Published in final edited form as:

Neuropharmacology. 2020 September 15; 175: 108176. doi:10.1016/j.neuropharm.2020.108176.

Different adaptations of dopamine release in Nucleus Accumbens shell and core of individual alcohol drinking groups of mice

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Abstract

Alcohol use disorder (AUD) places a tremendous burden on society, with approximately two billion alcohol users in the world. While most people drink alcohol recreationally, a subpopulation (3–5%) engages in reckless and compulsive drinking, leading to the development of AUD and alcohol dependence. The Ventral Tegmental Area (VTA)-Nucleus Accumbens (NAc) circuit has been shown to encode rewarding stimuli and drive individual alcohol drinking behavior. Our previous work successfully separated C57BL/6J isogenic mice into high or low alcohol drinking subgroups after a 12-day, two-bottle choice voluntary alcohol access paradigm. Electrophysiological studies revealed that low alcohol drinking mice exhibited elevated spontaneous and burst firing properties of their VTA dopamine (DA) neurons and specifically mimicking this pattern of activity in VTA-NAc neurons in high alcohol drinking mice using optogenetics decreased their alcohol preference. It is also known that VTA DA neurons encode the

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Authors contributions

Ming-Hu Han and Lu Zhang designed this study; Yutong Liu, Barbara Juarez, Erin S. Calipari, Carole Morel, Song Zhang performed the experiments and analyzed the data; Ming-Hu Han, Yutong Liu, Sarah Montgomery, Barbara Juarez and Erin Calipari interpreted the data and wrote the manuscript. Yimeng Kong helped to establish the electrode calibration system.

Declaration of competing interest

The authors have declared no conflict of interest.

salience and rewarding properties of external stimuli while also regulating downstream dopamine concentrations. Here, as a follow-up to this study, we utilized Fast Scan Cyclic Voltammetry (FSCV) to examine dopamine release in the NAc shell and core between alcohol drinking groups. We observed dynamic changes of dopamine release in the core of high drinking mice, but failed to see widely significant differences of dopamine release in the shell of both groups, when compared with ethanol-naïve controls. Overall, the present data suggest subregion-specific differences of evoked dopamine release in the NAc of low and high alcohol drinking mice, and may provide an anatomical substrate for individual alcohol drinking behavior.

This article is part of the special issue on Stress, Addiction and Plasticity.

Keywords

Individual alcohol drinking; Dopamine release; NAc core; NAc shell; Fast scan cyclic voltammetry; Stimulation patterns

1. Introduction

Alcohol is the most widely used psychoactive substance worldwide (Spoelder et al., 2017a, 2017b). High alcohol drinking and heavy intoxication can lead to alcohol dependence and diagnosis of alcohol-use disorder (AUD). Interestingly, a majority of alcohol drinkers consume alcohol frequently, but in a recreational manner, with a small subpopulation (3%–5%) who go on to drink pathologically (Grant et al., 2009; Spoelder et al., 2015). Based on this phenomenon, we previously hypothesized that individual alcohol drinking behaviors were a result of distinct neural adaptations within the mesolimbic dopamine (DA) system. To test this hypothesis, in our earlier work we separated C57BL/6J male mice into high and low alcohol drinking subgroups based on individual alcohol consumption and preference they displayed during a 12-day, two-bottle choice voluntary alcohol drinking paradigm (Juarez et al., 2017). After the establishment of individual alcohol drinking profile, we found elevated DA neuronal activity, including an increased firing rate and phasic (burst) firing properties in VTA dopaminergic neurons of the low alcohol-drinking group. Moreover, we demonstrated that by optogenetically increasing the phasic firing in VTA-NAc neurons of high alcohol drinking mice, we were able to significantly decrease alcohol preference. This suggests that the activity of VTA-NAc neurons plays a causal role in regulating individual differences underlying alcohol drinking behaviors (Juarez et al., 2017).

