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Altered Corticostriatal Connectivity and Exploration-Exploitation Imbalance Emerge as Intermediate Phenotypes for a Neonatal Dopamine Dysfunction

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ABSTRACT

Findings showing that neonatal lesions of the forebrain dopaminergic system in rodents lead to juvenile locomotor hyperactivity and learning deficits have been taken as evidence of face validity for the attention deficit hyperactivity disorder. But the core cognitive and physiological intermediate phenotypes underlying this rodent syndrome remain unknown. Here we show that early postnatal dopaminergic lesions cause long-lasting deficits in exploitation of shelter, social and nutritional resources, and an imbalanced exploratory behavior, where non-directed local exploration is exacerbated while sophisticated search behaviors involving sequences of goal directed actions are degraded. Importantly, some behavioral deficits do not diminish after adolescence but instead worsen or mutate, particularly, those related to the exploration of wide and spatially complex environments. In vivo electrophysiological recordings and morphological reconstructions of striatal medium spiny neurons reveal corticostriatal alterations associated to the behavioral phenotype. More specifically, an attenuation of corticostriatal functional connectivity affecting medial prefrontal inputs more markedly than cingulate and motor inputs is accompanied by a contraction of the dendritic arbor of striatal projection neurons in this animal model. Thus, dopaminergic neurons are essential during postnatal development for the functional and structural maturation of corticostriatal connections. From a bottom-up viewpoint, our findings suggest that neuropsychiatric conditions presumably linked to developmental alterations of the dopaminergic system should be evaluated for deficits in foraging decision making, alterations in the recruitment of corticostriatal circuits during foraging tasks, and structural disorganization of the frontostriatal connections.

Introduction

Early postnatal disturbances of forebrain dopamine (DA) neurotransmission are relevant to our understanding of neuropsychiatric conditions like the attention deficit hyperactivity disorder (ADHD) (Biederman and Faraone, 2005; Swanson et al., 2007). Patients diagnosed with ADHD usually show hyperactivity, impulsivity and inattention, but the clinical presentation of ADHD is highly heterogeneous. This heterogeneity relates to the main symptoms and also to the high rates of comorbidity with mood, impulse control and personality disorders. Symptoms usually diminish after adolescence, but children diagnosed with ADHD show significant psychosocial and educational impairment as adults and significant comorbidities through their life span (Biederman and Faraone, 2005; Wilens et al., 2009; Klein et al., 2012) . Although patients with ADHD show depressed DA activity in the caudate nucleus (Volkow et al., 2007), delayed maturation of caudate volume (Castellanos and Tannock, 2002), and respond to DA neurotransmission enhancing drugs (Biederman and Faraone, 2005), it is unclear how early dopaminergic alterations relate to symptoms. Findings showing that neonatal forebrain dopaminergic depletion leads to hyperactivity and learning deficits in rodents suggested that early dopaminergic manipulations may be useful to study behavioral phenotypes relevant to ADHD and related disorders (Shaywitz et al., 1976; Archer et al., 1988; Moy, 1995; Avale et al., 2004). However, the core cognitive alterations underlying this rodent syndrome remain unknown. Moreover, there is little evidence that any of the purported animal models of ADHD replicate the alterations of corticostriatal connectivity observed in patients.

Here we have re-examined the behavioral effects of a dopaminergic neuron (DAN) lesion induced by 6-hydroxydopamine (6-OHDA) in mice neonates, a purported model of ADHD, from a bottom-up perspective. In line with current views emphasizing the role of DA signals in orienting behavior towards the discovery of information (Bromberg-Martin and Hikosaka, 2009; Dolan and

Dayan, 2013; Pearson and Platt, 2013), we focused on the exploration and exploitation of information-rich novel environments rather than on conditions of instrumental learning favoring model-free cognitive control (Dolan and Dayan, 2013). Since information is rewarding by itself (Bromberg-Martin and Hikosaka, 2009) we speculate that developing without DANs leads to the acquisition of an imbalanced exploration-exploitation strategy, with enduring consequences on behavior. Moreover, because studies in children and adults with ADHD report structural and functional deficits in the associative corticostriatal circuits (Konrad and Eickhoff, 2010; Liston et al., 2011), and experimental evidence supporting that postnatal DA availability shapes corticostriatal connectivity is scarce (Galiñanes *et al*, 2009), we have performed in vivo electrophysiological studies of corticostriatal circuits and reconstructed the dendritic arbors of striatal neurons in neonatally DAN-lesioned mice.

Materials and Methods

Further information is available online in Supplemental Experimental Procedures.

Animals

CF-1 mice were used for behavioral and electrophysiological recordings and Drd1a-tomato/Drd2-eGFP double transgenic mice (Shuen et al., 2008) for morphological reconstructions of neurons. Mice were maintained under a 12:12 hours light:dark cycle with ad libitum food and water. All experimental procedures were in accordance with institutional (IACUC of the University of Buenos Aires School of Medicine) and government regulations (SENASA RS617/2002, Argentina)

Lesions

Bilateral DAN lesion was performed at PD2 following published protocols (Galiñanes *et al*, 2009). Pups received injections of 6-OHDA or vehicle in each lateral ventricle, after desipramine pretreatment, under hypothermal anesthesia. Control and DAN-lesioned mice were housed together.

Behavioral tests

DAN-lesioned and age-matched control mice (see Results) were subjected to the following tests: standard open field, activity in a small arena, marble burying, elevated plus maze, large open field, Y maze spontaneous alternation, hoarding behavior, social interaction, and nest-building test as previously described (Belforte et al., 2010) with some modifications. All findings were confirmed in at least two separate cohorts of animals.

