Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience and Biobehavioral Reviews 27 (2003) 623–637
NEUROSCIENCE AND BIOBEHAVIORAL REVIEWS
www.elsevier.com/locate/neubiorev

Review

Dopamine phenotype and behaviour in animal models: in relation to attention deficit hyperactivity disorder

Davide Viggiano, Lucia A. Ruocco, Adolfo G. Sadile*

Department of Experimental Medicine, Laboratory of Neurophysiology, Behavioral and Neural Networks, Second University of Naples, Naples, Italy

Received 15 March 2003; revised 20 August 2003; accepted 25 August 2003

Abstract

The phenotypic expression of behaviour is the outcome of interacting neuronal networks and is modulated by different subcortical systems. In the present paper the role of a major subcortical neurochemical system, dopamine (DA), is reviewed. In particular, knockout (KO) technology has given an overwhelming insight into the effects of specific component of the dopaminergic system. Therefore, the behavioural profile of dopamine transporter (DAT), tyrosine hydroxylase (TH), DA and cAMP-regulated phosphoprotein (DARPP 32), and D1, D2, D3, D4 and D5 dopamine receptors knockouts (and their combination) is reviewed.

TH, D1, D2, D4 KO mice exhibit decreased locomotor activity, perhaps due to decreased motivational level. D3 KO and DAT KO mice show an increase in basal and novelty-induced activity respectively. It is possible that the increased dopamine levels in DAT KO mice enhance motivation. These observations support the hyperDA hypothesis in hyperactive phenotypes. Moreover, they suggest that the inhibitory effect of psychostimulant drugs, such as methylphenidate and amphetamines, in Attention Deficit Hyperactivity Disorder may be the outcome of an altered balance between auto- and hetero-receptors. However, since KO technology is hampered by blockade of the target at early stages of development, some alternatives have been proposed, such as inducible mutagenesis and inhibitory small RNAs conveyed to target by viral vectors in adulthood.

Keywords: Dopamine receptors; Knockouts; Dopamine transporter; Tyrosine hydroxylase

Contents

1. Introduction

The phenotypic expression of behavioural traits is the outcome of interacting neuronal networks and is modulated by different subcortical systems [1].

* Corresponding author. Tel.: +39-81-5665827; fax: +39-81-5665820.
E-mail address: adolfo.sadile@unina2.it (A.G. Sadile).

0149-7634/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neubiorev.2003.08.006

Therefore, the relation between behaviour and lower systems, from neuronal networks to single neurons and genes is increasingly complex. A direct linear or monotonic relationship between the properties of lower systems and behaviour is unlikely. Therefore, the genes, that must be considered the lowest basic system, which subserve behaviour, are related to the highest behavioural traits in complex ways.

As a matter of fact, no single gene can be regarded as responsible for a certain behaviour, and a great number of genes seem to be necessary for different behavioural repertoires. A number of knockout mice for different genes appear indeed to share the same behavioural impairments. An example is the impairment of hippocampal functions and hippocampal-dependent learning, which appear to be present in a great number of mutant mice [2–4]. This might be due to (i) limited understanding of behavioural tasks and poor descriptive value of behavioural indices (ii) limited changes due to the restricted behavioural repertoire (iii) mechanisms of compensation during development [2–4].

Moreover, it is now well established that the genetic background interacts with the single knocked-out gene, leading to completely opposite behavioural traits.

However, the depicted scenario contrasts with some expected findings from the knockout technology. In particular, knockout of DA pathways and DA receptors yielded valuable insights into the regulation of dopamine system and behaviour.

In fact, all cortical networks and subcortical structures (basal ganglia, amygdala complex, hippocampus), are modulated by subcortical influences (see [5]), represented by cholinergic neurons of Meynert's basal nucleus, dopamine (DA) neurons in the Ventral Tegmental Area (VTA), serotonergic neurons in the Raphè nuclei, norepinephrine neurons in the *Locus coeruleus*, and histamine neurons in the posterior hypothalamus. Each subsystem is involved in different aspects of behavioural performance, i.e. accuracy for ACh, latency for DA, impulsivity for serotonin, and distractibility for norepinephrine [6].

Though behaviour emerges from complex interactions, the DA component is very important for the phenotypic expression of attention and reward (see below). Moreover, the involvement of DA in behaviour is also supported by neuropsychiatric disorders such as schizophrenia, maniac-depressive psychosis and Attention Deficit Hyperactivity Disorder (ADHD).

The pivotal role of dopamine systems in the regulation of motivation results in striking behavioural phenotypes, which confirm previous models of dopamine regulation.

This, in turn, is of relevance, as different neuropsychiatric disorders have been linked to a dysfunction of the dopamine system. Though this is mainly inferred 'post hoc' i.e. from the effectiveness of dopamine-regulating drugs on these disorders (see e.g. antipsychotic for schizophrenia, methylphenidate for ADHD, L-DOPA for Parkinson disease), the exact neurobiological substrate underlying each of these diseases is not completely elucidated.

In ADHD, for instance, there is still much debate whether the dopamine system is hyper or hypofunctioning. On the one hand, methylphenidate ameliorate ADHD symptoms, 6OHDA striatal lesions cause hyperactivity in rats, there are evidences for a hypofunctioning DA system in SHR rats [7–9] and for striatal hypoplasy in ADHD children [10].

On the other hand, methylphenidate stimulates normal children, many DA receptor KO mice are hypoactive, whereas DAT KO mice are hyperactive (see below), the basal tone of DA in SHR rats is high (see Carboni et al. this issue), some rat lines with hyperactive behaviour show morphological and biochemical evidences of increased DA innervation [11–13]. The overall picture simply underlines our incomplete understanding of a system that serves basic behavioural functions.

2. Dopamine networks

DA is a highly conserved neurotransmitter, present in invertebrates such as Drosophila M. [14,15] and *C. elegans* [16], as well as in all vertebrates [17]. Two main dopamine systems, a central and a peripheral one, are involved in a number of complex functions such as regulation of blood pressure, movements, goal-directed behaviour, cognition, attention and reward. In particular, a number of studies have shown the involvement of DA projections to the *nucleus accumbens* and frontal cortex in reward, motivation, consummatory behaviour and learning [18–23].

The central dopaminergic system has been associated with several neuropsychiatric disorders including Parkinson's disease (PD), which is the result of selective degeneration of mesostriatal (MS) DA neurons, schizophrenia and ADHD, both of which are associated with dysfunction of mesocorticolimbic (MCL) DA neurons. In fact, most of the anti-psychotic drugs used to treat schizophrenia act as DA receptors antagonists, whereas ADHD symptoms are generally alleviated by drugs that regulate DAergic transmission. Moreover, drugs of abuse such as cocaine, amphetamine, opiates, nicotine and alcohol, act by modifying DA neurotransmission [20,24,25].

The cell bodies of DA neurons are present all along the ventral part of the brain. Though isolated cells can be identified, the vast majority is grouped into functional and anatomical units and has been progressively numbered. In our study, we will focus on cellular groups in the ventral mesencephalon, which are numbered from A8 to A10 in the caudo-lateral to rostro-medial direction [26], and particularly on the VTA and *Substantia Nigra pars compacta* (SN), although other dopamine systems, such as the olfactory or hypothalamic one are also especially important in rodent behaviour.

Ontogenetic studies in the rat showed that midbrain dopamine neurons appear between embryonic days E 12–15, near the midbrain–hindbrain junction (rhombic isthmus) [27]. These neurons begin to express tyrosine hydroxylase (TH) by E12.5, and then extensively migrate from the rhombic isthmus in a rostro-ventral direction to the ventral midbrain [27]. The floor plate and the notochord could influence the development of particular neuronal classes. Indeed, the floor plate is involved in the specification of DA neuron fate along the dorso-ventral axis. This depends on molecular differentiation cascades ([28–30]; for a review see [31,32]), and requires the orchestration of a number of genes such as Sonic hedgehog [33], Engrailed1 [34,35], Engrailed2 [36], Pax2 [37], Pax5 [38], Wnt1 [34], and Fgf8 [29,30]. The two transcription factors Ptx3 and Nurr1 are implicated in specification of the mesencephalic DA system ([39] see also other articles in this issue), The homeobox gene Ptx3, whose expression in brain is restricted to DA neurons [40] and the orphan nuclear hormone receptor Nurr1, are in fact required for induction of the enzyme tyrosine hydroxylase, which catalyzes the initial step in DA biosynthesis [41,42]. Moreover, the LIM homeobox gene Lmx1b has been shown to take part to this phenomenon [43].

Sex hormones further influence the development of DA neurons [44] and the pruning of axonal arbors, whereas BDNF, retinoic acid are thought to be necessary for striatal innervation. The projection of these axons to the target site requires signals from the target as well as from intermediate regions. Indeed, the action of genes such as Semaphorins [45] and ephrins [46] is required. It is interesting to note that the expression of such genes continue throughout life and can change after cocaine treatment [46]. Finally, extracellular molecules such as perisynaptc chondroitin sulphates (so called perineuronal nets, see [47]), could be implicated in the final asset of the dopamine network, as suggested by developmental data [48], enzymatic treatments [49], and the evidence for a complementary disposition of chondroitin sulphates and TH immunoreactivity ([50]; see also companion paper in this issue).

On the postsynaptic site, D1, D2 and D3 receptors mRNA are the first dopamine signaling molecule to appear (around E14, though the protein is synthesized later; see below [51]). Notably, the specification of DA receptor subclasses appears to be independent on the synthesis of DA itself, as suggested by studies on thyrosine hydroxylase KO mice (see below).

The axons of SN and VTA neurons topographically project to the caudate nucleus and *Putamen* (dorsal *Striatum*, CPu), to the ventral *Striatum* including *nucleus accumbens*, and to most areas of neocortex, especially the prefrontal cortex (PFC) [52]. These three systems, namely mesostriatal (MS), mesolimbic (ML) and mesocortical (MC), are not strictly separated from anatomical perspective, since DA efferents from both the *Substantia Nigra* and VTA overlap in a large ventral and medial segment of the CPu [53].

The MS system is described as the projection originating in the SNpc (A9 cell group) and the retrorubral field (A8 cell group) and terminating in the CPu (dorsal *Striatum*). DA terminals in the CPu synapse mainly on GABA medium spiny neurons [26,54,55] which represent the major output of this system. Two different pathways have been described, originating from striatal neurons expressing D1 (named direct pathway) or D2 (indirect pathway) receptor types. They are anatomically and functionally independent, though substantial revisions to this model, as well as the anatomical strict segregation of the DA receptors, have been proposed. Another level of CPu organization consists of the heterogeneous distribution of DA innervation: a patch or striosomal DA innervation is, in fact, surrounded by a later developing diffuse matrix compartmental subdivision.

On the other hand, DA neurons in the VTA project mainly to the ventral *Striatum* (*nucleus accumbens* and olfactory tubercle complex) and to the PFC, giving rise to the mesolimbic (ML) and mesocortical branches of the MCL system. In fact, the medial ML system is primarily derived from the A10 neurons situated in the VTA from where axons arrive to the ventral *Striatum* and PFC [52,53].