Unraveling changes of dopamine release in the NAc, as well as its subregions, has been a long-time exploration in the field of addiction and alcohol research. Early studies used microdialysis to investigate extracellular dopamine concentrations in the NAc of rats exposed to various alcohol treatments such as intraperitoneal injection and in-situ perfusion (Yan, 1999; Yim and Gonzales, 2000; Yoshimoto et al., 1992). These studies showed increased dopamine release in the NAc in alcohol treated animals versus controls (Yan, 1999; Yim and Gonzales, 2000; Yoshimoto et al., 1992). But the NAc, as a key functional region in the reward system, can be divided into at least two major subregions, the shell and the core. These two structures are distinct from one another both anatomically and functionally (Siciliano et al., 2017). Although they both receive dopaminergic input from the

individually housed once the alcohol drinking paradigm began. All procedures were in accordance with the National Institutes of Health Guidelines for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the Icahn School of Medicine at Mount Sinai, New York.

2.2. Alcohol drinking paradigm

C57BL/6J mice at 7–8 weeks of age were housed individually in Plexiglas cages with corncob bedding at an ambient temperature of 21 ± 1 °C. All fluids were presented in 50 mL polypropylene conical tubes and fitted with rubber stoppers with a stainless-steel drinking spout inserted through wires in front of the cage. On the first day, mice were habituated to two 50 mL conical tubes of water as a 24-h acclimation. After the acclimation period, one 50-mL tube was filled with 3% ethanol (EtOH, v/v) solution instead, the other one refilled with fresh water. During the alcohol-drinking paradigm, mice were given a choice between water and an increasing concentration of ethanol, 3%, 6% and 10% EtOH (v/v), for a total of 12 days. Each EtOH % was offered for 4 days. On Day 12, the alcohol drinking behavior was determined, and mice were split into high alcohol drinking and low alcohol drinking groups (see below). Mice that exhibited middle alcohol drinking behavior were excluded. After the determination of alcohol drinking behavior, mice had continued access to 10% EtOH (v/v) and water until experimental recording day. All tubes were measured every day and tube sides were switched to avoid side preference during the experimental period. All mice were weighed on the first day when each EtOH concentration was replaced. The ethanol naive control group was given the choice between two 50 mL bottles of water, and handled the same as experimental animals in the alcohol group.

EtOH preference%: EtOH preference% was defined as (EtOH intake/total intake) * 100%. EtOH consumption was calculated as g(EtOH)/Kg (body weight)/24 h. Mice that met the criteria of both EtOH preference% > 60%, EtOH consumption >10 g/kg/24 h were labeled “high alcohol drinking mice”, and those who had EtOH preference% < 40%, EtOH consumption <10 g/kg/24 h, were labeled “low alcohol drinking mice”. Mice were excluded in experiments if they failed to meet the above criteria or altered their drinking behaviors more than two days after the 12-day paradigm.

2.3. Ex vivo FSCV dopamine signal recordings

Brain slice preparation: Mice were decapitated under isoflurane anesthesia. The brain was removed quickly and sectioned into 250- μ m-thick slices in iced-cold, carbogen-saturated (95% O₂, 5% CO₂) artificial cerebrospinal fluid (aCSF) (in mM) (pH 7.4): 126 NaCl, 2.5 KCl, 1.2 NaH₂PO₄, 2.4 CaCl₂, 1.2 MgCl₂, 25 NaHCO₃, 11 D-glucose, and 0.4 L-ascorbic acid ascorbic acid. Once sliced, the brain tissue which contained the NAc region was immediately placed to a recovery chamber filled with aCSF solution, bubbled with 95% O₂, 5% CO₂ for 1 h at room temperature. Once transferred into the recording chamber, slices were equilibrated for an additional 30 min with oxygenated aCSF perfused at a rate of 1.5 mL/min. Chamber temperatures were held at 32 °C.

Carbon Fiber Microelectrodes (CFM) preparation: CFM (7 μ m diameter) was loaded into a capillary tube under negative pressure and subsequently pulled to a taper. This carbon fiber

microelectrode was then placed under a microscope and cut to an exposed length of 100–200 μm . We then filled the CFM with 150 mM KCl and tested the quality of the CFM by placing it in regular aCSF solution in the recording chamber at the same temperature (32 °C) used during our experiment.

