

# Genetic Selection for Alcohol Preference in Mice Alters Dorsal Striatum Neurotransmission

Brandon M. Fritz , Braulio Muñoz, and Brady K. Atwood

**Background:** Although it is widely acknowledged that the risk of developing an alcohol use disorder (AUD) is strongly influenced by genetic factors, very little is known about how this genetic predisposition may alter neurotransmission in a way that promotes AUD susceptibility. The dorsal striatum has garnered increased attention as a brain region of interest in AUD development given its significant roles in goal-directed and habitual behavior.

**Methods:** In the present work, dorsal striatal neurotransmission parameters were measured in pre-clinical mouse models of high and low AUD risk. We performed brain slice whole-cell patch clamp electrophysiological recordings from medium spiny neurons (MSNs) in the dorsomedial (DMS) and dorsolateral (DLS) striatum of naive adult male and female selectively bred high- and low-alcohol-preferring lines of mice (HAP and LAP).

**Results:** We found that MSNs of HAP mice were significantly more excitable than those of LAP mice, specifically in the DLS. Additionally, the frequencies of spontaneous glutamate- and GABA-mediated currents were both elevated in HAP mice relative to LAP mice in both dorsal striatal subregions, whereas amplitude differences were more variable between lines and subregions. AMPAR/NMDAR current ratios were significantly lower in HAP mice in both DLS and DMS.

**Conclusions:** Collectively, these results suggest that genetic predisposition for high or low alcohol consumption produces significantly different basal functional states within both DLS and DMS which may be important factors in the behavioral phenotypes of HAP and LAP mice.

**Key Words:** Alcohol, Dorsal Striatum, Genetics, Glutamate, GABA, Mice.

AN INDIVIDUAL'S risk for developing an alcohol use disorder (AUD) is heavily influenced by his or her genetic background. In fact, the relative "density" or pervasiveness of AUD diagnosis in an individual's family is positively related to the likelihood of future problematic alcohol use (Kendler et al., 2018). This association is especially strong if 1 or both parents reach AUD diagnostic criteria. An analysis of a large U.S. sample determined that having 1 parent with AUD produced a 2.5-fold increase in risk for AUD development, whereas having both parents with AUD resulted in a 4.4-fold increase in risk (Yoon et al., 2013). This heavy genetic load, along with the inherent complexity of AUD, suggests that this predisposition likely manifests in neurobiological and neurophysiological alterations.

The dorsal striatum is a prominent brain region of interest in the etiology of AUD, composed of ~95% GABAergic medium spiny projection neurons (MSNs) (Tepper and Bolam, 2004), and is the primary input nucleus of the basal

ganglia. The dorsal striatum can be anatomically divided into medial (DMS) and lateral (DLS) subregions, each receiving unique excitatory glutamatergic inputs from diverse cortical regions and thalamic nuclei as well as from basolateral amygdala (Hunnicutt et al., 2016). Both human and animal studies demonstrate that the DMS and DLS are also functionally heterogeneous, with the DMS having a prominent role in goal-directed learning whereas the DLS is important in habit learning (Balleine and O'Doherty, 2010; Tricomi et al., 2009; Yin et al., 2004). Aberrant function in each of these regions has been hypothesized to have relevance for problematic drug and alcohol (ethanol) use, with the DMS promoting hyper goal-directed behavior aimed at substance use/seeking (Cheng et al., 2017; Nam et al., 2013), and the DLS driving the development of habitual use/seeking (Everitt and Robbins, 2016).

Human imaging studies reveal elevated blood oxygen levels (putative neural activity) in the dorsal striatum of heavy-drinking individuals (including those with AUD) relative to social drinkers in response to alcohol-related cues (Vollstadt-Klein et al., 2010). Furthermore, individuals with AUD demonstrate an overreliance on habit learning coupled with increased activation of the posterior putamen (analogous to the DLS) during an instrumental learning task (Sjorreds et al., 2013). Preclinical studies also demonstrate that the DMS regulates goal-directed alcohol seeking whereas the DLS regulates habitual alcohol seeking (Corbit et al., 2012, 2014). Furthermore, alcohol exposure can differentially influence synaptic input to and plasticity within both dorsal

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striatum subregions (Adermark et al., 2011; Munoz et al., 2018; Wang et al., 2010; Wilcox et al., 2014), thus having the potential to influence how an individual interacts with alcohol with repeated use.

