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α7 nicotinic acetylcholine receptor modulation of accumbal dopamine release covaries with novelty seeking

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Abstract

Heightened novelty seeking phenotypes are associated with a range of behavioral traits including susceptibility to drug use. These relationships are recapitulated in preclinical models, where rats that exhibit increased exploratory activity in novel environments (high responders- HR) acquire self-administration of psychostimulants more rapidly compared to rats that display low novelty exploration (low responders- LR). Dopamine release dynamics in the nucleus accumbens (NAc) covaries with response to novelty and differences in dopaminergic signaling are thought to be a major underlying driver of the link between novelty seeking and drug use vulnerability. Accumbal dopamine release is controlled by local microcircuits including modulation through glutamatergic and nicotinic acetylcholine receptor (nAChR) systems, but whether these mechanisms contribute to disparate dopamine signaling across novelty phenotypes is unclear. Here, we used *ex vivo* voltammetry in the NAc of rats to determine if α7 nAChRs contribute to differential dopamine dynamics associated with individual differences in novelty exploration. We found that blockade of α7 nAChRs attenuates tonic dopamine release evoked by low frequency stimulations across phenotypes, but that phasic release is decreased in LRs while HRs are unaffected. These stimulation frequency- and phenotype-dependent effects result in a decreased dynamic range of release exclusively in LRs. Furthermore, we found that differential α7 modulation of dopamine release in LRs is dependent on AMPA but not NMDA receptors. These results help to form an understanding of the local NAc microcircuitry, and provides a potential mechanism for covariance of dopamine dynamics and sensitivity to the reinforcing effects of drugs of abuse.

Graphical Abstract

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Author Contributions

Conceptualization, A.C.L., C.A.S., and M.J.F.; Formal analysis, A.C.L., E.G.P., and C.A.S.; Investigation, A.C.L. and C.A.S.; Resources, M.J.F.; Writing – Original Draft, A.C.L. and E.G.P.; Writing – Review & editing, A.C.L., E.G.P., C.A.S., and M.J.F.; Funding Acquisition, A.C.L. and M.J.F.

Competing Interests

The authors declare no competing interests.

Data Accessibility

Individual animal data are available on request from the corresponding author.

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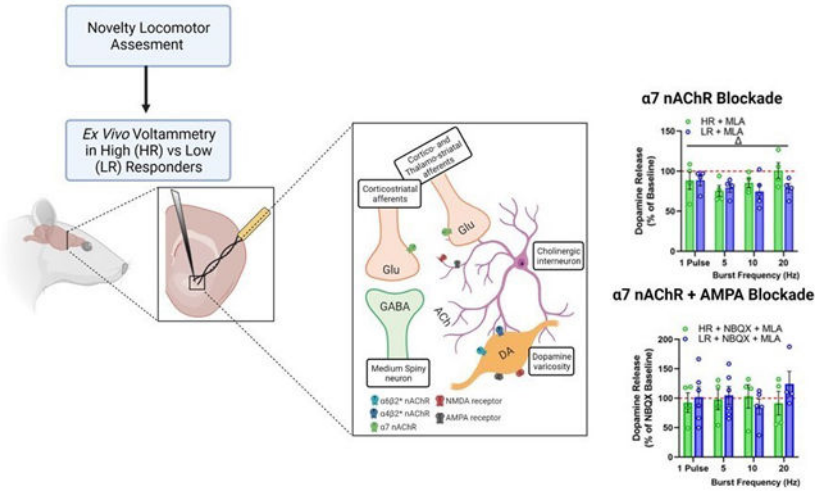
We examined the role of α7 nicotinic acetylcholine receptors (nAChRs) in differential dopamine signaling in the nucleus accumbens of rats with higher or lower novelty response. Using *ex vivo* voltammetry, we found that α7 nAChR blockade resulted in a phenotype- and stimulation frequency-dependent effect on dopamine release and that this modulation is dependent on AMPA receptor signaling.



Keywords

Individual differences; vulnerability; voltammetry; nicotine; SUD

Introduction

From 2015–2019, yearly survey data has shown that an estimated 70.5% of Americans aged 12 or older report having used an illicit substance at least once, while an estimated 10–20% of those individuals ultimately develop a substance use disorder (SUD) (SAMHSA, 2019). These trends highlight substantial individual variability in the risk of developing SUD following initial drug use. Identifying behavioral and neurochemical markers of increased vulnerability to initiation of drug use and the development of SUD provides a significant opportunity to identify at-risk populations and to aid in SUD prevention. Additionally, forming a more coherent understanding of the mechanisms that drive differences in vulnerability will enable the advancement of more effective treatment strategies for the millions of individuals in the United States already suffering from SUD.

In preclinical rodent models, locomotor response to an inescapable novel environment can predict drug use vulnerability. Rodents that demonstrate increased locomotor response (high responders; HR) acquire self-administration of many drugs of abuse, including psychostimulants such as cocaine and nicotine more rapidly and at lower doses than low responders (LR) (Ferris et al., 2013; Marinelli & White, 2000; Piazza et al., 1989; N. Suto et al., 2001). HR rats also have increased rates of responding and drug intake for cocaine, morphine, and ethanol across large dose ranges (Nadal et al., 2002; Kabbaj, 2006). Additionally, locomotor response following non-contingent administration

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of psychostimulants is greater in HR rats (Briegleb et al.,2004; Coolon & Cain, 2009; Ferris et al., 2013). Importantly, while the HR phenotype is predictive of the propensity to acquire and sustain self-administration of drug and of sensitivity to shifts in drug dose, it is dissociable from the vulnerability to shift to compulsive drug-taking which is better predicted by high impulsivity (Belin et al., 2008). It is also interesting to note that there is evidence to suggest that the HR trait may in fact confer resistance to some addiction-like behaviors assessed by the 3-Criteria Model (increased motivation to take drug, inability to refrain from drug-seeking, and maintained drug use despite aversive consequences) (Deroche-Gamonet et al., 2004; Belin et al., 2008; Belin et al., 2011; Fouyssac et al., 2021). Similarly in humans, the sensation seeking trait is associated with the initiation of substance use but does not seem to be an endophenotype for stimulant dependence (Ersche et al., 2010). This agrees with current views in substance abuse research that individual differences in the vulnerability to drug use and development of SUD are distinct dimensions of drug-taking and may have distinct underlying mechanisms. Thus, examination of locomotor response to novelty and associated neurochemical characteristics prior to any drug experience is a powerful model for investigating premorbid markers of drug use vulnerability.