Stimulation pattern with different frequencies (Fordahl and Jones, 2017; John and Jones, 2007): Electrodes were placed $\sim 100\ \mu\text{m}$ below the surface of slice in NAc shell or core. The tip of the CFM was placed 100 μm away from the stimulating electrode. Dopamine signal was evoked by a single electrical pulse (pulse amplitude: 0.35 mA, pulse width: 4 msec, pulse polarity: monophasic) at a constant interval of 180 s. Triangular waveform scanned between -0.4 and $1.2\ \text{V}$ and then back to $-0.4\ \text{V}$ vs Ag/AgCl, $400\ \text{V/s}$ was applied for the purpose of detecting extracellular dopamine release. When a stable baseline was established (3 collections within 10% variability), stimulation paradigm of 5 pulses at (pulse amplitude: 0.35 mA, pulse width: 4 msec, pulse polarity: monophasic) 5, 10, 20, or 100 Hz were applied to the brain slice and each stimulation session was separated by a 300 s interval. Collect duration was 30 s.

Multi-pulse stimulation pattern (Chen and Rice, 2002; Patel and Rice, 2013): Electrodes were first placed in the dorsolateral striatum region of the brain slice to find the “perimaximal intensity” (Patel and Rice, 2013)- the first stimulating pulse amplitude that gives the maximal dopamine release. It was examined by giving a series of sequentially increasing stimulating amplitudes (started with 0.2 mA and increased 0.05 mA steps each time) to the dorsolateral striatum region (which displays larger dopamine signals than the NAc) of each brain slice and then marked the first stimulating pulse amplitude which induced the maximal dopamine release as “perimaximal intensity”. Electrodes were then placed in NAc shell or core once perimaximal intensity had been determined. One single pulse and multi-pulse stimulation pattern (10 Hz with 5, 10 or 30 pulses) at the perimaximal intensity were then applied to the brain tissue. Pulse width was 4 msec (monophasic) and the same triangular waveform scanning protocol was used for dopamine detection. A constant interval of 180 s was used in single-pulse stimulation, while constant interval for each multi-pulse stimulation session was 600 s. 3 successive collections within 10% variation were considered constant.

Electrode calibration: After recording, the electrode was rinsed in deionized water and soaked in isopropyl alcohol overnight. Solutions of three known dopamine concentrations were used as follows: 0.5, 0.75, 1 μM . A slow bath calibration apparatus was applied and electrodes exhibited linear slopes that were close to 1 (range: 0.93–0.99). Calibration was used to convert electrical current to DA concentration.

2.4. Data analysis

SPSS was used to perform the statistics and analyze data sets. Alcohol drinking behaviors including EtOH preference (%) and EtOH consumption (g/kg/24h), total liquid intake and body weight were analyzed using a repeated measures ANOVA, followed by an Independent-samples *t*-test. For all analysis of FSCV data, Demon voltammetry and analysis software was used (Yorgason et al., 2011). Dopamine concentration data from EtOH naive,

low and high alcohol drinking group were also analyzed using a repeated measures ANOVA, followed by the LSD Post-Hoc Test.

3. Results

3.1. Segregation of mice into low and high alcohol drinking groups

To separate C57BL/6J mice into low and high alcohol drinking groups based on their individual alcohol consumption and preference, we performed a continued access, two-bottle choice alcohol drinking paradigm (Fig. 1A) (Juarez et al., 2017). Here, a 12-day procedure was carried out in 7–8-week old C57BL/6J male mice using elevated ethanol concentrations (each concentration was maintained for 4 days). Mice were offered a two-bottle choice between water and ethanol (3%, 6%, 10% v/v ethanol). On day 12, alcohol-drinking behaviors were determined and then animals were maintained on 10% ethanol and water until the day of recording. Animals that exhibited middle alcohol drinking behaviors or changed their preference more than 2 days after the 12-day paradigm were removed from the study.

