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Dopamine $D_{2/3}$ Receptor Availabilities and Evoked Dopamine Release in Striatum Differentially Predict Impulsivity and Novelty Preference in Roman High- and Low-Avoidance Rats

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Abstract

Background: Impulsivity and novelty preference are both associated with an increased propensity to develop addiction-like behaviors, but their relationship and respective underlying dopamine (DA) underpinnings are not fully elucidated.
Methods: We evaluated a large cohort (n = 49) of Roman high- and low-avoidance rats using single photon emission computed tomography to concurrently measure in vivo striatal $D_{2/3}$ receptor ($D_{2/3}R$) availability and amphetamine (AMPH)-induced DA release in relation to impulsivity and novelty preference using a within-subject design. To further examine the DA-dependent processes related to these traits, midbrain $D_{2/3}$-autoreceptor levels were measured using ex vivo autoradiography in the same animals.
Results: We replicated a robust inverse relationship between impulsivity, as measured with the 5-choice serial reaction time task, and $D_{2/3}R$ availability in ventral striatum and extended this relationship to $D_{2/3}R$ levels measured in dorsal striatum. Novelty preference was positively related to impulsivity and showed inverse associations with $D_{2/3}R$ availability in dorsal striatum and ventral striatum. A high magnitude of AMPH-induced DA release in striatum predicted both impulsivity and novelty preference, perhaps owing to the diminished midbrain $D_{2/3}$-autoreceptor availability measured in high-impulsive/novelty-preferring Roman high-avoidance animals that may amplify AMPH effect on DA transmission. Mediation analyses revealed that while $D_{2/3}R$ availability and AMPH-induced DA release in striatum are both significant predictors of impulsivity, the effect of striatal $D_{2/3}R$ availability on novelty preference is fully mediated by evoked striatal DA release.
Conclusions: Impulsivity and novelty preference are related but mediated by overlapping, yet dissociable, DA-dependent mechanisms in striatum that may interact to promote the emergence of an addiction-prone phenotype.

Key Words: Dopamine, D2/3 receptors, impulsivity, novelty preference

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Significance Statement

This study extends previous observations that impulsivity is inversely related to striatal $D_{2/3}$ receptor ($D_{2/3}R$) availability by examining the presynaptic aspect of dopamine (DA) function as well as determining the neurochemical underpinnings of novelty preference using a within-subject design in rats. Impulsivity was related to both a reduced striatal $D_{2/3}R$ availability and heightened amphetamine (AMPH)-induced DA release. Novelty preference was related to impulsivity. A high magnitude of AMPH-induced DA release in striatum predicted both impulsivity and novelty preference. Mediation analyses revealed that while striatal $D_{2/3}R$ availability and evoked DA release are both significant predictors of impulsivity, the effect of striatal $D_{2/3}R$ availability on novelty preference is fully mediated by an enhanced AMPH-induced DA release. As both impulsivity and novelty preference predict individual susceptibility to compulsive drug use, these data further our understanding of the molecular and cellular bases of linked phenotypes influencing vulnerability to addiction.

Introduction

Impulsivity and novelty/sensation seeking are prominent characteristics of human personality that have been associated with substance use disorders (Crawford et al., 2003; Ersche et al., 2010). In rats, impulsivity and novelty-related behaviors have been associated with an increased propensity to initiate or develop compulsive drug use (Belin et al., 2008, 2011). However, the relationship between these addiction-related traits is unclear. In rodents, novelty-related behaviors are commonly assessed as the locomotor response to an inescapable novel environment (i.e., novelty reactivity) or as the preference for an escapable novel environment (i.e., novelty preference). Although preference for and reactivity to novelty are both linked to an increased propensity to use drugs (Marinelli and White, 2000; Meyer et al., 2010), these 2 behaviors have different predictive implications in the addiction process, with novelty reactivity predicting initiation of drug use (Belin et al., 2008) and novelty preference predicting compulsive drug use (Belin et al., 2011). Moreover, these 2 measures of response to novelty are not correlated with each other (Lukkes et al., 2016; Hughson et al., 2019), suggesting that they are mediated by different neurobiological processes. Consistent with this distinction, while there is no association between novelty reactivity and impulsivity (Loos et al., 2009; Molander et al., 2011), impulsive rats display a higher preference for novel objects and environment (Molander et al., 2011), suggesting some association with novelty preference but not novelty reactivity. However, due to the paucity of studies, the relationship between impulsivity and novelty preference is still unclear. Despite this, the predictive value of impulsivity and novelty preference for developing compulsive drug use in rats (Belin et al., 2008, 2011) suggests the existence of common underlying neurobiological mechanisms. In the search for molecular markers of addiction-related phenotypic traits, impulsivity in rodents has been consistently linked to low levels of dopamine (DA) $D_{2/3}$ receptors ($D_{2/3}R$) in the striatum (Dalley et al., 2007; Jupp et al., 2013; Caprioli et al., 2013, 2015), and low striatal $D_{2/3}R$ availabilities are predictive of drug self-administration (Dalley et al., 2007), supporting the view that low $D_{2/3}R$-dependent DA signaling may represent a common mechanism underlying impulsivity and vulnerability to addiction (Trifilieff and Martinez, 2014). Strikingly, the majority of studies investigating the neurobiological underpinnings of impulsivity have focused on striatal postsynaptic $D_{2/3}R$, and few studies have examined the presynaptic aspect of DA signaling. Conversely, most studies investigating the molecular mechanisms of novelty-related behaviors have focused on novelty reactivity and presynaptic DA signaling to show that high novelty-reactive animals exhibit elevated basal levels of striatal DA and increased DA release in response to psychostimulants (Hooks et al., 1992; Verheij et al., 2008) compared with low novelty-reactive animals. To our knowledge, no study has yet investigated the dopaminergic underpinnings of novelty preference.

The selectively-bred Roman high- (RHA) and low-avoidance (RLA) rat lines exhibit innate variations in addiction-related traits, providing a valuable tool to investigate associations between addiction-related traits and their underlying molecular mechanisms. Differences in indices of DA signaling have been identified between these rat lines, which could play a role in the higher levels of impulsivity, novelty-related, and drug-seeking behaviors displayed by RHA relative to RLA rats (review in Giorgi et al., 2019). An approach enabling to concurrently measure indices of pre- and postsynaptic striatal DA function in relation to addiction-related traits would provide a greater understanding regarding the relationships between traits and their respective DAergic determinants. Here, we assessed a large cohort of RHA and RLA rats using single photon emission computed tomography (SPECT) and the $D_{2/3}R$ radiotracer [^{123}I]IBZM to concurrently measure, for the first time to our knowledge, in vivo striatal $D_{2/3}R$ availability and psychostimulant-induced DA release in relation to impulsivity and novelty preference using a within-subject design. In an effort to further examine DA-dependent processes related to these traits, we also evaluated, within the same animals, the associations between ex vivo measures of midbrain $D_{2/3}$-autoreceptor levels, in vivo SPECT measures of $D_{2/3}R$ and DA release in striatum, and addiction-related traits.

Methods

Animals

Male RHA (n = 25) and RLA (n = 24) rats, weighing 225–250 g, and bred in our permanent colony of outbred Roman rats at the Department of Psychiatry of the University Hospitals of Geneva were used. These animals are the direct offspring of the RHA/Verh and RLA/Verh rats that were originally psychogenetically selected and bred in Zurich based on their divergent performances in the acquisition of avoidant behavior in the shuttle-box (Driscoll et al., 1998). Animals were pair-housed under temperature-controlled conditions and on a reversed 12-hour-light/-dark cycle (lights off at 7:00 AM). Animals were food-restricted (85%–90% of free-feeding weight) from the beginning and throughout the experiment, but water was provided ad libitum. Experiments were conducted in accordance with the Swiss federal law on animal care and was approved by the animal ethics committee of the canton of Geneva.