The above findings inspire an important question: Does genetic predisposition for AUD manifest as aberrant dorsal striatum function in a manner that may increase the risk of AUD development? Human work has primarily focused on the ventral striatum, and altered function is indeed observed in children of 1 or more parents with AUD (Bjork et al., 2008; Heitzeg et al., 2010). However, whether functional differences in the dorsal striatum subregions exist *prior* to alcohol exposure in individuals predisposed to AUD development remains unclear. One challenge of human imaging work is the broad nature of its analysis. Preclinical techniques employing animal models (e.g., electrophysiology) allow for direct measurement of neural activity within the brain region(s) of interest and have the power to address mechanisms of functional differences. To address the question posed above, the current study comparatively assessed functional differences in DMS and DLS neurotransmission in mouse models of divergent genetic risk for AUD development.

## MATERIALS AND METHODS

### *Animals*

Naïve adult (postnatal day 60 to 115) mice from the genetically selected high- and low-alcohol-preferring mouse lines (HAP and LAP) were used. Detailed information regarding the derivation and drinking behavior of these lines can be found elsewhere (Matson and Grahame, 2013; Oberlin et al., 2011). Briefly, these lines have been selectively bred for many generations from a common outbred stock (HS/Ibg) for high or low alcohol preference in a 2-bottle choice drinking paradigm. HAP mice demonstrate nearly complete preference for the alcohol solution over water (~90 to 95% preference) and reach blood alcohol concentrations in excess of 80 mg/dl, whereas LAP mice are nearly complete abstainers (<5% preference). These lines thus model divergent genetic predisposition for excessive alcohol intake and avoidance. HAP mice also exhibit other AUD-relevant phenotypes relative to LAP mice such as heightened impulsivity (Oberlin and Grahame, 2009), enhanced alcohol tolerance capacity (Fritz and Boehm, 2014; Fritz et al., 2013), and a propensity for binge-like alcohol consumption (Linsenbardt and Boehm, 2015). In selective breeding studies, these associated phenotypes are referred to as “correlated responses” to selection. For the current work, mice from replicates 2 and 3 of selection were used (HAP2/HAP3 and LAP2/LAP3). A “replicate” line is one where selective breeding from the outbred stock was repeated to, in part, ensure that the selection phenotype (alcohol preference) is reliably produced. The observation of line differences within 1 or more replicate lines is considered evidence for a genetically correlated response (Crabbe et al., 1990), with more consistency across replicates providing increasingly strong evidence. Given that the LAP1 line is extinct, replicate 1 mice were not used in the current study because no genetic comparison could be made.

### *Electrophysiology*

Mice were deeply anesthetized via isoflurane and decapitated. Brain tissue was then rapidly excised and transferred to an ice-cold,

oxygenated (95% CO<sub>2</sub>/5% O<sub>2</sub> bubbled) cutting solution containing (in mM) 30 NaCl, 4.5 KCl, 1 MgCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 10 glucose, and 194 sucrose. 280- $\mu$ m coronal brain slices containing the striatum were prepared on a Leica VT1200S vibratome. Sections were rapidly transferred to 32°C, oxygenated artificial cerebrospinal fluid (aCSF) containing (in mM) 124 NaCl, 4.5 KCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, and 10 glucose.

Whole-cell voltage and current clamp recordings were made using a MultiClamp 700B amplifier and Digidata 1550B (Molecular Devices, Sunnyvale, CA). For recording, brain slices were moved to a recording chamber, held at 32°C, and continuously perfused with oxygenated aCSF at a rate of ~1.5 ml/min. Slices were visualized on an Olympus BX51WI microscope (Olympus Corporation of America, Center Valley, PA, USA). MSNs in the DMS and DLS were confirmed by their membrane resistance and capacitance. Borosilicate glass recording pipettes of 2 to 4 M $\Omega$  were filled with specific internal solutions (see below, adjusted to 295 to 310 mOsm) for whole-cell recordings. All recordings were filtered at 2.2 kHz and digitized at 10 kHz. Data were acquired using Clampex 10 software (Molecular Devices). Series resistance was continuously monitored, and only cells with a stable access resistance (<25 M $\Omega$  and that did not change >15%) were included for data analysis.