Criteria for alcohol drinking groups were based on EtOH preference% and EtOH consumption (g/kg/24h) were set as a cut off: mice displayed both EtOH preference (%) > 60% and EtOH consumption > 10 g/kg/24h were labeled “high alcohol drinking mice” (Fig. 1B) and those that showed EtOH preference (%) < 40% and EtOH consumption < 10 g/kg/24h were labeled “low alcohol drinking mice” (Fig. 1C). In addition, we found low alcohol-drinking mice depicted a significantly lower EtOH preference (Fig. 1D, repeated measures ANOVA: interaction effect $F_{(1.41, 25.31)} = 9.81$, $P = 0.002$; drinking group effect $F_{(1, 18)} = 218.24$, $P < 0.0001$. Independent-samples t -test, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$) and EtOH consumption (Fig. 1E, repeated measures ANOVA: interaction effect $F_{(1.43, 25.82)} = 5.70$, $P = 0.015$; drinking group effect $F_{(1, 18)} = 127.56$, $P < 0.0001$. Independent-samples t -test, $***P < 0.001$) than high alcohol drinking mice. Moreover, we also measured total liquid intake (g) (Fig. 1F, repeated measures ANOVA: interaction effect $F_{(1.36, 24.44)} = 1.71$, $P = 0.205$; drinking group effect $F_{(1, 18)} = 14.46$, $P = 0.001$. Independent-samples t -test, $P > 0.05$) and body weight (g) of the mice (Fig. 1G, repeated measures ANOVA: interaction effect $F_{(2, 36)} = 1.99$, $P = 0.152$; drinking group effect $F_{(1, 18)} = 0.59$, $P = 0.452$. Independent-samples t -test, $P > 0.05$). No significant differences were observed with these supplementary measurements between alcohol drinking groups.

These results demonstrate that stable, individual alcohol drinking behaviors can be displayed in isogenic C57BL/6J mice. After segregation into distinct groups, we examined dopamine release dynamics in the NAc shell and core of low and high alcohol drinking mice, with EtOH naive mice as control to further our understanding of the dopaminergic mechanisms underlying individual alcohol drinking groups.

3.2. Dopamine release in NAc shell in response to two different stimulation patterns between EtOH naive, low and high alcohol drinking groups

Next, we measured *ex vivo* dopamine release in the NAc core and shell in EtOH naive, low and high alcohol drinking mice by using the FSCV technique after the 12-day, two-bottle

choice alcohol drinking paradigm (Fig. 2A and B). We utilized varying frequencies and multi-pulse stimulation patterns to probe the tonic or phasic dopamine release properties and maximal amount of dopamine release in the shell, respectively.

Our results showed that there was no significant difference of dopamine release in NAc shell between three groups of mice when varying frequencies of the stimulation pattern was applied (Fig. 3A and B repeated measures ANOVA: Interaction effects: $F_{(2.51, 15.06)} = 0.38$, $P = 0.73$; drinking group effect: $F_{(2, 12)} = 1.52$, $P = 0.26$; frequency effect: $F_{(1.26, 15.06)} = 59.61$, $P < 0.0001$. LSD Post Hoc Test, $P > 0.05$). In addition, we only observed a significant elevated dopamine signal in low alcohol drinking mice (compared to EtOH naive controls) under the multi-pulse stimulation at 5 pulse, 10 Hz, while dopamine release in the higher drinking group under this stimulation paradigm was less affected compared to the controls (Fig. 3A, C, repeated measures ANOVA: Interaction effects: $F_{(2.26, 11.28)} = 1.53$, $P = 0.259$; drinking group effect: $F_{(2, 10)} = 2.06$, $P = 0.178$; pulse effect: $F_{(1.13, 11.28)} = 89.36$, $P < 0.0001$. LSD Post Hoc Test). However, the dopamine-signal difference between low alcohol drinking mice and EtOH-naive controls did not extend to the longer pulse-train (10 pulses or 30 pulses) stimulations. Due to the above observations, we next asked whether dopamine release in NAc core would be distinct from the shell.

