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Short communication

Early postnatal experience and *DRD2* genotype affect dopamine receptor expression in the rat ventral striatum☆

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HIGHLIGHTS

- We examine the interaction between variants in the gene coding for dopamine D2 receptor and early life adversity on D2 expression.
- We find that early life adversity, within one variant of the *DRD2* gene, affects D2 expression.
- We propose that early life adversity will alter dopamine transmission and behavior to a greater extent based on genetic background of individuals.

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ABSTRACT

Dopamine systems can be altered by experiences such as early life adversity. The intensity of these effects seems to vary as a function of interactions between genetic and environmental influences. In a series of experiments we have investigated the effects of genetic variants and early life adversity on several biobehavioral outcomes. Here we investigated the presence of single nucleotide polymorphisms (SNPs) in the gene coding for dopamine D2 receptors (*DRD2*) and the interaction between these variants with early life adversity on the expression of D2 receptors in the striatum. Time-mated pregnant female rats underwent restraint stress (gestational days 10–21) or were left undisturbed. Following parturition rat pups were maternally reared (MR) or artificially reared (AR). Subsequent to adult behavioral testing, rats were genotyped and their brains were processed (autoradiography) for D2 receptor expression. We found three variants in the *DRD2* gene and these variants interacted with early adversity to affect D2 receptor expression in the nucleus accumbens. Specifically, artificially reared rats with AG *DRD2* variant showed significantly higher D2 expression compared to mother reared rats with the AG *DRD2* variant as well as the artificially reared rats with a GG *DRD2* variant. These findings show that adult D2 expression is significantly influenced by the interaction of *DRD2* SNPs and early developmental factors. These finding may explain why there are significant individual differences in the impact of early life adversity on dopamine-dependent processes and disorder vulnerabilities.

Brain dopamine (DA) systems are functionally heterogeneous as they are involved in sensory, cognitive, motor, and motivational processes [see 22]. Significant adverse events in early life can alter these DA systems and subsequently change the expression of naturally occurring behaviors (e.g., maternal behavior) and increase pathology liability (e.g., addiction) [4,12,13,15,16]. For example, early life adversity, in the form of maternal deprivation, is associated with greater amphetamine and K^+ stimulated DA release in the nucleus accumbens (NAcc) [11]. Furthermore, we have found that rats reared in social isolation from the mother and littermates, using an artificial rearing (AR) procedure, show greater basal DA levels in the NAcc [1]. These adverse early life experiences also produce changes in DA-mediated behaviors. For example, maternally deprived rats show an increase in novelty and amphetamine-induced locomotor activity as well as an increase in impulsivity, responding for primary reinforcers, and cues associated with primary reinforcers [15–17]. However, the effects of early adversity on DA systems and related behaviors are variable [e.g., 8]. Further, animals that experience early adversity

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are characterized by greater variation in measures of DA function and DA-mediated behaviors. The variable effects of maternal deprivation or social isolation are likely due to complex interactions between the early experiences and genetic background variations [8,9].

An important source of genetic variability comes from single nucleotide polymorphisms (SNPs). Individuals in a population have different alleles coding for specific genes, such as for proteins forming neurotransmitter receptors. Through non-transcriptional mechanisms, such as mRNA stability, translational efficiency and regularity, SNPs can lead to subtle but significant changes in protein (e.g., neurotransmitter receptor) expression and structure [14,21].

DA acts on five receptor subtypes (D1–5) and indirectly influences other neurotransmitter systems, most notably glutamate [24]. The DA D2 receptor is a G-protein receptor coupled to inhibitory cyclic AMP. Pre-synaptic D2 receptor (autoreceptors) stimulation decreases neuronal firing, synthesis, and release of DA [25] while stimulation of the post-synaptic D2 receptors results in excitation (e.g., increase in locomotor activity) [2]. Hence, D2 receptors play a critical role regulating and mediating DA related activity. D2 receptors are most abundantly expressed in the dorsal and ventral striatum while lower levels are expressed elsewhere in the brain [19].