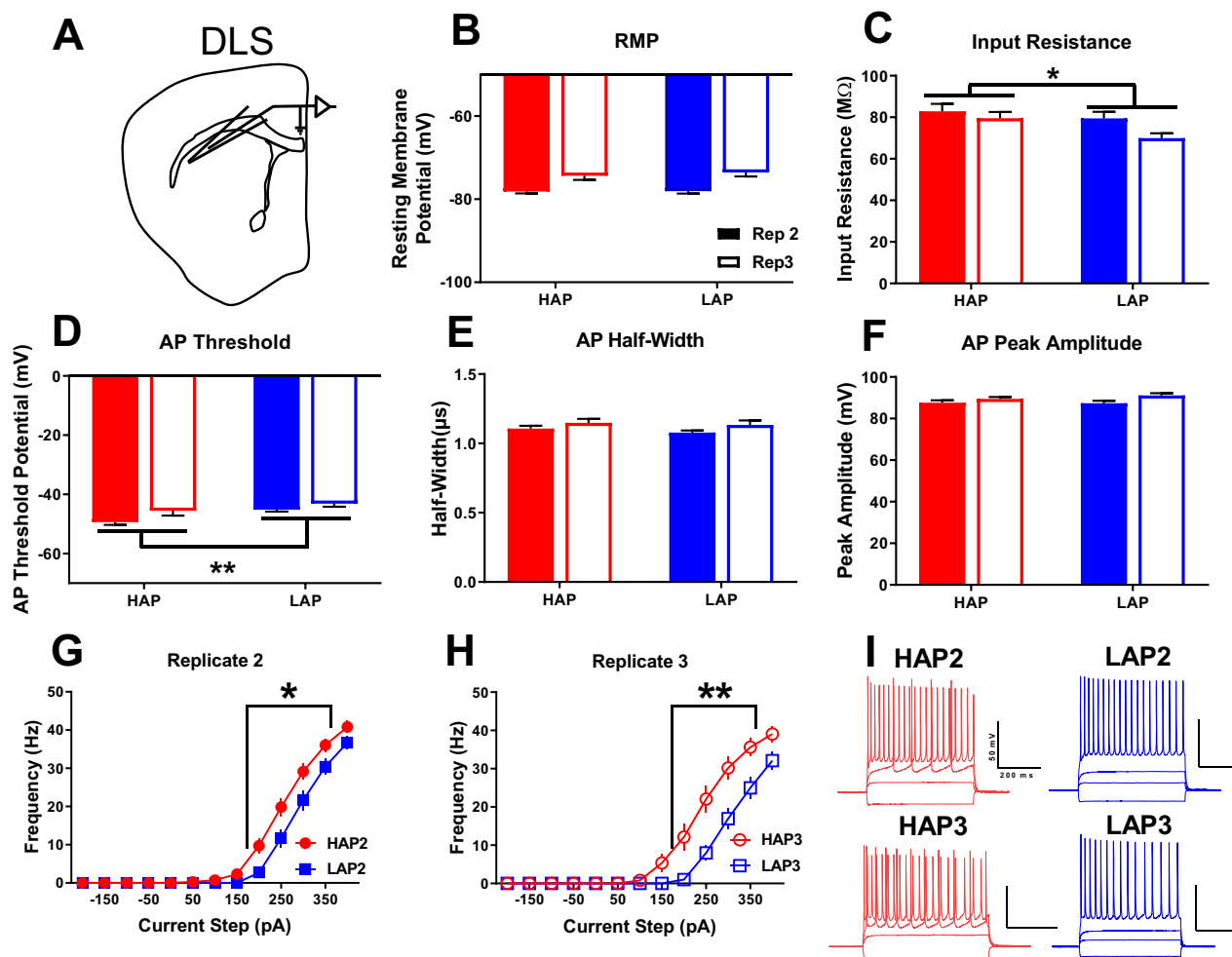
### *Excitability*

Cells were recorded in current clamp mode, allowing them to sit at their natural resting membrane potential. Increasing current steps (–200pA to +400pA in 50 pA increments) were injected for 500 ms every 10 seconds. The following excitability parameters were measured: resting membrane potential (RMP), input resistance, action potential (AP) threshold potential, AP peak, AP half-width, and AP frequency. For the AP threshold potential, AP peak, and AP half-width parameters, data from the first current step that produced APs were used. A K-gluconate internal solution was used and contained (in mM) 4 KCl, 10 HEPES, 4 MgATP, 0.3 NaGTP, 10 phosphocreatine, 126 K-gluconate.

### *Excitatory Transmission*

To isolate excitatory transmission, the aCSF contained 50  $\mu$ M picrotoxin. Recordings were conducted in voltage-clamp mode, and cells were held at –60 mV (unless otherwise indicated). The internal solution for excitatory recordings contained (in mM) 120 CsMeSO<sub>3</sub>, 5 NaCl, 10 TEA-Cl, 10 HEPES, 5 lidocaine bromide, 1.1 EGTA, 0.3 NaGTP, and 4 MgATP. After a stabilization period of at least 10 minutes, spontaneous excitatory postsynaptic currents (sEPSCs) were measured over the course of 5-minute gap-free recordings. Parameters measured included sEPSC amplitude, frequency, rise time, and decay constant.