3.3. Dopamine release in NAc core in response to two stimulation patterns between EtOH naive, low and high alcohol drinking groups

Within the NAc core, we noticed significant changes of dopamine release in high alcohol drinking mice, whereas dopamine release in low drinking mice was less affected by these two different stimulation patterns when compared with EtOH naive controls (Fig. 4A–C). Specifically, high alcohol drinking mice displayed attenuated dopamine signals at 5 pulses 10 and 20 Hz when a varying-frequency stimulus pattern was applied (Fig. 4B, repeated measures ANOVA: Interaction effects: $F_{(2.92, 36.51)} = 2.71$, $P = 0.061$; drinking group effect: $F_{(2, 25)} = 3.29$, $P = 0.054$; frequency effect: $F_{(1.46, 36.51)} = 65.07$, $P < 0.0001$. LSD Post Hoc Test, $*P < 0.05$). But surprisingly, when performing multi-pulse stimulations, with longer trains applied (10 Hz with 5, 10 and 30 pulses), the high alcohol drinking group showed a remarkable increase in dopamine release when compared to EtOH naive controls (Fig. 4C, repeated measures ANOVA: Interaction effects: $F_{(2.57, 12.84)} = 2.53$, $P = 0.11$; drinking group effect: $F_{(2, 10)} = 3.63$, $P = 0.065$; pulses effect: $F_{(1.28, 12.84)} = 73.23$, $P < 0.0001$. LSD Post Hoc Test, $*P < 0.05$). It is also interesting that the same stimulation pattern (5 Hz/5 Pulses) can induce distinct effects with different intensities (0.35 mA vs. perimaximum) (Fig. 4B and C). Moreover, both the low and high alcohol drinking groups failed to display significant difference in dopamine release at tonic single-pulse stimulation when compared to EtOH-naive controls (Fig. 4B and C).

4. Discussion

Delineating the circuit adaptations that underlie varying alcohol drinking behaviors will further our understanding of the mechanisms of the progression to AUD. Previous findings reported that while low alcohol drinking mice displayed increased VTA dopamine activity at the cell body, high alcohol drinking mice displayed firing activity similar to EtOH naive

mice. Here, we identified regionally divergent dopamine release in the NAc in individual alcohol drinking groups of mice. We showed that high alcohol drinking mice displayed significant changes of dopamine release in the core but not the shell under various stimulations, while low alcohol drinking mice did not show a widely significant difference of dopamine release under extra stimulations. Moreover, dopamine signals in the NAc core of high alcohol drinking mice showed different variations after applications of phasic and multi-pulse stimulations, respectively. Our findings indicate subregion-specific neural adaptations that may be causally related to distinct alcohol drinking behaviors and patterns of alcohol consumption.

The C57BL/6J mouse strain is reported to display a high alcohol preference and ability to drink to intoxication (Belknap et al., 1993; Linsenbardt and Boehm, 2015). In both voluntary and restricted alcohol access paradigms, these high alcohol drinking mice may exhibit “front-loading” drinking behavior, where the majority of alcohol is consumed within a short period of time, physiologically challenging the dopamine system via EtOH and leading to intoxication (Belknap et al., 1993; Linsenbardt and Boehm, 2015). A body of evidence have shown that reward-related behaviors are closely associated with VTA dopaminergic neuronal firing patterns and dopamine release in downstream regions such as the NAc (Bass et al., 2013; Calipari et al., 2017; Carelli and Wightman, 2004; Flagel et al., 2011; Salamone et al., 2016; Willuhn et al., 2010; Wook Koo et al., 2016). Phasic, but not tonic, firing is primarily responsible for mediating reward-related behaviors via large transient (phasic) dopamine release in the NAc (Grace, 2000; Tsai et al., 2009). Here we report that phasic stimulation (5-pulse, 20-Hz) evoked an increased dopamine release in the NAc core, not the shell, in high drinking mice when compared with the controls. Tonic stimulations (single-pulse and 5-pulse, 5-Hz) had little effect on dopamine release across the NAc in both low and high alcohol drinking mice. Interestingly, we previously found that phasic (5 spikes, 20 Hz), not tonic stimulation (either 0.5 Hz or 5 Hz) of VTA dopaminergic cell bodies decreased the alcohol drinking behaviors of high alcohol drinking mice (Juarez et al., 2017). It is important to note that our previous study found high drinking mice and EtOH naive controls exhibited similar firing activity at the soma within the VTA (Juarez et al., 2017). However, we currently observed a significant difference of dopamine release from the NAc terminals in the NAc core of high drinking mice when compared to EtOH naive controls. This shows a potential alcohol-induced presynaptic (local) adaptation in dopamine release in animals that consume high levels of ethanol that is only seen at high stimulation intensities. It is also consistent with the evidence showing that local mechanism in the NAc plays an important role in regulating the releasing property of VTA DA neurons (Walsh et al., 2014). Therefore, we hypothesize that the magnitude of phasically evoked dopamine release might be of great importance in mediating the rewarding properties of voluntary alcohol drinking behaviors in high drinking individuals.