Signaling of mesolimbic dopamine neurons that project from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) is fundamental for guiding behaviors implicated in SUD, including incentive value, and reward prediction error (Berridge, 2007; Schultz et al., 1997; Woolverton & Virus, 1989). Patterns of tonic (single spikes at ~4–5 Hz) and phasic (2–5 spikes at 20–100 Hz) firing encodes information about salient environmental stimuli and rewards (Marinelli & Mccutcheon, 2014; Tobler et al., 1995; Waelti et al., 2001). Importantly, differences between the dopaminergic systems of HR versus LR rats have been observed. For example, HR rats have increased extracellular dopamine levels following systemic cocaine injection compared to LR rats (Hooks et al., 1991; Nelson et al., 2009). HR rats also show higher dopamine transporter (DAT) levels, faster dopamine uptake, and increased phasic dopamine signaling to reward-predictive cues (Flagel et al., 2011; Nelson et al., 2009), suggesting heightened activity of the dopamine system. Additionally, heightened response to novelty has been shown to be dependent on the mesolimbic dopamine system (Hooks & Kalivas, 1995). Increased activity of the dopamine system in HRs may help explain higher locomotor responses following psychostimulant administration and also suggests that HRs may experience unique alterations within the mesolimbic system following drug exposure compared to LRs.

It is becoming increasingly evident that somatic action potential activity in dopamine neurons and release of dopamine from presynaptic terminals are dissociable and may contribute to distinct aspects of motivated behavior (Mohebi et al., 2018, Mohebi et al., 2019; Mohebi & Berke, 2020; Nolan et al., 2020). Perhaps most notably, cholinergic interneurons (CINs) in the striatum have direct, local influence over dopamine release via activation of nicotinic acetylcholine receptors (nAChRs) located on presynaptic DA varicosities (Cohen et al., 2012; Exley et al., 2008; Patel et al., 2017; Rice & Cragg, 2004; Threlfell et al., 2010; Zhang & Sulzer, 2004). In fact, CINs can trigger dopamine release independent of action potentials generated by dopamine neurons (Cachope et al., 2012; Threlfell et al., 2012). We have previously shown that locomotor response to a novel

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environment is predictive of differential nAChR modulation of phasic dopamine signals in the NAc (Siciliano et al., 2017). Specifically, desensitization or blockade of α6β2-containing (α6β2*) nAChRs within the NAc augments phasic dopamine signals in brain slices of HR rats, but reduces phasic dopamine signals in LRs.

However, there are several other nAChR subtypes that may contribute to individual differences in dopamine signaling in HRs versus LRs. Within the NAc, α7 nAChRs are located on striatal glutamate terminals and may modulate dopamine signaling through several mechanisms (Grady et al., 2007; Livingstone & Wonnacott, 2009; Marchi et al., 2002). It has previously been shown that cortical and thalamic glutamatergic inputs to the striatum can modulate dopamine release via activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) glutamate receptors on CINs (Kosillo et al., 2016). By promoting increased activity and acetylcholine release from CINs, these glutamatergic inputs indirectly drive dopamine release by activating nAChRs on dopamine axons. Furthermore, activation of α7 nAChRs has been shown to elicit glutamate release which in turn acts at putative ionotropic glutamate receptors on dopamine terminals to stimulate dopamine release (Desce et al., 1992; Kaiser & Wonnacott, 2000; Wang, 1991). Given this evidence that α7 nAChRs on glutamatergic terminals regulate dopamine release within the NAc, we sought to determine if α7 nAChR modulation of NAc dopamine signaling contributes to the underlying neurobiological features that distinguish the HR/LR phenotypes. To address this question, we used *ex vivo* fast-scan cyclic voltammetry (FSCV) to examine how antagonism of α7 nAChRs impacts dopamine release across a range of stimulation frequencies in the NAc core of HR and LR animals and examine what mechanisms may be mediating this effect.

Methods and materials

Animals

Adult male Sprague-Dawley rats (300 – 325 grams, Envigo, Dublin, VA) were pair housed and maintained on a reversed 12:12 hour light/dark cycle (4:00 a.m. lights off; 4:00 p.m. lights on) with food and water available *ad libitum*. All animals were maintained according to the National Institutes of Health guidelines in Association for Assessment and Accreditation of Laboratory Animal Care accredited facilities. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Wake Forest School of Medicine. A total of 32 rats were tested on their locomotor response to novelty. A cohort of 12 rats was initially used to examine the effects of α7 nAChR antagonism on dopamine release. A separate cohort of 20 rats was then used to examine AMPA/NMDA antagonism. Rats with total distance traveled in the top and bottom third of the distribution were included in the final voltammetry analysis.

Locomotor assessment

Rats were habituated to the housing environment for a minimum of one week prior to the start of experiments. All locomotor testing occurred during the dark/active cycle (9:00 a.m.; midway through dark cycle) to prevent sleep from contributing to variability in locomotor activity. Rats were first transferred to the dark locomotor testing room and allowed to

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habituate in their home cages for one hour. Animals were then placed in acrylic activity monitors (43 × 43 × 30 cm, Med Associates, St. Albans, Vermont) equipped with two infrared beam arrays. Horizontal activity was measured for 90 minutes by beam breaks, which were recorded by a computer.