The ratio of glutamate-driven  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor to *N*-methyl-D-aspartate (NMDA) receptor current was also assessed in voltage-clamp mode. A Teflon-coated bipolar stimulating electrode (PlasticsONE, Roanoke, VA) was placed at the border of the corpus callosum and the DMS or DLS (Figs 1A and 2A). Neurons were held at a –60 mV holding potential and EPSCs were evoked via a DS3 Isolated Current Stimulator (Digitimer, Ft. Lauderdale, FL), and the intensity was adjusted until a stable response between –200 and –400 pA was observed. The holding potential was then changed to –80 mV to isolate AMPA currents, and 10 EPSCs were evoked (20-second interstimulus interval). The AMPA receptor antagonist NBQX (Tocris Bioscience, Bristol, UK) was then perfused onto the slice at a concentration of 5  $\mu$ M until AMPA receptor-mediated currents were no longer evident. The holding potential was then adjusted to +60 mV to isolate NMDA receptor-driven currents, and 10 more EPSCs were evoked (20-second interstimulus interval). The



**Fig. 1.** Dorsolateral striatal medium spiny neurons in HAP mice are hyperexcitable relative to LAP mice. **(A)** Schematic of recording location for DLS MSNs. Measures of **(B)** resting membrane potential, **(C)** input resistance, **(D)** action potential threshold, **(E)** action potential half-width, **(F)** action potential peak amplitude, and **(G, H)** frequency of action potentials with **(I)** representative action potential traces from HAP (red) and LAP (blue) neurons stimulated with  $-100$  to  $200$  pA of currents steps, separated by replicate. Scale bars =  $200$  ms,  $50$  mV. Data represent mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$  for main effect of line. Statistical analyses of sex, replicate, and interactions may be found in the main body of the text.  $n = 7$  to  $14$  cells per line/replicate/sex combination from 3 to 4 animals per each combination.

ratio of mean AMPA receptor current peak was then compared to the mean NMDA receptor current peak.