In humans, the gene coding for the D2 receptor (*DRD2*) is found on chromosome 11q23.1 and variants of the human *DRD2* are associated with behavioral and pathology differences [14,21,23]. Until this report, it was unknown if similar variants in *DRD2* existed in rats and how such gene variations might interact with environmental events, such as early adversity, to produce changes in D2 expression or DA dependent behaviors. This brief report is part of a large multi-institutional and multi-disciplinary set of studies (MAVAN) aimed at investigating gene–environment interactions [see 3]. We report here that *DRD2* variants are present in a normal population of Sprague-Dawley rats and that these variants significantly interact with early adversity, in the form of early social isolation, to alter behavior and D2 expression in the ventral striatum.

Fifty-one male Sprague-Dawley rats were assessed. They were born and raised at the University of Toronto Mississauga (stock originally obtained from Charles River Farms, St. Constan, Quebec, Canada). Housing rooms were maintained on a 12:12 h light:dark cycle (lights on 0800 h) in a temperature (22 °C) and humidity (50–60%) controlled rooms. Beginning on postnatal day (PND) 21, rats were pair-housed in Plexiglas cages (20 cm × 43 cm × 22 cm), with food (Purina Rat Chow) and water available ad libitum.

Procedures employed here were previously described [7]. Briefly, 17 virgin female rats were time-mated and left undisturbed until gestational day (GD) 10. From GD 10 to 21, some of the dams (prenatal stress group—PS; $n = 9$) underwent restraint stress (4 h/day). PS dams were daily weighed and subsequently placed in a Plexiglas restrainer (8 cm diameter × 20 cm length) for 4 h at random times between 0900 and 1800 h. The length of the restrainers was adjustable in order to accommodate the size ranges of dams. This procedure was done with an aim of limiting, but not prohibiting, movement of pregnant rats. Importantly, the apparatus did not constrict dams' abdomen. Dams in the non-stressed group (NS) were left undisturbed ($n = 8$). On PND 0 litters were culled to 14 pups (7 males and 7 females). On PND 4, 3 male pups were removed from the nest and 2 were implanted with a cheek cannula and raised artificially. The third male was sham operated and returned to the mother (MR-SHAM). Remaining pups were not manipulated and constituted control subjects—mother reared control (MR-CON; only 1 subject/group/litter was used). MR-SHAM and MR-CON groups were not different from each other and were combined into one mother-reared group (MR; $n = 26$; see below).

Once the cheek cannulae were implanted, AR pups were individually housed in plastic cups (11 cm diameter × 15 cm deep), which floated in temperature controlled water bath (36–40 °C). Pups' tubing was connected to time-controlled infusion pumps (Harvard Apparatus Syringe, PHD 2000) which delivered milk (Messer diet) for 10 min every hour, 24 h/day. AR rats were randomly assigned to either AR-MIN ($n = 13$) AR-MAX ($n = 12$) groups. AR-MIN pups' anogenital region was stimulated with a wet camel-hair paintbrush twice a day for 30 s each to stimulate urination and defecation. AR-MAX pups also received the same anogenital stimulation regimen but received additional 8 (2 min) general body stimulations. Stimulations were carried out PND 4–16. If AR pups' cheek cannula became dislodged PND 17–21, they were individually placed in small mouse cages, kept on heating pads, provided with fresh milk formula and closely monitored for weight gain. Otherwise, their cheek cannula was removed on PND21. On PND 21, all rats were weaned and pair-housed with same age and sex rats (control) and left undisturbed until PND 60.

As adults rats were tested on several behavioral tests [see 7]. Given that these behavioral effects have been described previously and that the manipulations described in this report did not have a major impact on our behavioral endpoints, the focus here is on the expression of D2 receptors. Following termination of behavioral testing rats were rapidly decapitated and their brains and hearts (see DNA procedures below) were dissected out and flash frozen on dry ice until further processing. 20 μm brain sections throughout the striatum (+1.2 from Bregma [20]) were thaw mounted onto gelatin-coated slides and stored at −80 °C prior to further processing. Tissue sections were thawed and incubated for 10 min in assay buffer (50 mM Tris–HCl, 120 mM NaCl, 1 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, pH 7.4) at room temperature. Sections were incubated for 2 h with 1 nM [3H]YM-09151-2 (Perkin Elmer, 85.0 Ci/mmol) and 50 nM 18-OH-DAPT (5-HT1A receptor blocker, Sigma) for specific binding or with the addition of 1 μM (+)butaclamol (Sigma) for non-specific binding. Slides were then dipped in an ice-cold assay buffer, washed, dried, and exposed to tritium-sensitive Biomax MR film (Amersham) for 5 days before being developed. The level of D2 expression in the dorsal striatum (CPu) and NAcc shell and core regions was determined by measures of the optical density on autoradiograms using an MCID image analysis system (St Catherine, ON) and appropriate standards. For each rat, a minimum of 3–4 sections were analyzed per region of interest.