Slice preparation

At least 24 hours after locomotor assessment, rats were anesthetized with isoflurane and euthanized by rapid decapitation. As previously described, brains were rapidly removed and transferred into ice-cold, pre-oxygenated (95% O_2 / 5% CO_2) artificial cerebral spinal fluid (aCSF) containing (in mM): NaCl (126), KCl (2.5), monobasic NaH_2PO_4 (1.2), $CaCl_2$ (2.4), $MgCl_2$ (1.2), $NaHCO_3$ (25), dextrose (D-glucose) (11), and L-ascorbic acid (0.4) (Fennell et al., 2020; Ferris et al., 2012). Tissue was sectioned into 400 μm-thick coronal slices with a compresstome® VF-300 vibrating microtome (Precisionary Instruments, San Jose, California). Brain slices were placed in submersion recording chambers and perfused at 1 mL/minute with oxygenated aCSF at 32°C.

Ex vivo fast-scan cyclic voltammetry (FSCV)

FSCV was used to assess dopamine release in the NAc core of rat brain slices. A bipolar stimulating electrode was placed 100 – 150 μm from a carbon-fiber recording microelectrode (100 – 200 μm length, 7 μm diameter). Extracellular dopamine was recorded by applying a triangular waveform from −0.4 to 1.2 V and back to −0.4 (Ag vs AgCl) at a scan rate of 400 V/s.

Dopamine release was initially evoked by a single electrical pulse (750 μA, 2 msec, monophasic) applied to the tissue every 3 minutes. Once the extracellular dopamine response was stable (3 collections within < 10% variability), five-pulse stimulations were applied at varying burst frequencies (5, 10, or 20 Hz) to model the physiological range of dopamine neuron firing. After assessing the dopamine response to single and multi-pulse stimulations across a range of frequencies, the α7-selective nAChR antagonist MLA (30 nM), the AMPA receptor antagonist NBQX (5 μM), the NMDA receptor antagonist D-AP5 (30 μM), or a combination of NBQX and D-AP5 were bath applied to separate slices. Dopamine response was equilibrated to single electrical pulse stimulations and five-pulse stimulations were reassessed. MLA (30 nM) was then added to the baths containing NBQX, D-AP5, and NBQX / D-AP5 and dopamine response was equilibrated to single electrical pulse stimulations and five-pulse stimulations were once again reassessed (as above).

Drugs

All drugs were purchased from Cayman Chemical Company (Ann Arbor, MI). MLA (methyllycaconitine citrate; 20-ethyl-1α,6β,14α,16β-tetramethoxy-4-[[[2-[(3S)-3-methyl-2,5-dioxo-1-pyrrolidinyl]benzoyl]oxy]methyl]-aconitane-7,8-diol, 2-hydroxy-1,2,3-propanetricarboxylate) was dissolved in distilled water at 1mM concentration. NBQX (sodium salt) (1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide, disodium salt) and D-AP5 (5-phosphono-D-norvaline) were dissolved in DMSO at 1 mM and 10 mM concentrations, respectively. Aliquots were stored at −20°C and diluted with oxygenated aCSF to final concentration before bath application on slices.

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Data analysis

Demon Voltammetry and Analysis software was used to acquire and model FSCV data (Yorgason et al., 2011). Recording electrodes were calibrated by recording electrical current responses (in nA) to a known concentration of dopamine (3 μM) using a flow-injection system. This was used to convert electrical current to dopamine concentration. Michaelis-Menten kinetics were used to determine maximal rate of dopamine uptake (Vmax) (Ferris et al., 2013).

Statistical Analysis

Bivariate regression (correlation) was used to initially assess the relationship between locomotor response to novelty and α7 nAChR modulation of dopamine release. We performed a tertiary split of locomotor data (comparing top and bottom thirds of animals based on their locomotor data) in order to determine the differential effects of various drugs on dopamine release across stimulation parameters. Following euthanasia for voltammetry, multiple brain slices containing the NAc were utilized from each animal to test one drug or drug combination per slice. Differences in phasic/tonic ratios between HRs and LRs and percent changes in dopamine release following drug application and response to novelty were compared across stimulation frequencies using two-way mixed-factor ANOVAs. In the case of significant interactions, Bonferroni post-hoc comparisons were used. Effect sizes for significant results were calculated using Cohen's d where $d = \text{Mean}_1 - \text{Mean}_2 / \sigma_{pooled}$ (t-tests) or **partial eta squared (η_p^2) = SS_{effect} / (SS_{effect} + SS_{error}) (mixed ANOVAs)**. All statistics were performed using GraphPad Prism (version 9, La Jolla, CA) with $\alpha \leq 0.05$. In general, power was calculated for 80% ($\beta = 0.80$) to detect small- to medium-sized effects for correlations. Outliers were removed using Grubb's test on a normal distribution of dopamine release prior to data transformations and normalizations based on a distribution derived from raw dopamine release magnitude.

Data are presented as mean ± SEM across multiple variables or individual data points.

Results

α7 nAChR blockade has differential effects on dopamine release in animals with higher versus lower responses to novelty

Animals were classified as HR or LR by performing a tertiary split of the total distance traveled in a novel open field apparatus and comparing the upper (HR) and lower (LR) thirds of locomotor data. By definition and as expected, HR animals traveled significantly farther in the novel environment than LR animals across the 90 minute session (Fig. 1B; main effect of phenotype: $F_{(1,189)} = 220.5$, $p < 0.0001$, **$\eta_p^2 = 0.538$**). As expected, HR animals also travel a significantly greater cumulative distance than LR animals (Fig. 1C; $t_{15} = 9.386$, $p < 0.0001$, $d = 4.55$). Under drug-free conditions, HR and LR animals do not show differences in dopamine release (Fig. 1D; no main effect of phenotype: $F_{(1,48)} = 1.098$, $p = 0.2999$) or the maximal rate of dopamine uptake (V_{max}) (Fig. 1E; 1 Pulse: $t_{12} = 1.186$, $p = 0.2586$, $d = 0.634$; 5 Pulse- 20 Hz: $t_{12} = 1.155$, $p = 0.2706$, $d = 0.618$).