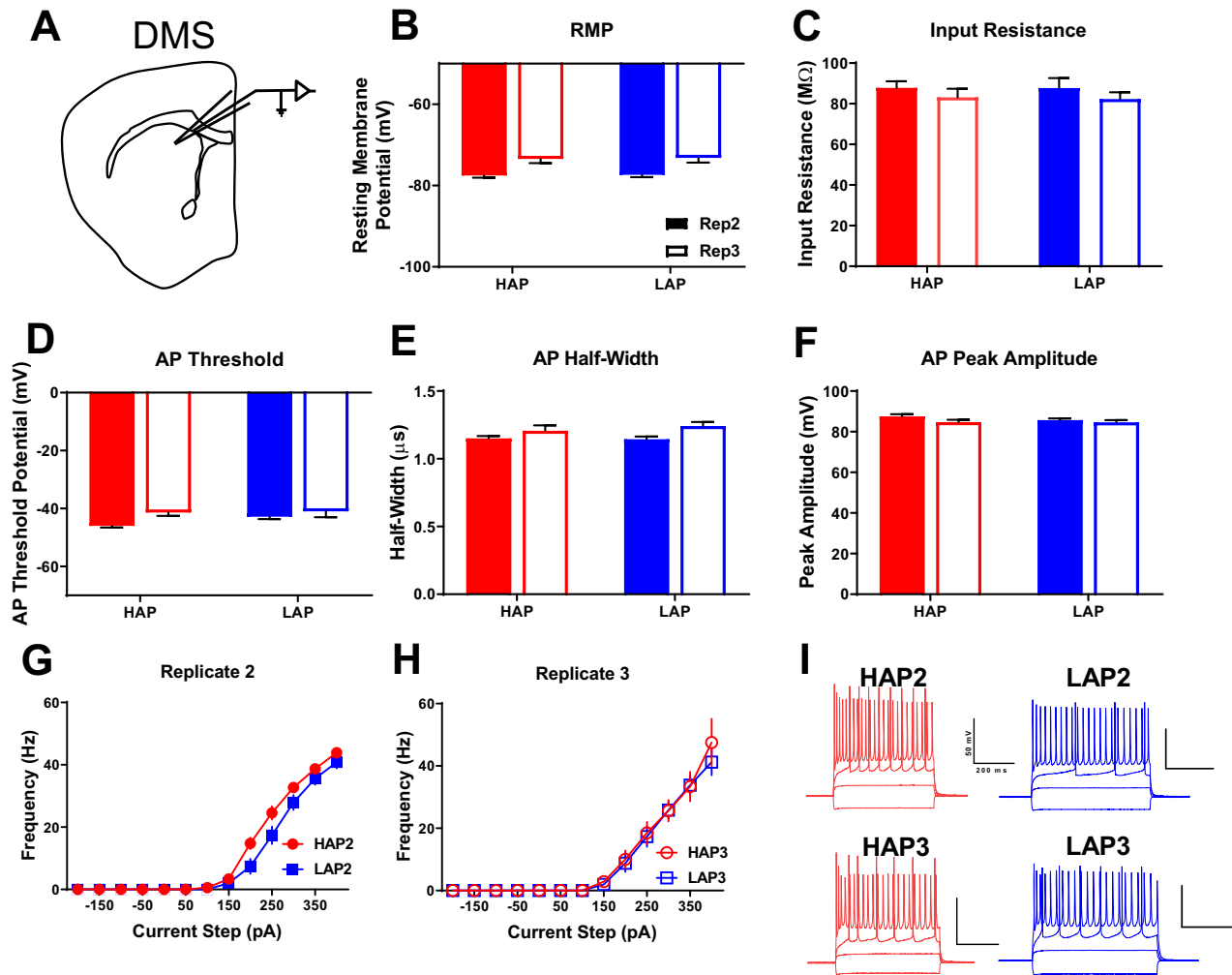
#### Inhibitory Transmission

To isolate inhibitory transmission, the aCSF contained  $5 \mu\text{M}$  NBQX and  $50 \mu\text{M}$  AP-5 (NMDA receptor antagonist; Tocris Bioscience, Bristol, UK). Inhibitory recordings were conducted in voltage-clamp mode, and cells were held at  $-60$  mV. After a stabilization period of at least 10 minutes, spontaneous inhibitory postsynaptic currents (sIPSCs) were measured over the course of 3-minute gap-free recordings. sIPSC recordings yielded many more postsynaptic events and were therefore shorter than sEPSC recordings. Parameters measured included sIPSC amplitude, frequency, rise time, and decay constant. The Cs-based internal solution of inhibitory recordings contained (in mM)  $120$  CsCl<sub>2</sub>,  $10$  HEPES,  $10$  EGTA,  $4$  MgCl<sub>2</sub>,  $2$  MgATP,  $0.5$  NaGTP, and  $5$  lidocaine.

#### Electrophysiology Data Processing and Statistical Analysis

All excitability and AMPA/NMDA ratio data were processed via pClamp 10.6 software (Molecular Devices, San Jose, CA, USA).

sEPSC and sIPSC data were processed via MiniAnalysis software (Synaptosoft Inc.). Statistical analyses were conducted via Statistica 7 software (StatSoft, Tulsa, OK). Statistical outliers were determined via Grubbs' test. Out of a total of 649 cells, 23 met criteria as outliers (3.5% of total population) and were removed from their respective analysis. DMS and DLS data were analyzed separately. Analyses of variance (ANOVAs) were used for all analyses and included line (HAP/LAP), sex (M/F), and replicate (2/3) as factors. Analyses were collapsed on sex where it was found to not be a significant factor. The significance level for all analyses was set at  $p < 0.05$ , and Tukey–Kramer post hoc statistics were run where applicable. Any main effects or possible interactions that are not reported did not reach statistical significance. It is important to bear in mind that main effects of replicate are both expected and not particularly relevant to our genetic hypothesis. Due to the logistical limitation of mouse colony size, genetic drift occurs over many generations in selected lines and some alleles not relevant to the selection phenotype (alcohol preference in this case) become fixed. As a result, replicates (e.g., replicate 2 vs. replicate 3) may be observed to differ in a variety of experimental measures. So long as there is no interaction with line, this effect has no relationship to the selection phenotype. As such, the details of all main effects of



**Fig. 2.** Dorsomedial striatal medium spiny neuron excitability does not differ between HAP and LAP mice. (A) Schematic of recording location for DMS MSNs. (B) Resting membrane potential, (C) input resistance, (D) action potential threshold, (E) action potential half-width, (F) action potential peak amplitude, and (G-H) frequency of action potentials. (I) Representative action potential traces from HAP (red) and LAP (blue) neurons stimulated with  $-100$  to  $200$  pA of currents steps. Scale bars =  $200$  ms,  $50$  mV. Data represent mean  $\pm$  SEM. Statistical analyses of sex, replicate, and interactions may be found in the main body of the text.  $n = 7$  to  $13$  cells per line/replicate/sex combination from  $3$  to  $4$  animals per each combination.

replicate can be found in Table S1 and are not noted in the figures. Finally, as our main hypotheses were driven by the behavioral genetics of HAP and LAP mice, figures are presented collapsed on sex to highlight where line differences occurred. Sex effects and interactions are also detailed in Table S1.

