Dysfunctions in Dopamine Systems and ADHD: Evidence from Animals and Modeling

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SUMMARY

Animal models are useful for characterizing neural substrates of neuropsychiatric disorders. Several models have been proposed for the study of Attention Deficit Hyperactivity Disorder (ADHD). The models can be divided into various groups: (i) genetically derived hyperactivity/inattention, (ii) animal models showing symptoms after pharmacological intervention, and (iii) those based on spontaneous variations in a random population. Spontaneously hypertensive (SHR) and Naples High Excitability (NHE) rats show behavioral traits featuring the main aspects of ADHD in humans but show different changes in dopamine (DA) systems. In fact, the enzyme tyrosine hydroxylase is hyperexpressed in NHE rats and hypoexpressed in SHR. The DA transporter is hyperexpressed in both lines, although in the SHR, DAT activity is low (reduced DA uptake). The DA levels in the striatum and prefrontal cortex are increased in the juvenile SHR, but are decreased in handled young and non-handled older animals. The mRNA of the D1 DA receptor is upregulated in the prefrontal cortex of SHR and down-regulated in NHE. The D2 DA receptors are likely to be hypofunctioning in SHR, although the experimental evidence is not univocal, whereas their mRNA is hyperexpressed in NHE. Thus, in SHR both the mesocortical and mesolimbic DA pathways appear to be involved, whereas in NHE only the mesocortical system. To understand the effects of methylphenidate, the elective ADHD drug treatment in humans, in a dysfunctioning DA system, we realized a simple mathematical model of DA regulation based on experimental data from electrophysiological, cyclic voltammetry, and microdialysis studies. This model allows the estimation of a higher firing frequency of DA neurons in SHR rats and suggests that methylphenidate increases attentive processes by regulating the firing rate of DA neurons.

KEYWORDS

ADHD, dopamine system, motor activity, attention, behavior, review, model

INTRODUCTION

The use of animal models in the study of neuropsychiatric disorders is useful in the
characterization of neurological substrates of the main features of a given disorder. Moreover, animal models can be necessary for testing new pharmacological treatments and for studying the mechanisms of action of already used drugs. To this aim, an animal model should generally reproduce symptomatic expression of the disease, its treatment responses, and pathophysiology. According to Davids et al. (2003), a model should have (a) face validity, that is display fundamental behavioral deficits found in the neuropsychiatric disease, (b) construct validity, that is to conform a theoretical rationale and (c) predictive validity or an ability to predict unknown aspects of the disease.

Attention Deficit Hyperactivity Disorder (ADHD) has been modeled using different strategies. Several reasons argue for the use of animal models in the study of ADHD.

1. First, the midbrain dopamine (DA) system, which includes the ventral tegmental area (A10, VTA) and the substantia nigra (A9, SN), thought to play a central role in the pathogenesis of ADHD, is relatively similar in different mammals.

2. Second, the molecular targets of methylphenidate, the main pharmacological treatment of ADHD, are highly conserved in rats and humans.

3. Finally, hyperactivity and inattention can be measured in small laboratory animals like rodents. Moreover, ADHD morphofunctional substrates are likely to be the same in rats and humans.

In particular, two main strategies have been adopted: (i) selection of animals based on the similarity of some of their behaviors to the human symptoms, and (ii) lesions thought to reproduce the pathogenesis of the human disease. Solanto (2000) proposed that valid models of clinical ADHD should include the following:

- a deficit in measures of attention and not only hyperactivity;

- an improvement of both cognitive and motor deficits by stimulants and other clinically effective treatments in clinically plausible doses,

- an immediate onset of action and lack of tolerance or sensitization with repeated administration of drugs used to treat ADHD, and

- an effect of therapeutic agents on both DA and norepinephrine (NE) systems.

The various animal models proposed for the study of ADHD can be divided into those displaying genetically derived hyperactivity/inattention, those acquiring these changes after pharmacological intervention, and those based on spontaneous variations in a random population. The models comprise mice, rats, and monkeys (see also Comings, 2001; Davies et al., 2001; Davids et al., 2003).

Most studies on animal models of ADHD focus on changes in the catecholamine (DA, NE) systems, but these may represent only part of the neurobiological changes. As a matter of fact, changes in other systems such as the hippocampus (Sadile, 1993), the hypothalamic-hypophyseal axis (Sadile, 1993; King et al., 2000), the NE system (Russell et al., 2000; see also companion paper by Viggiano et al., 2004 - this issue), cholinergic (Russell et al., 2000; Viggiano et al., 2003b), and serotonin systems (Gainetdinov et al., 1999; Adriani et al., 2003) have been reported in some animal models. In particular, many toxins that give rise to a hyperDArgic behavioral profile (see also Masuo et al., 2004 - this issue) are correlated to peculiar changes in the cerebellar vermis (Ferguson & Cada, 2003), which have been reported to be present also in human ADHD (Castellanos et al., 1996). In fact these models are grouped overall as models with 'cerebellar stunting' (Ferguson & Cada, 2003).