The ML DA system synapses on the shafts of the dendritic spines of medium spiny GABA neurons of *nucleus accumbens* [56], whereas glutamate inputs from a variety of cortical sources synapse on the heads of medium spiny neurons [57].

In CNS dopamine is a slow-acting molecule; however, the pattern of DA firing is functionally significant. It is possible to differentiate single-spike firing (which can be divided into regular and random types [58,59]), burst type firing, which releases a higher amount of DA [60] and colocalized peptides [61], and is able to activate early immediate genes in target regions [62], and tonic activity.

The phasic component has been subdivided into a fast (100–300 ms) and slow (s–min) components, the first being involved in reward prediction error, the second in reinforcement, sex, movement, punishment and stress [63]. An increased DA release has been shown in the *nucleus accumbens* during behavioural activation [64] and a previous study investigated the involvement of the MCL and MS DA systems in the control of activity, orienting, scanning times towards environmental stimuli and emotional reactivity in mouse model systems.

Anatomical, functional and neurochemical evidence all justify the division of the *nucleus Accumbens* in a rostral area, termed pole, a dorso-caudal area (*Accumbens*-core) and a ventro-caudal component (*Accumbens*-shell).

No anatomical separation exists between the *Accumbens*-core and the CPu: they are anatomically continuous, and both show a patch-matrix organization. Viceversa, the border between core and shell can be rapidly recognized using immunohistochemical markers such as substance P, calbindin, neurotensin, enkephalin [64].

Moreover, the core and shell connections are very different. The core region is involved in a corticostriatal circuitry with parts of the frontal lobe, and has reciprocal connections to the VTA (see [65]). The shell has a complex organization and could be included in a loop comprising the dorsal PFC, the lateral ventral *Pallidum* and *Substantia Nigra*.

Mesencephalic VTA DA neurons receive glutamate inputs mainly from the frontal cortex, and lead to reward effects, as demonstrated by electrical and pharmacological stimulation with phencyclidine of glutamate frontal neurons [57,66–68]. Other psychostimulants such as amphetamine and cocaine when injected into the *nucleus accumbens* are rewarding as they induce self-administration and place preference behaviour by interaction with presynaptic ML DA terminals [69–72].

3. Biosynthesis

The aminoacids phenylalanine and tyrosine are precursors for catecholamines (dopamine, norepinephrine and epinephrine). The sequence of enzymatic steps starts with the enzyme tyrosine hydroxylase, which converts the aminoacid L-tyrosine into 3,4-dihydroxyphenilalanine (L-DOPA). L-DOPA is then converted in dopamine and, in noradrenergic neurons, it is further oxidized to norepinephrine by dopamine-beta-hydroxylase (which is, therefore, a marker for noradrenergic neurons). Recently, a mouse knockout for TH has been generated [73,74]. Since TH is the rate-limiting enzyme for both DA and norepinephrine synthesis, these mice are DA and NE KO [73,74]. They survive embryogenesis, but die at 3–4 weeks of age because of the severe hypoactivity/hypophagic behaviour. However, by transgenic expression of TH under the control of the DA-beta-hydroxylase promoter the synthesis of NE can be normalized, generating in that way a pure DA KO mouse. This survives, displays normal norepinephrine synthesis, but makes no DA [75]. Mice lacking DA are severely hypoactive. Interestingly, the same DA KO mice displayed an enhanced behavioural response to D1-like or D2-like receptor agonists. The expression of D1-like and D2-like receptor, in fact, was normal in the DA KO *Striatum*, suggesting that DA is not required during embryonic and postnatal development for adequate expression of D1-like and D2-like receptors [74].

The expression of TH is finely regulated to modulate the rate of synthesis of catecholamines, despite the marked fluctuations in the activity of catecholamine-containing neurons. In fact, prolonged sympathetic neuronal activity increases also TH synthesis in neuronal perykarya. TH is modulated by (i) end-product inhibition and (ii) phosporylation-induced activation by depolarization of chatecolaminergic terminals [76]. The phosphorylation of TH is carried out by protein kinase C, cAMP-dependent protein kinase A and Ca/calmodulin protein kinases. An alteration of TH expression in basal state and after stimulation of the dopamine system has been recently observed in animal models of ADHD (see companion paper and papers by Carboni et al. and Leo et al. in this issue).

4. Dopamine release and reuptake

The conversion of tyrosine to L-DOPA and L-DOPA to DA occurs in the cytosol. DA is then taken up into the storage vesicles by the Vesicular Monoamine Transporter (VMAT-2), which is blocked by reserpine. Knockout mice lacking the VMAT2 dye at P6 and show alterations in the cortical organization [77]. However, VMAT2 is also responsible for storage of serotonin, which is important for the development and plasticity of the cerebral cortex [78]. An alteration of the storage of dopamine has been recently suggested in an animal model of ADHD (Carboni et al. this issue). The main point of release of dopamine from axon terminals is represented by regular enlargements of the axon, called axonal varicosities. DA varicosities contain vesicles that can store and release DA.

The vesicles then release their content through Ca-activated fusion with the neuronal membrane. Afterwards, the action of DA released at the synapse is terminated by diffusion and reuptake into presynaptic nerve terminals. In fact, the main mechanism for clearance of released DA is represented by the dopamine transporter (DAT) a membrane transporter that clear DA from the synaptic cleft [79,80]. DAT represents the major target for amphetamine and methylphenidate, the main pharmacological treatment for ADHD [81]. The DAT gene has 15 exons, and many polymorphisms. An association between ADHD and polymorphisms in the DAT gene has been reported [82–84]. In particular, the 10-repeat polymorphism has been associated with an increased dopamine reuptake [85].

The DAT KO mice have been generated. They are viable, but show growth retardation phenotype, that is anterior pituitary hypoplasia, dwarfism, lactation deficits, and high mortality [86]. A mutant mouse which expresses 10% of wild-type DAT levels (DAT knockdown) has also been developed [87]. These mice are in a chronic state of hyperDArgic activity and do not display the growth retardation phenotype.

The DAT KO and Knockdown mice display novelty-induced hyperactivity [88], that is likely to be due to higher levels of brain DA, due to absent clearance of DA from the synaptic cleft.

Therefore, hyperactivity may be related to increased responses to novelty [89] and decreased habituation [90–93].

Strikingly, the DAT KO reduces hyperactivity after treatment with psychostimulants, although they lack the target of psychostimulants (DAT). This suggests that psychostimulants such as methylphenidate could improve ADHD symptoms acting on non-DA sites, such

as the serotonin system or the result of an altered balance between autoreceptor and heteroreceptor functions.

5. DA receptors

Dopamine synapses in *Striatum* are 'open' synapses, i.e., synapses which favour diffusion of the transmitter into the surrounding ECF (volume transmission; [94]). Therefore, dopamine released from the axonal varicosity can reach targets far from the release site. As a matter of fact, there is evidence for abundance and wide distribution of dopamine receptors at various extrasynaptic sites in the *Striatum* and the *Substantia Nigra* [95]. Five dopamine receptors have been classified (see e.g. [96,97]). They can be subdivided into two subfamilies, the D1 like (D1, D5 receptors) and D2 like (D2, D3 and D4 receptors) on the basis of structure and pharmacological properties [98].

In the *Striatum* D1 and D2 receptors are expressed on two different populations: the striatonigral GABA neurons express D1 receptors, substance P (SP) and dynorphin, and project to entopeduncular nucleus and *Substantia Nigra pars reticulata*, whereas striatopallidal GABA neurons express D2 receptor and enkephalin. A third population coexpresses, D1Rs and D2Rs [99,100]. Similarly, in the *Accumbens* D1 expressing cells are SP positive, whereas D2 expressing ones are enkephalin and neurotensin positive.

DA neurons can also express D2-type receptors, which function as autoreceptors. The DA release associated with behavioural activation [64] is regulated by presynaptic DA acting at D2 and D3 autoreceptors and by blockade of the firing of DA neurons in the VTA by DA D2 autoreceptors. This short-term regulation of DA release is also controlled by afferent excitatory and inhibitory inputs from a variety of different neural systems.

D3–D2 interactions are very complex (see below for a discussion from the behavioural perspective). For example, D3 receptors in the *Accumbens*-shell activate neurotensin gene expression, whereas D2 in the *Accumbens*-pole inhibit neurotensin expression. Generally, D3–D2 receptors appear to be expressed in different locations.

D4 receptor distribution markedly differs from that of D2 and D3, and it has a high affinity for clozapine, which is used in schizophrenic patients treatment.

Below we analyze the distribution of DA receptors, along with functional aspects and their role on behaviour, reviewing data from knockout technology. Indeed, so far, mice lacking D1, D2, D3, D4 and D5 receptors or some of their combination have been produced [101,102].

5.1. D1R

The dopamine receptor 1 (D1 R) is the most widely distributed central DA receptor. In cerebral cortex it is largely represented in the frontal, anterior cingulate, orbital, insular, piriform, and entorhinal cortex, predominantly in layers V and VI, which are known to be the receptive layers for DA projections. However, they are also represented in cortical areas lacking of DA terminals, thus suggesting that other sources of DA may exist, such as norepinephrine terminals [103]. D1 receptor is also localized in the anterior olfactory nuclei, where an independent DA system has been largely described [32,104].

In the *Striatum* and *Accumbens* shell/pole D1 receptor mRNA expression is high and could modulate cortical activity, providing a functional interaction between basal ganglia and cerebral cortex [105]. In particular, a subpopulation of medium spiny neurons co-expresses also dynorphin and substance P and projects to the *Substantia Nigra* pars reticulata [100]. A subpopulation of medium spiny neurons also expresses substance P, enkephalin, and both D1-type and D2-type DA receptors [99,106].

D1 receptor is also expressed in amygdaloidal nuclei, where it might be important in alterations of motivational aspects of D1 KO mice, such as rearing frequency. There are high levels of D1 receptor binding in the SNpr, but not in the SNpc or VTA. This is in agreement with the primary postsynaptic function of D1 R. Interestingly, D1, D3 receptors have been described in the rat cerebellum, although no DA fibers have been detected in the same region in rats (but not in humans; see also D3 receptor [107]). This is notable because alterations in cerebellar development have been suggested in ADHD children [108]. Finally, cells expressing D1 receptor are also present in several other locations (e.g. hippocampus, *septum*, various thalamic and hypothalamic nuclei, and few hindbrain nuclei). In particular, it is expressed in *Locus Coeruleus* and dorsal Raphè where it could regulate norepinephrine and serotonin systems.