General Procedure

RHA and RLA rats were first tested using the novelty-induced place preference test. Following 2 days without tests, rats were trained in the 5-choice serial time task (5-CSRTT) and, once the animals had acquired the final learning criteria of the task, they were tested for impulsivity. Finally, animals were scanned with SPECT and the $D_{2/3}R$ antagonist radiotracer [^{123}I]IBZM to assess their striatal $D_{2/3}R$ density and capacity to release DA in response to amphetamine (AMPH). One week later, part of the animals (15 rats/line) were killed and the brains processed for ex vivo autoradiography of [^{3}H]-(+)-PHNO and $D_{2/3}R$ agonist-stimulated [^{35}S]GTPγS bindings.

Novelty-Induced Place Preference

The apparatus consisted of open fields (ActiMot, TSE Systems, Bad Homburg, Germany) divided into 2 equally sized compartments (23 × 46 × 40 cm), which differed in color, pattern walls, and floor texture. Both compartments were separated by a partition provided with a sliding door. On the first 4 days, rats were placed into 1 compartment (i.e., the familiar compartment) for a total of 15 min/d with the door between compartments closed. On the fifth day, rats were placed into the familiar compartment but with the door opened, allowing the rats to move freely between the 2 compartments for 10 minutes. The percentage of total time spent in the novel vs familiar compartment was used as the index of novelty preference.

Five-Choice Serial Reaction Time Task

Details on the 5-CSRTT apparatus and procedure are provided in the supplementary Methods. Briefly, rats were trained to initiate a trial by nose-poking into the food tray. After an inter-trial interval (ITI) of 2 seconds, 1 of the 5 response holes was pseudo-randomly illuminated for 30 seconds. A nose-poke into the illuminated response hole was rewarded with a pellet delivery. A nose-poke into any other response hole counted as incorrect and was punished with a time-out (TO). Missed trials (i.e., omissions) also resulted in a TO. Responses made during the ITI were counted as premature. Throughout the training period, the level of difficulty progressively increased by shortening the light stimulus duration and the time available for the rat to respond (limited hold) and by increasing the ITI over 8 training phases. Rats were trained until the final set of task parameters (stimulus duration, 1.5 seconds; limited hold, 5 seconds; ITI, 7 seconds) and fulfilled the criterion performance (>80% accuracy and <30% omissions) and stable baseline measures (<10% variation in accuracy over 3 consecutive days).

Performance measures of interest were (Bari et al., 2008):

- Percentage of premature responses [#premature/(#correct+#incorrect+#omission)] × 100, a measure of impulsivity
- Percentage accuracy: [#correct /(#correct+#incorrect)] × 100, a measure of attention
- Percentage omissions: [#omissions/#trials] × 100, a measure of attention and motivation
- TO responses, a measure of compulsivity

SPECT Imaging

Details on the SPECT data acquisition and analysis are provided in the supplementary Methods. Under isoflurane anesthesia, rats were injected i.v. with [^{123}I]IBZM and scanned for 132 minutes (33 frames of 4 minutes) using the U-SPECT-II imaging system (MiLabs, Utrecht, the Netherlands). AMPH (1.5 mg/kg i.v.) was injected at the beginning of the 18th frame (i.e., 72 minutes post-radiotracer injection). The first portion of SPECT acquisition (0–71 minutes) corresponded to the measure of [^{123}I]IBZM kinetics at baseline, and the second portion (72–133 minutes) measured the kinetics of [^{123}I]IBZM binding in response to AMPH.

Reconstructed SPECT images were processed using PMOD V3.9 software (PMOD Technologies Ltd, Zurich, Switzerland). SPECT images were co-registered to a magnetic resonance imaging atlas of the rat brain (Schweinhardt et al., 2003). A region of interest (ROI) template including the dorsal striatum (DST), ventral striatum (VST), and cerebellum was defined on the magnetic resonance imaging atlas and applied to the dynamic images to produce time-activity curves (TACs) for the target-rich (DST and VST) and reference (cerebellum) regions. Nonlinear least squares fitting analyses based on the linear extension of the simplified reference region model (LSSRM; Alpert et al., 2003) were applied to the regional TACs of [^{123}I]IBZM binding to estimate the nondisplaceable binding potential (BP_{ND}) as an index of $D_{2/3}R$ availability and gamma as an index of AMPH-induced DA release in DST and VST.

Briefly, the LSSRM takes into account temporal perturbations in radioligand-specific binding caused by pharmacological or nonpharmacological-induced changes in endogenous levels of neurotransmitter such as DA during a single-scan session (Alpert et al., 2003). The LSSRM assumes that a steady physiological state is disturbed at a certain time of the experiment and allows the dissociation rate of the radioligand from the receptor, k_{2a}, to change over time in response to local variation in the DA concentration ($k_{2a} = k_2/[1+BP_{ND}]$), where k_2 is the tissue-to-plasma efflux constant in the target region. Changes in BP_{ND} in competition studies are assumed to reflect inverse variations in the concentration of extracellular neurotransmitter (Ginovart, 2005). Competition between DA and radioligand for binding on receptors is reflected by a temporal change of k_{2a}, which is accounted for by a time-dependent parameter k_{2a}+gamma· h(t), where gamma represents the amplitude of the radioligand displacement and the function h(t) describes a rapid change following competition onset and dissipation over time. The decay function $h(t) = \exp[-\tau(t-T)]$ denotes temporal fluctuation in the model parameters, where τ controls the rate at which competition effects die away and T represents the time of competition onset. Therefore, an increase in k_{2a}, reflected by a decrease in BP_{ND} caused by an increase in AMPH-induced DA release, results in a positive value of gamma. Here, T was set to the time of AMPH injection, and τ was set to 0 min^{-1} as the decrease in BP_{ND} caused by AMPH administration is long-lasting and is unlikely to recover during the 60 minutes of post-AMPH SPECT acquisition used in our study. Indeed, previous studies showed that the decrease in BP_{ND} of $D_{2/3}R$ radioligands such as [^{11}C]raclopride (Narendran et al., 2007), [^{123}I]IBZM (Laruelle et al., 1997), and [^{11}C]-(+)-PHNO (Ginovart et al., 2006) is sustained for at least 3 hours following AMPH administration.

The standard error of BP_{ND} and gamma as estimated by the nonlinear least squares fitting was expressed in percent of the parameter value (%SE) and used to assess the parameter identifiability (Carson, 1986).