The correlation with such changes and the changes in the DA system is unclear and has never
been studied in detail. These changes, in fact, may arise as independent alterations, or are the result of a common cause or could be directly connected.

Here we review the neurophysiologic and biochemical evidence for an alteration of the DA system in two rat models of ADHD, spontaneously hypertensive rats (SHR) and Naples High Excitability (NHE) rats. Moreover, a unitary view of the effects of methylphenidate in a hypothesized dysfunction of DA system is addressed using a simple mathematical model of the regulation of DA at the synaptic cleft.

SPONTANEOUSLY HYPERTENSIVE AND NAPLES HIGH EXCITABILITY RATS

The SHR strain was selected for familial hypertension in Japan by Okamoto (1969) in the early 1960s. Interestingly, the selection process also resulted in behavioral hyperactivity, which was subsequently disentangled from hypertension by Hendley and Ohlsson (1991), producing the Wistar-Kyoto (WKY) hypertensive (WKY-HT) and WKY hyperactive (WKY-HA) strains.

The SHR rat strain shows increased locomotor activity compared with WKY rats during forced exploration, that is in open field conditions (Tilson et al., 1977; Hendley et al., 1985; Sagvolden et al., 1993) in their own home cage and in simple mazes (Låt maze) (Aspide et al., 1996). This hyperactivity appears to be modulated by environmental factors, as continuous handling can reduce the locomotor activity in SHR below the level of WKY controls (Ferguson & Cada, 2003). This behavior has received a number of different explanations, such as a loss of habituation in a novel environment, altered emotional reactivity, and delayed aversion, but its relevance has been recently challenged. Recent longitudinal studies by Ferguson et al. al. (2003) show normal locomotor activity in an open field. Nevertheless, the longitudinal design may impair the significance of these findings as perinatal manipulation and behavioral experience normally lead to reduced hyperactivity in novelty situations. Multiple evidence of the alteration of DA and NE (see accompanying paper) systems in SHR has emerged, although many reports show contrasting results (see below).

On the other hand, NHE rats have been selected for their higher exploration in the Låt maze. They do not display hyperactivity in their home cage (Sadile, 1993), whereas novelty induced hyperactivity increases as a function of the complexity of the environment (Sadile et al., 1988, 1993; Viggiano et al., 2002b, 2003b).

SYNTHESIS, CLEARANCE, CONCENTRATION, AND EFFECTS OF DA IN ANIMAL MODELS

The expression of the tyrosine hydroxylase (TH) gene encoding the rate-limiting enzyme in the synthesis of catecholamines, including DA, is normal in NHE and SHR rats (Fig. 1); the protein,
however, is upregulated in NHE and downregulated in SHR in the prefrontal cortex (PFC) (King et al., 2000; Leo et al., 2003), whereas in the striatum it is similar to their respective controls (King et al., 2000), under basal conditions. This difference was detected in young adult NHE rats (Viggiano & Sadile, 2000, Viggiano et al., 2002a,b; 2003a,b). Interestingly, TH mRNA is down-regulated in the striatum in a bounded postnatal period from P7 through P14 in SHR (Leo et al., 2003). In the ventral mesencephalon, the expression of TH mRNA is normal in both NHE and SHR rats (Fig 1) when compared with their respective controls.

The integral plasmalemmal protein dopamine transporter (DAT) responsible for DA clearance is hyperexpressed in both NHE (Viggiano et al., 2002b, 2003b) and SHR rats (Watanabe et al., 1997), in the PFC, and, at least for SHR animals, in the striatum. In synaptosomal preparations from the striatum, however, the reuptake of DA by the DAT is reduced in SHR compared with controls (Leo et al., 2003). This would suggest that the DAT is hypofunctioning in the SHR. Therefore, in studies involving DA release from slices, the lower reuptake leads to reduced DA release, thus mimicking hypofunctioning DA terminals (Russell, 2003).

As a consequence, less DA is cleared from the synaptic cleft and the tonic level of DA is higher, as showed by microdialysis studies in juvenile SHR animals (Howes et al., 1984; Carboni et al., 2003). In contrast, the levels of DA in the striatum are normal in NHE rats (Carboni et al. personal communication), whereas no data are available about the PFC.

Consistently, depletion of DA by 6-hydroxydopamine lesion of the substantia nigra of SHR decreases the magnitude of adult hypertension (van den Buuse et al., 1985, 1986; Linthorst et al., 1994; de Jong et al., 1995). Interestingly, intensive postnatal handling can reduce the difference between SHR and WKY in terms of the basal levels of DA and locomotor activity (Ferguson & Cada, 2003; Ferguson et al., 2003), possibly due to a reshaping of the neural networks (Sadile, 1999). Unfortunately, the electrophysiological response of DA neurons in the phasic and tonic mode has not yet been explored in hyperactive models.

