995), and might have potential as a model for investigating and correcting dysfunctions of the dopaminergic system. The absence of spontaneous catalepsy in D2 knock-out mice is confirmed by several groups. Kelly et al. (1997, 1998) reported an absence of spontaneous catalepsy in their line of dopamine D2 receptor −/− mice, and Boulay et al. (1999) and Baik et al. (1995) analyzed dopamine D2 receptor −/− and dopamine D2 receptor+/− mice generated from individuals issued from the same colony of mice and reported that their mice were not spontaneously cataleptic.

5.5. Dopamine D3 receptor knock-out and the mutant phenotype

Dopamine D3 receptor −/− mice are more active than wild-type mice in a novel environment (Xu et al., 1997a,b), exhibit enhanced behavioral sensitivity to Dopamine D1 receptor and dopamine D2 receptor agonists (Xu et al., 1997a,b), display increased locomotor activity and rearing behavior (Accili et al., 1996), and have reduced anxiety (Steiner et al., 1997). These observations suggest a role of the dopamine D3 receptor in modulating behaviors by inhibiting the cooperative effects of postsynaptic D1 and other D2 class receptors and involvement of dopamine D3 receptors in the regulation of anxiety. Analysis of dopamine D3 receptor mutants by Xu et al. (1997a,b) showed that D1, D2, D4 and D5 receptor ligand-binding sites were expressed in normal patterns in the brain, as were binding sites for the DAT. No abnormality in TH immunostaining patterns was observed. So, it is clear that no apparent compensatory mechanisms are detectable after functional loss of the dopamine D3 receptor.

5.6. Dopamine D4 receptor knock-out and the mutant phenotype

The human dopamine D4 receptor has received considerable attention because of its high affinity for the atypical antipsychotic drug, clozapine (Jardemark et al., 2002). To clarify the in vivo role of the dopamine D4 receptor, mutant mice lacking the dopamine D4 receptor were generated. Biochemical analysis revealed that dopamine synthesis and its conversion to DOPAC were elevated in the dorsal striatum of dopamine D4 receptor −/− mice, indicating that the overall dopamine turnover is enhanced. The animals show hypoactivity, but they display locomotor supersensitivity to ethanol, cocaine, metamphetamine (Rubinstein et al., 1997) and, interestingly, exhibit reduced behavioral responses to novelty (Dulawa et al., 1999). In conclusion, the dopamine D4 receptor is closely linked to motor output and is especially involved in drug-stimulated motor behaviors.

5.7. Dopamine D5 receptor knock-out and the mutant phenotype

The dopamine D5 receptors were generated to investigate the behavioral consequences of ablation of this receptor (Holmes et al., 2001). It was shown that they are born normally and are of good health. Under baseline conditions these mice were normal on locomotor activity, the rotarod test, the acoustic startle response, the pre-pulse inhibition test, elevated plus maze, light/dark exploration, Morris water mace test and cued and contextual fear conditioning. Although there were some small effects on certain anti depressant drugs, it was concluded that the dopamine D5 receptor is not essential for many dopamine-mediated behaviors. In addition, Hollon et al. (2002) showed clearly that the dopamine D5 receptor knock-out animals display a higher sympathetic tone and are hypertensive. It is discussed that this defect has its origin in the central nervous system.

5.8. Tyrosine hydroxylase (TH) knock-out and the mutant phenotype

Tyrosine hydroxylase catalyses the conversion of tyrosine into L-DOPA, the initial rate-limiting step in the catecholamine biosynthetic pathway. The gene encoding TH becomes transcriptionally active in developing neuroblasts during mid-gestation of rodent embryos, before the onset of neurotransmission. Inactivation of the TH gene results in mid-gestational lethality apparently of cardiovascular failure, indicating the unequivocal requirement for catecholamines during embryonic development (Zhou et al., 1995; Kobayashi et al., 1995). Administration of L-DOPA to pregnant females results in complete rescue of mutant mice in utero (Zhou et al., 1995; Rios et al., 1999). Once born, TH −/− pups can survive without further

