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INCREASED IMPULSIVE BEHAVIOR AND RISK PRONENESS FOLLOWING LENTIVIRUS-MEDIATED DOPAMINE TRANSPORTER OVER-EXPRESSION IN RATS' NUCLEUS ACCUMBENS

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Abstract—Multiple theories have been proposed for sensation seeking and vulnerability to impulse-control disorders [Zuckerman M, Kuhlman DM (2000) Personality and risk-taking: Common biosocial factors. J Pers 68:999–1029], and many of these rely on a dopamine system deficit. Available animal models reproduce only some behavioral symptoms and seem devoid of construct validity. We used lentivirus tools for over-expressing or silencing the dopamine transporter (DAT) and we evaluated the resulting behavioral profiles in terms of motivation and self-control. Wistar adult rats received stereotaxic inoculation of a lentivirus that allowed localized intra-accumbens delivery of a DAT gene enhancer/silencer, or the green fluorescent protein, GFP. These animals were studied for intolerance to delay, risk proneness and novelty seeking. As expected, controls shifted their demanding from a large reward toward a small one when the delivery of the former was increasingly delayed (or uncertain). Interestingly, in the absence of general locomotor effects, DAT over-expressing rats showed increased impulsivity (i.e. a more marked shift of demanding from the large/delayed toward the small/soon reward), and increased risk proneness (i.e. a less marked shift from the large/uncertain toward the small/sure reward), compared with controls. Rats with enhanced or silenced DAT expression did not show any significant preference for a novel environment. In summary, consistent with literature on comorbidity between attention-deficit/hyperactivity disorder and pathological gambling, we demonstrate that DAT over-expression in rats' nucleus accumbens leads to impulsive and risk prone phenotype. Thus, a reduced dopaminergic tone following altered accumbal DAT function may subserve a sensation-seeker phenotype and the vulnerability to impulse-control disorders.

Key words: dopamine transporter, sensation seeking, intolerance to delay, probabilistic delivery.

Sensation seeking is a supertrait expressed by some individuals who are often involved in very extreme behaviors and risky activities, such as exaggerate sexual behavior, reckless driving, drug misuse, and pathological gambling (PG) (Zuckerman and Kuhlman 2000). Noteworthy, there is substantial genetic influence in these sensation-seeking traits: for instance, the dopamine (DA) D4 receptor is the prototypic polymorphic gene subserving a background for novelty seeking (Ebstein et al., 1996), drug abuse (Laucht et al., 2005) and vulnerability to attention-deficit hyperactivity disorder (ADHD) (Faraone et al., 2005) and PG (Comings et al., 1999).

ADHD is heterogeneous, is highly heritable, and results from complex gene–gene and gene–environment interactions. ADHD affects 1–4% of children, representing a social burden (Biederman, 1998). Beside core symptoms of hyperactivity, impulsivity and impaired sustained attention, which are also found in other syndromes, ADHD children often display accompanying behavioral difficulties, including a disinhibited conduct and obsessive–compulsive symptoms (Snyder et al., 2002). According to the dominant model, ADHD is viewed as an executive dysfunction (Doyle, 2006; Castellanos et al., 2006; Willcutt et al., 2005), but alternative accounts present ADHD as a motivational dysfunction (Sonuga-Barke, 2005), arising from altered reward processes within fronto-striatal circuits (Sagvolden and Sergeant, 1998; Oades, 1998). Very frequently comorbid with ADHD (Sood et al., 2003), PG is a chronic, progressive disorder with a prevalence of 1–4%, and is rapidly emerging as a mental health concern among Western civilizations. PG is essentially an impulse-control disorder, commonly comorbid to compulsive buying and compulsive sexual behavior (Black and Moyer, 1998). PG may also be conceptualized as an addictive disorder and/or as part of the obsessive–compulsive spectrum (Lowengrub et al., 2006): in fact, more than half of PG patients have an obsessive–compulsive, schizotypal and paranoid personality, as well as substance abuse/dependence problems (Hollander et al., 2000, 2005).

The deficits in cognitive control and/or motivation seen in sensation seeking, ADHD and PG highlight the importance of DA and 5-HT systems' disruption in these disorders. Indeed, 5-HT subserves (dis)inhibition via cortical control over behavioral initiation, which is important for the difficulty in controlling instinctive reactions and temptations (Hollander et al., 2000). DA systems are involved in many reinforcement-related processes: 1) they subserve motivation to sustain effort toward positive (attracting) and negative (avoided) stimuli, and may subserve the addictive/

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Abbreviations: ADHD, attention-deficit/hyperactivity disorder; DA, dopamine; DAT, dopamine transporter; GFP, green fluorescent protein; LL, large/late; LLL, large/luck-linked; NAcc, nucleus accumbens; NOP, non-operated; PG, pathological gambling; SIL, silenced; SS, small/soon or small/sure; TBST, 10 mM Tris, 150 mM NaCl, and 0.1% Tween-20.

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compulsive component of these disorders (Hollander et al., 2000); 2) they are involved in forming predictions about future outcomes, in that DA neuronal firing is linked to detecting discrepancies between actual and expected outcomes (Schultz et al., 1997). Learning when, or in which contexts, to expect what, is a critical event for adjusting behavior appropriately (Casey and Durston, 2006) when predicted outcomes are violated. Likewise, even if the ability to approach/avoid salient stimuli and/or to predict/detect (ir)regularities in the environment is intact, any inefficient top-down inhibitory control could result in poor feedback regulation of behavior. The variability of ADHD sub-populations reported in the medical literature may be partly due to differences in the relative dysfunction between DA and 5-HT systems (Sagvolden and Sergeant, 1998; Oades 1998).