The DA receptors also show peculiar changes in these hyperactive animals. The D1 DA receptor is postsynaptic; therefore, its expression level can be related to the effects of DA (Jackson et al., 1994; Missale et al., 1998). D2 DA receptors are both post and presynaptic, therefore related to inhibitory and feedback effects. Strikingly, the pattern of expression of the D1-D2 receptors is very different in SHR and NHE rats. The D1 receptor protein and mRNA are hyperexpressed in SHR (striatum and PFC) (Lim et al., 1989; Kirouac & Ganguly, 1993; Watanabe et al., 1997; Sadile, 1999), whereas in NHE rats D1 mRNA is hypoexpressed in the PFC (Fig. 1) (Viggiano et al., 2002b). The D2 receptors have been reported to be hyper- (see also Fig. 1), hypo-, or normoexpressed (Lim et al., 1989; Watanabe et al., 1989; Kirouac & Ganguly, 1993; Linthorst et al., 1993; Sadile, 1999; Vaughan et al., 1999; Russell et al., 2000) in the striatum of SHR, whereas their mRNA is hyperexpressed in the striatum of NHE rats, without changes in the PFC (Fig. 1). Some of the conflicting results on the SHR (see also Table 1) may be explainable by different experimental setups or the age of the animals. The high genetic heterogeneity of the WKY strain among different commercial suppliers should also be considered (Samani et al., 1989).

Therefore, the higher DA release in SHR is accompanied by enhanced effects on a postsynaptic site (D1), although whether D1 receptors are normofunctioning in SHR is still being debated. Conversely, in NHE rats, a higher DA release is accompanied by lower postsynaptic effects (D1) and enhanced feedback inhibition (D2).

Finally, the DA branches appear to be differentially involved in SHR and NHE rats. In the
# Table I

## SHR

| Target                          | Change                                                                 | Brain region |
|--------------------------------|------------------------------------------------------------------------|--------------|
| Locomotor activity             | >(Hellstrand, 1980; Fuller, 1983; Ueno et al., 2002 2002; Fujita, 2003) |              |
|                                 | - (Ferguson, 2003; Yang et al., 2003)                                  |              |
|                                 | < (Ferguson, 2003)                                                    |              |
| Attention                       | < (Ueno et al., 2002)                                                 |              |
| TH                             | < (King et al., 2000; Leo et al., 2003)                                | PFC          |
|                                 | - (King et al., 2000)                                                 | Striatum     |
| DAT                            | > (Watanabe et al., 1997)                                             | Striatum     |
| DAT function (reuptake)         | < (Leo et al., 2003; Russell, 2003)                                   | Striatum     |
| DA                             | Juvenile animals, basal conditions:                                   | Striatum     |
|                                 | > (Howes et al., 2002b, 2003b 1984; Carboni et al., 2003)              |              |
|                                 | older animals or after handling:                                      |              |
|                                 | < (Linthorst et al., 1991; Sutoo, 1993; Nakamura, 2001; Fujita, 2003) |              |
|                                 | - (Fuller, 1983; Yu, 1990; Inada, 1992; Ferguson, 2003)                |              |
|                                 | > (Carboni et al., 2003)                                              | PFC          |
| DA stimulated release           | < (van den Buuse et al., 1991; Youisi-Alaoui, 2001; Russell, 2003)   | PFC, striatum |
| D1 R                           | > (Lim et al., 1989; Kirovoc & Ganguly, 1993; Watanabe et al., 1997; Sadile, 1999) | Striatum     |
|                                 | - (Hellstrand, 1980; Watanabe et al., 1989; Linthorst et al., 1993)   | Striatum     |
| D2 R (presynaptic)             | > (Lim et al., 1989; Kirovoc & Ganguly, 1993; Vaughan et al., 1999; Russell et al., 2000) | Striatum     |
|                                 | < (Sadile, 1999)                                                     | Striatum     |
|                                 | - (Watanabe et al., 1989; Linthorst et al., 1993)                     | Striatum     |
| Mesolimbic projection           | - (King et al., 2000)                                                |              |
| Mesocortical projection         | < (King et al., 2000)                                                |              |
| Hyperactivity after MPH         | - (Yang et al., 2003)                                                |              |
|                                 | < (Ueno et al., 2002)                                                |              |