Animals lacking the D1 receptor show an approximately 30% reduced body weight and a smaller brain [109]. They show normal or increased horizontal locomotor activity when tested in a standard rat cage with photocells [110–113]. However, the rearing rate in these animals is strongly decreased [109,112–114], as well as grooming sequences. The decrease in rearing frequency could indicate an alteration in motivational or attentive aspects of behaviour. Moreover, sniffing sequences did not differ from wild type animals, in contrast with the effects of D1 antagonists, which reduce both rearing and sniffing behaviours. There is also evidence of retarded habituation in several tasks in the same KO mice with a different genetic background, thus raising the problem of the interaction of phenotype with background genes [112,114].

The effect of a different background on D1 KOs (KO) could be explained in terms of basal state of the DA system. In fact, recent evidences show that the modulation of D1 receptor using selective agonists or antagonists, gives different results in normal rats and in hyperdopaminergic, hyperactive rats or mice such as the NHE rats (see companion paper in this issue). It is also possible that the D1 KO has compensatory changes or the effects of D1 receptors in the wild type animal are interactive on a neural

network basis. This issue remains to be resolved, perhaps by development of conditional postnatal D1 receptor KO mice.

5.2. D2R

The activity of DA neurons in the midbrain is modulated by the release or exogenous DA, that interacts with a subclass of DA receptors that act as 'autoreceptors' and belong to the D2 receptor family [115–122]. They regulate the firing rate of DA neurons in the short term (depolarization block [123]), and are involved in hormonal [124–126] and motor activity control [115–117]. In fact, DA D2 receptors represent the major target of antipsychotic drugs and are involved in various neuropathological conditions, including Parkinson's disease, Tourette's syndrome, and drug addiction [115,127,128].

By alternative splicing, the D2 receptor gene encodes two molecularly distinct isoforms [127], named long (D2L) and short (D2S) [129]. They differ by an insertion of 29 aminoacids in the third intracellular loop of the D2L receptor, and are co- expressed in a ratio favouring the long isoform. D2L mainly acts at postsynaptic sites and D2S serves presynaptic autoreceptor functions [121,130–133].

D2 receptors are widely expressed in CNS. The limbic cortices (anterior cingulate, orbital, and insular) express high levels of D2 receptor mRNA, but it is mainly expressed in the neostriatum, particularly in large cells of the external *globus pallidus*, pole and core of the *nucleus accumbens* and olfactory tubercle. Indeed, the D2-type receptors are largely restricted to a subpopulation of medium spiny neurons expressing enkephalin and projecting primarily to the *pallidum* ([134]; see also above, D1 receptors).

D2 receptor in the midbrain and hindbrain is likely to be involved in a host of autonomic functions and in the regulation of DA release. Here cells expressing D2 receptor mRNA are detectable in the DAergic cells of the *Substantia Nigra* pars compacta, the VTA, and in the magnocellular cells of the red nucleus that are part of the rubrospinal pathway.

In the dorsal mesencephalon, cells expressing D2 receptor mRNA are localized in the intermediate and deep layers of the superior colliculus and in the periaqueductal gray, where they may be important in modulating analgesic responses. Morphine-induced analgesia could be related to D2 R expressed in midbrain and pontine nuclei. In Raphè nuclei D2 R may regulate serotonin release. In the hypothalamus (lateral preoptic area, anterior and lateral hypothalamic area) D2 is likely to be involved in the regulation of pituitary hormones.

Several other regions also express the D2 R, such as *septum*, the diagonal band of Broca, hippocampus, basomedial amygdala, brainstem nuclei [104].

A linkage between D2 gene and ADHD has been reported by some authors [135].

The D2L KO [121,126] and the combined D2L + S [116] KO mice have been generated and could be studied in our laboratory, in collaboration with H. Westphal and E. Borrelli, in different behavioural paradigms [116,136]. In particular, we have studied behavioural activation in novelty situations [137].

In the latter the D2R KO demonstrated a lower horizontal locomotor activity than wild type littermates [116,136,138,139].

Moreover, in novel situations also the rearing frequency was lower, with increased scanning durations relative to the WT group. Therefore, the D2 receptor appears to be important in the modulation of scanning phase of attention. Moreover, these data suggest that D2 receptors control activity, as D2R KO mice could represent an animal model of Parkinson's disease [116].

Interestingly, for this last variable the heterozygotes D2 KO mice showed the highest score and the homozygotes an intermediate, thus revealing a non-linear relation between the number of normal alleles and the duration of the rearings.

The absence of depolarization-block due to the lack of mesencephalic autoreceptors in DA neurons is likely to increase DA release and subsensitivity of D1 receptors. This leads to reduced firing of thalamo-cortical neurons. It is interesting to note that the lack of D2L R subtype is able to reduce locomotor activity and rearings as well [139]. Moreover, the interaction of D2L with D1 receptor might be selectively involved in rearing behaviour, whereas D2S with D1 in stereotypy [140].

However, the interpretation of these KO mice is complicated by a large reshaping of DA network, as suggested by their hyper-DA-innervation and the increased DAT expression [141].

5.3. D3R

The dopamine D3 receptor, cloned by Sokoloff and colleagues [142], is mainly expressed in limbic areas [142] and regulates the inhibitory effect of 7-OH-DPAT to produce hyperlocomotion [143,144]. Examination of DA3 receptor in the rat brain indicates that the distribution is distinct from D2 receptor, more localized and less expressed. It is expressed on post- and presynaptic sites, where it can function as autoreceptor, though (i) studies on KO mice and (ii) the absence of D3R in midbrain areas (though this is still controversial [145,142]) would suggest that D2R is the only release regulating autoreceptor [146].

Cells expressing D3 receptor mRNAs are not detected in either neocortical or palleocortical areas, but are predominantly in the ventral *Striatum*, in particular in the *nucleus accumbens* shell and septal pole. The expression of D3 receptor mRNA in the islands of Calleja is the highest observed in the CNS and appears to be selective for D3 (the same region display no expression of D1 and D2 receptors). The localization of D3R in these regions might be responsible for the hyperactive behaviour of D3 KO mice.

Other brain regions that express D3R are the *septum*, hippocampus, geniculate bodies and various hypothalamic regions, suggesting a role in hypothalamic regulation.

Low levels of D2 receptor mRNA expression are also seen in the cerebellum, in large Purkinje cells. In fact, the lobules 9 and 10 of the cerebellum contain high densities of dopamine D3 receptors and almost no D2 receptors [107]. D3 receptor in cerebellum has been shown to modulate locomotor activity, a function similar but weaker than the D3 receptor system in the *Accumbens* [107].

The D3R KO mice [147,148] have been studied in different behavioural paradigms. Here we review their response to behavioural activation in novelty situations using the same experimental paradigm already described (see D2 receptors).

The heterozygous D3 demonstrated a biphasic time-dependent locomotor activation, as there was an increase in the first 15 minutes of exposure to novelty, followed by a steep decrease in the second 15 minutes of the test. This contrasts with a monotonic decline in the WT group and the absence of significant habituation for the D3 homozygous KO mutant mice. The frequency of rearings of the heterozygous and homozygous D3R groups was higher and lower respectively, as compared to WT (see also [147]).

In summary, D3R KO heterozygous mice were more active than D3R KO homozygous and WT animals, which contrasts with previous observations [147]. In other words, D3R heterozygous apparently show a cognitive defect, whereas the homozygous are slightly better in comparison to heterozygous and control mice (see also [148]).

The non-selective attention of D3R mutants was not different from WT; therefore, the D3 receptor appears not to be involved in the modulation of scanning phase of attention.

5.4. D2–D1 and D2–D3 double knockouts

The study of D1, D2 and D3 KO raise the question of the possible interaction of these receptors in modulating complex behavioural phenomena (Table 1).

A double KO D1/D3 R has been previously characterized. This mice display a summation of the behavioural profiles of D1 and D3 KO mice, e.g. increased locomotor activity (see D3 KO) and reduced rearing frequency (see D1 KO) [149].

Table 1
Summary of behavioural data on dopamine knockouts

Target	Rearing frequency	Locomotor activity
TH	L [73–75]	L
DAT	N [86–88]	H only in novel situations
D1	L [112,113,109]	N [112,113]; H [111]
D2 L + S	L [137,139]	L [137,139,116]
D3	H [137,147,148]	H [137,147,148]
D4	N	L [109,159]
D5	N [187]	N [187]
D1 + D3	L [149]	H [149]
D2 + D3	L [137]	L [137]

N = normal; H = high; L = low.

Moreover, in our laboratory we have previously characterized double homozygous D2/D3 (D2 −/−; D3 −/−) or double heterozygous D2/D3 (D2 +/−; D3 +/−) mutants and WT (D2 +/+; D3 +/+). The double mutant mice were then tested in the Làt maze (spatial novelty).

The D2/D3 −/− double mutants were less active than the WT littermate group. In particular, the activity decline was significant only between WT and double homozygous mutants D2/D3 −/− as assessed by regression analysis.

Only the homozygous double D2/D3 −/− mutant groups were significantly less active than WT in the first part as well as in the second part of the testing period.

The double homozygote D2/D3 −/− mice presented a lower rearing frequency than wild type controls over the entire testing period.

The homozygous D2/D3 KO mice displayed significantly lower scanning times as compared to control mice in the first part of the test. In addition, the homozygous and the controls prolonged rearing duration in the second part as compared to the first part of the testing period.

Therefore, for the double mutant D2R/D3R phenotype, the D2/D3 −/− were less active than WT mice. Thus, in the interaction between D2 and D3 receptor subtypes the D2R phenotype seems predominant.

Further, the D2R/D3R −/− double mutants demonstrated shorter scanning times compared to WT controls but limited to the first part of the test.

The double D2R/D3R mutants indicate an interaction between these two receptor subtypes and prevalence D2R on D3R gene expression.

5.5. D4R

The gene for D4 receptor (D4R) is mapped on chromosome 11p15.5 and presents several insertions in the functionally significant third intracellular loop. A 48-base pair sequence in this region is present in single or multiple (4, 7 or 2 most commonly) copies [97]. The D4R has received great attention because of reports that specific tandem repeat polymorphisms of the human D4R gene correlate with a higher than average novelty-seeking scores on questionnaires [150–154]. Indeed, the 7-repeat form codes for a subsensitive postsynaptic receptor [97] and has been linked to ADHD [155–158].

The D4R plays a role in modulating approach-avoidance responses in general and novelty- related exploration in particular [159], as suggested in KO mice studies, and by the distribution in brain areas that could mediate the observed reductions in behavioural responses to novelty.

Glutamatergic pyramidal neurons of frontal cortex [106, 160,161] that project to the CPu and the *Substantia Nigra* [162] display high expression of D4 R [163]. This receptor

in the frontal cortex is likely to be under influence of both noradrenergic inputs and DAergic inputs. In fact, norepinephrine is only fivefold less potent at this receptor than DA [164]. This circuit plays an important role in regulating cognitive processes and emotional status and is, in fact, one of the main targets of antipsychotic drugs.

D4 receptor is also highly expressed in amygdala, hippocampus, hypothalamus and *globus pallidus*. It is also represented in *Substantia Nigra pars reticulata* [97].