Ex Vivo Autoradiography of [^{3}H]-(+)-PHNO and [^{35}S]GTPγS Bindings

At 1 week following SPECT scanning, part of the animals (15 rats per line) were injected with [^{3}H]-(+)-PHNO i.v (Movarek

Biochemical, Brea, CA), killed by decapitation 60 minutes later, and brains removed. Series of five 20-µM-thick adjacent sections, taken at 300-µm intervals, were selected to cover most of the rostro-caudal extension of the caudate-putamen, nucleus accumbens, substantia nigra (SN), ventral tegmental area (VTA), and cerebellum. The first sections were stained for acetylcholinesterase as histological reference. The second and third to fifth sections were processed for [^{3}H]-(+)-PHNO autoradiography and for $D_{2/3}$R-stimulated G-protein activation using [^{35}S]GTPγS autoradiography, respectively, as detailed in the supplementary Methods. Ex vivo binding of [^{3}H]-(+)-PHNO in target ROI was quantified using the specific binding ratio (SBR), which is defined as SBR=(ROI – cerebellum)/cerebellum using the cerebellum as reference (Tournier et al., 2013). Agonist-stimulated [^{35}S]GTPγS binding was expressed as the percentage increase in [^{35}S]GTPγS binding in the presence vs the absence of the $D_{2/3}$R agonist quinelorane in the incubation milieu. As previously reported, basal [^{35}S]GTPγS binding in VTA was extremely low, if any (Wang et al., 2014), and highly variable following quinelorane stimulation (Selley et al., 2019), precluding accurate measurements of [^{35}S]GTPγS binding in VTA. VTA was therefore excluded from the analysis.

Statistical Analysis

The sample size (24–25 animals per line) was calculated based on previous results obtained in our group using [^{123}I]IBZM and SPECT in rats (Tsartsalis et al., 2018) to provide the statistical power to detect a 20% between-line difference in $D_{2/3}$R availability in VST (90% power, 2-sided 5% significance level).

Normality of data distribution was tested using the Shapiro-Wilk test. Prior to ANOVA and multiple regression analyses, non-normal data were subjected to appropriate log10 or square root transformation. If mathematical transformations were unable to normalize the data, nonparametric tests were used.

Pairwise comparisons between lines were conducted using 2-tailed independent Student's *t* tests for normally distributed data and Mann-Whitney U-tests for non-normally distributed data. Between-line differences in [^{3}H]-(+)-PHNO and [^{35}S]GTPγS binding were analyzed with a repeated-measures ANOVA, with the region as the within-subject factor and line as the between-subject factor, followed by a LSD post-hoc test when appropriate. Relationships between behavioral and SPECT variables were tested using Pearson's correlation coefficient for normally distributed data and Spearman's rank correlation coefficient for non-normally distributed data.

Stepwise multiple linear regression analyses were conducted to determine how much of the variance of premature responding and novelty preference was accounted for by in vivo [^{123}I]IBZM BP_{ND} in DST and VST, and [^{123}I]IBZM gamma in DST.

Mediation analyses were performed to investigate the extent to which AMPH-induced DA release accounted for links between DST $D_{2/3}$R availability, premature responding, and novelty preference. The models included [^{123}I]IBZM BP_{ND} value as the independent variable and premature responding in the 5-CSRTT or percentage of time spent in the novel compartment in the novelty-induced place preference test as the dependent variable, with [^{123}I]IBZM gamma entered as the mediator. Analyses were performed according to the procedure by Baron and Kenny (1986), which indicates that a variable is considered as a mediator when the following criteria are met: the independent variable must be significantly related to both the mediator (path a) and the dependent variable (path c; total effect), the mediator must be significantly related to the dependent variable after controlling for the independent variable (path b), and the previously significant relationship between the independent variable and the dependent variable must be either reduced but still significant (partial mediation) or no longer significant (full mediation) when the mediator is controlled for (path c'; direct effect). Significance of the estimated mediations was assessed using the Sobel test (Sobel, 1982) and a nonparametric bias-corrected bootstrapping procedure, which does not impose the assumption of normality in the sampling distribution (Preacher and Hayes, 2008). Both tests determine the significance of the indirect effect (product of path coefficients a and b) of the independent variable on the dependent variable through the mediator. Nonparametric bootstrapping procedure was based on 5000 bootstrapped re-samples to provide stable estimates of direct, indirect, and total effects and was performed using the SPSS PROCESS macro, version 3.4, developed by Hayes (Hayes, 2017). The indirect effect in the bootstrapping procedure is considered significant if the upper and lower bounds of the 95% bias-corrected bootstrap confidence intervals (BC-CI) do not include zero (Preacher and Hayes, 2008).

Data were analyzed using SPSS statistics (IBM, version 25.0) and Stata (Stat Corp, version 15.1).

Results

Novelty Preference

RHA rats displayed a higher preference towards the novel compartment compared with RLA rats (t test =5.97, df=47, $P<.001$; Figure 1A) and were also faster to initiate exploratory behavior in the novel compartment (t test=−2.88, df=47, $P<.01$; Figure 1B). However, the number of visits in the novel compartment was similar between the 2 lines (Mann–Whitney U=213, z=1.73, group 25-24, $P>.05$; Figure 1C).

5-Choice Serial Time Task

RHA and RLA rats acquired the task at similar rate up to training phase 4, requiring a similar number of training sessions to progress to the next phase. With increasing task demands, differences between lines emerged from training phase 5, with RLAs requiring a significantly higher number of sessions than RHAs to reach the required learning criteria (Figure 2A). As previously observed (Moreno et al., 2010), this difference resulted from

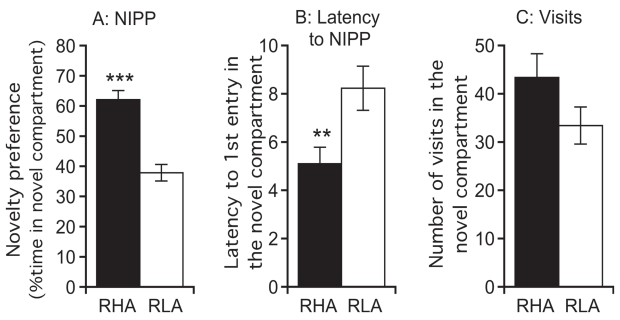

Figure 1. Novelty preference in RHA (n=25) and RLA (n=24) rats. In the NIPP test, (A) RHA rats displayed a higher percentage of time spent in the novel compartment, (B) a lower latency to first entry in the novel compartment, but (C) a similar number of visits in the novel compartment relative to RLA rats. Data are presented as mean ± SEM. Significantly different from RLA rats at **$P<.01$ and ***$P<.001$ using a 2-tailed independent Student's *t* test for normally distributed data and Mann-Whitney U test for non-normally distributed data.

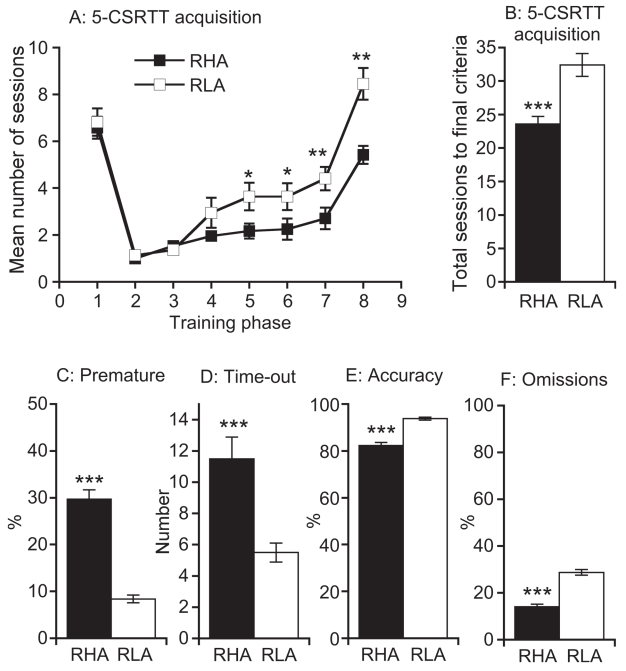

Figure 2. The 5-CSRTT performances in RHA (n=25) and RLA (n=24) rats. (A) During 5-CSRTT training, the number of cumulative sessions required to reach the required learning criteria in each training phase of the task was similar between both lines up to training phase 4 but differed from training phase 5, with RLA rats requiring a significantly higher number of sessions than RHAs to reach the required learning criteria. (B) The total number of 5-CSRTT training sessions needed to reach phase 8 criteria before premature responding could be tested was higher in RLA than in RHA rats. (C) The percentage of premature responses and (D) number of time-out responses (defined as responses during time-out periods) made during 3 stable sessions was higher in RHA than in RLA rats. (E) The percentage accuracy, and (F) percentage of omissions were lower in RHA than in RLA rats. Data are presented as mean ± SEM. Significantly different from RLA rats at ***$P<.001$ using a 2-tailed independent Student's *t* test for normally distributed data and Mann-Whitney U test for non-normally distributed data.