In vivo electrophysiology

Striatal recordings were performed with 24-channel two-shank or 32-channel four-shank silicon probes under urethane anesthesia with the animal fixed to a stereotaxic frame (Galiñanes et al., 2009, 2011). Cortical electrical stimulation was applied through concentric bipolar electrodes placed into prelimbic (Prl), cingulate and motor areas of the frontal cortex. Field potentials were analyzed offline with custom-made Matlab routines.

Morphological analysis

Fixed brain slices (200 μm-thick) were used for iontophoretical neuronal labeling with Lucifer yellow (LY) following published protocols (Suárez et al., 2014). After injections, LY was detected using colorimetric immunohistochemistry. Morphological reconstructions were made under transmitted light at 100x using Mercator software.

Histology and immunohistochemistry

Electrode location was histologically verified as in (Galiñanes et al., 2011). Striatal depletion of tyrosine hydroxylase (TH) immunoreactive terminals was quantified as described in (Galiñanes et al., 2009).

Statistical analysis

Statistical analysis was performed using SigmaPlot 11.0 or InfoStat 2014.

Results

Setting-dependent alteration of exploratory behavior after neonatal dopaminergic neuron lesion

Early dopaminergic lesions produce juvenile locomotor hyperactivity that wanes in adults, presumably because compensatory mechanisms are called into play (Shaywitz et al., 1976; Avale et al., 2004; Galiñanes et al., 2009). However, certain forms of hyperactivity can persist in the adult, especially after extensive dopaminergic denervation (Miller et al., 1981; Luthman et al., 1997)(Miller et al., 1981; Luthman et al., 1997). Hyperactivity and repetitive behaviors distinctive of human neuropsychiatric disorders may emerge only in specific settings, and symptoms observed in juvenile patients can metamorphose and persist in the adult. Therefore, we asked whether adult mice that were DAN-lesioned as neonates exhibit hyperactivity and repetitive behaviors under the appropriate conditions.

DAN-lesioned and age-matched control animals were subjected to a battery of behavioral tests after reaching adulthood (>12 weeks old). As expected, when tested in an open-field arena of standard size (40 x 40 cm), DAN-lesioned mice did not show hyperactivity (Figure 1A). But, in a smaller setting designed to score repetitive behaviors they displayed increased vertical

exploratory activity (RM-ANOVA, p<0.001); Figure 1B; Movies S1-S2). To assess self-oriented perseveration, mice were sprayed with water mist to induce grooming. Induced grooming was reduced in DAN-lesioned mice (RM-ANOVA, p<0.001). Figure 1B). Moreover, in the marble-burying test, which evaluates object-oriented perseveration, they did not show interest in burying marbles (RM-ANOVA, p<0.001; Figure 1C; Movies S3-S4). The expression of repetitive behaviors may be modified by anxiety; however, anxiety-related measures in the open field were normal in DAN-lesioned mice (Figure 1A). Moreover, their phenotype in the elevated plus maze was not typical of changes in anxiety levels (Figure 1D). DAN-lesioned mice entered fewer times to the open than the closed arms of an elevated plus maze than control mice (RM-ANOVA, p=0.002, but spent more time in the open arms (RM-ANOVA, p<0.001) displaying increased local exploratory behaviors (head dippings, Mann-Whitney test, p<0.001; Figure 1D). Thus, although the influence of anxiety in certain phenotypes cannot be completely excluded, we favor the view that DAN-lesioned mice experience an abnormal mode of exploratory behavior rather than locomotor hyperactivity or stereotyped repetitive behaviors.

To further evaluate the effect of setting on exploratory behavior, we exposed DAN-lesioned mice to a large-size open arena (1.25 m in diameter) where control mice exhibit a compound strategy involving wide-field ambulation and local exploration based on swaying head movements (Movies S5-S6). In this setting, DAN-lesioned animals take 10 times longer to leave the starting quadrant and five times longer to visit the four quadrants of the arena than control mice (Mann-Whitney test, p<0.001). Moreover, the total distance travelled was markedly reduced in DAN-lesioned mice (Mann-Whitney test, p<0.001; Figure 1E). Conversely, local exploration achieved by continuous swaying head movements was significantly increased (RM-ANOVA, p<0.001; Movie S6), suggesting an imbalanced exploratory strategy.

To further study this possibility we exposed the mice to a Y maze, a test assessing the natural tendency of mice to alternate their choice of maze arms in order to maximize information gathering in this environment (Figure 1F). DAN-lesioned animals visited less times the maze arms (Mann-Whitney test, p=0.005), stayed for longer in the first visited arm (t test, p=0.01) and took longer to visit all three arms (Mann-Whitney test, p=0.032). The Y maze revealed also a deficient spontaneous alternation (sequential exploration of the three arms). While reliable alternation was observed in control animals, DAN-lesioned mice displayed a significant reduction to the chance level (t test, p=0.02; Figure 1F). Although this would be interpreted as a working memory deficit, here it could relate to the higher memory load resulting from the longer time the mice spent in each arm. Thus, the neonatal dopaminergic lesion produces pervasive alterations of exploratory behavior in settings where costly search strategies are required to gather information.