To determine detail subregion-specific dopamine release in NAc from high and low alcohol-drinking animals, we utilized FSCV to simulate the physiological range of dopamine neural firing that may occur *in vivo*. Stimulation patterns with different frequencies are usually used to mimic tonic and phasic firing modes of dopaminergic neurons, or to assess the frequency dependence of dopamine release properties (Bao et al., 2010; Yorgason et al., 2014; Rice and Cragg, 2004; Siciliano et al., 2017). Further, multi-pulse stimulation patterns

evoked higher dopamine release in contrast with varying-frequency stimulations (Threlfell and Cragg, 2007). Another advantage of multi-pulse stimulation patterns here is that perimaximal stimulation induces the maximal dopamine release and can mask regulatory processes that might decrease DA release (Patel and Rice, 2013). Moreover, multi-pulse stimulations at 10 Hz had significant effects on evoked dopamine release as compared to those at lower frequencies (pulses with frequencies lower than 10 Hz), specifically a 30-pulse train at 10 Hz would evoke the greatest dopamine signal (Chen and Rice, 2002; Threlfell and Cragg, 2007)- higher stimulation frequencies did not evoke a greater dopamine signal (Rice et al., 1997).

Using these stimulus paradigms, we observed the most significant dynamic changes of dopamine release in the NAc core from high drinking mice. They displayed a reduced dopamine signal in contrast with EtOH naive controls at phasic-frequency stimulation but shifted to an enhanced signal when multi-pulse stimulations were applied. Our previous work also found that high drinking mice displayed a dramatically reduced excitatory I_h current—an excitatory hyperpolarization-activated current in the VTA—and a blunted response to the excitatory properties of EtOH in VTA-NAc dopamine neurons when compared to low alcohol drinking mice and EtOH-naive controls (Juarez et al., 2017). These previous findings may explain the observations that varying-frequency stimulation induces a reduction of dopamine release (Fig. 4B). Other factors such as nicotinic acetylcholine receptors (nAChRs), especially the $\alpha 6^*$ -nAChRs, which is functionally operational in NAc core for modulating evoked dopamine release, might also possibly contribute to this decreased dopamine release (Schilaty et al., 2014). However, although high drinking mice exhibit reduced excitatory I_h current and a blunted response to EtOH, when under the multi-pulse stimulation paradigm, a reversal of dopamine release was observed in the core (Fig. 4C). Thus, we hypothesize that the longer stimulating durations as well as the perimaximal stimulation induced a maximal release of dopamine transmissions into the cleft from the terminals, which is possibly associated with the functioning of releasable vesicles in VTA terminals of high drinking mice (Nolan et al., 2020). Notably, it seems that only the high alcohol-drinking mice displayed a crosscurrent in dopamine release under the two different stimulation patterns. EtOH-naive mice showed a similar amount of dopamine release at the same 5-pulse, 10 Hz stimulation parameters under these two patterns, suggesting that the stimulation patterns we applied are less likely to drive the dynamic changes in dopamine release in high drinking mice.