difficulties for RLA rats to meet the ≤30% of omission criteria to progress to the next phase. RLA rats required significantly more cumulative sessions than RHA rats to acquire the task before impulsivity could be measured (Figure 2B; Mann–Whitney U=113, z=–3.75, group 25-24, $P<.001$). On reaching stable performance to the final 5-CSRTT criteria, RHAs committed more premature responding (Mann–Whitney U=7, z=5.86, group 25-24, $P<.001$; Figure 2C) and TO responses (Mann–Whitney U=115, z=–3.42, group 25-24, $P<.001$; Figure 2D) than RLAs, indicating greater impulsivity and compulsivity, respectively, in the former line. RHA rats also displayed lower accuracy of responding than RLAs (Mann–Whitney U=24, z=–5.51, group 25-24, $P<.001$; Figure 2E). Consistent with previous reports (Dalley et al., 2008), accuracy inversely correlated with premature responding (Spearman's rho=–0.79; $P<.001$). In contrast, RHAs committed lower levels of omissions than RLAs (*t* test =–8.88, df=47, $P<.001$; Figure 2F). Results from other 5-CSRTT variables measured are shown in supplementary Table 1.

In Vivo Binding of [^{123}I]IBZM in RHA and RLA Rats

Compared with RLA rats, RHA rats displayed lower [^{123}I]IBZM BP_{ND} in both DST (–34.0%; *t* test=–7.44, df=47, $P<.001$; Figure 3A) and VST (–35.9%; *t* test=–6.98, df=47, $P<.001$; Figure 3A), indicating lower densities of striatal $D_{2/3}R$ in RHA vs RLA rats. BP_{ND} values in both DST and VST were identified with excellent precision, with %SE values lower than 4% (Figure 3A).

In contrast, RHA rats showed higher [^{123}I]IBZM gamma in DST (+95.6%; Mann–Whitney U=35, z=–5.21, group 25-23, $P<.001$; Figure 3B), indicating a higher capacity to release DA in response to AMPH compared with RLA rats. In VST, [^{123}I]IBZM gamma did not differ between lines (Figure 3B). However, while [^{123}I]IBZM gamma in DST was estimated with good precision, with %SE values lower than 20% (Figure 3B) (Carson, 1986), [^{123}I]IBZM gamma in VST showed poor identifiability with %SE values exceeding 70% and 130% in RLA and RHA rats, respectively (Figure 3B). [^{123}I]IBZM gamma in VST were thus treated as unreliable and excluded from further analysis.

[^{123}I]IBZM BP_{ND} inversely correlated with [^{123}I]IBZM gamma in DST (Spearman's rho=–0.70, $P<.001$; Figure 3C), indicating that in vivo postsynaptic $D_{2/3}R$ availability in striatum is inversely related to the presynaptic capacity to release DA in response to AMPH.

Relationships Between [^{123}I]IBZM Binding Measures and Behavioral Traits

Premature responding was negatively correlated with $D_{2/3}R$ availability (i.e., BP_{ND}) measured in both DST (Spearman's rho=–0.70, $P<.001$; Figure 4A) and VST (Spearman's rho=–0.66, $P<.001$; Figure 4B) but positively correlated with AMPH-induced DA release (i.e., gamma) in DST (Spearman's rho=0.64, $P<.001$; Figure 4C). Similarly, novelty preference was negatively correlated with $D_{2/3}R$ availability measured in both DST (Pearson's r=–0.43, $P=.002$; Figure 4D) and VST (Pearson's r=–0.42, $P=.002$; Figure 4E) but positively correlated with AMPH-induced DA release in DST (Spearman's rho=0.61, $P<.001$; Figure 4F). All correlations remained significant when a Bonferroni correction for multiple comparisons was applied (0.05/6=0.008).

As premature responding was positively correlated with novelty preference (Spearman's rho=0.53, $P<.001$), stepwise multiple regression analyses were used to determine whether the 3 indices of DA function (BP_{ND} in DST, gamma in DST, and BP_{ND} in VST) explained the same or nonoverlapping proportions of the variance in premature responding and novelty preference. Multicollinearity, as assessed with the variance inflation factor and condition index, was evidenced between BP_{ND} in DST and BP_{ND} in VST, and this latter variable was therefore excluded from the regression models. BP_{ND} in DST explained 46.8% ($P<.001$) of the variance of premature responding, and gamma explained an additional 6.3 % ($P=.02$) of the variance. As expected, in a separate analysis, the inclusion of BP_{ND} in VST did not significantly increase the variance accounted for in the model. Conversely, gamma in DST explained 35.8% ($P<.001$) of the variance of novelty preference, and adding BP_{ND} in DST did not increase the variance accounted for in the model ($P=.862$).

Mediation analyses were conducted to investigate the extent to which [^{123}I]IBZM gamma accounted for the associations between BP_{ND} in DST and our behavioral trait variables. Analyses indicated that AMPH-induced DA release in DST partially mediated the relationship between $D_{2/3}R$ availability and premature responding (Sobel z=–1.97; $P<.05$; BC-CI = –0.39 to –0.01; Figure 5A) but fully mediated the relationship between $D_{2/3}R$ availability and novelty preference (Sobel z=–2.99; $P<.01$; BC-CI = –0.61 to –0.17; Figure 5B).

Ex Vivo Binding of [^{3}H]-(+)-PHNO in RHA and RLA Rats

Significant main effects of rat line ($F_{1,111}=42.4$, $P<.001$) and brain region ($F_{3,111}=895.1$, $P<.001$) were found in [^{3}H]-(+)-PHNO binding as well as a significant interaction between line and brain region