Reduced exploitation of opportunities in dopaminergic neuron-lesioned animals

Optimal utilization of environmental opportunities, including social, nutritional and shelter resources, depends on foraging decision making (Cohen et al., 2007; Adams et al., 2012). Foraging decisions involve choosing between exploiting a local resource and searching for alternative resources in other places. DAN-lesioned animals may be impaired at making foraging decisions, as they fail to bury strange objects, to seek refuge in risky environments, and to adaptively explore large arenas and maze arms (Figure 1). To investigate whether DAN-lesioned animals are impaired at exploiting primary resources, we studied their performance in a series of ethologically based tests assessing social, shelter and nutritional behaviors spontaneously emitted by mice (Deacon, 2012).

In a three-chamber social interaction test that evaluates the natural tendency of mice to explore a novel conspecific over a novel object, DAN-lesioned mice show a significant decrease in social

approach behavior directed to the mouse (RM-ANOVA, interaction: p=0.088, treatment: p=0.046, chamber: p<0.001; Figure 2A). Moreover, they fail to show an increase in social approach towards a novel conspecific when paired with a familiar one during the second part of the test (Figure 2A; RM-ANOVA interaction: p=0.007). Thus, DAN-lesioned mice exhibit deficits in social exploratory behavior and at discriminating social novelty.

Nest building behavior is connected with exploitation of natural resources to secure shelter, facilitate heat conservation and promote social behaviors (Deacon, 2012). To minimize the effect of exploration deficiencies this test was conducted in the mice home cage. Most control mice formed a nest using a cotton nestlet provided as starting material the evening before, but DAN-lesioned mice had not built clear nests by the following morning (t test, p<0.001; Figure 2B) consistently with the idea of decreased exploitation of primary resources.

To directly assess exploration/exploitation balance we adapted a test that examines hoarding behavior (Deacon, 2012) to increase exploration load (Figure 2C). Mice were individually housed in a modified home cage that connects with two 50 cm long wire mesh tubes extending in opposite directions. After 24 hours of habituation to the central cage animals were allowed to freely explore the tubes during the entire night under infrared video surveillance. One tube contained 100 g of regular food pellets and the other one was empty. Control mice hoarded most of the food during the first 4 hours whereas the DAN-lesioned mice hoarded on average 36±13 g along the 14 hours of the test (t test, p<0.05). Moreover, control and DAN-lesioned mice spent the same time in the loaded and unloaded tubes (RM-ANOVA), indicating that the DAN-lesioned animals make a less efficient use of their time in the loaded tube. Finally, control animals entered more times to the unloaded tube than DAN-lesioned mice, thus being more efficient in updating cognitive maps of the environment.

Overall, in adulthood, neonatally DAN-lesioned animals are impaired at foraging for information and at exploiting primary environmental resources (Figure S1).

Exploration/exploitation deficits emerge early and worsen with age

Since previous studies have not examined the exploration/exploitation phenotype induced by neonatal DAN lesions so extensively, we asked whether the deficits we observe in the adult emerge during the juvenile period. Juvenile mice (30-50 day-old) neonatally depleted of DANs show exacerbated vertical exploration (t test, p=0.004; Figure 3A) and deficits in exploitation as assessed in the marble-burying and nest building tests (RM-ANOVA, p<0.001; Figure 3B-C). A subtle increase in local exploration was also observed in the large circular arena, but globally, juvenile mice exhibited normal wide-field exploration in this setting (Figure 3D). In contrast, the cognitively more demanding Y maze revealed navigation deficits qualitatively similar to those observed in adults (Figure 3E). Remarkably, at difference with the adults (Figure 1E), alternation in the Y maze was preserved in juvenile mice (t test, p=0.1, Figure 3E). This dissociation observed in juvenile mice, of alternation performance from navigation deficits, suggests that the deficient alternation observed in adults does represent a working memory deficit. Finally, in the modified hoarding behavior test, juvenile mice showed deficits qualitatively similar to those observed in adults (Figure 3F). Thus, alterations in the exploration/exploitation balance emerge early, but symptoms persist and worsen in adults.

Reduced prefrontostriatal functional connectivity in dopaminergic neuron-lesioned animals

Several factors suggested an involvement of associative corticostriatal circuits in the behavioral deficits observed in DAN-lesioned mice. First, this animal model shows maximal depletion of dopaminergic nerve terminals in the medial part of the dorsal striatum and less compromise of the dorsolateral striatum and nucleus accumbens (Galiñanes *et al*, 2009). Second, foraging decisions involve neuronal activity in associative corticostriatal circuits (Daw et al., 2006; Hayden

et al., 2011). Third, patients with ADHD and related disorders show functional alterations in the associative corticostriatal circuits (Konrad and Eickhoff, 2010; Liston *et al*, 2011).

To assess corticostriatal functional connectivity in vivo we extracellularly recorded the striatal response to prelimbic cortex (Prl) stimulation through a multisite electrode located in the dorsomedial striatum, where Prl stimulation evokes high amplitude responses in the anesthetized mouse (Galiñanes *et al*, 2011). Prl stimulation evokes a well characterized striatal field wave (Figure 4A) whose amplitude is linearly related to that of depolarizing postsynaptic potentials and population spike responses of medium spiny projection neurons (MSNs) (Galiñanes et al., 2011). Recordings from adults showed smaller responses in DAN-lesioned mice, as shown by a significant leftward shift of the frequency distribution of field wave amplitudes (Kolmogorov-Smirnov test, $p < 0.001$) and by a 42% reduction of the maximal response attained within the more responsive striatal area (RM-ANOVA, $p < 0.001$; Figure 4B). Juvenile mice showed less marked alterations. There was a small but significant leftward shift in the distribution of response amplitudes (Kolmogorov-Smirnov test, $p < 0.001$), but maximal field responses did not significantly differ between DAN-lesioned and control mice (Figure 4C-D). Besides, although juvenile mice show depressed population spike responses to Prl stimulation, which is in agreement with previous findings (Galiñanes et al., 2009), the population spike response is more markedly depressed in adults (Figure S2). To determine if reduced occupancy of DA receptors (DAR) during the recordings can per se induce functional deficits similar to those induced by neonatal DAN lesions, we assessed the effect of DAR antagonists on striatal responses in control mice. Acute DAR blockade did not modify the striatal field and population spike response in adult control animals (Figure 4E and S2). Thus, the neonatal DAN lesion induces early alterations of prefrontostriatal functional connectivity that evolve into a marked reduction in functional connectivity in the adult.

