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Research Report

Motivational effects on interval timing in dopamine transporter (DAT) knockdown mice

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ABSTRACT

We examined interval timing in mice that underexpress the dopamine transporter (DAT) and have chronically higher levels of extracellular dopamine (Zhuang et al., 2001). The dopaminergic system has been proposed as a neural substrate for an internal clock, with transient elevations of dopaminergic activity producing underestimation of temporal intervals. A group of DAT knockdown (KD) and littermate wild type (WT) mice were tested with a dual peak procedure. Mice obtained reinforcement by pressing one of two levers after a fixed amount of time (30 or 45 s) had elapsed since lever extension. Only one lever was available at a time, and each lever was associated with a single duration. On occasional probe trials, the DAT KD mice began responding earlier in the interval than WT mice, but showed maximal responding and terminated responding around the same time as the WT mice. Administration of raclopride (0.2, 0.6, and 1.2 mg/kg), a D2 antagonist, eliminated most of the differences between DAT KD and WT mice, suggesting that the effects of chronic DAT downregulation on interval timing were mediated by the D2 receptors. Another cohort of DAT KD mice was trained on a visual attention task, and no deficits were observed, confirming that the changes in timed behavior were not attentionally mediated. Our data are consistent with the view that tonic dopamine affects the sensitivity of an organism to external reward signals, and that this increased motivation for reward of DAT KD mice lowers the threshold for initiating responding in a timing task.

1. Introduction

The neuromodulator dopamine (DA) plays a fundamental role in many critical functions, including learning (Schultz et al., 1997), motivation (Salamone et al., 1994; Niv et al., 2006), and interval timing (e.g., Meck, 1996; Balci et al., 2008a,b). For example, interval timing is altered in several disorders associated with pathological dopaminergic function, including

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Table 1 – ANOVA results for Experiment 1—Phase 1.

Measure	Genotype $F(1,14)$	FI $F(1,14)$	Genotype×FI $F(1,14)$
Start	5.45 *	68.67 ***	0.20 NS
Stop	3.87 NS	141.25 ***	0.48 NS
Peak	4.23 NS	124.39 ***	0.10 NS
Spread	2.07 NS	43.78 ***	0.61 NS
Stop–start	4.04 NS	67.34 ***	1.62 NS
Response rate	19.52 ***	8.57 *	1.53 NS
Start/FI	5.32 *	0.008 NS	2.51 NS

Note. NS: $p \geq 0.05$, *: $p < 0.05$; ***: $p < 0.001$.

schizophrenia (Elvevag et al., 2003), Parkinson's disease (Malapani et al., 1998), Huntington's disease (e.g. Balci et al., 2009a; Paulsen et al., 2004), and ADHD (Yang et al., 2007) (for a review see Balci et al., 2009c). Furthermore, acute administration of dopamine receptor (DAR) agonists often leads to earlier timed responding (e.g., Maricq et al., 1981; Matell et al., 2006), although high doses can be disruptive (Abner et al., 2001; Balci et al, 2008a, b), whereas DAR antagonists produce the converse result (Maricq and Church, 1983). Here, we evaluate interval timing in mice that underexpress the DA transporter (DAT), which have chronically higher levels of tonic DA than appropriate controls (Zhuang et al., 2001).

One explanation for the observed changes in temporally controlled responses following acute administration of direct or indirect DAR agonists is that DA directly drives the speed of an internal clock (e.g., Meck, 1996). Under this *DA-clock* hypothesis, DAR agonists increase, and antagonists decrease, the speed of an internal clock, producing a transitory overestimation or underestimation of elapsing time, respectively (Meck, 1996). This DA-clock hypothesis remains a controversial proposal, which has only received mixed empirical support (for a sampling, see Balci et al., 2008a,b; Odum et al., 2002; Cheng et al., 2007, Tables 1 and 2). For instance, amphetamine and methamphetamine, two dopaminergic agonists often explored in the interval timing literature, induced changes consistent with a faster clock, but they also caused temporal dysregulation at higher doses (e.g., Abner et al., 2001; Balci et al., 2008a,b; Cheng et al., 2007; Maricq and Church, 1983; Saulsgiver et al., 2006). Other recent evidence suggests that DAR agonists might instead act on a decision criterion for response initiation, rather than directly on clock speed (Taylor et al., 2007). This latter finding suggests that the effect of DAR agonists on interval timing might be mediated via the differential coding of reinforcement signals under these agents. This interpretation is also consistent with the observed effects of reward magnitude on response initiation in the peak procedure (Ludvig et al., 2007; Galtress and Kirkpatrick, 2009).