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Given evidence that α7 nAChRs localized to glutamatergic terminals within the NAc indirectly modulate dopamine release through signaling of AMPA and NMDA receptors (Kaiser & Wonnacott, 2000) and our previous study demonstrating differential dopamine regulation by β2-containing nAChRs localized to dopamine terminals (Siciliano et al., 2017), we first tested whether α7 nAChR modulation of dopamine release is predicted by response to novelty by utilizing the α7 nAChR selective antagonist MLA. Fig. 2A depicts the NAc circuitry that was pharmacologically investigated in this study as well as localization of nAChR subtypes within the NAc. To determine the relationship between response to novelty and α7 nAChR-mediated modulation of dopamine signaling, frequency-response curves were assessed at baseline and after bath application of MLA (30 nM). MLA has been shown to be selective for α7 nAChRs up to at least 50 nM (Klink et al., 2001; Mogg et al., 2002), thus we used a 30nM concentration to ensure clear interpretation of the effects of MLA on dopamine release.

We found that at single pulse stimulation, MLA decreased dopamine release compared to drug-free baseline (Fig. 2B, inset: $t_{11} = 3.19$, $p = 0.0086$), but did not differentially impact release in animals with higher or lower responses to novelty (Fig. 2B: $r^2 = 0.01$, $p = 0.79$). However, at phasic-like stimulations of 5 pulse 20 Hz, we found that response to novelty positively predicted dopamine release magnitude (Fig. 2C: $r^2 = 0.44$, $p = 0.02$), such that only animals with a lower locomotor response demonstrated a decrease in release magnitude following application of MLA. Splitting the data into HR and LR groups (Fig. 2D) showed that HRs had significantly higher dopamine release following application of MLA compared to LRs (phenotype x stimulation frequency interaction: $F_{(3,18)} = 3.172$, $p = 0.0495$, $\eta_p^2 = 0.346$). These data suggest that α7 nAChRs exert frequency-dependent inhibitory control over local dopamine release, and that phasic dopamine release in HR animals is insensitive to α7 modulation. Calculating the ratio of dopamine release from phasic-like stimulation frequencies to release from tonic-like stimulation captures a “signal-to-noise” ratio (T. Zhang et al., 2009). There were no significant differences in phasic/tonic ratios under drug-free conditions or following application of MLA in LRs compared to HRs. (Fig. 2E; no main effect of phenotype: $F_{(1,6)} = 2.197$, $p = 0.2442$).

Blockade of AMPA or NMDA receptors differentially impacts the α7 nAChR-mediated effect on dopamine release

Given the localization of α7 nAChRs to glutamatergic terminals and the absence of this subtype on dopamine terminals (Livingstone & Wonnacott, 2009), we next sought to determine if the relationship between α7 nAChR modulation of dopamine release and response to novelty is dependent on glutamate signaling through AMPA or NMDA receptors. To determine the contribution of AMPA receptors, frequency response curves were assessed following bath application of NBQX (5 μM). We found that blocking AMPA receptors with NBQX did not differentially affect HRs compared to LRs at either tonic- or phasic-like stimulation parameters (Fig. 3A; no significant phenotype x stimulation frequency interaction: $F_{(3,25)} = 1.322$, $p = 0.2893$). Next, we reassessed frequency response curves after MLA (30 nM) was added to the bath containing NBQX to examine whether blockade of AMPA receptors masks the differential effects of MLA on dopamine release observed in HRs versus LRs. We found that addition of MLA no longer resulted in the

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previously observed interaction effect (Fig. 3B; no significant phenotype x stimulation frequency interaction: $F_{(3,21)} = 1.589$, $p = 0.2218$). Additionally, there were no significant differences in phasic/tonic ratios observed following blockade of AMPA receptors or following the addition of MLA (Fig 3C; no main effect of phenotype: $F_{(1,6)} = 2.197$, $p = 0.9605$).

We next investigated the contribution of NMDA receptors to these effects via bath application of the selective antagonist D-AP5 (30 μM). We found that D-AP5 did not significantly affect release in either HRs or LRs across the examined range of frequencies (Fig. 3D; no significant phenotype x stimulation frequency interaction: $F_{(3,27)} = 0.3883$, $p = 0.7623$). We next reexamined frequency response curves following bath application of MLA to determine if D-AP5 blocks the individual differences in dopamine release seen after application of MLA alone. Here, we found that release following α7 nAChR blockade was dependent on stimulation parameter (Fig. 3E; main effect of frequency: $F_{(3,22)} = 3.774$, $p = 0.0252$, $\eta_p^2 = 0.340$). **This suggests that while the phenotype-dependent effect of α7 nAChR antagonism is blocked by D-AP5, the stimulation frequency-dependent effect is not.** Interestingly, the frequency-dependent effect following MLA appeared to be driven by HR rats, which was not observed with NBQX or MLA alone. While D-AP5 or MLA did not affect the phasic/tonic ratio, there was a significant difference in this measure between HRs and LRs in the animals that were tested (Fig. 3F; main effect of phenotype: $F_{(1,9)} = 6.333$, $p = 0.033$, $\eta_p^2 = 0.413$).

Similarly to when NMDA and AMPA receptors were blocked separately, when they were simultaneously blocked, there were no observed differential effects on dopamine release between HRs versus LRs (Fig. 4A; no significant phenotype x stimulation frequency interaction: $F_{(3, 29)} = 1.287$, $p = 0.2974$). As predicted by the effect of AMPA receptor blockade, the combination of AMPA and NMDA receptor antagonism blocked the phenotype-dependent effects of α7 nAChR antagonism with MLA (Fig 4B; no significant phenotype x stimulation frequency interaction: $F_{(3, 25)} = 1.665$, $p = 0.2001$). Here we found that phasic/tonic ratios were modulated by an interaction between application of NBQX/D-AP5 and MLA and novelty response (Fig. 4C; phenotype x drug interaction: $F_{(2, 17)} = 5.133$, $p = 0.018$, $\eta_p^2 = 0.377$).