## NHE rats

see (Viggiano & Sadile; 2000; Viggiano et al., 2002a,b, 2003a,b) and Fig 1

| Target                          | Change                                                                 | Brain region |
|--------------------------------|------------------------------------------------------------------------|--------------|
| TH                             | >                                                                     | PFC          |
|                                 | -                                                                     | Striatum     |
| DAT                            | >                                                                     | PFC          |
| DA                             | -                                                                     | Striatum     |
| D1                             | < (mRNA)                                                             | PFC          |
|                                 | - (mRNA)                                                             | Striatum     |
| D2                             | - (mRNA)                                                             | PFC          |
|                                 | > (mRNA)                                                             | Striatum     |
| Mesolimbic projection           | -                                                                     |              |
| Mesocortical projection         | >                                                                     |              |

<: decreased; >: increased; -: unchanged
former, the mesolimbic branch has received more attention, showing an anterior segmental defect (Sadile, 2000), although the mesocortical one might be involved as well (King et al., 2000). In NHE rats, only the mesocortical branch appears to be involved, being hypertrophic (Viggiano & Sadile, 2000; Viggiano et al., 2002a,b; 2003a,b). Therefore, different changes in the DA machinery can be correlated to hyperactivity or to different types of hyperactivity. A direct translation of neuro-biological changes into behavioral correlates is difficult, however, given our poor understanding of the actual gears of this machinery.

The psychostimulant drug methylphenidate used in the treatment of ADHD has been widely studied in these animal models (Wultz et al., 1990; Sadile, 1999; Aspide et al., 2000; Russell et al., 2000; Andersen et al., 2002; Fox et al., 2002; Carboni et al., 2003; Ferguson & Cada, 2003; Yang et al., 2003). Some investigators have postulated that mesencephalic presynaptic D2 receptors in normal animals are more sensitive to low doses of direct agonists (Skirboll et al., 1979; Carlson et al., 1987; Piercey et al., 1996). As a consequence, a biphasic response to methylphenidate results as low doses of DA agonists would reduce tonic spiking and decrease motor behavior (Carlsson, 1975; Strombom, 1975; Doare et al., 1986), whereas high doses are sufficient to activate directly post-synaptic receptors, thereby increasing motor activity. Therapeutic doses of methylphenidate, which are very low, should act to decrease DA-catecholamine transmission (Seeman & Madras, 1998; Solanto, 1998). Nevertheless, some authors reported that the indirect-acting stimulants methylphenidate (Ruskin et al., 2001) and amphetamine (Piercey et al., 1996) do not have a preferential action on D2 auto-receptors. In fact, the injection of methylphenidate leads to a dose-dependent decrease in the firing rate of DA neurons, which can be reversed by the inhibition of D2 receptors (Ruskin et al., 2001). Moreover, methylphenidate also increases the excitability of post-synaptic neurons (Ruskin et al., 2001). In vivo methyl-phenidate increases the release of DA in target regions (Kuczenski & Segal, 1989; Pehek et al., 1990; Carboni et al., 2003).

In the next session, we will address the effects of methylphenidate using a modeling approach.

MODELING THE REGULATION OF DOPAMINE AT SYNAPTIC SITES AND THE EFFECTS OF METHYLPHENIDATE

Several models of the DA system have been proposed. Higher level models are mainly based on the experiments by Schultz and collaborators (Schultz et al., 1992) showing that DA neurons increase their firing rate during unexpected rewards (Schultz et al., 1997). Besides, biophysical models of the regulation of DA release have been proposed (Cragg et al., 2001; Schmitz et al., 2001; Schonfuss et al., 2001; Venton et al., 2003; Viggiano et al. 2004), but they do not address the effects on the firing of DA neurons, as discussed below. The latter regulation, in fact, is important when considering changes in the brain of hyper-active animals and the effects of methylphenidate.

In the striatum, the resting levels of extracellular DA are 2 to 6 nM (Huff & Davies, 2002). This concentration results from the balance between the opposing processes of release and uptake (Wightman, 1988, 1990). The general equation describing this relation is given by (Garris et al., 1994; Wu et al., 2001):

\[ \frac{d[DA]}{dt} = \frac{d[DA]_{release}}{dt} - \frac{d[DA]_{uptake}}{dt} \quad (1) \]

where \( \frac{d[DA]}{dt} \) is the rate of change of extracellular DA, \( \frac{d[DA]_{release}}{dt} \) is the release rate, and \( \frac{d[DA]_{uptake}}{dt} \) is the uptake rate by the DA transporter.

The release of DA can be treated as a discrete process, every firing event being associated with
the release of a constant amount of DA, resulting in an instantaneous increase in [DA]. Therefore, the rate of DA release is determined by the firing rate (f) of DA neurons. Each spike will release a constant (quantum) amount of DA ([DA]p). Thus:

\[ \frac{d[DA]_{\text{release}}}{dt} = [DA]_p \ast f \] (2)

[DA]p represents the concentration of DA after a single spike.