## RESULTS

### Excitability

Within the DLS (Fig. 1A), analysis determined no effect of line on MSN RMP ( $p > 0.05$ ; Fig. 1B). Replicate,  $F(1, 66) = 41.95$ ,  $p < 0.001$ ; Table S1, and sex,  $F(1, 66) = 5.07$ ,  $p < 0.05$ ; Table S1, were significant factors with female and replicate 3 cells exhibiting more depolarized RMPs. A significant line  $\times$  replicate  $\times$  sex interaction,  $F(1, 66) = 5.1$ ,  $p < 0.05$ ; Table S1, also indicated that within HAP3 mice, female DLS cells sat at a more depolarized RMP relative to male cells. HAP MSNs exhibited significantly greater input

resistance compared to LAP cells,  $F(1, 66) = 3.978$ ,  $p = 0.05$ ; Fig. 1C, indicating that current was translated into larger changes in voltage across the membrane. Increasingly positive current steps also revealed that HAP MSNs fired APs at a significantly lower AP threshold potential compared to LAP MSNs,  $F(1, 66) = 8.682$ ,  $p < 0.01$ ; Fig. 1D. Cells from replicate 2 animals also exhibited a lower AP threshold potential than those from replicate 3,  $F(1, 66) = 8.684$ ,  $p < 0.01$ ; Table S1. No line differences were found for AP half-width or peak amplitude ( $p$ 's  $> 0.05$ ; Fig. 1E,F), although APs from replicate 3 cells had a slightly larger peak amplitude,  $F(1, 66) = 5.23$ ,  $p < 0.05$ ; Table S1. Throughout the positive current steps ( $+50$ – $400$  pA), significantly more frequent APs were generated in HAP MSNs relative to LAP MSNs,  $F(1, 66) = 16.773$ ,  $p < 0.001$ ; Fig. 1G,H.

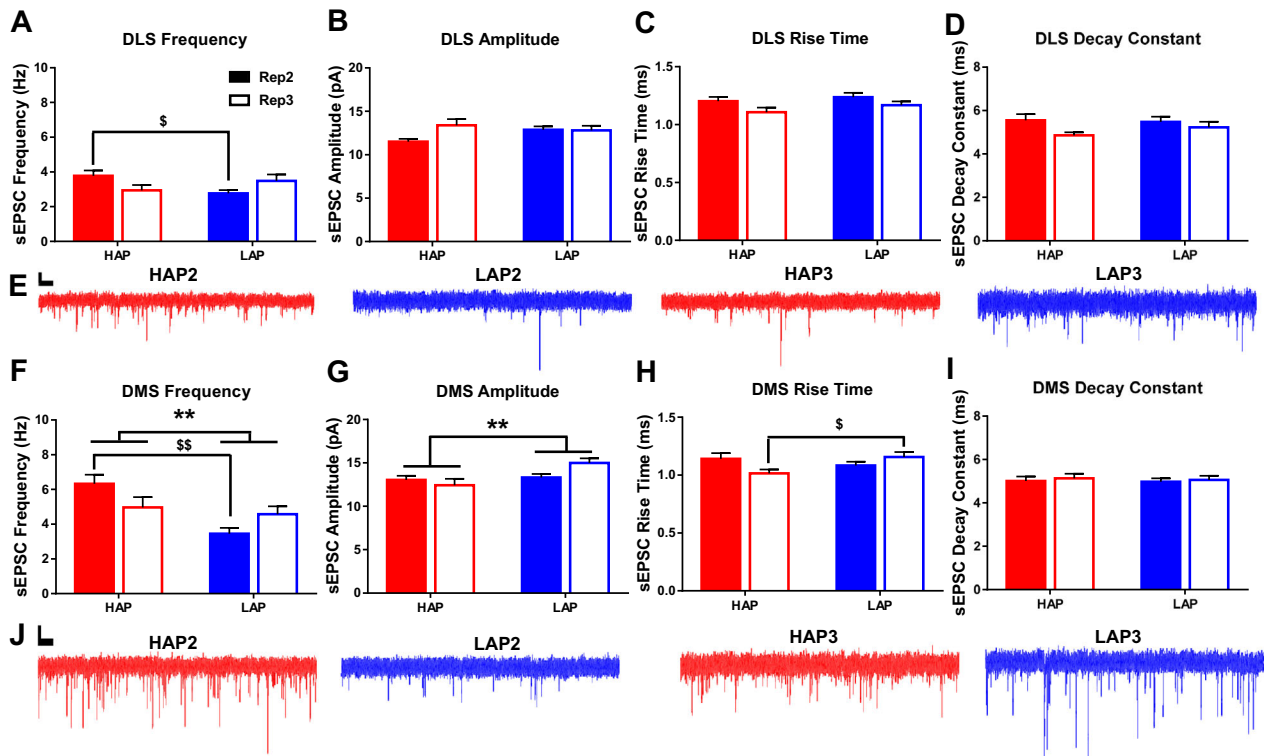
Within the DMS (recording location depicted in Fig. 2A), no clear line differences in excitability were observed. RMP was similar in both lines ( $p > 0.05$ ; Fig. 2B), although a main