Genomic DNA was extracted from 25 mg of heart tissue for each of the 51 rats using Sigma's GenElute Mammalian Genomic DNA Miniprep kit (St. Louis, MO, USA) according to the manufacturer's instruction. DNA amounts were determined using Nanodrop ND-1000 spectrophotometer (Wilmington, DE, USA). A specific primer pair, forward primer 5′-TCTGCTAGCTAGCTCTTGGG-3′/reverse primer 5′-GTGGTCTGCAAAGCCTTCTC-3′, was designed to amplify a 580 bp region in the rat DA receptor gene, *DRD2*. This region included exon 2 and upstream SNPs described in NCBI as G/A, rs13448058 and rs13448059, respectively. PCR amplification of the 580 bp region was performed as described in [3]. The final PCR products were then purified using Qiaquick PCR Purification Kit (Qiagen, Maryland, USA). See [3] for DNA sequencing and genotyping conditions.

Rats were polymorphic for the rs13448058 site with the AA genotype displaying the most infrequent group (only 3 observations). Rats with this genotype were excluded from the analyses (see below). Furthermore, we identified a new single nucleotide polymorphism 10 bp upstream of rs13448058 due to an insertion/deletion of adenine (A). This SNP was linked to the rs13448058 SNP. The only known SNP (a synonymous C/T SNP identified as

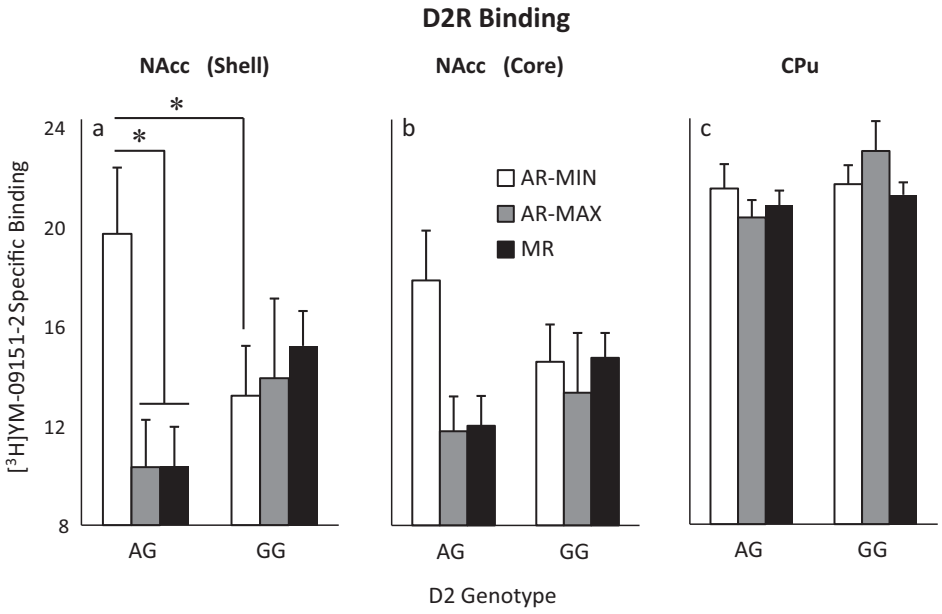

Fig. 1. D2 receptor binding (mean + sem) in 3 areas of the striatum across 3 postnatal treatment groups and *DRD2* variants. (a) For NAcc shell there was a significant postnatal treatment × SNP interaction. *One way ANOVAs, within each genotype group, and subsequent post hoc tests revealed that AR-MIN rats, within the AG SNP group, have significantly greater D2 expression then AR-MAX and MR rats ($p < 0.05$). *Planed comparison also revealed significant group differences between AR-MIN rats carrying different *DRD2* variants ($p < 0.05$). (b) D2 expression in NAcc core; and (c) D2 expression in CPu.

rs8154872) found in the coding region of *DRD2*, which is located in exon 7, did not show variation in the rat population tested here.