DAT is the primary mechanism for DA clearance from synapses. DAT KD mice express fewer DATs than normal (around 90% less than WT mice) and, as a result, have chronically elevated (70% more) levels of tonic DA in the striatum (Zhuang et al., 2001). Motivation for reward, in particular "wanting" but not "liking", seems to be increased in these mice, with little change in learning processes (Cagniard et al., 2006; Peciña et al., 2003). For example, Cagniard et al. (2006b) reported that following the induction of the DAT knockdown by doxicycline, performance on a goal-directed, operant-responding task acquired prior to the induction was enhanced in the absence of new learning. Similarly, Yin et al. (2006) found no disruption in instrumental learning, but a reduction in stimulus control over responding, in DAT KD mice which they interpreted as increased incentive motivation.

DAT regulation also plays a role in DA-related disorders, such as ADHD. Compounds that block DAT and increase extracellular DA (e.g., methylphenidate) are effective in treating ADHD (Biederman and Faraone, 2005)—a disorder in which timing deficits are observed in addition to the more prominent attentional deficits (Barkley et al., 1997; Rommelse et al., 2008; Toplak et al., 2006; Yang et al., 2007). Furthermore, an association between human DAT gene polymorphisms and ADHD has been reported (e.g., Cook et al., 1995). Following this rationale, we tested DAT KD mice in two different cognitive domains: interval timing and visual attention.

Interval timing was tested using a *dual peak procedure*—a task that permits the simultaneous assessment of temporal accuracy and temporal uncertainty with two different time intervals (Catania, 1970; Drew et al., 2003; Roberts, 1981). For this procedure, mice were trained to respond for reinforcement on two different levers, each associated with a different fixed time interval (FI). On FI training trials, mice were reinforced the first time they pressed the lever after the FI for that lever elapsed. Interspersed among these training trials were occasional peak (test) trials, when no reward was available which lasted substantially longer than normal trials and thus allowed the observation of the change in responding as a function of trial time, well past the ordinary time of reinforcement. We used a dual rather than the single peak procedure in order to eliminate or delay the development of possible habitual or automatic temporal processing and concomitant loss of cognitive control over anticipatory

Table 2 – ANOVA results for Experiment 1—Phase 2.

Measure	Genotype $F(1,13)$	FI $F(1,13)$	Dose $F(1,13)$	Block $F(2, 25)$	Genotype×Dose $F(1,13)$	Genotype×FI $F(1,13)$	Genotype×FI×Dose $F(2,13)$	Genotype×Block×Dose $F(4, 25)$	Genotype×FI×Block×Dose $F(4,25)$
Start	13.82 **	86.52 ***	15.46 **	0.65 NS	4.65 NS	0.68 NS	0.23 NS	3.05 *,#	0.10 NS
Stop	1.75 NS	324.57 ***	0.03 NS	2.35 NS	0.82 NS	0.47 NS	0.32 NS	2.20 NS	0.62 NS
Peak	7.17 *	227.14 ***	3.51 NS	1.08 NS	2.92 NS	1.77 NS	0.94 NS	2.51 NS	0.31 NS
Spread	7.15 *	60.85 ***	5.57 *	3.77 *	0.10 NS	1.85 NS	0.98 NS	2.91 *,#	2.30 NS
Response rate	39.50 ***	29.14 ***	46.40 ***	3.10 NS	1.29 NS	6.04 *	1.41 NS	10.79 ***,#	0.04 NS

Note. NS: $p \geq 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; # see simple effects in Table 3.