The D4R KO mouse [109,159] show reduced behavioural responses to novelty [159]. This is consistent with the hypothesis that a lack of D4R function may lead to decreased novelty-seeking in humans [150,151]. D4 KO mice show also increased locomotor response to ethanol, cocaine, and methamphetamine [161]. These mice have also increased DA synthesis and its conversion to DOPAC in the CPu [161].

This phenotype can be explained observing that the stimulation of MS pathway [165] by glutamate induces DA release [166].

Thus, frontal D4Rs may alter the activity of MS DA neurons by modulating the release of glutamate onto these neurons.

An association between polymorphisms of the D4R gene and personality profile of the novelty-seeking trait [150,151] is in agreement with D4R role in modulating behavioural responses to novelty.

Moreover, behavioural disorders, such as drug abuse [167,168], pathological gambling [169], and ADHD [158, 170] have been recently correlated to the same D4R alleles which are associated to novelty-seeking.

Interestingly, in humans 2% of the population has a null allele for the D4R [171], though no behavioural reports are available.

5.6. D5R

The D5 DA receptor (D5 DAR) is functionally coupled to the activation of adenylate cyclase, and GABAA receptor-mediated activity through both second messenger cascades [172] as well as through direct receptor–receptor interactions [173]. It has a high affinity for DA, compared with other DA Rs, and has constitutive activity [174,175], suggesting that the D5 DAR may be activated in the absence or presence of low concentrations of endogenous agonist. Interestingly, recent reports have suggested a possible association of the D5 DAR gene with schizophrenia [176], substance abuse [177], ADHD [178–180].

The physiological and behavioural roles of the D5 receptor have been difficult to characterize, due to overlapping pharmacological properties of the D1 and D5 receptors. There are few ligands selective for either subtype [98], and DA is one, demonstrating ~10-fold higher affinity at the D5 DAR compared with the D1. The D5 receptor mRNA has very restricted expression, in the hippocampus (where it is more expressed than D1), enthorinal and prefrontal cortex, basal ganglia [106,181] and to specific thalamic (lateral nucleus) and hypothalamic (lateral mammillary nucleus [175], diagonal band of Broca) nuclei. Characteristically, D5 receptors are localized on large cholinergic interneurons. In the hypothalamus, D5R may regulate circadian rhythms [182] and female sexual behaviours [183,184].

Within the periphery, D5 R have been found in adrenal tissue [185], kidney, and also the gastrointestinal tract, where they may exert a protective effect on the intestinal mucosa [186].

Approaches to the problem of D5R roles include the use of antisense technologies to downregulate D1 or D5 DAR expression as well as the creation of D1 DAR- deficient mice [101,102,187].

The antisense ‘knock-down’ of D5 receptor expression have suggested a role for the D5 DAR in regulating female sexual behaviours [183,184] and locomotor responses to DAergic agonists [188].

The D5 DAR-KO mice are viable, with normal development, and are fertile [187]. This contrasts with antisense studies [183,184] that described a suppression of lordosis behaviour in D5 DAR knockdown receptive females. D5 KO animals were hypertensive, exhibiting significantly elevated blood pressures [187]. This can be attributable to increased sympathetic tone, possibly of central origin. In fact, D5 receptor deletion results in an oxytocin-dependent sensitization of V1 vasopressin and non-NMDA glutamatergic receptor-mediated pathways, potentially within the medulla, leading to increased sympathetic outflow [187].

6. Transduction mechanisms

D1-like receptors have been shown to couple the stimulation of adenylate cyclase activity, whereas D2-like subfamily can reduce cAMP production as well as regulate the activity of various ion channels.

In fact, systemic administration of D1 but not D2 agonists induces enhanced expression of immediate-early genes c-fos and zif268 in the cerebral cortex and *Striatum*. Concomitant D1 and D2 receptor stimulation appear to produce a synergistic effect on c-fos expression, modulating cognitive, sensorimotor, and neuroendocrine phenomena.

DA and numerous other neurotransmitters may alter the phosphorylation and/or dephosphorylation state of a cAMP-regulated protein named DARPP-32 (DA and cyclic adenosine 3′, 5′-monophosphate-regulated phosphoprotein). Indeed, DARPP plays a central role in the biology of dopaminoceptive neurons. Its phosphorylated form is an extremely potent inhibitor of protein phosphatase-1 (PP-1), a major multifunctional serine/threonine protein phosphatase in the brain. This, in turn, regulates phosphorylation and activity of many physiological effectors, such as voltage-gated ion channels and neurotransmitter.

Mice lacking the DARPP-32 gene in the basal state showed hyperlocomotion and decreased grooming in females and a reduced number of rearings in both genders [189]. Moreover, pharmacological evidences suggest DARPP-32 to mediate the biological effects of presynaptic D2-like autoreceptor and specific behaviour. In fact, the developmental absence of DARPP-32 appears to be associated with compensatory processes that maintain some topographies of spontaneous and agonist-induced DAergic function, while other topographies remain impaired [189]. These studies have also shown that the DARPP-32/PP-1 cascade is a major target for psychostimulants and antipsychotic drugs (for a review see e.g. [190]).

7. DA receptor phenotype and behaviour

Several studies have shown an increased DA release in the *nucleus accumbens* associated with behavioural activation [65]. When an animal is introduced in a non-familiar environment, novelty triggers an array of behavioural traits: walking about, rearings on the hindlimbs, leanings against walls and sniffing (see e.g. [191]). Walking and rearing have been genetically dissociated in mice [192] and rats [193], suggesting that different genes control these behavioural traits.

Recent studies demonstrated that the duration of rearing episodes in a novelty situation index the level of non-selective attention towards environmental stimuli [194,195].

The novelty-induced DA release can be controlled by the activation of presynaptic DA D2 autoreceptors and by the blockade of the firing of DA neurons in the VTA (VTA), wherein DA is also released at somatodendritic level, activating D2 autoreceptors, which hyperpolarize membrane potential. In addition, this short-term regulation of DA release is controlled by afferent excitatory and inhibitory inputs from Raphè serotonin (5HT), *Locus Coeruleus* norepinephrine (NE) neurons, GABA VTA interneurons, *Accumbens* medium spiny neurons and glutamate frontal neurons in a complex manner [196].

The participation of each DA receptor in such processes is of interest because their selective regulation could be useful in the treatment of several psychiatric disorders. In particular, ADHD has been hypothesized to be underlined by a DA dysfunction on the bases of theoretical considerations, and experimental and clinical observations (for a review see e.g. [108]). In fact, ADHD has a substantial genetic component, with a heritability of 0.75–0.91 [87], and recent studies have indicated an association between a polymorphism in the human DAT gene and ADHD [83,84,108].

The KO technology has been useful to study the role of each component of the dopamine system in behaviour. The studies reviewed here suggest that D1 and D4 receptors could be directly involved in the pathogenesis of hyperactivity and lack of attention, and that D2 receptors might be important for the action of some therapeutic drugs such as methylphenidate.

Taken together, results from both DA KO, DA receptors KO and the DAT KO and knockdown mice support the hyperDArgic hypothesis for ADHD. In fact, DAT KO and knockdown mice are hyperdopaminergic and hyperactive, whereas DA KO mice are severely hypoactive. Moreover, all DA receptors KO mice are hypoactive in different tasks, with the exclusion of DA D3 mice.

By the time DA terminals complete their development under the guidance of Eph/Semaphorins, a hyper DA state might lead to DA-induced neurotoxicity. DA itself, its oxidation and formation of free radicals, which might lead to neuronal death via apoptosis and/or necrosis at low or high dopamine level, respectively, can bring this about. This will eventually lead to an altered network asset with estrogens having a neuroprotective role, partially explaining the different F/M ration in the incidence of ADHD.

Unfortunately, KO mice studies are hampered by the blockade of the expression of a given receptor at early stages of development. In fact, DA systems that develop in absence of the deleted receptor might undergo compensatory changes, if the deletion is not lethal. An alternative strategy is represented by inducible mutagenesis that allows blocking in the adult organism the expression of a given protein. The main disadvantage of the latter is tissue responsiveness as, for instance, skin responds in 100% of the cases, whereas the brain in 10–15% only.

Therefore, other strategies have been proposed. These include injection of antisense oligonucleotides in brain regions, the use of sequence-selective ribozymes, RNA interference (RNAi), transcriptional regulators (reviewed in [197]).

Antisense oligonucleotides are thought to work by several mechanisms, including RNAase H digestion of mRNA made double stranded by hybridizing to antisense DNA or RNA. An advantage is the ease with which naked antisense oligonucleotides seem to enter brain neurons for reasons which are not fully understood [197].

Ribozymes consist of a conserved catalytic domain between targeting sequences in the ribozyme-RNA molecule, which may be applied exogenously or synthesized from a suitable vector in the cell. This method is still being developed [197].

The RNAi method is based on a defense mechanism against viruses and transposomes, with emerging regulatory role as well. The presence of double-stranded RNA of a defined sequence activates a ribonuclease III, termed dicer, which chops into small pieces (small interfering RNAs, si RNAs). These, in turn, become incorporated into a RNA–protein complex termed RISC (RNAi-induced silencing complex), which recognizes and degrades mRNA of the same sequence. It has been successfully used ‘in vitro’ and ‘in vivo’ only in Drosophila and *C. elegans* [197].

Transcriptional regulators require the knowledge of the promoter of the target gene and the relative silencing elements.

A problem common to most of the methods discussed above, is getting exogenous DNA, RNA or analogue into the target region. Cationic lipid-based transfection reagents or various alternatives generally have reasonable efficiency but have transient effects. An alternative is represented by viral vectors such as those based on adenovirus, adeno-associated virus, herpes virus, lentiviruses etc. [188,198–200].

Acknowledgements

This paper has been supported by COFIN MIUR 2001–2002 and by Ministero della Sanità Fondi Speciali 2002–2004.