MR-SHAM and MR-CON groups were not statistically different from each other on any measures of interest and were thus combined into one group: mother-reared (MR). D2 receptor expression and locomotor activity data were analyzed using 3 way ANOVAs (prenatal stress × postnatal treatment × *DRD2* SNPs) and where appropriate, followed by one-way ANOVAs and Tukey post hoc tests. The level of statistical significance was set at $\alpha < 0.05$.

Of the 51 Sprague-Dawley rats assessed, 24 (47.1%) were homozygous for the G allele (referred to here as GG rats; coming from 13 different litters), 3 (5.8%) were homozygous for the A allele (AA; all from the same litter), and 24 (47.1%) were heterozygous (AG; coming from 12 different litters). Therefore, we have observed 3 genetic variants of *DRD2* with GG and AG variants being predominant in our sample of rats. Minority (35%) of dams produced pups with concordant DRD2 SNPs (in terms of the pups sampled), while the rest of the dams produced pups with discordant SNPs.

Univariate ANOVAs (prenatal stress × postnatal rearing × SNPs) were utilized to assess group differences in D2 binding in the NAcc shell and core, and caudate nucleus (CPu). For the NAcc shell, we did not find significant main effects of any factor but there was a significant postnatal rearing × *DRD2* SNPs interaction [$F_{(2,24)} = 5.39$, $p < 0.05$]. As shown in Fig. 1a, within the GG genotype, AR-MIN rats have significantly higher D2 expression ($p < 0.05$) than do MR or AR-MAX rats. We also performed a planned comparison between AG and GG rats, within the AR-MIN group. No group differences were found within the AG genotype. Within the NAcc core there were no significant main effects or interactions; however, as shown in Fig. 1b the pattern of effects was similar to that observed for the NAcc shell [postnatal rearing × SNPs interaction, $F_{(2,24)} = 2.5$, $p = 0.1$]. Finally, with respect to the D2 levels in the CPu there were no significant main effects; however there was a prenatal stress × postnatal rearing interaction [$F_{(2,24)} = 3.56$, $p < 0.05$]. Follow-up ANOVAs for postnatal group differences within each of the prenatal stress conditions did not reveal any significant group differences (Fig. 1c). See Fig. 2 for photomicrographs of the D2 binding in striatum.

The purpose of the present experiment was to investigate the presence of *DRD2* variants in Sprague-Dawley rats and how these variants might interact with early life adversity to affect of D2 expression. We report several novel findings. First, we found 3 variants of the gene coding for the D2 receptor in a normal population of Sprague-Dawley rats. Secondly, we found that postnatal, but not prenatal, adversity interacts with *DRD2* SNPs in producing changes in adult D2 expression in the NAcc—a site critically involved in reward learning processes. Additional somatosensory stimulation provided to AR-MAX pups reversed the effect of postnatal adversity as observed in AR-MIN rats.

Our finding that D2 expression varies as a function of *DRD2* genotype corresponds with a previous study in healthy human subjects where *DRD2* polymorphisms accounted for individual differences in D2 densities [14]. Hence, irrespective of environmental events, *DRD2* polymorphisms are associated with changes in D2 densities. The precise mechanism of these effects is unknown, but it has been hypothesized that different *DRD2* versions can, through non-transcriptional mechanism, such as mRNA stability, translational efficiency and regularity, lead to subtle but significant changes in receptor expression and structure [14,21].

D2 receptors are located both pre- and post-synaptically and one of the shortcomings of the above-mentioned human studies as well as our study is the lack of procedural differentiation of pre- and post-synaptic D2 densities. Stimulation of pre-synaptic D2 receptors tends to reduce synthesis and release of DA from the pre-synaptic neuron and hence has a general inhibitory function. Conversely, stimulation of the post-synaptic D2 receptors, which are often linked with D1 receptors, tends to have stimulating effects (e.g., increased behavioral output). AR-MIN rats show a behavioral profile consistent with increased DA transmission as they are more impulsive and more attracted to reward-related cues—a process mediated by DA transmission in the NAcc [10,15,17]. Secondly, AR-MIN rats are more sensitive to the locomotor-inducing effects of amphetamine (DA releaser) but not methylphenidate (DA