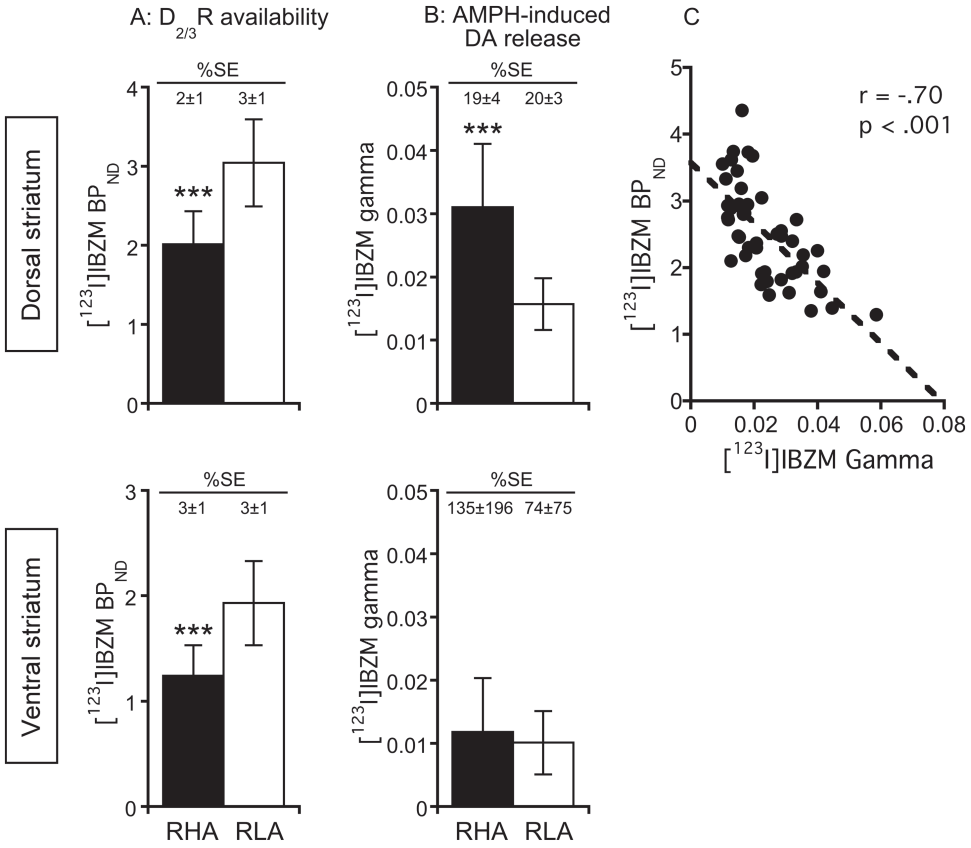

Figure 3. High-impulsive/high novelty-preferring RHA rats (n = 25) exhibited reduced striatal $D_{2/3}$R availabilities and heightened amphetamine (AMPH)-induced dopamine release compared with low-impulsive/low novelty-preferring RLA rats (n = 24). (A) Nondisplaceable binding potential (BP_{ND}) of the $D_{2/3}$R antagonist [^{123}I]IBZM, an index of $D_{2/3}$R availability, in the dorsal (top) and ventral (bottom) striatum in RHA compared with RLA rats. (B) [^{123}I]IBZM gamma values, an index of AMPH-induced DA release, in the dorsal (top) and ventral (bottom) striatum in RHA compared with RLA rats. %SE represents the mean percent standard error of BP_{ND} and gamma values, as given by nonlinear least squares fittings of [^{123}I]IBZM kinetics using the linear extension of the simplified reference region model, and it was used to assess parameter identifiability. With %SE ranging between 74% and 135%, [^{123}I]IBZM gamma in VST was poorly identified by the model and treated as unreliable. (C) Postsynaptic $D_{2/3}$R availability in dorsal striatum was positively correlated with the presynaptic capacity to release DA in response to a challenge dose of AMPH (1.5 mg/kg i.v.). Data are presented as mean ± SD. Significantly different from RLA rats at ***$P < .001$ using a 2-tailed independent Student's *t* test for normally distributed data and Mann-Whitney U test for non-normally distributed data.

($F_{3, 111} = 8.0$, $P < .001$). Post-hoc analysis showed that RHA rats displayed significantly lower levels of [^{3}H]-(+)-PHNO SBR in DST, VST, SN, and VTA compared with RLA rats (Figure 6A).

In midbrain, wherein $D_{2/3}$R function as autoreceptors, [^{3}H]-(+)-PHNO SBR in SN was inversely correlated with [^{123}I]IBZM gamma in DST (Spearman's rho = –0.42; $P < .05$; Figure 6B), indicating that a lower $D_{2/3}$- autoreceptor availability in midbrain is associated with a hightened capacity to evoke striatal DA release in response to AMPH.

[^{3}H]-(+)-PHNO SBR in SN significantly correlated with premature responding (Spearman's rho = –0.37; $P < .05$; Figure 6C) and novelty preference (Pearson's r = –0.49; $P < .01$; Figure 6E), whereas [^{3}H]-(+)-PHNO SBR in VTA correlated with novelty preference (Pearson's r = –0.56; $P = .001$; Figure 6F) but not with premature responding (Spearman's rho = –0.32; $P > .05$; Figure 6D).

$D_{2/3}$R-Stimulated G Protein Activation in RHA and RLA Rats

A repeated-measures ANOVA revealed no significant main effect of line ($F_{1, 28} = 1.31$, $P = .26$) and no line × region interaction ($F_{2, 56} = 0.02$, $P = .98$) on basal [^{35}S]GTPγS binding (Figure 7A), indicating that basal [^{35}S]GTPγS binding did not differ across lines in any brain region. Similarly, a significant main effect of region ($F_{2, 56} = 22.1$, $P < .001$) but no main effect of line ($F_{1, 28} = 1.26$, $P = .27$) or line × region interaction ($F_{2, 56} = 0.31$, $P = .73$) was found on quinelorane-stimulated [^{35}S]GTPγS binding (Figure 7B), indicating similar $D_{2/3}$R-stimulated [^{35}S]GTPγS binding in RHA and RLA rats.

Discussion

Here, we replicated a robust inverse relationship between impulsivity and $D_{2/3}$R availability in VST (Dalley et al., 2007) and extended this relationship to $D_{2/3}$R levels measured in DST. Novelty preference was related to impulsivity and also showed strong associations with the availability of $D_{2/3}$R in DST and VST. A high magnitude of AMPH-induced DA release in striatum predicted both impulsivity and novelty preference, perhaps owing to the diminished availability of midbrain $D_{2/3}$-autoreceptor measured in high-impulsive/novelty-preferring RHA animals that may amplify the effect of AMPH on DA transmission. The strong inverse association found between striatal $D_{2/3}$R availabilities and evoked DA release suggests that the deficits in striatal postsynaptic $D_{2/3}$R associated with impulsivity and novelty preference may arise, at least in part, from a compensatory downregulation mechanism to excessive dopaminergic transmission. Further analyses revealed that while $D_{2/3}$R availability