Studies in rodents (Kasanetz et al., 2008; Mailly et al., 2013) and primates (Haber and Knutson, 2010) show that functionally-related corticostriatal projections partially overlap. To assess whether neonatal DAN-depletion affects corticostriatal integration that could be crucial for decision making (Haber and Knutson, 2010), we placed a multisite electrode array (32 sites in 4 shanks) spanning most of the dorsal striatum to record responses to electrical stimulation of Prl, cingulate and motor cortices, in control and DA-lesioned mice under anesthesia (Figure 5A). In control mice, Prl and cingulate stimulation evokes reliable responses in the three more medial shanks with a topography that recapitulates rodent anatomy of corticostriatal projections (Figure 5B-C; (Mailly *et al*, 2013). Conversely, responses to motor cortex stimulation are maximal in the lateral shank and negligible in the medial one. This organized pattern is degraded in DAN-lesioned mice, which show attenuated responses to Prl stimulation across the whole span of the Prl projection field and a distorted striatal representation of cingulate and motor cortex inputs (Figure 5D).

Contraction of medium spiny neuron dendritic fields in neonatally dopaminergic neuron-lesioned animals

The more marked attenuation of prefrontostriatal connectivity observed in adult than juvenile mice suggested that the lesion induces progressive corticostriatal alterations. During adolescence, the volume of the human caudate nucleus decreases (Castellanos and Tannock, 2002) through a process that is regulated by the DA transporter and D4 receptor genes (Durston *et al*, 2005) and that has been speculatively attributed to synaptic pruning (Toga et al., 2006). Consequently, we looked for a morphological correlate of the diminished functional connectivity observed in adult DAN-lesioned mice.

We used BAC-transgenic mice that allow visualizing direct and indirect pathway MSNs (dMSNs and iMSNs) (Shuen *et al*, 2008) to label and subsequently reconstruct using an image analysis

system MSNs located in the dorsomedial striatum (Figure 6A), where depletion of dopaminergic terminals is maximal in this animal model (Figure 6B). The number of primary dendrites, of branching points in the dendrites, and the cell body area, were not affected by the DAN lesion (Fig S3). However, MSNs showed a significant reduction of total dendritic length in DAN-lesioned mice (Figure 6C; two-way ANOVA, $p<0.05$ for treatment), without changes in spine density (Figure 6D). Sholl analysis of dendritic arbors revealed that iMSN dendrites located >55 µm away from the soma consistently show reduced length and fewer intersections in DAN-lesioned mice (RM-ANOVA interactions, $p<0.001$; Figure 6E). Fewer intersections were also detected in dMSNs (RM-ANOVA interaction, $p=0.027$), but the Sholl analysis did not reveal changes in dendritic length, suggesting less robust effects of the DAN lesion in dMSNs (Figure 6E). Thus, the span of the dendritic arbor of MSNs is reduced in neonatally DAN-lesioned animals, more markedly in iMSNs than dMSNs.

Discussion

Animals facing novel environments have to decide whether to exploit what is immediately available or to search new locations (Adams *et al*, 2012; Cohen *et al*, 2007). Search processes may involve sophisticated sequences of goal directed actions relying on mechanisms of cognitive control comparable to those involved in exploitation (Cohen *et al*, 2007; Daw *et al*, 2006; Hayden *et al*, 2011). Moreover, recent studies have shown that information is rewarding for animals and that DANs encode advanced information about rewards as if it were a primary reward (Bromberg-Martin and Hikosaka, 2009). Thus, exploitation and search seem to share a common drive, the salience of primary (e.g., food) and abstract (e.g., information) rewards (Pearson and Platt, 2013). Thus, DAN-lesioned mice seem to have a general deficiency in processing salience, as they fail both to exploit and to forage for information in novel settings. This is best seen in a

hoarding behavior test adapted to increase information needs. Control mice were more efficient at hoarding food and also at updating information about the environment than DAN-lesioned mice. Although the ultimate mechanisms underlying deficient salience processing in this animal model remain uncertain, DAN-lesioned animals exhibit a disorganized corticostriatal functional topography affecting more markedly projections from medial prefrontal cortex than those from lateral frontal areas. The more prominent functional connectivity alterations observed in adult than juvenile mice, and the atrophy observed in the dendrites of MSNs, support that corticostriatal connectivity is shaped by DANs during postnatal development (Galiñanes et al., 2009).