The uptake of DA can be treated as a continuous process following Michaelis-Menten kinetics. The reaction scheme can be represented with

\[
\begin{align*}
DA + DAT &\rightarrow DA-DAT \rightarrow DAT + DA_i \\
k_1 \quad k_2 &\quad k_1
\end{align*}
\]

where DAi represents the concentration of intracellular DA. The above reaction can also follow the opposite direction, with DAT acting by extrusion of DA into the extracellular space (Falkenburger et al., 2001). This might take place on DA neuron dendrites, where a special dendro-dendritic communication has been shown.

Using the Michaelis-Menten law in a quasi-steady-state approximation, we get:

\[ \frac{d[DA]}{dt} = \frac{V_{\text{max}} \ast [DA]}{[DA] + K_m} \] (3)

where Km is equal to:

\[ \text{Km} = \frac{(k_1 + k_2)}{k_1} \]

and is related to the affinity of DA for the transporter and to its turnover rate, whereas Vmax is a constant equal to:

\[ V_{\text{max}} = k_2 [DAT]_{\text{TOT}} \]

reflecting the number of uptake or transporters sites.

Here [DAT]_{TOT} represents the total amount of enzyme and is equal to:

\[ [DAT]_{\text{TOT}} = [DAT] + [DA-DAT] \]

Because the rate of formation of DAi is equal to the rate of DA internalization, we can write:

\[ \frac{d[DA]}{dt} = -\frac{d[DA]}{dt} = -\frac{V_{\text{max}} \ast [DA]}{[DA] + K_m} \] (4)

Recent data suggest that the DAT also elicits ion-channel-like currents, increasing the firing rate of DA neurons in vitro after blockade of D1, D2, and adrenergic receptors (Ingram et al., 2002). The relevance of such a system in vivo is still being debated. Moreover, the DAT can be regulated by D2 receptors (Wu et al., 2002). In fact, the inhibition of D2 receptors decreases the rate of clearance of DA, but this effect is not evident after DAT blockade. It should be noted that the clearance of DA also depends on diffusion, as shown by voltammetry studies in vivo after DAT blockade. This mechanism of clearance is dependent on the initial concentration of DA/D2 receptors and the firing rate, and is important just after the release of DA, when DA reaches concentrations in the micromolar range in the synapse for very short times. The diffusion of DA has been previously modeled (see e.g. Garris et al., 1997; Cragg et al., 2001; Schonfuss et al., 2001; Venton et al., 2003) and must be taken into account if considering DA at the single synapse on a very short time scale (after 40 microseconds) more than 96% of DA has diffused out of the synaptic site (Garris et al., 1994). However, here we will focus on a greater space and longer time-scales. Finally, some authors (Mercuri et al., 1997) suggested that another important mechanism of DA clearance is represented by monoamine oxidase (MAO-a and MAO-b).

As here we were mainly interested in the effects of methylphenidate, we did not include diffusion, MAO, or DAT-linked channels in the present simulation. The rate of extracellular DA change during activity can be described by combining Eqs. (2) and (4) (see also Garris et al., 1994; Wu et al., 2001):

\[ \frac{d[DA]}{dt} = ([(DA]_p \ast f) - (V_{\text{max}} \ast [DA] / ([DA] + K_m)) \]
Estimations for $[DA]_p$, $V_{max}$, and $K_m$ have been previously reported. The $K_m$ and $V_{max}$ for DAT have been estimated using synaptosome preparations from different brain regions. Interestingly, the $K_m$ is about four times higher in the striatum than in the median eminence (Annunziato et al., 1980, 1981, 1984). Similarly, the $V_{max}$ is about five times smaller in the striatum than in the median eminence (Annunziato et al., 1980). Here we will analyze the striatal interface, where $K_m$ has been estimated in a range from 0.03 micromolar up to 2.3 micromolar (Coyle, 1969; Annunziato et al., 1980; Paton, 1980; Sarkar et al., 1983; Near et al., 1988; Horn, 1990; Jones et al., 1995; Zahniser et al., 1999; Wu et al., 2001), although values up to 8 micromolar have been reported (Stamford et al., 1984). Such a wide range can be explained by different experimental sets. For the actual simulation we used a value $K_m = 0.22$ micromolar, which is within the range reported by most authors. Similarly, the $V_{max}$ of the DAT has different values according to the brain region (Wu et al., 2001). We used a value of $V_{max} = 3.8$ micromolar/s, as reported by Wu et al. (2001).

At the steady state $d[DA]/dt = 0$.