effect of replicate indicated that cells from replicate 3 animals sat at a more depolarized RMP,  $F(1, 63) = 24.72$ ,  $p < 0.001$ ; Table S1. Line and sex were not significant factors for input resistance ( $p$ 's  $> 0.05$ ; Fig. 2C); however, a significant line  $\times$  sex  $\times$  replicate interaction revealed that within LAP2 cells, those from female mice exhibited greater input resistance relative to male cells,  $F(1, 63) = 7.626$ ,  $p < 0.01$ ; Table S1. No line or sex differences were found in AP threshold ( $p$ 's  $> 0.05$ ; Fig. 2D); however, replicate 2 cells exhibited a significantly lower AP threshold than those from replicate 3,  $F(1, 63) = 5.446$ ,  $p < 0.05$ ; Table S1. No main effects of line or sex were found for AP half-width or peak amplitude ( $p$ 's  $> 0.05$ ; Fig. 2E,F), although replicate 3 cells demonstrated a wider AP half-width than replicate 2 cells,  $F(1, 63) = 8.665$ ,  $p < 0.01$ ; Table S1. In addition, a complex line  $\times$  sex  $\times$  replicate interaction revealed that within replicate 3 male cells, the AP half-width in LAP cells was significantly wider than in HAP cells,  $F(1, 63) = 6.981$ ,  $p < 0.05$ ; Table S1. Analysis of AP frequency yielded no main effects of line, sex, or replicate ( $p$ 's  $> 0.05$ ; Fig. 2G,H). A significant line  $\times$  sex  $\times$  replicate interaction, however, found that within HAP3 cells, those from female mice fired significantly more frequent APs in response to current injection compared to those from male mice,  $F(1, 63) = 8.362$ ,  $p < 0.01$ ; Table S1.

### Excitatory Transmission

Spontaneous glutamate-driven currents were measured to evaluate excitatory input to MSNs. Within DLS, a line  $\times$  replicate interaction for sEPSC frequency identified significantly higher frequencies in HAP2 MSNs compared to LAP2 MSNs,  $F(1, 75) = 6.572$ ,  $p < 0.05$ ; Fig. 3A. Neither line nor sex was a significant factor for sEPSC amplitude, rise time, or decay ( $p$ 's  $> 0.05$ ; Fig. 3B–D), although replicate 2 cells were found to have a significantly slower rise time compared to those from replicate 3,  $F(1, 75) = 4.22$ ,  $p < 0.05$ ; Table S1.

sEPSC frequency within DMS was significantly greater in HAP cells compared to LAP cells,  $F(1, 69) = 12.306$ ,  $p < 0.001$ ; Fig. 3F. A significant line  $\times$  replicate interaction indicated that this difference was particularly large between HAP2 and LAP2 MSNs,  $F(1, 69) = 7.048$ ;  $p < 0.01$ ; Table S1. A line difference was also found for sEPSC amplitude with significantly lower amplitudes in HAP compared to LAP cells,  $F(1, 69) = 7.242$ ,  $p < 0.01$ ; Fig. 3G. Analysis of rise time yielded a significant line  $\times$  replicate interaction with HAP3 cells having a significantly shorter rise time than LAP3 cells,  $F(1, 69) = 5.938$ ,  $p < 0.05$ ; Fig. 3H. No main effects or interactions reached statistical significance for decay ( $p > 0.05$ ; Fig. 3I).



**Fig. 3.** Dorsal striatal basal glutamate transmission differs between HAP and LAP mice. Measures of spontaneous excitatory postsynaptic current (sEPSC): **A, F**) frequency, **B, G**) amplitude, **C, H**) rise time, and **D, I**) decay constant. **E, J**) Representative sEPSC traces from HAP (red) and LAP (blue) MSNs. Scale bars = 500 ms, 10 pA. Bar graphs represent mean  $\pm$  SEM. \*\* $p < 0.01$  for main effect of line; \$ $p < 0.05$  and \$\$ $p < 0.01$  for line  $\times$  replicate interaction. Statistical analyses of sex, replicate, and other interactions may be found in the main body of the text.  $n = 10$  to 14 cells per line/replicate/sex combination from 3 to 5 animals per each combination.

AMPA/NMDA ratios measured in the DLS,  $F(1, 64) = 11.719$ ,  $p < 0.001$ ; Fig. 4A,C, and DMS,  $F(1, 65) = 4.748$ ;  $p < 0.05$ ; Fig. 4B,D, of HAP mice were significantly lower than those observed in LAP mice. In addition, a significant line  $\times$  sex interaction for DMS data indicated that this difference was particularly strong in male animals,  $F(1, 65) = 5.027$ ,  $p < 0.05$ ; Table S1.