Although both the NAc core and the shell receive dopaminergic innervations from the VTA, dopamine release does not appear to be homogenous across the NAc and may possibly show a different contribution to aspects of reward-related behaviors (Calipari et al., 2012). Here we showed dopamine release in the NAc shell was less affected by extra stimulations in both the low and high drinking mice. Conversely, dopamine release in the NAc core was distinctly different from the shell, displaying a pulse number-, but limited frequency-dependence in high drinking mice. These observations may indicate that NAc core seems easier to be affected by various stimulations than the shell. The NAc core has been reported to play a crucial role in mediating spatial learning, conditioned responses, responses to motivational stimuli and impulsive choices (Salgado and Kaplitt, 2015). Further, high drinking in the dark (DID) mice display compulsivity/impulsivity traits concomitant with a

decreased basal Orexin Receptor 1 (OX1r) and OX2r mRNA expression in the NAc core, suggesting molecular changes specifically in the NAc core in high drinking mice (Alcaraz-Iborra et al., 2017), which is in consistent with our findings. In addition, NAc contains D1 or D2-expressing medium spiny neurons (MSNs), which have divergent projection targets and exert antagonistic effects in reward-related behaviors (Hearing et al., 2016). Since these two sub-populations display a differential topographical distribution in NAc subregions and D1 receptors are considered more readily to detect phasic dopamine activity and release (Gangarossa et al., 2013; Richfield et al., 1989; Soares-Cunha et al., 2016) within the context of our results we suggest that there may be alcohol-induced neuroadaptations specifically in D1 expressing MSNs in the core that may regulate high alcohol drinking behaviors. Additional work is needed in future to directly investigate this possibility.

In summary, we demonstrate that individual alcohol drinking behaviors cause subregion-specific adaptations in dopamine release across the NAc core and shell. Consequently, the core shows more sensitivity to the dynamic range of applied stimulations than the shell, implying an existence of region-specific neural adaptations in the NAc. This study reveals an anatomical substrate for individual alcohol drinking behavior, and future experiments will be necessary to examine the expression of presynaptic related transporters, the molecular regulation of DA vesicle release and D2 auto-receptor sensitivity within this rodent alcohol-drinking model.

Acknowledgements

We thank Jingqi Gong for helpful suggestions and discussion, and Tomas Fanutza for technical assistance.

Funding sources

This work was supported by grants from the National Institute on Alcohol Abuse and Alcoholism (R01 AA022445, MHH; F31 AA022862, BJ), Natural Science Foundation of China (81971297, 81571860), Colleges Pearl River Scholar Funded Scheme (GDUPS2015), Natural Science Foundation of Guangdong Province (2018B030311062) and Program for Changjiang Scholars and Innovative Research Team in University (IRT 16R37). China Postdoctoral Science Foundation Grant (2019M652962).

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HIGHLIGHTS

- Mice were split into groups based on their low and high alcohol drinking behaviors.
- Dopamine release between alcohol drinking mice varied in the NAc core and the shell.
- High alcohol drinking mice exhibited significant evoked dopamine release in NAc core.

Fig. 1.

C57BL/6J male mice were segregated into low or high alcohol drinking groups. (A) Timeline of the 2-bottle choice alcohol-drinking paradigm. Mice were exposed to a continuous, 12-day, 2-bottle choice alcohol drinking paradigm (4 days each concentration: 3%, 6%, 10% v/v ethanol [EtOH]). (B) Scatterplot of individual EtOH preferences across days of paradigm (EtOH preference (%) on the 4th day of each EtOH concentration). (C) Scatterplot of individual alcohol consumption (g/kg/24 h on the 4th day of each EtOH concentration)). (D) EtOH preference (%) of low alcohol drinking mice was lower when compared with high alcohol drinking mice on the day of 4th, 8th and 12th ($n_{\text{HAD}} = 16$; $n_{\text{LAD}} = 4$). (E) EtOH consumption (g/kg/24 h) of low alcohol drinking group (LAD) was significantly lower than high alcohol drinking group (HAD) on the days 4, 8 and 12 ($n_{\text{HAD}} = 16$; $n_{\text{LAD}} = 4$). (F) Total liquid intake between low and high alcohol drinking mice. (n