The concentration of DA in WKY rats has been evaluated equal to $[DA] = 5.17nM$ (Carboni et al., 2003) in the striatum, whereas the firing is $f = 4.5Hz$ (Ruskin et al., 2001), it is possible to calculate $[DA]_p = 17nM$. This value is below the range of 89-250nM reported using cyclic voltammetry. This technique is based on microsensors of 15 micrometers diameter and takes record of more than one axonal varicosity that have a density of $10^8$ synapses $/\text{mm}^3$; (Pickel et al., 1981; Garris et al., 1994). It allows the determination of DA concentration released after the artificial stimulation of DA fibers (thus setting $f$ to a fixed value), thus making possible to evaluate $[DA]_p$. However, on the one hand, the artificial stimulation of neurons with an extensive arborization, such as DA and NE, leads to a failure of release at individual synapses 99% of the time (Cunnane & Stjarnes, 1984), thus preventing a direct comparison between the frequency of excitation and the natural firing frequency of DA neurons. On the other hand, synapses fire more asynchronously in the unstimulated animal than in the stimulated one, causing a more rapid dilution in the extrasynaptic space (Kawagoe et al., 1992; Garris et al., 1994). Moreover, $[DA]_p$ has been suggested to change in relation to VMAT2 expression, D2 receptor stimulation, DAT activity, and firing frequency (Garris et al., 1994; Pothis et al., 2000; Ingram et al., 2002; Wu et al., 2002). These effects might explain the difference in $[DA]_p$ calculated in our model or after artificial stimulation (such as in voltammetry studies).

As a matter of fact, the firing rate of DA neurons ($f$) changes in vivo from pacemaker, to random, to burst modes (Schultz, 2002). During the burst mode, a transient rate exceeding 30 Hz (Wightman & Robinson, 2002), a large, phasic increase of DA is evident, whereas the tonic DA release is due to random and pacemaker modes (Paladini et al., 2003). The firing rate is also regulated by the activation of D2 autoreceptors (Schmitz et al., 2003). Dopamine binds to D2 autoreceptors forming the complex DA.D2, a reaction that, at equilibrium, respects the Law of mass:

$$[DA.D2]_{eq} = B_{max} [DA] / (K_d + [DA])$$  \hspace{1cm} (6)$$

where $B_{max} = [DA]+[D2]$ represents the total number of receptors, and $K_d$ is the concentration of DA required to occupy 50% of the receptors. Estimates for $B_{max}$ and $K_d$ in rat striatum are $B_{max} = 0.5-2.3 \text{ pmol mg}^{-1} \text{ protein}$ or 100 micromolar (Matres et al., 1985; Boyson et al., 1986; Joyce & Marshall, 1987; Richfield et al., 1989; Albert et al., 1990). Estimations for $K_d$ are 7.4 to 43 nanomolar (Seeman et al., 1985; Richfield et al., 1989; Albert et al., 1990) in the high-affinity state, which comprises 74% of the binding sites.
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(Richfield et al., 1989), and 4550 to 4300 nanomolar in the low affinity state (Seeman et al., 1985; Richfield et al., 1989).

The DA-D2 receptor complex has multiple effects, such as: (i) decreased amount of DA released after a spike (Garris et al., 1994), (ii) increased activity of the DAT (Cass & Gerhard, 1994; Schmitz et al., 2001, 2002; Wu et al., 2002), (iii) regulation of potassium channels (Uchimura et al., 1986; Lacey et al., 1988), and voltage-dependent calcium channels (Cardozo & Bean, 1995), which in turn hyperpolarize the cell membrane, thus decreasing the probability of DA release and the firing rate of DA neurons (Einhorn et al., 1988; Lacey et al., 1988; Mercuri et al., 1997; Ruskin et al., 2001; Ingram et al., 2002; Paladini et al., 2003). The first two effects are presynaptic and more evident at target sites (e.g. the neostriatum, accumbens, PFc). The third one takes place in the nuclei of origin (VTA, SN) and is due to D2 autoreceptors on the soma and dendrites of DA cells (Carlsson, 1975; Starke, 2001). In this case, DA derives from axon collaterals, which form a feedback, or from the same dendrites (Falkenburger et al., 2001). Some have suggested that small doses of D2 agonist would act primarily on these autoreceptors, thus inhibiting the firing rate, whereas D2 receptors on target sites would be activated by higher doses of D2 agonists (Skirboll et al., 1979; Ruskin et al., 2001).

We restricted the analysis to the effects on the firing rate, as we were interested in low doses of MPH:

\[
\frac{df}{dt} = -f([DA.D2])
\]  

(7)

Experimental data (Skirboll et al., 1979; Einhorn et al., 1988) would suggest, indeed, that the spontaneous firing rate decays linearly with the external concentration of DA. Therefore, the above formula can be empirically simplified with:

\[
\frac{df}{dt} = -k*[DA]
\]  

(8)

The firing rate of DA neurons is also regulated in vivo by a complex neuronal network comprising GABA, glutamate, NE, acetylcholine, serotonin, and nitric oxide influences (see e.g. (West & Grace, 2000; Grillner, 2002)). For instance, inactivation of the ventral pallidum enhances DA release, resetting the steady state level to a new point (Floresco et al., 2003). These influences are not completely described from a quantitative perspective. By blocking the D2 autoreceptors (setting \(k=0\)), however, it is possible to study \(df/dt\), deriving empirically the sum of all these influences. After blockade of D2 autoreceptor the firing rate of DA neurons increases initially almost linearly, until a new steady state is reached (Einhorn et al., 1988; Ruskin et al., 2001).