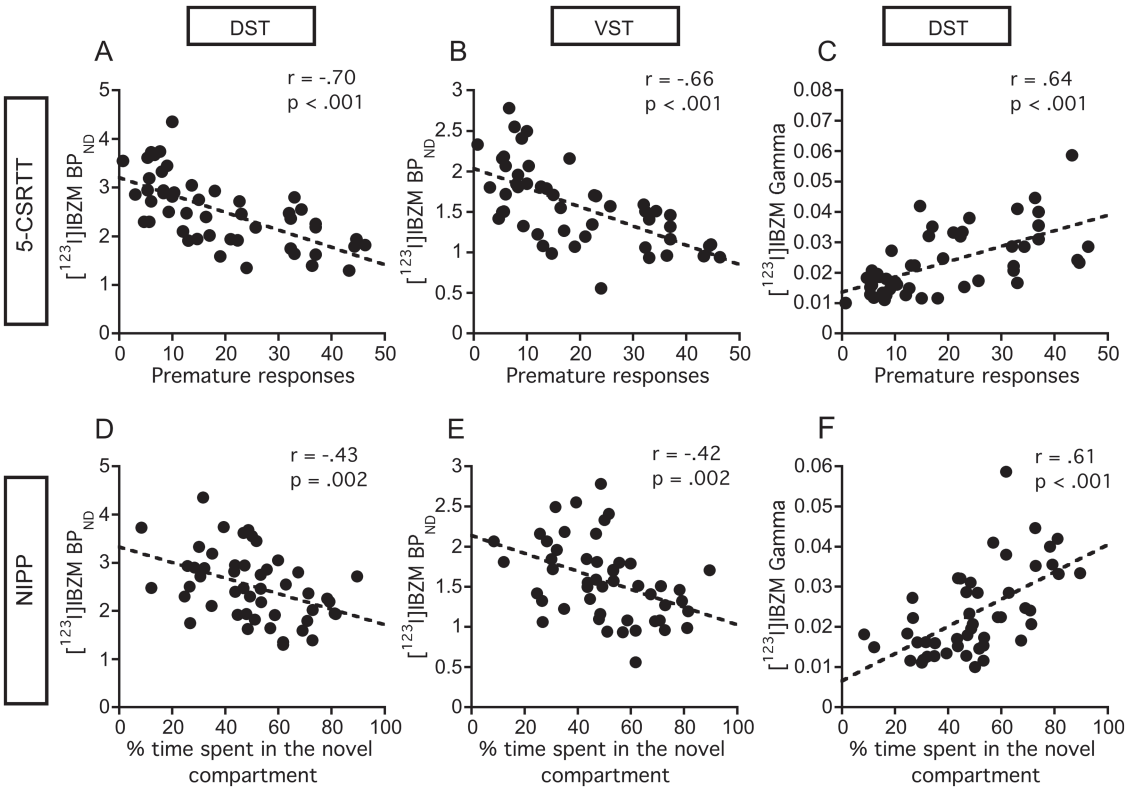

Figure 4. Relationships between premature responding, novelty preference, and $D_{2/3}R$-mediated indexes of DA signaling. (A) $D_{2/3}R$ availability (i.e., BP_{ND}) in dorsal and (B) ventral striatum negatively correlated with premature responding in the 5-CSRTT. (C) AMPH-induced DA release in dorsal striatum (i.e., gamma) positively correlated with both premature responding. (D) $D_{2/3}R$ availability (i.e., BP_{ND}) in dorsal and (E) ventral striatum negatively correlated with novelty preference and (F) novelty preference.

and evoked DA release in striatum are both significant predictors of impulsivity, the effect of striatal $D_{2/3}R$ availability on novelty preference is fully mediated by evoked striatal DA release. Thus, while the main locus of dopaminergic abnormality in novelty preference appears to be predominantly presynaptic, impulsivity appears to result not only from downstream abnormalities at $D_{2/3}R$ on postsynaptic striatal neurons but also from presynaptic events.

The lower $D_{2/3}R$ availabilities (i.e., BP_{ND}) measured in RHAs vs RLAs may have resulted from radioligand displacement due to elevated levels of endogenous DA competing with the radiotracer in RHA rats. However, previous studies failed to detect RHA vs RLA differences in baseline DA levels in striatum (Giorgi et al., 2007; Moreno et al., 2010), and lower striatal $D_{2/3}R$ availabilities are still measured in RHAs vs RLAs even after removal of endogenous DA (Tournier et al., 2013). Intriguingly, this difference did not translate into lower receptor-mediated G-protein activation in RHAs, suggesting a more efficient coupling of $D_{2/3}R$ to their downstream G proteins in this line. Confirming previous reports (Moreno et al., 2010; Klein et al., 2014), RHAs were more impulsive in the 5-CSRTT than RLAs. Importantly, an inverse relationship was observed between $D_{2/3}R$ availability in both DST and VST and impulsivity, extending previous results on a similar relationship but observed in only VST (Dalley et al., 2007). Contrasting with our data, previous studies failed to reveal alteration in $D_{2/3}R$ availabilities in DST in relation to impulsivity, highlighting the VST as a key structure of premature response control (Dalley et al., 2007; Jupp et al., 2013; Caprioli et al., 2013, 2015). The reason for these contrasting results is unclear but may be related to different strains of rats or to insufficient statistical power due to the small group size (n = 6–8/group) used in previous studies. By using 24/25 animals per group, our study may have overcome this limitation. Our data add to previous evidence implicating the DST in premature responding. Lesion (Rogers et al., 2001), or infusion of a $D_{2/3}R$ agonist (Agnoli et al., 2013) into the dorsomedial striatum increases 5-CSRTT premature responding while infusion of a $D_{2/3}R$ antagonist into this structure reversed premature responding induced by medial prefrontal cortex (mPFC) lesioning (Agnoli et al., 2013). Taken together, these findings thus question the prevailing hypothesis that impulsive action in the 5-CSRTT is mainly controlled by the mesolimbic and not the nigrostriatal DA system. Interestingly, studies in humans have also identified $D_{2/3}R$ deficits in DST in relation to impulsivity as assessed with self-rating scales (Lee et al., 2009) or the stop-signal task (Robertson et al., 2015). The dissociation between the locus of $D_{2/3}R$ dysmodulation (e.g., VST vs DST) has lend support to the view that the neural substrate and circuitry putatively associated with "waiting impulsivity," as measured with the 5-CSRTT, and "stopping impulsivity," as measured with the stop-signal task, may be subserved by different functional striatal subdivisions (Robinson et al., 2009; Dalley and Robbins, 2017). By showing that $D_{2/3}R$ abnormalities associated with 5-CSRTT impulsivity involve both the DST and VST, our study suggests that the neural dichotomy between "waiting" and "stopping" impulsivity may not be as clear and that both forms of motor impulsivity may share overlapping neural substrates within the striatum.

Few studies have investigated the presynaptic aspect of DA signaling in relation to impulsivity. Compared with RLAs, not only did high-impulsive RHA rats display lower striatal $D_{2/3}R$, but they concomitantly showed enhanced AMPH-induced DA release. Importantly, this heightened presynaptic DAergic responsivity

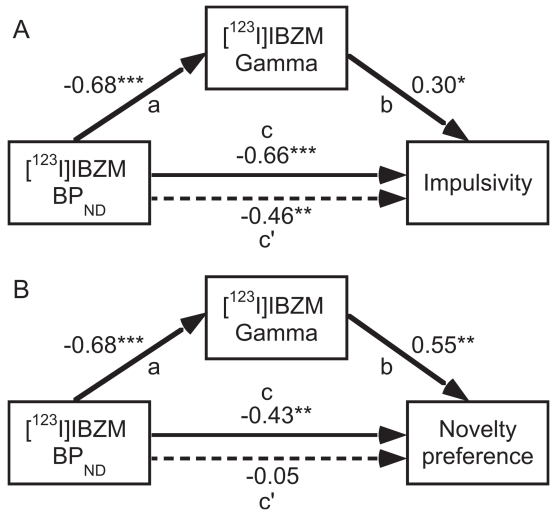

Figure 5. Premature responding and novelty preference were differentially predicted by striatal $D_{2/3}$R availability and amphetamine (AMPH)-induced DA release. (A) Mediational model of [123I]IBZM gamma as the mediator between [123I]IBZM BP_{ND} in dorsal striatum and impulsivity. Mediation analyses indicated that the impact of $D_{2/3}$R availability on premature responding is partially mediated through an influence of AMPH-induced DA release (Sobel test $z = -1.97$; $P < 0.05$; BC-CI = −0.39 to −0.01). (B) Mediational model of [123I]IBZM gamma as the mediator between [123I]IBZM BP_{ND} in dorsal striatum and novelty preference. Mediation analyses indicated that AMPH-induced DA release fully mediated the relationship between $D_{2/3}$R availability and novelty seeking (Sobel test $z = -2.99$; $P < .01$; BC-CI = −0.61 to −0.17). Path a shows the standardized coefficient for the impact of $D_{2/3}$R availability on AMPH-striatal DA release. Path b shows the standardized coefficient for the effect of striatal DA release on premature responding and novelty preference in their respective model. Paths c (total effect) and c' (indirect effect) show coefficients for the effects of $D_{2/3}$R availability on premature responding and novelty preference in their respective model. All coefficients are standardized.