Neonatal DAN depletion has been evaluated as an ADHD model from a top-down perspective. In addition to hyperactivity, this rodent model exhibits learning deficits in tasks with dissimilar neural substrates such as lever-pressing operant conditioning (Heffner and Seiden, 1983), active avoidance learning (Smith et al., 1973) and the Morris water-maze (Archer *et al*, 1988). Remarkably, learning is not completely impaired in DAN-lesioned mice. Although DAN-lesioned animals are usually outperformed by control animals, in some tasks they show no impairment (Levine et al., 1980) or perform better than controls by using novel strategies (Pearson et al., 1984). Moreover, they exhibit normal motivation (Moy, 1995; Pearson *et al*, 1984) and normal reward effectiveness in self-stimulation tasks (Takeichi et al., 1986; Stellar et al., 1988). Thus, no clear description of the core cognitive alteration underlying the learning deficits emerged from these studies (Moy, 1995), nor a hypothesis linking learning deficits to hyperactivity (Heffner and Seiden, 1983; Pearson et al., 1984). Deficient salience processing, resulting in reduced interest in exploiting and discovering, would very likely affect most kinds of learning irrespective of the neural substrates required by each specific task.

The hyperactivity of DAN-lesioned mice takes the form of different local exploratory behaviors adapted to each setting (i.e., vertical exploration, swaying head movements, head dippings). However, in complex environments like the Y maze, where rodents display very specialized search routines, the total travelled distance is shorter in DAN-lesioned, which rules out a general form of hyperactivity in this model. We propose that DAN-lesioned mice are impaired at making transitions from local exploration to global search. The decision to engage in a costly search is driven by the possibility that richer resources will be found in other parts of the environment and is thought to depend on incentive signals carried by DA and other neuromodulators (Berridge and Robinson, 1998; Cohen et al., 2007; Adams et al., 2012; Dolan and Dayan, 2013). Likely, a deficiency in attributing salience to environments carrying high uncertainty would produce symptoms only in those environments, where less efficient exploratory behaviors would substitute for the required specialized search strategies.

The behavioral syndrome induced by neonatal DAN lesion is unlikely to be related to DA deficiency during behavioral testing. Acute DAR blockade in adults induces akinesia and rigidity instead of an exploration/exploitation imbalance. Moreover, neonatally DAN-lesioned mice are relatively insensitive to DAR antagonists (Duncan et al., 1987). Finally, preliminary data suggest that psychostimulants do not reverse the symptoms in adult DAN-lesioned mice (Fig. S4). On the other hand, DA has effects on corticostriatal plasticity that could shape corticostriatal connectivity (Kreitzer and Malenka, 2008; Surmeier et al., 2009) and could have long-lasting effects on behavior (Durston et al., 2005; Cohen et al., 2009). Previously we found a delayed maturation of corticostriatal physiology in this animal model, but the activity of individual MSNs was almost normal in the adult (Galiñanes *et al*, 2009), contrasting with the marked behavioral alterations reported here. Indeed, we find that some behavioral alterations emerge or are exacerbated in adulthood. Adults use suboptimal search strategies in simple environments and

show deficient alternation in the Y maze indicative of a working memory impairment, and these deficits are not present in juvenile mice. Here, by using local field potential recordings to more globally assess corticostriatal connectivity, we reveal a marked reduction of prefrontostriatal connectivity and a topographical disorganization of cingulostriatal projections in the adult. Additionally, we found a putative morphological correlate of these alterations in the contraction of the dendritic arbor of iMSNs and, to a lesser degree, dMSNs. In contrast, we find more subtle alterations of prefrontostriatal connectivity in juvenile mice, in line with our previous study revealing a delayed maturation of corticostriatal synchronization in this model (Galiñanes et al., 2009). Importantly, prefrontostriatal connectivity may be crucial for working memory and decision making. While prefrontal cortex contains the mechanisms maintaining working memory contents, basal ganglia circuits may be responsible for updating those contents (Chatham et al., 2014). Moreover, binary decisions like those involved in foraging behaviors are thought to be implemented by a striatal thresholding mechanism (Hayden et al., 2011; Ding and Gold, 2013). Thus, the persistence and aggravation of some symptoms in DAN-lesioned mice may partly result from progressive alterations in the connections linking associative frontal areas with the dorsomedial striatum.

At difference with nigrostriatal lesion induced in the adult, which reportedly produces spine depletion accompanied or not by dendritic shrinkage (Day et al., 2006; Villalba et al., 2009; Zhang et al., 2013; Suárez et al., 2014), MSNs do not show significant spine pruning in our neonatal lesion model. While several factors can account for the observed differences (e.g., lesion extent), early lesions could trigger compensations that are available only at certain developmental stages but not after circuit has completed its maturation. Of note, in addition to direct effects of dopamine on spine formation (Fasano et al., 2013), complex network effects (Kozorovitskiy et al., 2012) and other factors expressed by DANs, like brain-derived neurotrophic

factor, may differentially modulate corticostriatal connectivity along postnatal development. Thus, multiple mechanisms may contribute to the alterations of corticostriatal connectivity after early dopaminergic lesions.

There are several parallels between the behavioral alterations observed in DAN-lesioned mice and those of patients with ADHD, including setting-dependent hyperactivity and impaired working memory, social behavior and learning. Moreover, patients with ADHD show altered functional connectivity between frontal association cortex and caudate nucleus and reduced caudate volume (Konrad and Eickhoff, 2010; Liston *et al*, 2011). Though, it is not clear that an early hypodopaminergic state can account for the attention deficit and impulsivity, which are crucial for ADHD diagnosis. Attention is regarded as a mechanism that optimizes information-seeking (Gottlieb, 2012) and as a form of foraging (Manohar and Husain, 2013). Accordingly, "exploratory attention" is guided by uncertainty, novelty and surprise, whereas "targeted attention" is guided by rewards. Thus, the salience and reward signals conveyed by DANs may have a role in attention (Redgrave and Gurney, 2006; Gottlieb, 2012). Some forms of impulsivity have also been related to delay aversion and DA modulation (Sagvolden et al., 2005; Sonuga-Barke, 2005). However, in ADHD the attention deficit and the impulsivity may relate to impaired behavioral inhibition and altered noradrenergic modulation of the prefrontal cortex (Castellanos and Tannock, 2002; Arnsten, 2011). Our findings suggest that ADHD and other neuropsychiatric conditions presumably linked to developmental alterations of the dopaminergic system should be examined for alterations in foraging decision making.