References

[1] Robbins TW, Everitt BJ. Arousal systems and attention. In: Gazzaniga MS, editor. The cognitive neurosciences. Cambridge, MA: The MIT Press; 1994. p. 703–20.
[2] Wolfer DP, Lipp HP. Dissecting the behaviour of transgenic mice: is it the mutation, the genetic background, or the environment? Exp Physiol 2000;85:627–34.
[3] Lipp HP, Wolfer DP. Genetically modified mice and cognition. Curr Opin Neurobiol 1998;8:272–80.
[4] Wolfer DP, Stagljar-Bozicevic M, Errington ML, Lipp HP. Spatial memory and learning in transgenic mice: fact or artifact? News Physiol Sci 1998;13:118–23.
[5] Robbins TW, Everitt BJ. Motivation and reward. In: Zigmond MJ, Bloom FE, Landis SC, Roberts JL, Squire LR, editors. Fundamental neuroscience. New York: Academic Press; 1999. p. 1245–77.
[6] Wikstrom M, Krab K, Saraste M. Hypertrophic A10 dopamine neurons in a rat model of Attention-Deficit Hyperactivity Disorder (ADHD). New York: Academic Press; 1981.
[7] Sadile AG. Modeling ADHD in rodent mutants. Neurosci Biobehav Rev 2000;24:1–169.
[8] Sagvolden T, Metzger MA, Schiorbeck HK, Rugland AL, Spinnangr I, Sagvolden G. The spontaneously hypertensive rat (SHR) as an animal model of childhood hyperactivity (ADHD): changed reactivity to reinforcers and to psychomotor stimulants. Behav Neural Biol 1992;58:103–12.
[9] King JA, Barkley RA, Delville Y, Ferris CF. Early androgen treatment decreases cognitive function and catecholamine innervation in an animal model of ADHD. Behav Brain Res 2000;107:35–43.
[10] Castellanos FX, Giedd JN, Marsh WL, Hamburger SD, Vaituzis AC, Dickstein DP, Sarfatti SE, Vauss YC, Snell JW, Lange N, Kaysen D, Krain AL, Ritchie GF, Rajapakse JC, Rapoport JL. Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder. Arch Gen Psychiatry 1996;53:607–16.
[11] Sadile AG, Viggiano D. Incresed number of varicosities of mesocorticolimbic terminals in the frontal cortex in the naples high exitability rats. Soc Neurosci Abstr 2001;27:2628.
[12] Sadile AG, Ruocco LA, Viggiano D, Hendley ED. Activity, orienting frequency and scanning time in the Wistar-Kyoto hyperactive and the Wistar-Kyoto hypertensive rats. Soc Neurosci Abstr 2002;28.
[13] Viggiano D, Sadile AG. Hypertrophic A10 dopamine neurons in a rat model of Attention-Deficit Hyperactivity Disorder (ADHD). Neurorep 2000;11:3677–80.
[14] Nassel DR. Neuropeptides, amines and amino acids in an elementary insect ganglion: functional and chemical anatomy of the unfused abdominal ganglion. Prog Neurobiol 1996;48:325–420.
[15] Neckameyer WS. Dopamine in Drosophila: neuronal and developmental roles. In: di Porzio U, Pernas-Alonso R, Perrone-Capano C, editors. Development of dopaminergic neurons. Austin, Texas: Landes; 1999. p. 157–74.
[16] Lints R, Emmons SW. Genetic analysis of dopaminergic neurons in the nematode *Caenorabditis elegans*. In: di Porzio U, Pernas-Alonso R, Perrone-Capano C, editors. Development of dopaminergic neurons. Austin, Texas: Landes; 1999. p. 175–90.
[17] Le Crom S, Kapsimali M, Barome PO, Vernier P. Dopamine receptors for every species: gene duplications and functional diversification in Craniates. J Struct Funct Genom 2003;3:161–76.
[18] Fibiger HC, Phillips AG. Reward, motivation, cognition, psychobiology of mesotelencephalic dopamine systems. In: Bloom FE, editor. Handbook of Physiology-The Nervous System IV. Baltimore, MD: Williams and Wilkins; 1986.
[19] Canavan AGM, Passingham RE, Marsden CD, Quinn N, Wyke M, Polkey CE. The performance on learning tasks of patients in the early stages of Parkinson's disease. Neuropsychology 1989;27:141–56.
[20] Wise RA, Hoffman DC. Localization of drug reward mechanisms by intracranial injections. Synapse 1992;10:247–63.
[21] Robinson TE, Berridge KC. The neural basis for drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 1993;18:247–91.
[22] Knowlton BJ, Mangels JA, Squire LR. A neostriatal habit learning system in humans. Science 1996;273:1399–402.
[23] Robbins TW, Everitt BJ. Neurobehavioural mechanisms of reward and motivation. Curr Opin Neurobiol 1996;6:228–36.
[24] Wise RA. Neurobiology of addiction. Curr Opin Neurobiol 1996;6:243–51.
[25] Wise RA. Brain reward circuitry: insights from unsensed incentives. Neuron 2002;36:229–40.
[26] Andeìn NE, Fuxe K, Hamberger B, Hokfelt T. A quantitative study on the nigro-neostriatal dopamine neurons. Acta Physiol Scand 1966;67:306–12.
[27] Hynes M, Rosenthal A. Specification of dopaminergic neurons: inductive factors and their receptors. In: di Porzio U, Pernas-Alonso R, Perrone-Capano C, editors. Development of dopaminergic neurons. Austin, Texas: Landes; 1999. p. 15–36.
[28] Hynes M, Rosenthal A. Specification of dopaminergic and serotonergic neurons in the vertebrate CNS. Curr Opin Neurobiol 1999;9:26–36.
[29] Ye W, Shimamura K, Rubenstein JL, Hynes MA, Rosenthal A. FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. Cell 1998;93:755–66.
[30] Shamim H, Mahmood R, Logan C, Doherty P, Lumsden A, Mason I. Sequential roles for Fgf4, En1 and Fgf8 in specification and regionalisation of the midbrain. Development 1999;126:945–59.
[31] Perrone-Capano C, di Porzio U. Genetic and epigenetic control of midbrain dopaminergic neuron development. Int J Dev Biol 2000;44:679–87.
[32] di Porzio U, Pernas-Alonso R, Perrone-Capano C. Development of Dopaminergic Neurons. Austin, Texas: Landes; 1999.
[33] Hynes M. Induction of midbrain dopaminergic neurons by Sonic hedgehog. Neuron 1995;15:35–44.
[34] Danielian PS, McMahon AP. Engrailed-1 as a target of the Wnt-1 signalling pathway in vertebrate midbrain development. Nature 1996;383:332–4.
[35] Wurst W, Auerbach ABJAL. Multiple developmental defects in Engrailed-1 mutant mice: an early mid-hindbrain deletion and

patterning defects in forelimbs and sternum. Development 1994;120: 2065–75.
[36] Hanks M, Wurst W, Anson-Cartwright L, Auerbach AB, Joyner AL. Rescue of the En-1 mutant phenotype by replacement of En-1 with En-2. Science 1995;269:679–82.
[37] Favor J. The mouse Pax2(1Neu) mutation is identical to a human PAX2 mutation in a family with renal-coloboma syndrome and results in developmental defects of the brain, ear, eye, and kidney. Proc Natl Acad Sci USA 1996;93:13870–5.
[38] Urbanek P, Fetka I, Meisler MH, Busslinger M. Cooperation of Pax2 and Pax5 in midbrain and cerebellum development. Proc Natl Acad Sci USA 1997;94:5703–8.
[39] Perrone-Capano C, Da Pozzo P, di Porzio U. Epigenetic cues in midbrain dopaminergic neuron development. Neurosci Biobehav Rev 2000;24:119–24.
[40] Smidt M. A novel homeodomain gene Ptx3 has higly restricted brain expression in mesencephalic dopaminergic neurons. Proc Natl Acad Sci USA 1997;94:13305–10.
[41] Zetterstrom RH, Solomin L, Jansson L, Hoffer BJ, Olson L, Perlmann T. Dopamine neuron agenesis in Nurr1-deficient mice. Science 1997;276:248–50.
[42] Saucedo-Cardenas O, Quintana-Hau JD, Le WD, Smidt MP, Cox JJ, De Mayo F, Burbach JP, Conneely OM. Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons. Proc Natl Acad Sci USA 1998;95:4013–8.
[43] Smidt MP, Asbreuk CH, Cox JJ, Chen H, Johnson RL, Burbach JP. A second independent pathway for development of mesencephalic dopaminergic neurons requires Lmx1b. Nat Neurosci 2000;3: 337–41.
[44] Pilgrim C, Beyer C, Ingrid R. The effects of sex and sex hormones on the development of dopaminergic neurons. In: di Porzio U, Pernas-Alonso R, Perrone-Capano C, editors. Development of dopaminergic neurons. Austin, Texas: Landes; 1999. p. 75–86.
[45] Sahay A, Molliver ME, Ginty DD, Kolodkin AL. Semaphorin 3F is critical for development of limbic system circuitry and is required in neurons for selective CNS axon guidance events. J Neurosci 2003; 23:6671–80.
[46] Yue Y, Widmer DA, Halladay AK, Cerretti DP, Wagner GC, Dreyer JL, Zhou R. Specification of distinct dopaminergic neural pathways: roles of the Eph family receptor EphB1 and ligand ephrin-B2. J Neurosci 1999;19:2090–101.
[47] Viggiano D, Ibrahim M, Celio MR. Relationship between glia and the perineuronal nets of extracellular matrix in the rat cerebral cortex: importance for volume trasmission in the brain. Prog Brain Res 2000;125:193–7.
[48] Charvet I, Hemming FJ, Feuerstein C, Saxod R. Transient compartmental expression of neurocan in the developing Striatum of the rat. J Neurosci Res 1998;51:612–8.
[49] Moon LD, Asher RA, Fawcett JW. Limited growth of severed CNS axons after treatment of adult rat brain with hyaluronidase. J Neurosci Res 2003;71:23–37.
[50] Hobohm C, Hartig W, Brauer K, Bruckner G. Low expression of extracellular matrix components in rat brain stem regions containing modulatory aminergic neurons. J Chem Neuroanat 1998;15:135–42.
[51] Liu F-C, Graybiel I. Neural development of the striatal dopamine system. In: di Porzio U, Pernas-Alonso R, Perrone-Capano C, editors. Development of dopaminergic system. Austin, Texas: Landes; 1999. p. 87–100.
[52] Kalivas PW, Nakamura M. Neural systems for behavioural activation and reward. Curr Opin Neurobiol 1999;9:223–7.
[53] Deutch AY, Bourdelais AJ, Zahm DS. The nucleus Accumbens core and shell: accumbal compartments and their functional attributes. In: Kalivas PW, Barnes CD, editors. Limbic circuits and neuropsychiatry. London: CRC press; 1993. p. 45–88.
[54] Dahlstrom A, Fuxe K. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in cell bodies of brain stem neurons. Acta Physiol Scand 1964;62(Suppl 232):1–55.
[55] Freund TF, Powell JF, Smith AD. Tyrosine hydroxylase-immunoreactive boutons in synaptic contact with identified striatonigral neurons, with particular reference to dendritic spines. Neuroscience 1984;13:1189–215.
[56] Bouyer JJ, Park DH, Joh TH, Pickel VM. Chemical and structural analysis of the relation between cortical inputs and tyrosine hydroxylase-containing terminals in rat neoStriatum. Brain Res 1984;302:267–75.
[57] Carlezon Jr WA, Wise RA. Microinjections of phencyclidine (PCP) and related drugs into nucleus Accumbens shell potentiate medial forebrain bundle brain stimulation reward. Psychopharmacology (Berl.) 1996;128:413–20.
[58] Wilson CJ, Young SJ, Groves PM. Statistical properties of neuronal spike trains in the Substantia Nigra: cell types and their interactions. Brain Res 1977;136:243–60.
[59] Tepper JM, Martin LP, Anderson DR. GABAA receptor-mediated inhibition of rat Substantia Nigra dopaminergic neurons by pars reticulata projection neurons. J Neurosci 1995;15:3092–103.
[60] Gonon FG. Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry. Neuroscience 1988;24:19–28.
[61] Ip NY, Zigmond RE. Pattern of presynaptic nerve activity can determine the type of neurotransmitter regulating a postsynaptic event. Nature 1984;311:472–4.
[62] Chergui K, Nomikos GG, Mathe JM, Gonon F, Svensson TH. Burst stimulation of the medial forebrain bundle selectively increase Fos-like immunoreactivity in the limbic forebrain of the rat. Neuroscience 1996;72:141–56.
[63] Schultz W. Getting formal with dopamine and reward. Neuron 2002; 36:241–63.
[64] Zahm DS. An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus Accumbens. Neurosci Biobehav Rev 2000;24: 85–105.
[65] Zahm DS. An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbes. Neurosci Biobehav Rev 2000;24:85–106.
[66] Routtenberg A. Intracranial chemical injection and behaviour: a critical review. Behav Biol 1972;7:601–41.
[67] Corbett D, Silva LR, Stellar JR. An investigation of the factors affecting development of frontal cortex self-stimulation. Physiol Behav 1985;34:89–95.
[68] You Z-B, Tzschentke TM, Brodin E, Wise RA. Electrical stimulation of the prefrontal cortex increases cholecystokinin, glutamate, and dopamine release in the nucleus Accumbens: an in vivo microdialysis study in freely moving rats. J Neurosci 1998;18: 6492–500.
[69] Hoebel BG, Monaco AP, Hernandez L, Aulisi EF, Stanley BG, Lenard L. Self-injection of amphetamine directly into the brain. Psychopharmacology (Berl.) 1983;81:158–63.
[70] Carr GD, White NM. Conditioned place preference from intra-Accumbens but not intra-caudate amphetamine injections. Life Sci 1983;33:2551–7.
[71] Carlezon Jr WA, Devine DP, Wise RA. Habit-forming actions of nomifensine in nucleus Accumbens. Psychopharmacology (Berl.) 1995;122:194–7.
[72] Fallon JH, Loughlin SE. Monoamine innervation of the forebrain: collateralization. Brain Res Bull 1982;9:295–307.
[73] Kobayashi K, Morita S, Sawada H, Mizuguchi T, Yamada K, Nagatsu I, Hata T, Fujita K, Watanabe Y, Nagatsu T. Targeted disruption of the tyrosine hydroxylase locus results in severe catecholamine depletion and perinatal lethality in mice. J Biol Chem 1995;270:27235–43.
[74] Zhou QY, Palmiter RD. Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. Cell 1995;83:1197–209.