These two systems are indeed strong candidates for pathogenesis of sensation seeking, ADHD and PG, but there is not a universally valid animal model yet (Russell et al., 2005; Sagvolden et al., 2005). Current animal models mimic distinct behavioral characteristics of these disorders, bearing different neural defects, and are mostly genetic, like the spontaneously hypertensive rats (SHR), the dopamine transporter (DAT) knockout mice, the synaptosomal-associated protein 25 kDa mutant mice, or mice expressing a mutant thyroid receptor. The validity of these models is however limited, due to lack of construct validity, thus promoting the efforts to identify novel animal models. Many studies have described modifications on DAT expression at least in ADHD (Fischman et al., 1998; Dougherty et al., 1999; Jucaite et al., 2005; Bannon 2005). We have tested the efficacy of lentiviral tools, driving the expression of DAT or siRNAs targeted against DAT mRNA in rats. It is well known that DAT gene is expressed in the midbrain (A9 and A10 areas), and proteins are then transported into the dorsal striatum and nucleus accumbens (NAcc). The aim of this study was to investigate behavioral effects of over-expression and/or down-regulation of DAT in this specific brain area, driving motivated behavior and impulsivity, rather than in the whole dopaminergic system. As a matter of fact, it is well established that DAT mRNA can be found in the NAcc (Maggos et al., 1997). Moreover, the capacity of lentiviruses to be retro-transported is well established (Szulc et al., 2006). We have demonstrated previously that lentivirus-transfected genes undergo a retrograde transport from the NAcc (Boyer and Dreyer, 2008), thus enabling DAT gene and siRNA expression within the ventral tegmental area (VTA). The *in vivo* efficacy of our DAT enhancer and of the three silencing siRNAs has been demonstrated as well (Boyer and Dreyer, 2008). DAT protein product is then transported and expressed within the NAcc (Boyer and Dreyer, 2008). Such an approach enabled the evaluation of behavioral changes, associated with focal meso-limbic DAT over-expression and/or suppression (Mazei-Robinson and Blakely, 2006).

We investigated here the relevant behavioral symptoms produced by enduring alteration of DAT function (Madras et al., 2005; Spencer et al., 2005; Thapar et al., 2005). Accumbal DAT suppression was expected to enhance tonic DA transmission, compared with controls, whereas its over-expression would drastically reduce synaptic DA levels. The NAcc was selected as the inoculation site since its lesions are known to cause hyperactivity and impulsive choice in rats (Cardinal et al., 2004). Our aim was to establish whether modulation of genetic DAT levels would lead rats to exhibit a deficit in motivational and/or impulse-control endpoints. Thus, to probe the resulting phenotype, animals were tested for their drive to seek for novelty, intolerance to delay, and temptation to gamble (Laviola et al., 2003; Adriani and Laviola, 2006). We demonstrate here that a peculiar behavioral profile was specific of DAT+ rats. To investigate the molecular correlates of such profile, NAcc samples, collected at sacrifice of DAT+ and control animals, were processed for DAT gene expression and protein density.

EXPERIMENTAL PROCEDURES

Animal experimental protocols were approved by institutional Animal Survey Board, on behalf of Ministry of Health, and were in close agreement with European Community Directives and Italian law. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to use alternatives to *in vivo* testing.

Subjects, breeding, and rearing conditions

Upon arrival, Wistar male rats (Harlan, Correzzana, Italy) weighing 200–250 g were housed in pairs, inside Plexiglas Macrolon III cages with metal tops and a sawdust bedding. Rats were located in an air-conditioned room (temperature 21±1 °C, relative humidity 60±10%), with a 12-h light/dark cycle (lights off from 21:00–09:00 h). Water and food (Enriched Standard Diet, Mucedola, Settimo Milanese, Italy) were available *ad libitum*. After 2 weeks of acclimation, animals were inoculated with lentiviral vectors for gene transfer. After 2 weeks of post-surgical recovery, they were tested with the impulsivity (i.e. delay-intolerance) and risk-proneness (i.e. probabilistic-delivery) operant tasks and for novelty seeking.

Construction of Lenti-DAT, stereotaxic surgery

The DAT cDNA (GenBank accession no. 012694) was amplified by reverse transcription. The cDNA was then PCR amplified from pCMV5rDAT as a template and the following primers: 5′-CC*G TTA AC*A TG**G ACT ACA AAG ACG ATG ACG ATA AG**C CAG TAA GAG CAA ATG C-3′ and 5′-CCG *CTC GAG* CGG TTA CAG CAA CAG CCA GTG ACG-3′. The forward primer contains an *Hpa*I restriction site followed by a FLAG epitope sequence (in bold) and the rat 5′ DAT cDNA specific sequence, the reverse primer contains the rat 3′ DAT cDNA specific sequence, a stop codon and a *Xho*I restriction site. The PCR product was digested with *Hpa*I and *Xho*I and cloned into *Hpa*I/*Xho*I restriction sites into pTK431 (Bahi et al., 2005a,b), which expresses the gene of interest under control of a Doxy-Off promoter: namely, doxycycline can switch off the Lenti-DAT-induced over-expression. To silence the DAT expression *in vivo*, three targets were designed according to the DAT mRNA sequence, and similarly cloned into pTK431. The following targets within the DAT sequence were selected: 1st target, bp 19–47; 2nd target, bp 864–890; 3rd target, bp 1827–1854. This selection (http://katahdin.cshl.org:9331/RNAi/html/rnai.html) was based on the Hannon's design criterion. To each oligo, a *Xho*I restriction site was added at 3′ and a U6-3′-specific 10mer at 5′. Using the pSilencer 1.0-U6 (Ambion, UK) as a template and a U6 promoter-specific forward primer containing *Bam*HI restriction site (5′-GC*G GAT CC*C GCT CTA GAA CTA GTG C-3′), each siRNA target was added to the mouse U6