Discussion

We utilized *ex vivo* FSCV to compare α7 nAChR and glutamate-mediated modulation of dopamine release in the NAc core of adult rats previously assessed on locomotor response to novelty. Response to novelty is used as an antecedent model of vulnerability to increased drug use as rats that exhibit increased activity in response to a novel environment acquire self-administration of drugs more rapidly and at lower doses than their lower responding counterparts (Blanchard et al., 2009; Ferris et al., 2013; Piazza et al., 1989).

Although drug acquisition rates were not measured in the current investigation, rats in our previous published studies that were exposed to identical housing and experimental conditions demonstrated this predictive relationship (Ferris et al., 2013). Differences between HRs and LRs in the mesolimbic dopamine system have also been well documented

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by our lab and others (Flagel et al., 2011; Hooks et al., 1991; Marinelli & White, 2000; Nelson et al., 2009). Investigations of the mechanisms driving these individual differences in dopamine signaling have found nAChRs on dopamine cell bodies and terminals to be important modulators of differential dopamine signaling (Fagen et al., 2007; Siciliano et al., 2017). However, whether nAChR subtypes within the NAc that are not localized to dopamine terminals play a role in differential dopamine signaling remains unclear. Thus, our study aimed to further characterize individual differences in NAc local circuitry and its regulation of dopamine signaling by comparing modulation of dopamine release by α7 nAChRs and glutamate receptors in rats with high and low locomotor responses to novelty.

As expected from our previous study (Siciliano et al., 2017), dopamine release was not predicted by HR/LR phenotype under drug-free conditions, but differences in release were shown following nAChR modulation. This further supports the hypothesis that the contribution of axonal modulation by nAChRs to the overall dopamine signal varies between individuals and in a manner that can be predicted by locomotor response to a novel environment. Given that differences in dopamine signaling between phenotypes are not seen at baseline, it is also likely that there is some degree of compensation in NAc inputs (such as glutamate or GABAergic interneurons) in order for HR/LR differences to not be present under drug-free conditions. We found that antagonism of α7 nAChRs by MLA resulted in a significant interaction between phenotype and stimulation frequency. This suggests that in addition to nAChR subtypes localized to dopamine terminals within the NAc (i.e., β2*), α7 nAChRs likely contribute to differences in dopamine signaling that are observed in HRs compared to LRs. In contrast to the facilitation in dopamine release that is seen following application of a β2*-selective nAChR antagonist (Siciliano et al., 2017), we found that α7 nAChR blockade attenuates dopamine release. These differential effects likely arise from disparate localization of these nAChR subtypes. Unlike β2* nAChRs, α7 nAChRs are not located on DA terminals, and so perhaps reducing α7-mediated glutamatergic drive onto CINs may reduce ACh, but not be sufficient to completely block CIN influence over DA terminals. This blockade, therefore, may not be sufficient to induce the facilitation with phasic-like stimulation parameters that is observed with β2* antagonists.

To further investigate the role of NAc glutamatergic signaling in the α7 nAChR-mediated effects on dopamine release, we next tested the effect of blocking AMPA and NMDA receptors on α7-mediated differences in dopamine release. **Here we found that blockade of either AMPA or NMDA receptors did not differentially impact dopamine release in HRs versus LRs. However, we showed that blockade of AMPA (i.e., NBQX), but not NMDA (i.e., D-AP5), receptors was sufficient to occlude the phenotype-dependent effects of α7 nAChR antagonism (i.e., MLA alone) on dopamine release. Specifically, NBQX, but not D-AP5, when given in combination with MLA, prevented the differential effects between HR and LR rats observed following MLA alone. These results indicate that α7 nAChRs in the NAc are another important source of local modulation that contribute to differential dopamine release in HRs versus LRs and that the effects of α7 nAChRs on dopamine may be mediated differentially through AMPA and NMDA receptors.** At least one study has demonstrated that HRs do not differ from LRs in extracellular glutamate levels at baseline or following acute cocaine

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administration (Mabrouk et al., 2018). However, this study used microdialysis which does not fully capture rapid changes in neurotransmitter release or presynaptic modulation of release that can be measured with FSCV (Ferris et al., 2013). It is possible that while cumulative extracellular levels of glutamate may not significantly differ between HR and LR phenotypes that differential terminal modulation of glutamate release is present and contributes to differential drug-induced synaptic plasticity experienced by HRs and LRs. To our knowledge, no studies to date have directly assessed whether basal differences in either number or functional activity of α7 nAChRs, NMDA, or AMPA receptors exist in HRs versus LRs. However, there is evidence that nAChRs play a role in exploratory and novelty-seeking behaviors and that mesocorticolimbic glutamate signaling modulates aspects of individual differences in sensitivity to reward-paired cues and vulnerability to increased ethanol intake (Chabout et al., 2013; Michaelides et al., 2013; Morganstern et al., 2012) Given this and the results of our current study, we find further examination of this question to be a compelling avenue for future studies.

We also examined the effect of α7 nAChR and glutamate antagonism on phasic/tonic ratios. **While we did not find any significant drug-dependent effects on this measure following antagonism of α7 nAChRs or following AMPA or NMDA receptor antagonism, there was a significant interaction between phenotype and drug effect following the combination of AMPA/NMDA receptor antagonism. These dissimilar results suggest that application of multiple antagonists may result in effects within the NAc local circuitry that are not restricted to only CIN/glutamate/dopamine signaling.**