[75] Zhou QY, Quaife CJ, Palmiter RD. Targeted disruption of the tyrosine hydroxylase gene reveals that catecholamines are required for mouse fetal development. Nature 1995;374:640–3.
[76] Kuhar MJ, Couceyro PR, Lambert PD. Catecholamines. In: Siegel GJ, editor. Basic neurochemistry. New York: Lippincott Williams and Williams; 1999. p. 243–62.
[77] Takahashi N, Miner LL, Sora I, Ujike H, Revay RS, Kostic V, Jackson-Lewis V, Przedborski S, Uhl GR. VMAT2 knockout mice: heterozygotes display reduced amphetamine-conditioned reward, enhanced amphetamine locomotion, and enhanced MPTP toxicity. Proc Natl Acad Sci USA 1997;94:9938–43.
[78] Alvarez C, Vitalis T, Fon EA, Hanoun N, Hamon M, Seif I, Edwards R, Gaspar P, Cases O. Effects of genetic depletion of monoamines on somatosensory cortical development. Neuroscience 2002;115: 753–64.
[79] Jones SR, Gainetdinov RR, Jaber M, Giros B, Wightman RM, Caron MG. Profound neuronal plasticity in response to inactivation of the dopamine transporter. Proc Natl Acad Sci USA 1998;95:4029–34.
[80] Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 1996;379:606–12.
[81] Swanson JM, Sergeant JA, Taylor E, Sonuga-Barke EJS, Jensen PS, Cantwell DP. Attention deficit hyperactivity disorder and hyperkinetic disorder. Lancet 1998;351:329–43.
[82] Cook Jr EH, Stein MA, Krasowski MD, Cox NJ, Olkon DM, Kieffer JE, Leventhal BL. Association of attention-deficit disorder and the dopamine transporter gene. Am J Hum Genet 1995;56:993–8.
[83] Gill M, Daly G, Heron S, Hawi Z, Fitzgerald M. Confirmation of association between attention deficit hyperactivity disorder and a dopamine transporter polymorphism. Mol Psychiatry 1997;2:311–3.
[84] Waldman ID, Rowe DC, Abramowitz A, Kozel ST, Mohr JH, Sherman SL, Cleveland HH, Sanders ML, Gard JM, Stever C. Association and linkage of the dopamine transporter gene and attention-deficit hyperactivity disorder in children: heterogeneity owing to diagnostic subtype and severity. Am J Hum Genet 1998;63: 1767–76.
[85] Swanson JM, Flodman P, Kennedy J, Spence MA, Moyzis R, Schuck S, Murias M, Moriarity J, Barr C, Smith M, Posner M. Dopamine Genes and ADHD. Neurosci Biobehav Rev 2000;24:21–6.
[86] Bosse R, Fumagalli F, Jaber M, Giros B, Gainetdinov RR, Wetsel WC, Missale C, Caron MG. Anterior pituitary hypoplasia and dwarfism in mice lacking the dopamine transporter. Neuron 1997;19: 127–38.
[87] Zhuang X, Oosting RS, Jones RS, Gainetdinov RR, Miller GW, Caron MG, Hen R. Hyperactivity and impaired response habituation in hyperdopaminergic mice. PNAS 2001;98:1982–7.
[88] Gainetdinov R, Wetsel W, Jones S, Levin E, Jaber M, Caron M. Role of serotonine in the paradoxical calming effect of psychostimulants on hyperactivity. Science 1999;283:397–401.
[89] Crabbe JC. Genetic differences in locomotor activation in mice. Pharmacol Biochem Behav 1986;25:289–92.
[90] Laviola G, Renna G, Bignami G, Cuomo V. Ontogenetic and pharmacological dissociation of various components of locomotor activity and habituation in the rat. Int J Dev Neurosci 1988;6:431–8.
[91] Hendley ED, Ohlsson WG. Two new inbred rat strains derived from SHR: WKHA, hyperactive, and WKHT, hypertensive, rats. Am J Physiol 1991;261:H583–9.
[92] Hendley ED, Ohlsson WG. Two new inbred rat strains derived from SHR: WKHA, hyperactive, and WKHT, hypertensive, rats. Am J Physiol 1991;261:H583–9.
[93] Gerlai R, McNamara A, Choi-Lundberg DL, Armanini M, Ross J, Powell-Braxton L, Phillips HS. Impaired water maze learning performance without altered dopaminergic function in mice heterozygous for the GDNF mutation. Eur J Neurosci 2001;14:1153–63.
[94] Zoli M, Torri C, Ferrari R, Jansson A, Zini I, Fuxe K, Agnati LF. The emergence of the volume transmission concept. Brain Res Brain Res Rev 1998;26:136–47.
[95] Caille I, Dumartin B, Bloch B. Ultrastructural localization of D1 dopamine receptor immunoreactivity in rat striatonigral neurons and its relation with dopaminergic innervation. Brain Res 1996; 730:17–31.
[96] Jackson DM, Westlind-Danielsson A. Dopamine receptors: molecular biology, biochemistry and behavioural aspects. Pharmacol Ther 1994;64:291–370.
[97] Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. Physiol Rev 1998;78:189–225.
[98] Neve KA, Neve RL. In: Totowa NJ, editor. The dopamine receptors. 1997.
[99] Gerfen CR. The neostriatal mosaic: multiple levels of compartmental organization. Trends Neurosci 1992;15:133–9.
[100] Aizman O, Brismar H, Uhlen P, Zettergren E, Levey AI, Forssberg H, Greengard P, Aperia A. Anatomical and physiological evidence for D1 and D2 dopamine receptor colocalization in neostriatal neurons. Nat Neurosci 2000;3:226–30.
[101] Sibley DR. New insights into dopamine rgic receptor function using antisense and genetically altered animals. Annu Rev Pharmacol Toxicol 1999;39:313–41.
[102] Glickstein SB, Schmauss C. Dopamine receptor function: lessons from knockout mice. Pharmacol Ther 2001;91:63–83.
[103] Devoto P, Flore G, Pani L, Gessa GL. Evidence for co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex. Mol Psychiatry 2001;6:657–64.
[104] Weiner DM, Levey AI, Sunahara RK, Hyman BN, O'Dowd BF, Seeman P, Brann MR. D1 and D2 dopamine receptor mRNA in rat brain. Proc Natl Acad Sci USA 1991;88:1859–63.
[105] Blandini F, Fancellu R, Orzi F, Conti G, Greco R, Tassorelli C, Nappi G. Selective stimulation of striatal dopamine receptors of the D1- or D2-class causes opposite changes of fos expression in the rat cerebral cortex. Eur J Neurosci 2003;17:763–70.
[106] Ariano MA, Wang J, Noblett KL, Larson ER, Sibley D. Cellular distribution of the rat D4 dopamine receptor protein in the CNS using anti-receptor antisera. Brain Res 1997;752:26–34.
[107] Barik S, de Beaurepaire R. Evidence for a functional role of the dopamine D3 receptors in the cerebellum. Brain Res 1996;737: 347–50.
[108] Castellanos FX, Tannock R. Neuroscience of attention-deficit/hyperactivity disorder: the search for endophenotypes. Nat Rev 2002;3:617–28.
[109] Drago J, Padungchichot P, Accili D, Fuchs S. Dopamine receptors and dopamine transporter in brain function and addictive behaviours: insight from targeted mouse mutants. Dev Neurosci 1998;20: 188–203.
[110] Xu M, Hu XT, Cooper DC, Moratalla R, Graybiel AM, White FJ, Tonegawa S. Elimination of cocaine-induced hyperactivity and dopamine-mediated neurophysiological effects in dopamine D1 receptor mutant mice [see comments]. Cell 1994;79:945–55.
[111] Xu M, Moratalla R, Gold LH, Hiroi N, Koob GF, Graybiel AM, Tonegawa S. Dopamine D1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioural responses. Cell 1994;79:729–42.
[112] Drago J, Gerfen CR, Lachowicz JE, Steiner H, Hollon TR, Love PE, Ooi GT, Grinberg A, Lee EJ, Huang SP, Bartlett PF, Jose PA, Sibley DR, Westphal H. Altered striatal function in a mutant mouse lacking D1A dopamine receptors. Proc Natl Acad Sci USA 1994;91: 12564–8.
[113] Drago J, Gerfen CR, Westphal H, Steiner H. D1 dopamine receptor-deficient mouse: cocaine-induced regulation of immediate-early gene and substance P expression in the Striatum. Neuroscience 1996; 74:813–23.
[114] McNamara FN, Clifford JJ, Tighe O, Kinsella A, Drago J, Croke DT, Waddington JL. Congenic D1A dopamine receptor mutants: ethologically based resolution of behavioural topography indicates genetic background as a determinant of knockout phenotype. Neuropsychopharmacology 2003;28:86–99.