to AMPH also predicted higher 5-CSRTT impulsivity. This is consistent with prior human work showing that trait impulsivity is positively associated with AMPH-induced DA release in striatum (Buckholtz et al., 2010) but conflicts with other studies showing either a negative association with trait impulsivity in humans (Oswald et al., 2007) or lower AMPH-induced striatal DA release in high-impulsive rats (Zeeb et al., 2016). However, other evidence suggesting excessive DA signaling in impulsivity comes from studies showing increases in 5-CSRTT impulsivity following systemic or intra-accumbens AMPH administration (Cole and Robbins, 1987; van Gaalen et al., 2006). Taken together, these studies suggest that premature responding may result from excessive DA surges in the striatum and that the striatal $D_{2/3}$R deficits observed in high-impulsive rats may be consecutive to a homeostatic postsynaptic $D_{2/3}$R downregulation. This hypothesis is reinforced by the strong negative association found here between presynaptic measures of AMPH-induced DA release and postsynaptic measures of striatal $D_{2/3}$R availability.

The mechanism underlying an increased DA response to AMPH in impulsivity is unclear but may result from reduced availability of midbrain $D_{2/3}$-autoreceptors, whose function is to retroinhibit DA cell excitability, DA synthesis, and release (Ford, 2014). Lower $D_{2/3}$R levels were found in SN and VTA of high-impulsive RHA vs low-impulsive RLA rats, confirming an interrelationship between $D_{2/3}$R availabilities found in midbrain and striatum (Zald et al., 2010). Importantly, midbrain $D_{2/3}$R availabilities were inversely related to the magnitude of AMPH-induced DA release and to 5-CSRTT impulsivity, a finding that accords well with human data using PET and self-reported measures of impulsivity (Buckholtz et al., 2010). Here, we thus provide a second line of evidence suggesting that a reduced $D_{2/3}$-autoreceptor function, and hence reduced inhibitory feedback control over DA release, could lead to aberrant evoked DA release in striatum and ultimately to impulsivity. Interestingly, desensitization of midbrain $D_{2/3}$-autoreceptors plays a key role in stimulant sensitization (Henry et al., 1998), and mice lacking D_2-autoreceptors showed an enhanced propensity to self-administer cocaine (Holroyd et al., 2015). Along these lines, RHAs displayed a higher propensity to develop stimulant sensitization (Tournier et al., 2013) and to self-administer cocaine than RLAs (Dimiziani et al., 2019). It is thus possible that by dysmodulating DA response of midbrain DA neurons, $D_{2/3}$-autoreceptor hypofunction in impulsive individuals could contribute to enhanced vulnerability to drug dependence.

To our knowledge, only 1 study has investigated the association between novelty preference and 5-CSRTT impulsivity, with negative results (Molander et al., 2011). In contrast, our results clearly indicated a strong association between 5-CSRTT impulsivity and novelty preference in RHA and RLA rats, indicating that both behavioral traits are related in these lines. Similar to impulsivity, novelty preference showed concurrent associations with lower striatal $D_{2/3}$R availabilities and higher amounts of AMPH-induced DA release. This is consistent with previous independent studies in humans showing that high-novelty/sensation seeking is associated with fewer striatal $D_{2/3}$R (Gjedde et al., 2010) and with higher psychostimulant-induced striatal DA release (Leyton et al., 2002; Jaworska et al., 2017) and that low levels of midbrain inhibitory $D_{2/3}$-autoreceptors correlate with increased novelty/sensation seeking (Zald et al., 2008; Savage et al., 2014), thus paralleling similar findings described above for impulsivity. Of particular interest, we found that our DA-related variables, while being correlated, predicted somewhat different proportions of the variance of impulsivity and novelty preference. While $D_{2/3}$R availability in DST was the strongest predictor of impulsivity, the magnitude of AMPH-induced DA release emerged as the unique predictor of novelty preference. Thus, although novelty preference and impulsivity are interrelated and co-segregate with similar pre- and postsynaptic indices of striatal DA function, both behavioral traits likely depend on overlapping but not identical neurochemical mechanisms in striatum.

Besides impulsivity, RHAs exhibited poorer response accuracy than did low-impulsive RLAs, suggesting alterations in attentional processing in RHA rats. This is congruent with the overall poorer cognitive abilities of RHA vs RLA rats in several tasks of learning and memory (review in Giorgi et al., 2019) and the reported reduction in mPFC volume in RHAs (Rio-Alamos et al., 2019). Interestingly, mPFC damage increases premature responding and decreases attentional accuracy in the 5-CSRTT, which can both be reversed by intra-striatal infusion of a $D_{2/3}$R antagonist (Pezze et al., 2009; Agnoli et al., 2013). As both impulsive and compulsive behaviors have been proposed to be controlled by overlapping cortico-striatal circuits (Dalley et al., 2011), an impaired "top-down" control exerted by the PFC over striatal functioning may thus be involved in the impulsive (premature responses) and compulsive (TO responses) responding observed in RHA rats. Moreover, besides DA, other neurotransmitters such as noradrenaline and serotonin also contribute to impulsivity in the 5-CSRTT (Robbins, 2018). In addition to enhanced mesolimbic activity, RHA rats display different cortical densities of serotonin $5\text{-}HT_{2A}$ receptors but lower expression of mGlu2 receptors than RLAs (Fomsgaard et al., 2018), and a positive association has been