We assumed that all these influences could be described using a single parameter, DF (Driving Force), which increases linearly the firing rate when D2 autoreceptors are blocked:

\[
\frac{df}{dt} = DF
\]  

(9)

Combining equations (8) and (9) we get:

\[
\frac{df}{dt} = DF - k*[DA]
\]  

(10)

The appropriate value for DF can be empirically derived by studying the rate of change of DA neurons firing blocking D2 autoreceptors. In our model we set DF= 1.5 spikes/s² (Ruskin et al., 2001). Moreover, the basal firing rate of DA neurons can be calculated from slice experiments, in which all the connections are cut, in the presence of a D2 inhibitor (DF=0, DA.D2=0, \(f=\) const). Under these conditions, \(f = 1.2±0.2Hz\) (Ingram et al., 2002). Without D2 inhibition, in the presence of DA, the firing rate rapidly drops to 0.1±0.1Hz, as expected by Eq. (8). In vivo, where DAT, D2, and DF are present at the same time, the typical basal firing rate is about 5 Hz (Ruskin et al., 2001; Xu & Shen, 2001).

The differential Eqs. (5) and (10) have been solved in the Matlab environment, based on an
explicit Runge-Kutta (Forsythe et al., 1977) formula. The system reaches a steady state very rapidly. Because we were interested in the steady state responses after blockade of the DAT by MPH, we changed the parameter $K_m$ and calculated the new steady state for $f$ and $[DA]$. In fact, to simulate the effects of methylphenidate injection, we considered the maximal concentration of methylphenidate in the brain and in the blood after i.p. injection using published data (Wargin et al., 1983; Aoyama et al., 1997; Huff 7 Davies, 2002; Swanson & Volkow et al., 2002, 2003). The blood concentration of methylphenidate is approximately linear to the injection dose (expressed in mg/kg body weight), although the ratio between dose and blood concentration is about 10 for an oral dose, but 1 for i.p injections. The brain concentration of methylphenidate has been considered here as approximately equal to the blood concentration, as suggested by data from Huff and Davies (2002).

The inhibition constant ($K_i$) of methylphenidate has been reported to be $41.3\pm73.8$ nM (Aoyama et al., 1997). Methylphenidate has chemical and structural properties similar to those of cocaine (Schweri et al., 2002), and, at least for its methylated derivative, has been reported to bind to the WIN site of DAT, increasing its $K_m$ but leaving the $V_{max}$ unchanged, acting in this way as a competitive inhibitor (Keener & Sneyd, 1998; Schweri et al., 2002):

$$K_m=0.22(1+([MPH]_{blood}/K_i))$$ (11)

We simulated the steady state concentration of DA following stepwise increases of methylphenidate (0.1 to 40 mg/kg). The percent of firing rate and DA level were calculated and reported on semi log scale (Fig. 2). As shown in figure, the model reproduces the experimental data reported by (Ruskin et al., 2001). In fact, the blockade of the re-uptake increases extracellular DA, which acts on D2 autoreceptors, thus reducing DA neuron firing. Methylphenidate has been also described to increase DA release in rats (Kalivas, 1989; Carboni et al., 2003) and humans as well (Seeman, 2002). This effect suggests that methylphenidate changes also the stimulation of DA neurons ($D_F$), since, at the steady state, the level of DA is regulated by $D_F$ and $k$. In fact, $D_AT$

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![Graph A](image1.png)
![Graph B](image2.png)

**Fig. 2:** Model of dopamine regulation; relationship between dose of methylphenidate (MPH) and firing frequency of dopamine neurons (A) or extracellular dopamine concentration (B). O, experimental data. X, simulated data for control rats (WKY). +, simulated data for SHR.
knockout mice, which lack the molecular target of MPH, still respond to this psychostimulant (Gainetdinov et al., 1999). It can be assumed that methylphenidate also increases DF. Data from literature (Carboni et al., 2003) allow to estimate such effect. The normal resting level of extracellular DA is approximately 4nM (Garris et al., 1994; Seeman & Madras, 2002), and 5.7nM in WKY rats (Carboni et al., 2003). This concentration can transiently rise of at least 60-fold to about 250 nM during a normal nerve impulse (phasic activity). The transiently elevated level of extracellular DA goes back to 4nM by diffusion, DAT activity, enzymatic degradation and autoxidation (Garris et al., 1994).