[115] Jackson DJ, Westlind-Danielsson A. Dopamine receptors: Molecular biology, biochemistry and behavioural aspects. Pharmacol Ther 1994;64:291–369.
[116] Baik JH, Picetti R, Saiardi A, Thiriet G, Dierich A, Depaulis A, Le Meur M, Borrelli E. Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. Nature 1995;377:424–8.
[117] Kelly MA, Rubinstein M, Phillips TJ, Lessov CN, Burkhart-Kasch S, Zhang G, Bunzow JR, Fang Y, Gerhardt GA, Grandy DK, Low MJ. Locomotor activity in D2 dopamine receptor-deficient mice is determined by gene dosage, genetic background, and developmental adaptations. J Neurosci 1998;18:3470–9.
[118] Mercuri NB, Saiardi A, Bonci A, Picetti R, Calabresi P, Bernardi G, Borrelli E. Loss of autoreceptor function in dopaminergic neurons from dopamine D2 receptor deficient mice. Neuroscience 1997;79: 323–7.
[119] Koeltzow TE, Xu M, Cooper DC, Hu XT, Tonegawa S, Wolf ME, White FJ. Alterations in dopamine release but not dopamine autoreceptor function in dopamine D3 receptor mutant mice. J Neurosci 1998;18:2231–8.
[120] Mansour A, Meador-Woodruff JH, Bunzow JR, Civelli O, Akil H, Watson SJ. Localization of dopamine D2 receptor mRNA and D1 and D2 receptor binding in the rat brain and pituitary: an in situ hybridization-receptor autoradiographic analysis. J Neurosci 1990; 10:2587–600.
[121] Usiello A, Baik JH, Rouge-Pont F, Picetti R, Dierich A, Lemeur M, Piazza PV, Borrelli E. Distinct functions of the two isoforms of dopamine D2 receptors. Nature 2000;408:199–203.
[122] Rouge-Pont F, Usiello A, Benoit-Marand M, Gonon F, Piazza PV, Borrelli E. Changes in extracellular dopamine induced by morphine and cocaine: crucial control by D2 receptors. J Neurosci 2002;22: 3293–301.
[123] Grace AA, Bunnney BS, Moore H, Todd CL. Dopamine-cell depolarization block as a model for the therapeutic actions of antipsychotic drugs. Trends Neurosci 1997;20:31–7.
[124] Saiardi A, Bozzi Y, Baik JH, Borrelli E. Antiproliferative role of dopamine: loss of D2 receptors causes hormonal dysfunction and pituitary hyperplasia. Neuron 1997;19:115–26.
[125] Doppler W. Regulation of gene expression by prolactin. Rev Physiol Biochem Pharmacol 1994;124:93–130.
[126] Kelly MA, Rubinstein M, Asa SL, Zhang G, Saez C, Bunzow JR, Allen RG, Hnasko R, Ben-Jonathan N, Randy DK, Low MJ. Pituitary lactotroph hyperplasia and chronic hyperprolactinemia in dopamine D2 receptor-deficient mice. Neuron 1997;19:103–13.
[127] Picetti R, Saiardi A, Samad TA, Bozzi Y, Baik J-H, Borrelli E. Dopamine D2 receptors in signal transduction and behaviour. Crit Rev Neurobiol 1997;11:121–42.
[128] Di Chiara G. The role of dopamine in drug abuse viewed from the perspective of its role in motivation. Drug Alcohol Depend 1995;38: 95–137.
[129] Da lToso R, Sommer B, Ewert M, Herb A, Pritchett DB, Bach A, Shivers BD, Seeburg PH. The dopamine D2 receptor: two molecular forms generated by alternative splicing. EMBO J 1989;8:4025–34.
[130] Kahn RA, Weinberger J, Panah M. How selective is 7-nitroindazole, an inhibitor of neuronal nitric oxide synthase? Response Anesth Analg 1998;86:679–80.
[131] Weiger TM, Holmqvist MH, Levitan IB, Clark FT, Sprague S, Huang WJ, Ge P, Wang C, Lawson D, Jurman ME, Glucksmann MA, Silos-Santiago I, DiStefano PS, Curtis R. A novel nervous system beta subunit that downregulates human large conductance calcium-dependent potassium channels. J Neurosci 2000;20: 3563–70.
[132] Montmayeur J-P, Borrelli E. Transcription mediated by a cAMP-responsive promoter element is reduced upon activation of dopamine D2 receptors. Proc Natl Acad Sci USA 1991;88:3135–9.
[133] Devine DP, Wise RA. Self-administration of morphine, dopamine MGO, and DPDPE into the ventral tegmental area of rats. J Neurosci 1994;14:1978–84.
[134] Gerfen CR. The neostriatal mosaic: multiple levels of compartmental organization. Trends Neurosci 1992;15:133–9.
[135] Comings DE, Wu S, Chiu C, Ring RH, Gade R, Ahn C, MacMurray JP, Dietz G, Muhleman D. Polygenic inheritance of Tourette syndrome, stuttering, attention deficit hyperactivity, conduct, and oppositional defiant disorder: the additive and subtractive effect of the three dopaminergic genes-DRD2, D beta H, and DAT1. Am J Med Genet 1996;67:264–88.
[136] Jung MY, Skryabin BV, Arai M, Abbondanzo S, Fu D, Brosius J, Robakis NK, Polites HG, Pintar JE, Schmauss C. Potentiation of the D2 mutant motor phenotype in mice lacking dopamine D2 and D3 receptors. Neuroscience 1999;91:911–24.
[137] Vallone D, Pignatelli M, Grammatikopoulus G, Rocco L, Bozzi Y, Westphal H, Borrelli E, Sadile A. Activity, non-selective attention and emotionally in dopamine D-2/D-3 receptor knock out mice. Behav Brain Res 2002;130:141–8.
[138] Clifford JJ, Usiello A, Vallone D, Kinsella A, Borrelli E, Waddington JL. Topographical evaluation of behavioural phenotype in a line of mice with targeted gene deletion of the D2 dopamine receptor. Neuropharmacology 2000;39:382–90.
[139] Wang Y, Xu R, Sasaoka T, Tonegawa S, Kung M-P, Sankoorikal E-B. Dopamine D2 long receptor-deficient mice display alterations in Striatum-dependent functions. J Neurosci 2000;20:8305–14.
[140] Fetsko LA, Xu R, Wang Y. Alterations in D1/D2 synergism may account for enhanced stereotypy and reduced climbing in mice lacking dopamine D2L receptor. Brain Res 2003;967:191–200.
[141] Parish CL, Finkelstein DI, Drago J, Borrelli E, Horne MK. The role of dopamine receptors in regulating the size of axonal arbors. J Neurosci 2001;21:5147–57.
[142] Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature 1990;347:146–51.
[143] Suzuki T, Maeda J, Funada M, Misawa M. The D3-receptor agonist (+/−)-7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) attenuates morphine-induced hyperlocomotion in mice. Neurosci Lett 1995;187:45–8.
[144] De Boer P, Enrico P, Wright J, Wise LD, Timmerman W, Moor E, Dijkstra D, Wikstrom HV, Westerink BH. Characterization of the effect of dopamine D3 receptor stimulation on locomotion and striatal dopamine levels. Brain Res 1997;758:83–91.
[145] Bouthenet ML, Souil E, Martres MP, Sokoloff P, Giros B, Schwartz JC. Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D2 receptor mRNA. Brain Res 1991;564:203–19.
[146] L'hirondel M, Cheramy A, Godeheu G, Artaud F, Saiardi A, Borrelli E, Glowinski J. Lack of autoreceptor-mediated inhibitory control of dopamine release in striatal synaptosomes of D2 receptor-deficient mice. Brain Res 1998;792:253–62.
[147] Accili D, Fishburn CS, Drago J, Steiner H, Lachowicz JE, Park B-H, Gauda EB, Lee EJ, Cool MH, Sibley DR, Gerfen CR, Westphal H, Fuchs S. A targeted mutation of the D3 dopamine receptor gene is associated with hyperactivity in mice. Proc Natl Acad Sci USA 1996;93:1945–9.
[148] Xu M, Koeltzow TE, Santiago GT, Moratalla R, Cooper DC, Hu XT, White NM, Graybiel AM, White FJ, Tonegawa S. Dopamine D3 receptor mutant mice exhibit increased behavioural sensitivity to concurrent stimulation of D1 and D2 receptors. Neuron 1997;19: 837–48.
[149] Wong JY, Clifford JJ, Massalas JS, Kinsella A, Waddington JL, Drago J. Essential conservation of D(1) mutant phenotype at the level of individual topographies of behaviour in mice lacking both D(1) and D(3) dopamine receptors. Psychopharmacology (Berl.) 2003;167:167–73.
[150] Benjamin J, Li L, Patterson C, Greenberg BD, Murphy DL, Hamer DH. Population and familial association between the D4 dopamine receptor gene and measures of novelty seeking. Nat Genet 1996;12: 81–4.