treatment until weaning. However, examination of pigmented TH −/− mice, which have not been supplemented with catecholamine precursors, showed that catecholamines were detected. Analysis of albino TH −/− mice, which lack an enzyme called tyrosinase that converts tyrosine to L-DOPA during melanin synthesis, showed that no catecholamines were detected in these mice (Rios et al., 1999). The comparison of TH −/− mice on a pigmented and an albino background indicated that tyrosinase serves as an alternate pathway to supply catecholamines. To prevent perinatal lethality and cardiac dysfunction in TH −/− mice, norepinephrine and epinephrine synthesis was restored by a transgenic rescue approach (Zhou and Palmiter, 1995; Nishii et al., 1998; Kim et al., 2000). These dopamine-deficient (dopamine −/−) mice were born at expected frequency but became hypoactive and stopped feeding a few weeks after birth. Administration of L-DOPA resulted in nearly normal growth. The locomotor activity response of these mutants to dopamine D1 or D2 receptor agonist and L-DOPA was 3- to 13-fold greater than the response elicited from wild-type mice, whereas no changes in steady-state levels of dopamine receptors and the DAT were observed as measured by ligand binding. Lack of TH expression in the cells that normally express the dopaminergic phenotype resulted in a marked reduction of dopamine accumulation in the tissues, which led to multiple behavioral abnormalities at the juvenile stage. These abnormalities were characterized by a reduction in spontaneous locomotor activity, blockade of methamphetamine-induced hyperactivity, cataleptic behavior, and defects in active avoidance learning. However, mesencephalic dopaminergic neurons, their projections and most of the characteristics of their target neurons in the striatum appeared normal, indicating that dopamine is essential for movement, feeding and associative learning, but is not required for the development of neural circuits that control these behaviors.

6. Conclusion

Recent data from molecular studies on mesencephalic dopaminergic neurons point out several important implications of gene cascades in development of dopamine systems. First, mesencephalic dopaminergic neurons are distinguished from olfactory and diencephalic dopamine neurons at the level of transcription factors they express. Although all these separate dopamine neurons arise at intersections of expression of the growth factors Fgf8 and Shh, mesencephalic dopaminergic neurons employ their own specific set of transcription factors, of which Lmx1b, Nurr1, Pitx3, Engrailed-1 and -2 are only exemplary.

Second, transcription factors in mesencephalic dopaminergic neurons are organized in at least three gene cascades, each serving separate properties of mesencephalic dopaminergic neurons. Nurr1 seems strictly coupled to transmitter synthesis, but does not affect Pitx3 or Lmx1b. In contrast, Lmx1b is not required for TH expression, but is necessary for the expression of Pitx3. Thus, three cascades exist: a Nurr1-dopamine synthesis cascade, an Lmx1b–Pitx3 cascade and an En1-maintenance pathway. Although without Lmx1b differentiating TH-positive neurons rapidly die, current efforts aim to detail the functions of the Lmx1b–Pitx3 cascade by focusing on functions mediated by Pitx3, the latest expressed transcription factor in this cascade. The remarkable phenotype of the Aphakia mouse suggests that a subset of mesencephalic dopaminergic neurons fails to adopt a mesencephalic dopaminergic phenotype during the induction phase at E11.5–E12.5 and is eliminated from further development.

Third, the data presented here on the regulation of dopamine homeostasis show clearly that the mesencephalic dopaminergic system can compensate on numerous methods including several molecular players in the dopamine producing neurons and in the receptive networks. Crucial in the adaptation strategy of the system is the function of the reuptake transporter the dopamine transporter. By regulating the level of the protein and therefore its activity, it is possible for the system to fine-tune the effect of the releasable pool of dopamine. In addition, the turnover of dopamine can be adjusted depending on the need for release. Finally, the dopamine receptors seem to be tightly coupled to specific functions and do not show many plasticity.

Finally, with gene cascades in mesencephalic dopaminergic neurons identified, it is challenging to envisage how these can be employed in neurological and mental disorders associated with the mesencephalic dopaminergic system. Clearly, in the case of Parkinson's disease, transcription factors need to be tested for their ability to protect mesencephalic dopaminergic neurons against progressive neurodegeneration and to provide undifferentiated cells with properties of mesencephalic dopaminergic neurons concerning dopamine synthesis as well as outgrowth and appropriate pathfinding. Such tests may be developed into rational new strategies in the treatment of Parkinson's disease. Concerning psychiatric disorders, it will be necessary to survey molecular genetic association of mesencephalic dopaminergic-related transcription factor genes with (subtypes of) schizophrenia and other affective disorders. Clear indications are presented here that small genetic differences in the Engrailed, Lmx1b, Nurr1 and Pitx3 genes may contribute to the overall state of the mesencephalic dopaminergic system and should therefore be incorporated in genetic analysis of psychiatric and Parkinson's patients.

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