It should be noted that in SHR, the in vivo basal level of DA in the striatum is increased to 6.35 nM versus 5.17 nM in WKY rats (Carboni et al., 2003). This 20% difference can be modified by environmental factors, as suggested by Ferguson and Gough (2003). Moreover, aged, hypertensive animals might show reduced striatal DA (Linthorst et al., 1991). The enhanced DA outflow may result from increased DA release or decreased uptake or both. In fact, synaptosome preparations from SHR rats suggested a reduction in the uptake by 28% compared to WKY (Leo et al., 2003), although the number of DAT binding might be increased (Watanabe et al., 1997).

Because at the steady state \( \frac{d[D]}{dt=0} = 0 \) \( [D] = D_0 \frac{DF}{k} \), the increase of \([D]\) might be due to a decreased effect of D2 receptors on the firing rate \( k \), as suggested by autoradiography studies (Sadile, 1999) and by lower responsivity of SHR to D2 blockers (van den Buuse et al., 1992).

Moreover, the induction of DA release by depolarization with \( K^+ \) or electrical stimulation leads to a greater increase of DA in WKY rats than in SHR (Russell et al., 2000; Carboni et al., 2003). This can be modeled by a decrease in \([D]_0\) in SHR, as suggested by the previously observed reduction in TH levels (King et al., 2000; Leo et al., 2003; Masuo et al., 2004 - this issue). Thus, at the steady state \( \frac{d[D]}{dt=0} = 0 \), the firing rate of DA neurons is predicted to be higher in SHR than in WKY rats. Methylphenidate (1 mg kg\(^{-1}\)) increases the steady state DA level to a greater extent in SHR than in WKY rats, suggesting a different response to methylphenidate in these animals. In fact, methylphenidate elevates the steady-state level of DA up to 7.5nM in WKY (150%) and up to 13.97 nM in SHR (220%) (Carboni et al., 2003) (see also Fig 2). This tonic increase in DA produced by methylphenidate is negligible with respect to the concentrations during the burst activity that reaches the micromolar range. Recent evidence (see Wightman et al., 2002) demonstrated that discrete, phasic DA signals accompany rewarding or alerting stimuli. Therefore, the effects of low doses of methylphenidate on alert and attention are possibly due to a different mechanism. In fact, simulated and experimental data suggest that the firing rate is strongly decreased during the tonic and phasic discharge after methylphenidate treatment (Einhorn et al., 1988; Ruskin et al., 2001). Low doses of methylphenidate would decrease the firing rate in SHR to the level of WKY, whereas higher doses are predicted to decrease the firing rate well below the 4Hz of WKY (Ueno et al., 2002; Yang et al., 2003). It is likely to hypothesize that the latter effect eventually impairs the responsivity of the system to salient novel stimuli.

Recently Volkow et al., 2002 (2002) suggested that individual responses to methylphenidate are due in part to individual differences in DA release, so that for an equivalent level of DAT blockade, methylphenidate would induce smaller DA changes in subjects with low DA than in those with high DA cell activity. Taken altogether, however, the data suggest that in a hyperDArgic system small doses of methylphenidate could actually have positive effects by reducing the firing rate of DA neurons, with small changes in the elevated extracellular DA.

As a matter of fact, the firing of DA neurons
has behavioral relevance, whereas the amount of tonic DA release is of great importance for its neurotoxicity and locomotor activity. Consistently, high doses of methylphenidate increase locomotor activity (Drolet et al., 2002). Moreover, elevated resting levels of DA in SHR rats are associated to a segmental defect consisting of a change in D1, D3, and CAMK-II levels in a restricted segment of the anterior forebrain (Sadile, 1999). This change is likely to be due to the neurotoxic effects of DA in the rostral striatum. This defect could be reverted by subchronic treatment with methylphenidate or postnatal stimulation during the 5th and 6th week of postnatal life. The effect of methylphenidate was transient, however, as the modification reversed following drug withdrawal. Conversely, the effect of postnatal stimulation was permanent. These beneficial effects are consistent with decreased DA after postnatal handling (Ferguson & Cada, 2003). Conversely, the long-term effects of methylphenidate are likely to involve changes in the DA machinery (Porrino & Lucignan, 1987; Andersen et al., 2002; Yang et al., 2003) (which are not included in our model) and await further investigation.

ACKNOWLEDGMENTS

We are grateful to Dr. Lars Schwabe for the modeling approach and to Dr. Xiuxia Du for helpful comments. We are also grateful to Dr. Nick Foulkes for critical reading. This research was supported by a grant from MIUR-COFIN 2001/2002 and Ministry of Health-Special Funds.

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