In summary, deficits in foraging decision making and alterations of frontostriatal connectivity emerge as candidate intermediate phenotypes for deficient dopaminergic neuron function beginning in early life.

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Supplementary information is available at the *Neuropsychopharmacology* website.

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Figure legends

Figure 1. **Setting-dependent alteration of exploratory behavior in adulthood after neonatal DAN lesion. A.** Standard open field. No difference was observed in total traveled distance (t test, $t_{31}=-0.16$, $p=0.9$), distance in the center (t test, $t_{31}=0.6$, $p=0.6$) and maximal velocity (Mann-Whitney $U=116$, $p=0.5$). **B.** Small arena. DAN-lesioned mice showed normal grooming but lower grooming induction (RM ANOVA, interaction: $F_{1, 55}=7$, $p=0.01$, *$p<0.05$ Tukey post hoc test). Vertical activity was increased in both conditions (RM ANOVA, treatment effect: $F_{1, 55}=15$, *$p<0.001$). **C.** Marble Burying. DAN-lesioned mice showed lower levels of burying across time (RM ANOVA, interaction: $F_{6, 131}=8.5$, $p<0.001$, Tukey post hoc test *$p<0.05$ **$p<0.001$). Representative pictures at the end of the test are shown. **D.** Elevated plus maze. DAN-lesioned mice traveled less along the maze (Mann-Whitney $U=53$, **$p<0.001$), spent more time in the open arms (RM ANOVA, interaction: $F_{1, 87}=26$, $p<0.001$, Tukey post hoc test **$p<0.001$), entered fewer times to the maze arms (RM ANOVA, interaction: $F_{1, 87}=11$, $p=0.002$, Tukey post hoc test *$p<0.05$ **$p<0.001$) and exhibited more (Mann-Whitney $U=58$, **$p<0.001$) and longer head dippings (Mann-Whitney $U=20$, **$p<0.001$). **E.** Large arena. Total distance traveled was lower (Mann-Whitney $U=0$, **$p<0.001$) while latency to exit the first quadrant (Mann-Whitney $U=1$, **$p<0.001$) and to visit all quadrants was higher for the lesioned mice (Mann-Whitney $U=0$, **$p<0.001$). Right: DAN-lesioned mice showed increased local exploration in the form of a higher distance traveled by the head relative to the body (RM ANOVA, interaction: $F_{5, 89}=9$, $p<0.001$, Tukey post hoc test *$p<0.05$ **$p<0.001$). **F.** Spontaneous alternation in Y maze. Total distance traveled in Y maze was lower (t test, $t_{31}=2.7$, *$p=0.01$) while latency to leave the first arm (Mann-Whitney $U=25$, **$p<0.001$) and to visit all arms (Mann-Whitney $U=76$, *$p=0.032$) was longer in lesioned than control mice. Moreover, number of entries to arms (Mann-Whitney $U=59$,

*p=0.005) and alternation (t test, t_{31}=2.4, *p=0.02) were lower in lesioned mice. Data are mean ± SEM, or median ± interquartile ranges and 10-90th percentiles; animal numbers are indicated in parentheses.

Figure 2. Reduced exploitation in neonatally DAN-depleted animals when tested as adults. **A.** Social interaction test. DAN-depleted mice showed lower sniffing levels directed to the unfamiliar mouse A (left; RM ANOVA, treatment factor: $F_{1, 47}$=4.5, *p=0.046, chamber factor: $F_{1, 47}$=23, **p<0.001) and failed to show an increase in social approach towards a novel conspecific (mouse B) (right; RM ANOVA, interaction: $F_{1, 47}$=8.9, df=1, p=0.007; Tukey post hoc test *p<0.05 **p<0.001). **B.** Nesting. DAN-depleted mice used less material from the cotton nestlet (arrow) provided for the test (t test, t_{23}=3.6, **p=0.001). **C.** Hoarding behavior. Photo of the behavioral setup: modified home cage connected with two 50 cm long tubes (see Results). From left to right: DAN-depleted mice hoarded less food (t test, t_{13}=3.1, *p=0.009), took longer to hoard all available pellets (Mann-Whitney U=7.5, *p=0.01) and visited fewer times the food and empty tubes (RM ANOVA, treatment effect: $F_{1, 29}$=43, *p<0.001), but spent the same time in each tube than control mice (RM ANOVA). Data are mean ± SEM; animal numbers are indicated in parentheses.