Overall, our results indicate that while α7 nAChRs the NAc do not regulate dopamine release in the same manner as β2-containing nAChRs on dopamine terminals, they do appear to contribute to the differences in dopamine signaling observed in HRs versus LRs. It is possible that the mechanism by which dopamine is differentially modulated here is ultimately the same mechanism by which β2* nAChRs modulate dopamine, namely that acetylcholine is reduced via blockade of excitatory inputs to CINs and this reduction in CIN activity has a similar (but not identical) net effect as blocking nAChRs directly on dopamine terminals. The more pronounced differences seen between phenotypes in our previous study (Siciliano et al., 2017) compared to the current study may be due to the fact that reduced excitation of CINs via glutamate receptor blockade is not necessarily a complete blockade of downstream nAChRs (see Fig. 2A for schematic of NAc circuitry). Our results are consistent with evidence that glutamatergic projections from the prefrontal cortex and amygdala to the ventral tegmental area and NAc play critical roles in synaptic changes associated with compulsive responding for drugs (Koob & Volkow, 2016). Our results from utilizing pharmacological blockade of α7 nAChRs and NMDA/AMPA receptors are also consistent with studies demonstrating that α7 nAChRs are localized to glutamatergic terminals in the NAc (Grady et al., 2007; Livingstone & Wonnacott, 2009; Marchi et al., 2002). Whether the effects observed in the current study are exerted through glutamatergic receptors on dopamine terminals or more indirectly through glutamate receptors on CINs remains unclear and provides an interesting avenue for future studies. Our current study further demonstrates that nAChR-mediated dopamine release varies significantly among individuals and these differences in dopamine release are predicted by locomotor response to novelty. Further characterizing the differences in local NAc circuitry between HRs and

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LRs enables us to better understand the differential effects of drugs of abuse between these populations and provides a potential underlying mechanism for increased acquisition rates of drug self-administration in HR animals.

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Abbreviations

AMPA	A-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
D-AP5	5-phosphono-D-norvaline
FSCV	Fast scan cyclic voltammetry
MLA	Methyllycaconitine citrate
NBQX	1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide, disodium salt
nAChR	Nicotinic acetylcholine receptor
NMDA	N-methyl-D-aspartate
NAc	Nucleus accumbens
SUD	Substance use disorder

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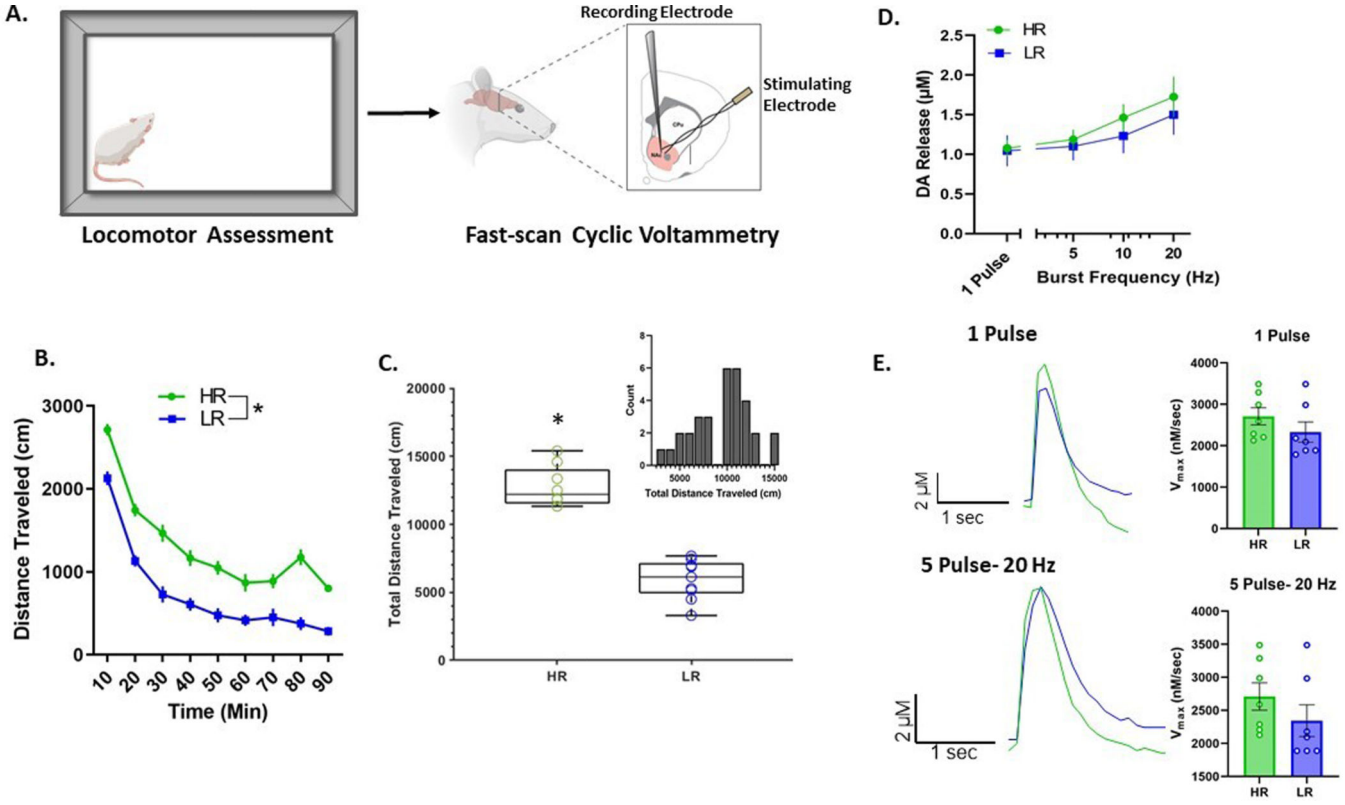

Figure 1.

HR and LR animals do not show differences in baseline dopamine release. (A) All animals were assessed for locomotor response to a novel environment prior to examining dopamine dynamics with voltammetry. (B) Locomotor activity over a 90 minute session in a novel environment. Data represented are from the upper (HR, n = 11) and lower (LR, n = 12) thirds of total distances traveled by all animals. Data points and bars represent mean ± SEM. (C) Sum of distance traveled for each group. Whiskers indicate minimum and maximum values. Inset shows total distances traveled for all tested animals. (D) HR and LR phenotypes do not differ in dopamine release across stimulation parameters. (E) Left: Representative traces showing baseline dopamine response at single pulse and 5 pulse 20 Hz stimulations in HR (green) and LR (blue) animals Right: Maximal rate of dopamine uptake (V_{max}) does not differ between HR and LR animals at either single pulse or 5 pulse 20 Hz stimulations. *$p < 0.05$.