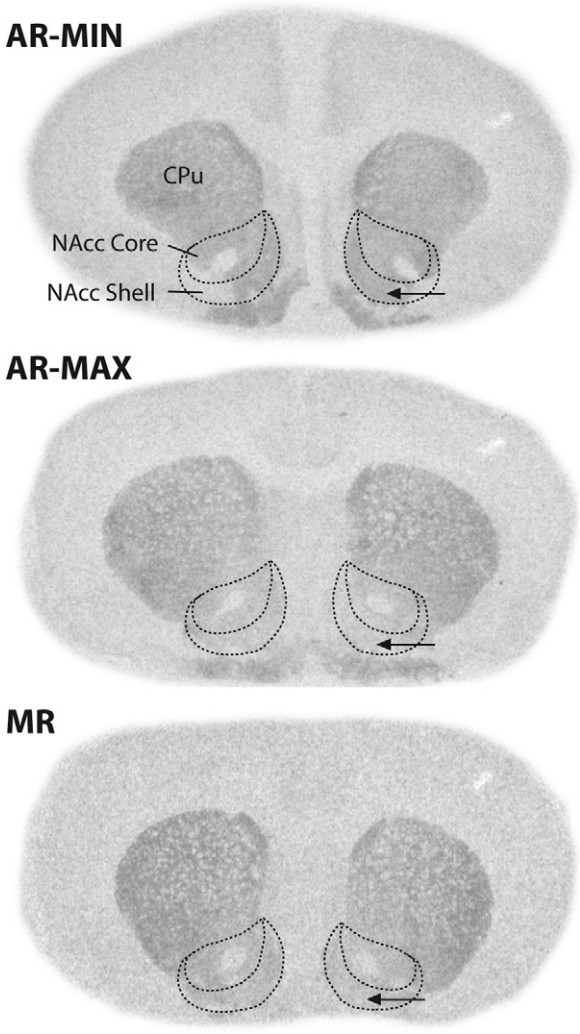

Fig. 2. Photomicrographs showing D2 binding in striatum in representative AR-MIN, AR-MAX and MR rats within AG *DRD2* genotype. Note the location of areas of interest (arrows) and greater intensity (darker) staining in the NAcc shell of AR-MIN representative compared to AR-MAX and MR representatives.

transporter blocker) [16]. Lastly, AR-MIN rats have greater baseline DA levels in the NAcc [1]. These findings suggest that early adversity rats (i.e., AR-MIN treatment) have poorer DA negative feedback, mediated by presynaptic D2 receptors. Our report here shows that AR-MIN rats' D2 receptor densities vary considerably based on their *DRD2* variants. The interacting effects between *DRD2* variants and early life treatments might explain our observations of greater variability in DA-mediated behavior in AR-MIN rats.

How might early adversity interact with DA genetics to produce enduring changes in this system? DA neurons begin to form during early-to-mid gestation (embryonic day 10). Soon after, these neurons start producing DA precursors (tyrosine hydroxylase, L-tyrosine, L-DOPA) and eventually DA. These neurons undergo two phases of apoptosis: within a day or two of birth and around PND 12 [5]. We have previously found indirect evidence that the AR procedure disrupts apoptotic processes (AR rats have greater number of neurons) [6]; therefore, early life adversity may reduce apoptosis of DA neurons and synaptic pruning and subsequently increase D2 expression. Additional somatosensory stimulation provided to the pups may help reverse the effects of early adversity on D2 expression by increasing apoptosis since we have previously found that additional stroking stimulation increases the number of apoptotic cells in several brain regions [6]. Secondly, adverse early life events, such as maternal deprivation and social isolation, potentiate glucocorticoid (GC) activity and GCs have been found to upregulate tyrosine hydroxylase synthesis and DA production [18,19]. Thus early life adversity may produce a hyperdopaminergic state resulting in subsequent alterations in DA receptors.

Given dopamine's role in motivated behaviors our findings are of interest to cases of aberrant motivational states. Early life adversity significantly increases the liability for several behavioral disorders such as addiction. However, while some individuals that experience early life adversity do become addicted many do not. These differences in genes coding for the D2 receptor might account for some variabilities in disorder liabilities.

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