Figure 3. Exploration/exploitation deficits emerge in juvenile animals. A. Small arena. DAN-lesioned mice showed an increase in spontaneous vertical activity (t test, t_{18}=-3.2, *p=0.004). **B.** Marble Burying. DAN-depleted mice showed lower levels of burying across time (RM ANOVA, interaction: $F_{3, 87}$=9.9, p<0.001, Tukey post hoc test *p<0.05 **p<0.001). **C.** Nesting. DAN-lesioned mice used less material from the cotton nestlet provided for the test (Mann-Whitney, U=3, *p=0.001). **D.** Large arena. No differences were observed in total traveled distance (t test, t_{23}=0.4, p=0.7), latency to exit the first quadrant (t test, t_{23}=-1.3, p=0.2) or latency to visit all quadrants (Mann-Whitney, U=47, p=0.1). DAN-lesioned mice showed increased local exploration

in the first 5 minutes of the test (RM ANOVA, interaction: $F_{5, 89}$=5.3, p<0.001, Tukey post hoc test *p<0.05). **E.** Y maze. Total distance traveled was lower (Mann-Whitney, U=7, **p<0.001) and latency to visit all arms longer (t test, t_{18}=-2.1, *p=0.047) in DAN-lesioned than control mice. The number of entries to arms were lower in lesioned mice (t test, t_{19}=5.6, p<0.001), and there was no difference in alternation between groups (t test, t_{19}=-1.7, p=0.1). **F.** Hoarding behavior. From left to right: DAN-depleted mice hoarded less food (t test, t_{13}=3.1, *p=0.009), visited fewer times both tubes (RM ANOVA, treatment effect: $F_{1, 31}$= 9.9, *p=0.007, tube effect: $F_{1, 31}$=6.7, p=0.02). The time spent in the tubes was lower in lesioned mice and both groups preferred the food arm (RM ANOVA, treatment effect: $F_{1, 31}$=10.6, *p=0.006, tube effect: $F_{1, 31}$=5.8, p=0.031). Data are mean ± SEM, or median ± interquartile ranges and 10-90th percentiles animal numbers are indicated in parentheses.

Figure 4. Functional corticostriatal disconnection in neonatally dopamine depleted animals. A. Representative histological sections showing the location of cortical stimulation (left) and striatal recording (right) electrodes and representative trace of a striatal response evoked by cortical stimulation (aca: anterior commissure; St, striatum; cc: corpus callosum). Field potential amplitude was measured between the N2 and P2 peaks. Arrow in A points to mark left by the stimulating electrode. **B.** Corticostriatal connectivity in adults. Top: Topographical reconstruction of recording sites. Colors represent the average field potential amplitude evoked by a cortical stimulation intensity of 300 µA. Middle: Cumulative frequency distribution and box and whisker plots (median with interquartile ranges and 10–90th percentiles) of evoked field potential amplitudes induced by 300 µA stimuli applied to the prelimbic cortex (Kolmogorov-Smirnov test **p<0.001). Bottom: Amplitude of the striatal field response (averaged for channels within the circle shown at the top) as a function of stimulation current intensity was diminished in adult DAN-lesioned mice (RM-ANOVA, interaction: $F_{6, 132}$: 5.7, p<0.001, *p<0.05 **p<0.001 Tukey post

hoc test). **C**. Corticostriatal connectivity in juvenile mice. Top, middle and bottom panels as in B. Midlle, **p<0.001 Kolmogorov-Smirnov test). **D.** Representative traces of maximal local field potential responses (20 individual trials overlapped in gray, average trace in black). **E.** Amplitude of the striatal field response was not affected by DA receptor antagonists (Eticlopride 0.25 mg/kg and SCH23390 0.25 mg/kg, i.p.) (RM ANOVA). Data are mean ± SEM, or median ± interquartile ranges and 10-90th percentiles animal numbers are indicated in parentheses.

Figure 5. Disorganized response pattern to cortical inputs in dopamine neuron depleted mice. A. Schematic diagram of the positioning of cortical stimulation electrodes and the striatal recording electrode (four shanks, eight channels per shank, channel separation: 100 μm). **B-C.** Amplitude of the field potential responses evoked from prelimbic, cingulate and motor cortex stimulation (700 μA) in different striatal regions (shanks) in control (B) and DAN-lesioned mice (C). Responses of different animals were aligned at the site of maximal response within each shank. Positive distances are dorsal to the maximum. **D.** Maximal amplitude of the response evoked at each shank by stimulation (700 μA) of each cortical area in control and DAN-depleted lesioned mice. In DAN-depleted-lesioned mice the responses to prelimbic stimulation were reduced at all shanks and the pattern of responses to cingulate and motor stimulation was degraded (two-way ANOVAs, #p<0.05 treatment main effect; *p<0.05 control vs. DAN-depleted-lesioned, Tukey post hoc test after significant treatment per shank interaction). Data are mean ± SEM; animal numbers are indicated in parentheses.

Figure 6. Contraction of medium spiny neuron dendritic fields in DAN-lesioned animals. A. Photomicrograph of representative control direct and indirect pathway MSNs, high magnification images of dendrites and examples of reconstructions of the dendritic tree. **B.** Striatal section immunostained for TH in a representative DAN-depleted mice (aca: anterior commissure; mSt, medial striatum; lSt: lateral striatum; cc: corpus callosum). In DAN-