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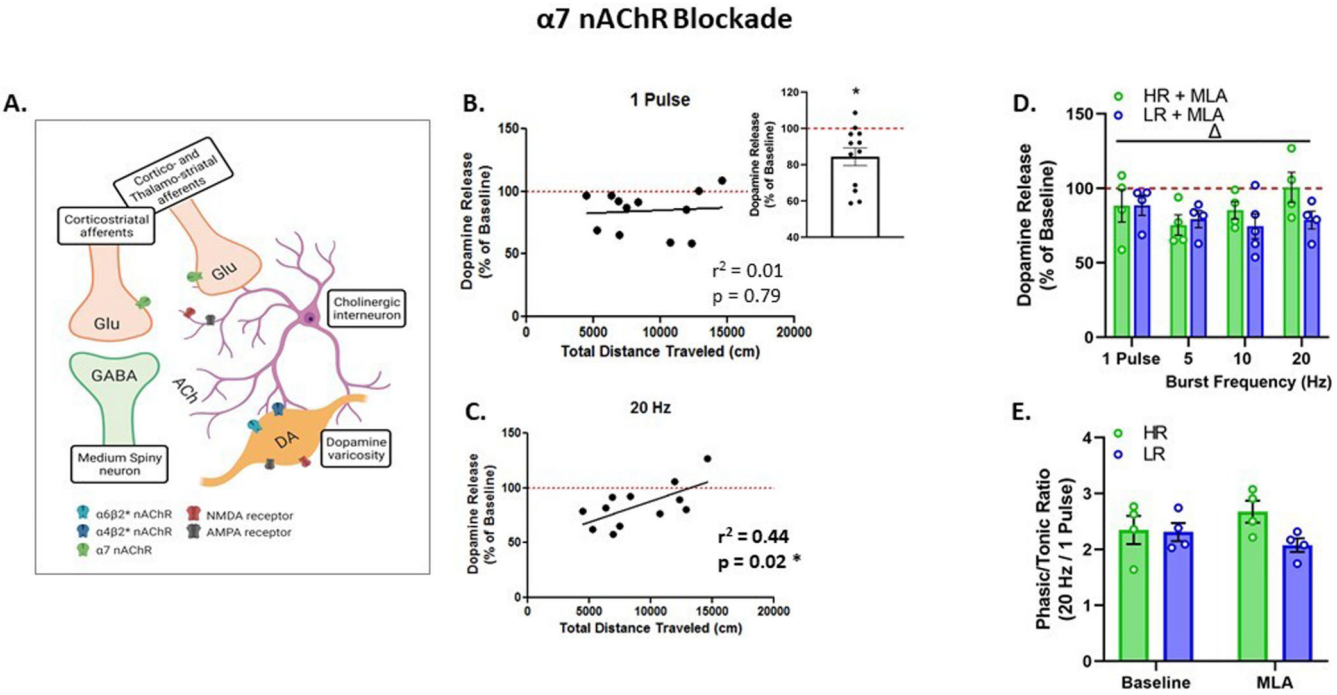

Figure 2.

α7 nAChR blockade has differential effects on dopamine release in animals with higher versus lower responses to novelty. (A) Schematic of NAc circuitry and nAChRs assessed using *ex vivo* voltammetry to determine effects of α7 nAChR modulation on dopamine release in rats with varying responses to novelty. (B) Bath application of 30 nM MLA decreased dopamine release at single pulse tonic-like stimulations across the spectrum of locomotor responses to novelty. Inset shows dopamine release following MLA application across all animals. (C) At phasic-like stimulation of 5 pulse 20 Hz, response to novelty positively predicted the effects of MLA on dopamine release. (D) Tertiary split of the data into HRs and LRs revealed a significant interaction effect of phenotype and stimulation frequency. (E) MLA application did not significantly affect phasic/tonic ratio in HRs or LRs. *$p < 0.05$. Δ $p < 0.05$, phenotype x stimulation frequency interaction.