[151] Ebstein RP, Novick O, Umansky R, Priel B, Osher Y, Blaine D, Bennett ER, Nemanov L, Katz M, Belmaker RH. dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of novelty seeking. Nat Genet 1996;12:78–80.
[152] Malhotra AK, Virkkunen M, Rooney W, Eggert M, Linnoila M, Goldman D. The association between the dopamine D4 receptor (D4DR) 16 amino acid repeat polymorphism and novelty seeking. Mol Psychiatry 1996;1:388–91.
[153] Vandenbergh DJ, Zonderman AB, Wang J, Uhl GR, Costa PT. No association between novelty-seeking and dopamine D4 receptor (D4DR) exon III seven repeat alleles in Baltimore Longitudinal Study of Aging participants. Mol Psychiatry 1997;2:417–9.
[154] Kuhn K, Meyer K, Nothen MM, Gansicke M, Papassotiropoulos A, Maier W. Allelic variants of dopamine receptor D4 (D4DR) and serotonin receptor 5HT2c (HTR2c) and temperament factors: replication tests. Am J Med Genet 1999;88:168–72.
[155] Barr CL, Wigg KG, Bloom S, Schachar R, Tannock R, Roberts W, Malone M, Kennedy JL. Further evidence from haplotype analysis for linkage of the dopamine D4 receptor gene and attention-deficit hyperactivity disorder. Am J Med Genet 2000;96:262–7.
[156] Holmes J, Payton A, Barrett J, Harrington R, McGuffin P, Owen M, Ollier W, Worthington J, Gill M, Kirley A, Hawi Z, Fitzgerald M, Asherson P, Curran S, Mill J, Gould A, Taylor E, Kent L, Craddock N, Thapar A. Association of DRD4 in children with ADHD and comorbid conduct problems. Am J Med Genet 2002;114:150–3.
[157] Manor I, Tyano S, Eisenberg J, Bachner-Melman R, Kotler M, Ebstein RP. The short DRD4 repeats confer risk to attention deficit hyperactivity disorder in a family-based design and impair performance on a continuous performance test (TOVA). Mol Psychiatry 2002;7:790–4.
[158] Faraone SV, Biederman J, Weiffenbach B, Keith T, Chu MP, Weaver A, Spencer TJ, Wilens TE, Cleves M, Sakai J. dopamine D4 gene 7-repeat allele and attention deficit hyperactivity disorder. Am J Psychiatry 1999;156:768–70.
[159] Dulawa SC, Grandy DK, Low MJ, Paulus MP, Geyer MA. Dopamine D4 receptor-knock-out mice exhibit reduced exploration of novel stimuli. JNeurosci 1999;19:9550–6.
[160] Mrzljak L, Bergson C, Pappy M, Huff R, Levenson R, Goldman-Rakic PS. Localization of dopamine D4 receptors in GABAergic neurons of the primate brain. Nature 1996;381:245–8.
[161] Rubinstein M, Phillips TJ, Bunzow JR, Bunzow JR, Falzone TL, Dziewczapolski G, Zhang G, Fang Y, Larson JL, McDougall JA, Chester JA, Saez C, Pugsley TA, Gershanik O, Low MJ, Grandy DK. Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. Cell 1997;90:991–1001.
[162] Carter CJ. Topographical distribution of possible glutamatergic pathways from the frontal cortex to the Striatum and Substantia Nigra in rats. Neuropharmacology 1982;21:379–83.
[163] Suzuki T, Kobayashi K, Nagatsu T. Genomic structure and tissue distribution of the mouse dopamine D4 receptor. Neurosci Lett 1995; 199:69–72.
[164] Lanau F, Zenner M, Civelli O, Hartman D. Epinephrine and norepinephrine act as potent agonists at the recombinant human dopamine D4 receptor. J Neurochem 1997;68:804–12.
[165] Westerink BHC, Santiago M, De Vries JB. The release of dopamine from nerve terminals and dendrites of nigrostriatal neurons induced by excitatory amino acids in the conscious rat. Naunyn Schmiedebergs Arch Pharmacol 1992;345:523–9.
[166] Overton P, Clark D. Electrophysiological evidence that intrastriatally administered *N*-methyl-D-aspartate augments striatal dopamine tone in the rat. J Neural Transm 1992;4:1–14.
[167] Kotler M, Cohen H, Segman R, Gritsenko I, Nemanov L, Lerer B, Kramer I, Zer-Zion M, Kletz I, Ebstein RP. Excess dopamine D4 receptor (D4DR) exon III seven repeat allele in opioid-dependent subjects. Mol Psychiatry 1997;2:251–4.
[168] Shields PG, Lerman C, Audrain J, Bowman ED, Main D, Boyd NR, Caporaso NE. dopamine D4 receptors and the risk of cigarette smoking in African–Americans and Caucasians. Cancer Epidemiol Biomarkers Prev 1998;7:453–8.
[169] de Castro PI, Torres P, Saiz-Ruiz J, Fernandez-Piqueras J. Genetic association study between pathological gambling and a functional DNA polymorphism at the D4 receptor gene. Pharmacogenetics 1997;7:345–8.
[170] LaHoste GJ, Swanson JM, Wigal SB, Glabe C, Wigal T, King N, Kennedy JL. Dopamine D4 receptor gene polymorphism is associated with attention deficit hyperactivity disorder. Mol Psychiatry 1996;1:121–4.
[171] Nothen MM, Cichon S, Hemmer S, Hebebrand J, Remschmidt H, Lehmkuhl G, Poutska F, Schmidt M, Catalano M, Fimmers R, Korner J, Rietschel M, Propping P. Human dopamine D4 receptor gene: frequent occurrence of a null allele and observation of homozygosity. Hum Mol Genet 1994;3–2207.
[172] Yan Z, Surmeier DJ. D5 dopamine receptors regulate Zn2 + -sensitive GABAA currents in striatal cholinergic interneurons through a PKA/PP1 cascade. Neuron 1997;19:1115–26.
[173] Liu F, Wan Q, Pristupa ZB, Yu XM, Wang YT, Niznik HB. Direct protein-protein coupling enables cross-talk between dopamine D5 and -aminobutyric acid A receptors. Nature 2000;403: 274–80.
[174] Sunahara RK, Guan HC, O'Dowd BF, Seeman P, Laurier LG, Ng G, George SR, Torchia J, Van Tol HHM, Niznik HG. Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. Nature 1991;150:614–9.
[175] Tiberi M, Caron MG. High agonist-independent activity is a distinguishing feature of the dopamine D1B receptor subtype. J Biol Chem 1994;269:27925–31.
[176] Muir WJ, Thomson ML, McKeon P, Mynett-Johnson L, Whitton C, Evans KL, Porteous DJ, Blackwood DH. Markers close to the dopamine D5 receptor gene (DRD5) show significant association with schizophrenia but not bipolar disorder. Am J Med Genet 2001; 105:152–8.
[177] Vanyukov MM, Moss HB, Gioio AE, Hughes HB, Kaplan BB, Tarter RE. An association between a microsatellite polymorphism at the DRD5 gene and the liability to substance abuse: pilot study. Behav Genet 1998;28:75–82.
[178] Tahir E, Yazgan Y, Cirakoglu B, Ozbay F, Waldman I, Asherson PJ. Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children. Mol Psychiatry 2000;5:396–404.
[179] Fisher SE, Francks C, McCracken JT, McGough JJ, Marlow AJ, MacPhie IL, Newbury DF, Crawford LR, Palmer CG, Woodward JA, Del'Homme M, Cantwell DP, Nelson SF, Monaco AP, Smalley SL. A genomewide scan for loci involved in attention-deficit/ hyperactivity disorder. Am J Hum Genet 2002;70:1183–96.
[180] Hawi Z, Lowe N, Kirley A, Gruenhage F, Nothen M, Greenwood T, Kelsoe J, Fitzgerald M, Gill M. Linkage disequilibrium mapping at DAT1, DRD5 and DBH narrows the search for ADHD susceptibility alleles at these loci. Mol Psychiatry 2003;8:299–308.
[181] Ciliax DJ, Nash N, Heilman C, Sunahara R, Hartney A, Tiberi M, Rye DB, Caron MG, Niznik HB, Levey AI. Dopamine D5 receptor immunolocalization in rat and monkey brain. Synapse 2000;37: 125–45.
[182] Rivkees S, Lachowicz JE. Functional D1 and D5 dopamine receptors are expressed in the suprachiasmatic, supraoptic and paraventricular nuclei of primates. Synapse 1997;26:1–10.
[183] Apostolakis EM, Gara J, Fox C, Smith CL, Watson SJ, Clark JH, O'Malley BW. dopamine rgic regulation of progesterone receptors: brain D5 dopamine receptors mediate induction of lordosis by D1-like agonists in rats. J Neurosci 1996;16:4823–34.
[184] Apostolakis EM, Garai J, O'Malley BW, Clark JH. In vivo regulation of central nervous system progesterone receptors: cocaine induces steroid-dependent behaviour through dopamine transporter modulation of D5 receptors in rats. Mol Endocrinol 1996;10: 1595–604.

[185] Dahmer MK, Senogles SE. dopaminergic inhibition of catecholamine secretion from chromaffin cells: evidence that inhibition is mediated by D4 and D5 dopamine receptors. J Neurochem 1996;66: 222–32.
[186] Mezey E, Eisenhofer G, Harta G, Hansson S, Gould L, Hunyady B, Hoffman BJ. A novel nonneuronal catecholaminergic system: exocrine pancreas synthesizes and releases dopamine. Proc Natl Acad Sci USA 1996;93:10377–82.
[187] Hollon TR, Bek MJ, Lachowicz JE, Ariano MA, Mezey E, Ramachandran R, Wersinger SR, Soares da Silva P, Jose PA, Sibley DR, Liu ZF, Grinberg A, Drago J, Young WSI, Westphal H. Mice lacking D5 dopamine receptors have increased sympathetic tone and are hypertensive. J Neurosci 2002;22:10801–11.
[188] Dziewczapolski G, Menalled LB, Garcia MC, Mora MA, Gershanik OS, Rubinstein M. Opposite roles of D1 and D5 dopamine receptors in locomotion revealed by selective antisense oligonucleotides. Neurorep 1998;9:1–5.
[189] Nally RE, McNamara FN, Clifford JJ, Kinsella A, Tighe O, Croke DT, Fienberg AA, Greengard P, Waddington JL. Topographical assessment of ethological and dopamine receptor agonist-induced behavioural phenotype in mutants with congenic DARPP-32 'Knockout'. Neuropsychopharmacology 2003;.
[190] Greengard P, Allen PB, Nairn AC. Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. Neuron 1999;23: 435–47.
[191] Sadile AG. Long-term habituation of theta-related activity components of albino rats in the Làt-maze. In: Sanberg PR, Ossenkopp KP, Kavaliers M, editors. Motor activity and movement disorders: measurement and analysis. New York: Humana Press; 1996. p. 1–54.
[192] Van Abeelen JHF. Genetics of rearing behaviour in mice. Behav Genet 1970;1:71–6.
[193] Sanders DC. The Bethlem lines: genetic selection for high and low rearing activity in rats. Behav Genet 1981;11:491–503.
[194] Aspide R, Fresiello A, De Filippis G, Gironi Carnevale UA, Sadile AG. Non selective attention in a rat model of hyperactivity and attention deficit: subchronic methylphenidate and nitric oxide synthesis inhibitor treatment. Neurosci Biobehav Rev 2000;24: 59–71.
[195] Aspide R, Gironi Carnevale UA, Sergeant JA, Sadile AG. Non-selective attention and nitric oxide in putative animal models of Attention-Deficit Hyperactivity Disorder. Behav Brain Res 1998;95: 123–33.
[196] Grillner P, Mercuri NB. Intrinsic membrane properties and synaptic inputs regulating the firing activity of the dopamine neurons. Behav Brain Res 2002;130:149–69.
[197] Burt DR, Reducing GABA. Receptors. Life Sci 2003;73:1741–58.
[198] Chiasson BJ, Armstrong JN, Hooper ML, Murphy PR, Robertson HA. The application of antisense oligonucleotide technology to the brain: some pitfalls. Cell Mol Neurobiol 1994;14:507–22.
[199] Landgraf R, Naruo T, Vecsernyes M, Neumann I. Neuroendocrine and behavioural effects of antisense oligonucleotides. Eur J Endocrinol 1997;137:326–35.
[200] Menalled LB, Dziewczapolski G, Garcia MC, Rubinstein M, Gershanik OS. D3 receptor knockdown through antisense oligonucleotide administration supports its inhibitory role in locomotion. Neurorep 1999;10:3131–6.