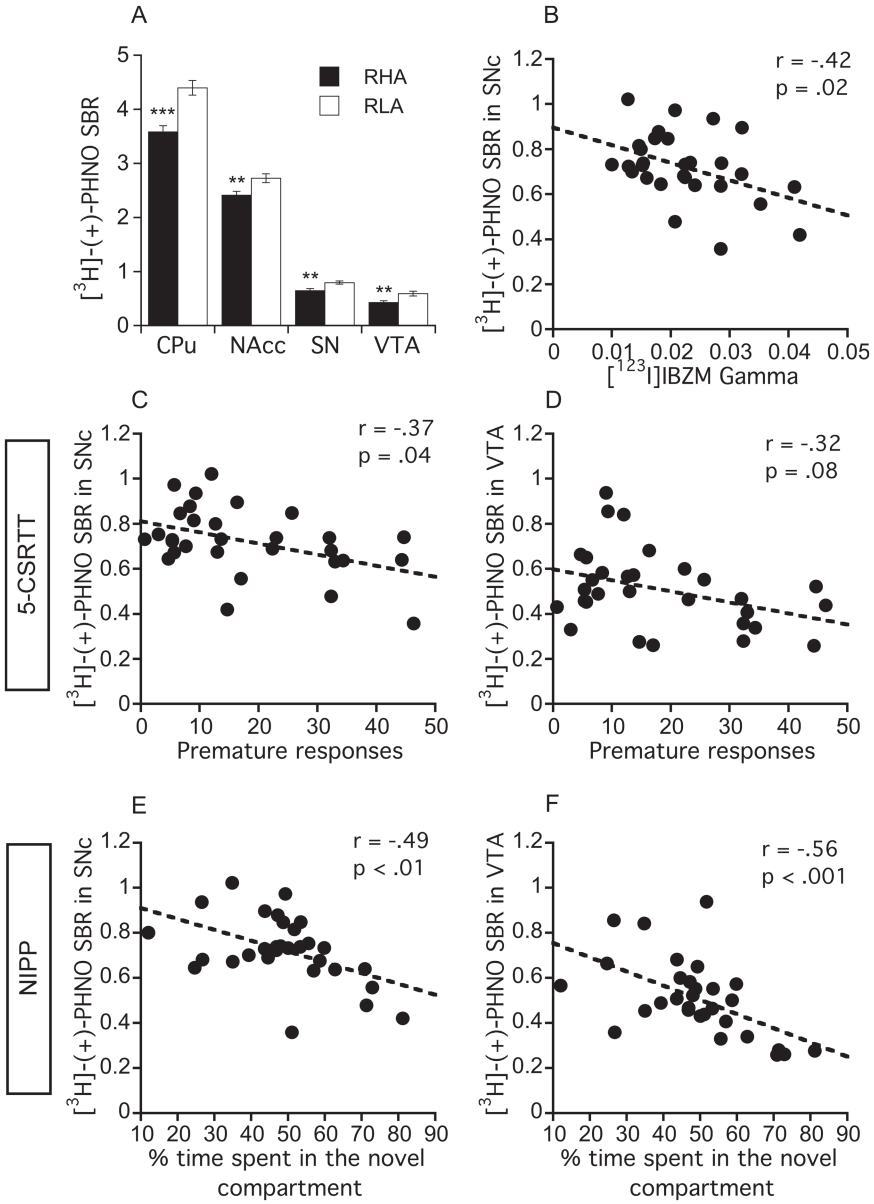

Figure 6. Reduced $D_{2/3}$-autoreceptor availability in midbrain predicted heightened amphetamine (AMPH)-induced DA release, heightened impulsivity, and heightened novelty preference. (A) $D_{2/3}R$ availability as measured by ex vivo autoradiography quantification of [^{3}H]-(+)-PHNO specific binding ratio (SBR) in RHA and RLA rats in the dorsal striatum (DST), ventral striatum (VST), and ventral tegmental area (VTA). (B) $D_{2/3}$-autoreceptor availability in SN_c was negatively correlated with AMPH-induced DA release in dorsal striatum. (C) $D_{2/3}$-autoreceptor availability in SN_c but (D) not in VTA was inversely correlated with premature responding in the 5-CSRTT. (E) $D_{2/3}$-autoreceptor availability in SN_c and (F) in VTA was inversely correlated with novelty preference. Data are presented as mean ± SD. Significantly different from RLA rats at $^{**}P < .01$ and $^{***}P < .001$ using a repeated 2-way ANOVA followed by a LSD post-hoc test. Correlations were tested using Pearson's correlation coefficient for normally distributed data and Spearman's rank correlation coefficient for non-normally distributed data.

found between 5-HT_{2A} receptor density and premature responding in RHAs (Klein et al., 2014). Moreover, in addition to differing in impulsivity and novelty preference, the Roman rat line also differs in a number of other behavioral and neurobiological aspects, including anxiety, emotionality, response to stress, spatial memory, prepulse inhibition of the startle reflex, and Pavlovian aversive conditioning (Giorgi et al., 2007, 2019). Our findings thus do not preclude the possibility that, besides differences in $D_{2/3}R$-mediated signaling, between-line differences in other neurotransmitter system signaling could also contribute to impulsivity in RHAs and RLAs, or that striatal $D_{2/3}R$ availability may also correlate with other behavioral traits in Roman rats.

Quantification of DA release using PET or SPECT imaging is usually done using a dual scan approach that requires 2 separate scan sessions, 1 performed at baseline and the other under activation conditions, and activation-induced DA release is estimated from a between-scan change in BP_{ND}. In addition to requiring 2 radiochemical syntheses and administrations, thus increasing radiation exposure in human studies, a drawback of this approach is the variability of endogenous DA levels between the baseline and activation scans, which are usually performed on 2 separate days, thereby introducing test-retest variability in the PET/SPECT measure. The use of LSSRM mitigates these limitations as it requires only a single scan, thereby reducing the source

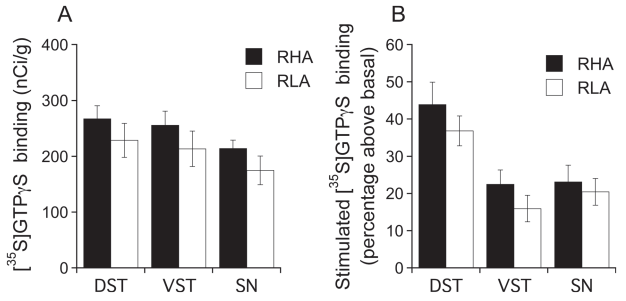

Figure 7. RHA and RLA rats displayed similar $D_{2/3}$R-mediated G-protein activation in the mesostriatal system. (A) Basal [^{35}S]GTPγS binding and (B) quinelorane-stimulated [^{35}S]GTPγS binding were similar in the dorsal striatum (DST), ventral striatum (VST), and substantia nigra (SN) of RHA and RLA rats. Data are presented as means ± SEM.

of experimental error due to between-scan variability and enhancing the sensitivity for detection of radioligand displacement induced by the activation. However, some limitations have to be acknowledged. In addition to the relatively low resolution of SPECT that precludes $D_{2/3}$R imaging in small-sized regions of the midbrain, a second limitation of our study was the inability to obtain reliable estimates of [^{123}I]IBZM gamma in VST using the LSSRM. The LSSRM has the advantage of requiring a single-scan approach, but stability of the LSSRM can be compromised by alterations in regional cerebral blood flow (rCBF) coincident to the AMPH activation. Increases in rCBF resulting from larger variations in K_1 than in k_2, the rate constants for radiotracer transport from plasma to tissue and back from tissue to plasma, respectively, result in TAC variations that have an opposite direction to TAC changes induced by increased DA levels (Pappata et al., 2002; Alpert et al., 2003). When these 2 effects occur simultaneously as a result of the activation, they counteract each other, and the amplitude of DA release (i.e., gamma) estimated by the LSSRM is underestimated and unreliable (Normandin et al., 2012). Whereas AMPH is well-known to enhance striatal rCBF (Hartvig et al., 1997), its effects on K_1 and k_2 in the different striatal subdivisions are unclear. However, 1 study showed that AMPH induced a transient initial increase in K_1 in VST but not in DST in baboons (Price et al., 2002). It is thus conceivable that AMPH-induced rCBF changes may have affected tracer delivery in VST and DST differentially, leading to model violation and gamma uncertain estimation in VST but not DST. Additional work is required to further establish the robustness of LSSRM toward changes in rCBF occurring during AMPH activation in the different subdivisions of the striatum. As a further limitation, our study included male rats only, thus potentially limiting the generalizability of the study to females.

In conclusion, this study contributes important information to our understanding of the neurochemical underpinnings of impulsivity and novelty preference by showing that both behavioral traits appear to depend on partially overlapping neurochemical mechanisms. We found that impulsivity is directly related to both a reduced $D_{2/3}$R availability and heightened capacity to evoke DA release in striatum, while novelty preference is mainly related to the latter. As both impulsivity and novelty preference predict and may interact to enhance individual susceptibility to compulsive drug use (Belin and Deroche-Gamonet, 2012), these data further our understanding of the molecular and cellular bases of linked phenotypes influencing vulnerability to addiction.

Supplementary Materials

Supplementary data are available at *International Journal of Neuropsychopharmacology (IJNPPY)* online.

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Statement of Interest

The authors declare no conflict of interest.

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