lesioned mice, the percentage of immunoreactive TH area was lower in the mSt compared to the lSt (RM ANOVA, interaction: $F_{1, 27}$=17.1, p=0.001; *p<0.05 Tukey comparisons). Grid of MSNs injected with Lucifer yellow (LY) and immunostained with antibodies against the dye (right). **C-D.** DAN-depleted mice showed a reduction in total dendritic length in both types of MSN (C; two-way ANOVA, treatment effect: $F_{1, 42}$=7.4, *p=0.009; MSN type effect: $F_{1,42}$=6.0, *p=0.02) without changes in spine density (D; two-way ANOVA). **E.** Sholl analysis: dendritic length at different distances from the soma (upper panel) and number of dendritic branches intersecting circular rings drawn around the soma (lower panel). Distal dendritic length was reduced in iMSNs (RM ANOVA, interaction $F_{10,230}$=6.1, p<0.001) but not dMSNs ($F_{10,230}$=1.5, NS), whereas intersections were reduced in both (RM ANOVA interactions, iMSNs: $F_{10,230}$=3.9, p<0.001 dMSNs: $F_{10,230}$=2.1, p=0.027). *p<0.05 Tukey post hoc test after significant interaction). Data are mean ± SEM; animal numbers are indicated in parentheses; scale bar for A: 10 µm, scale bar for B: 100 µm.
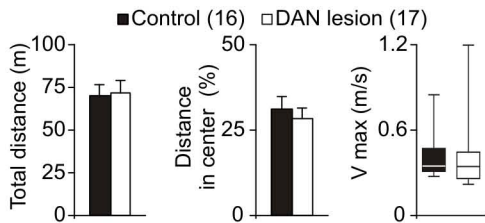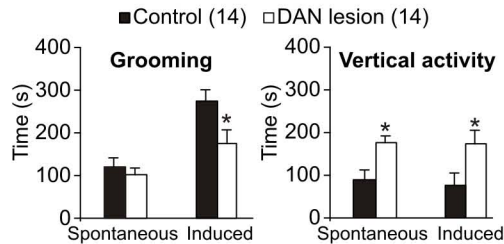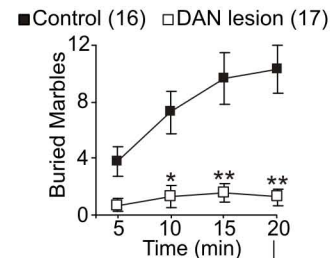
A. Standard Open Field

■ Control (16) □ DAN lesion (17)

Total distance (m) / Distance in center (%) / V max (m/s)

B. Small Arena

■ Control (14) □ DAN lesion (14)

Grooming / Vertical activity

Time (s): Spontaneous, Induced *

Vertical activity: Spontaneous *, Induced *

C. Marble Burying

■ Control (16) □ DAN lesion (17)

Buried Marbles vs Time (min): 5, 10 *, 15 **, 20 **

20 th min.

Control   DAN lesion

D. Elevated Plus Maze

■ Control (22) □ DAN lesion (22)

Total distance (m) *

Time (s): Open arms **, Closed arms **

Entries (counts): Open arms *, Closed arms **

Head dipping time (s) **

Head dipping duration (s) **

E. Large Open Field

■ Control (8) □ DAN lesion (8)

Total distance (m) **

Latency (s): Exit first quadrant **, Visit all quadrants **

Head-body index vs Time (m): * , *

F. Y maze

■ Control (16) □ DAN lesion (17)

Total distance (m) *

Latency (s): Exit first arm **, Visit all arms *

Arm entries (counts) *

Alternation (%) *

A. Social interaction test

■ Control (11) □ DAN lesion (13)

I. First stage

II. Social Novelty

B. Nesting

■ Control (11) □ DAN lesion (13)

C. Hoarding behavior

A. Small Arena

■ Control (9)
□ DAN lesion (11)

Time (s) / Vertical activity

B. Marble Burying

■ Control (10)
□ DAN lesion (12)

Buried Marbles vs Time (min)

C. Nesting

■ Control (8)
□ DAN lesion (8)

Used nestlet (g)

D. Large Open Field

■ Control (11) □ DAN lesion (14)

Total distance (m)

Latency (s) — Exit first quadrant / Visit all quadrants

Head-body index vs Time (m)

E. Y maze

■ Control (9) □ DAN lesion (11)

Total distance (m)

Latency (s) — Exit first arm / Visit all arms

Arm entries (counts)

Alternation (%)

F. Hoarding behavior

■ Control (8) □ DAN lesion (8)

Food hoarded (%)

Arm entries first hour — Food / Empty

Time in arm first hour (m) — Food / Empty

A

Stimulation

Prl Cg Mot

B +1.94

Recording

Shank 1
Shank 2
Shank 3
Shank 4

B +1.0/+0.6

B Control (8)

Prelimbic stimulation

Cingulate stimulation

Motor stimulation

Shank 1
Shank 2
Shank 3

Shank 1
Shank 2
Shank 3

Shank 2
Shank 3
Shank 4

Evoked field potential amplitude (mV)

Distance from maximum response recording site (mm)

C DAN lesion (5)

Prelimbic stimulation

Cingulate stimulation

Motor stimulation

Shank 1
Shank 2
Shank 3

Shank 1
Shank 2
Shank 3

Shank 2
Shank 3
Shank 4

Evoked field potential amplitude (mV)

Distance from maximum response recording site (mm)

D Maximum response recording site

Prelimbic stimulation

Cingulate stimulation

Motor stimulation

Evoked field potential amplitude (mV)

Control   DAN lesion

Control   DAN lesion

Control   DAN lesion

A

Control
D1 MSN   D2 MSN

Control
D1   D2

Control   DAN lesion

D1

D2

DAN lesion
D1   D2

C

Total dendritic lenght (μm)

D1   D2

D

Spines / 10 μm

D1   D2

B

αTH, DAN lesion

cc
mSt
lSt
aca

■ Control (7)
□ DAN lesion (7)

Immunoreactive TH area (%)

Medial   Lateral

MSNs injected with LY

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E

■ Control D1 (10)
□ DAN lesion D1 (11)

Lenght (μm)

Distance from soma (μm)

■ Control D2 (11)
□ DAN lesion D2 (11)

Lenght (μm)

Distance from soma (μm)

■ Control D1 (10)
□ DAN lesion D1 (11)

Number of intersections

Distance from soma (μm)

■ Control D2 (11)
□ DAN lesion D2 (11)

Number of intersections

Distance from soma (μm)