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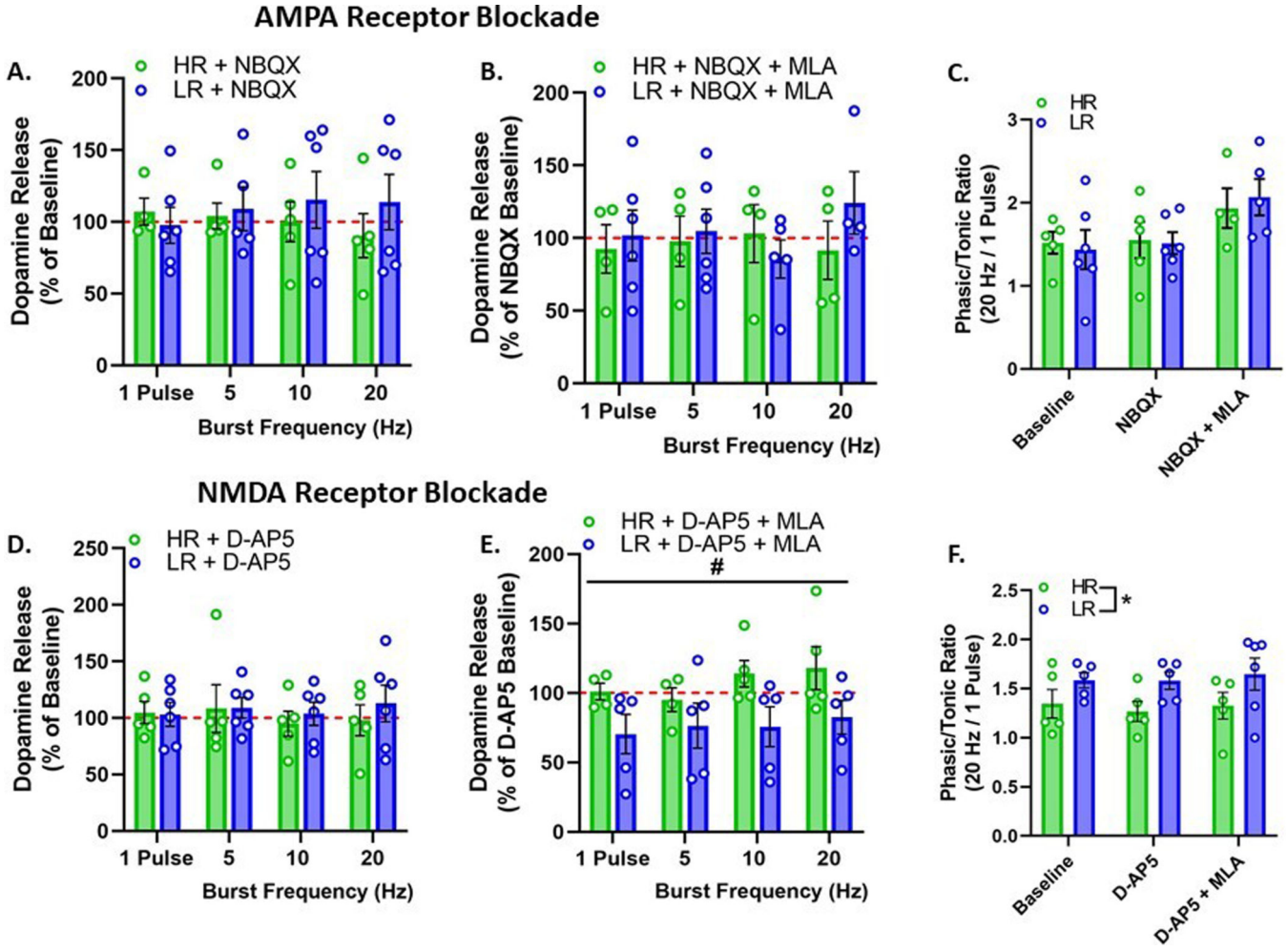

Figure 3.

Differential effects of α7 nAChR modulation on dopamine release are dependent on AMPA and NMDA receptors. (A) Grouped HR and LR data show that NBQX does not differentially affect dopamine release in HRs vs LRs. (B) NBQX blocks the interaction effect that was observed following application of MLA alone. (C) Phasic/tonic ratios were not significantly different between HRs and LRs following NBQX or MLA application. (D) D-AP5 alone does not significantly impact dopamine release in either HRs or LRs. (E) MLA following D-AP5 resulted in a significant main effect of frequency. (F) Phasic/tonic ratios are significantly different in HRs vs LRs following NMDA receptor and α7 nAChR blockade. *$p < 0.05$ main effect of phenotype, # $p < 0.05$, main effect of frequency.

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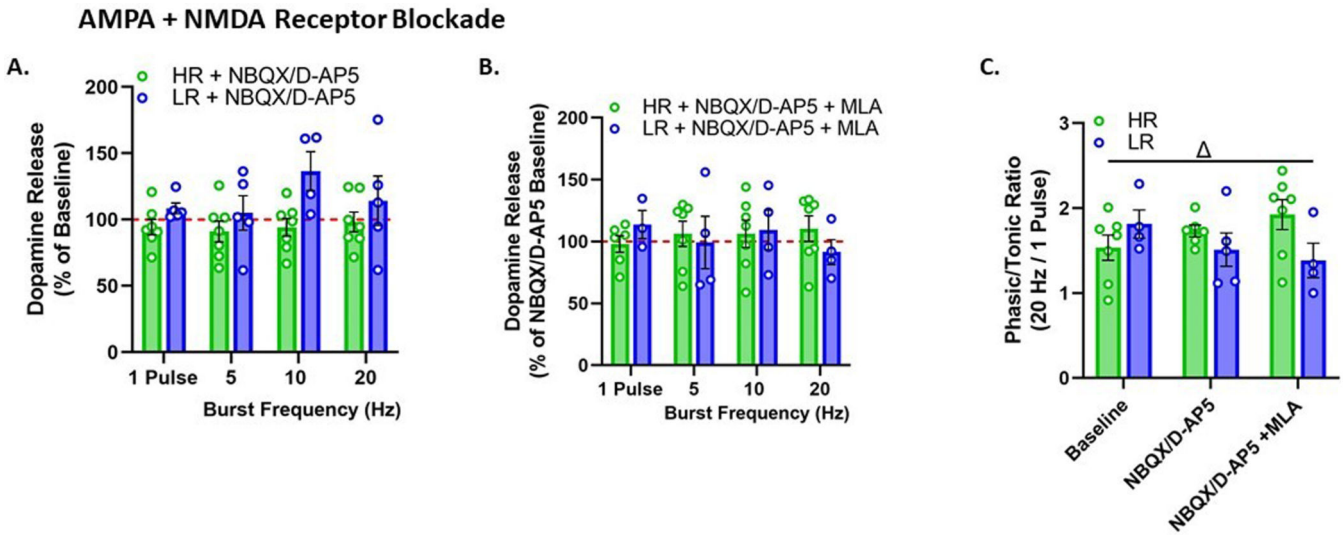

Figure 4.

Simultaneous blockade of AMPA/NMDA receptors blocks the effects of α7 nAChR antagonism. (A) Grouped HR and LR data show that the combination of NBQX/D-AP5 does not differentially affect dopamine release in HRs vs LRs. (B) Similar to NBQX alone, NBQX/D-AP5 blocks the phenotype-dependent effects of MLA on dopamine release. (C) Phasic/tonic ratios are modulated by both response to novelty and drug application. Δ $p <$ 0.05, phenotype x drug interaction.