(HAD) = 16, $n_{\text{(LAD)}} = 4$). (G) Body weight of two alcohol drinking groups ($n_{\text{(HAD)}} = 16$; $n_{\text{(LAD)}} = 4$). Data represented as mean $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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Fig. 2. FSCV recording schedule and anatomical recording regions of NAc shell or NAc core. (A) Experimental arrangement and stimulation patterns on FSCV recordings. Followed by the 12-day alcohol drinking paradigm, individual alcohol drinking behaviors were determined on the day 12th. FSCV *ex vivo* recordings were performed during the following two weeks. Dopamine signal was evoked by applying either varying-frequency stimulus (1 pulse or 5 pulses with 5, 10, 20, 100 Hz at a constant 0.35 mA pulse intensity) or multi-pulse stimulus (1 pulse or 10 Hz with 5, 10, 30 pulses at a perimaximal intensity). (B) Anatomic recording regions of NAc shell or NAc core. Recording electrodes were placed ~100 μ m below the surface of slice in NAc shell or core. The tip of the recording electrode was placed 100 μ m away from the stimulating electrode.

Fig. 3.

Dopamine release in response to different stimulation patterns in NAc shell of alcohol naive, low and high alcohol drinking mice. (A) Representative *ex vivo* traces (concentration-time) of dopamine signals in NAc shell of EtOH naive, low alcohol drinking mice and high alcohol drinking mice. Top, sample traces of DA signals evoked by a 5-pulse train at 10 Hz in NAc shell at either a constant 0.35 mA stimulation intensity (left) or perimaximal intensity (right); Bottom, corresponding dopamine signals shown in the current-time colorplots of NAc shell at 0.35 mA-constant stimulating intensity (left) and perimaximal intensity (right) after a 5-pulse, 10 Hz stimulation in three groups of mice. (B) No difference in NAc shell dopamine release between EtOH naive, low and high alcohol drinking mice when given a single-pulse stimulation or various frequencies (5, 10, 20 and 100 Hz) with 5 pulses at the constant stimulation intensity of 0.35 mA ($n_{\text{EtOH naive}} = 4$; $n_{\text{LAD}} = 3$; $n_{\text{HAD}} = 8$). (C) Low alcohol drinking mice displayed an increased dopamine release when compared to alcohol naive mice at a 5-pulse, 10 Hz stimulation pattern when the perimaximal intensity paradigm was applied in NAc shell ($n_{\text{EtOH naive}} = 5$; $n_{\text{LAD}} = 3$; $n_{\text{HAD}} = 5$). Data represented as mean $\pm$ S.E.M. * $P < 0.05$.

Fig. 4.

Dopamine release in response to different stimulation patterns in NAc core of alcohol naive, low and high alcohol drinking mice. (A) Representative *ex vivo* traces (concentration-time) of dopamine signals in NAc core of three groups of mice. Top, sample traces of dopamine signals evoked by a 5-pulse, 10 Hz stimulation with either the constant 0.35 mA stimulus intensity (left) or the perimaximal intensity (right) in NAc core of three mice groups; Bottom, representative dopamine release current-time color plots in the NAc core of alcohol naive, low and high alcohol drinking groups after a 5-pulse, 10-Hz stimulation at the constant stimulation intensity (left), or the perimaximal intensity (right). (B) High alcohol drinking group showed a decrease of dopamine release when 5 pulses with frequencies of 10 Hz and 20 Hz were applied at 0.35 mA stimulation intensity in NAc core as compared to alcohol naive mice ($n_{\text{(EtOH naive)}} = 8$, $n_{\text{(LAD)}} = 4$, $n_{\text{(HAD)}} = 16$). (C). High alcohol drinking group depicted a dramatically elevated dopamine release in NAc core when compared to the EtOH naive group when an increasing stimulation pulses was applied at a perimaximal intensity ($n_{\text{(EtOH naive)}} = 5$, $n_{\text{(LAD)}} = 3$, $n_{\text{(HAD)}} = 5$). Data represented as mean $\pm$ S.E.M. * $P < 0.05$.