### Inhibitory Transmission

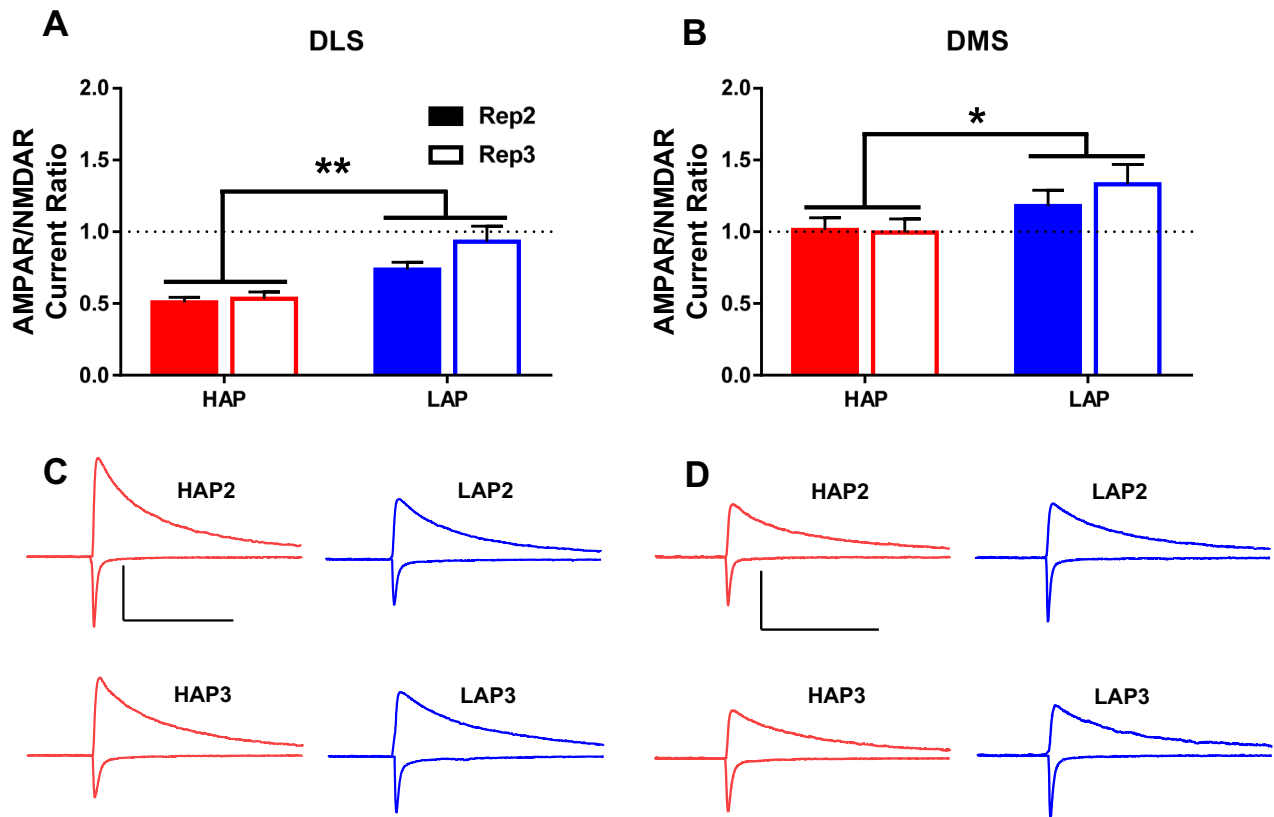
Within the DLS, HAP MSNs received more frequent inhibitory input compared to LAP MSNs,  $F(1, 78) = 5.125$ ,  $p < 0.05$ ; Fig. 5A. sIPSC frequency was also significantly greater in replicate 2 cells relative to replicate 3,  $F(1, 78) = 13.897$ ;  $p < 0.001$ ; Table S1. The amplitude of sIPSCs in HAP cells was significantly larger than that seen in LAP MSNs,  $F(1, 78) = 5.413$ ,  $p < 0.05$ ; Fig. 5B. Analyses of sIPSC rise time and decay yielded no main effect of line ( $p$ 's  $> 0.05$ ; Fig. 5C,D). Significant replicate  $\times$  sex interactions for rise time and decay indicated significantly slower rise times for cells from replicate 2 male mice compared to those from replicate 2 females,  $F(1, 78) = 10.253$ ,  $p < 0.01$ ; Table S1, and significantly shorter decay times for replicate 2 female cells compared

to replicate 3 female cells,  $F(1, 78) = 4.529$ ,  $p < 0.05$ ; Table S1.

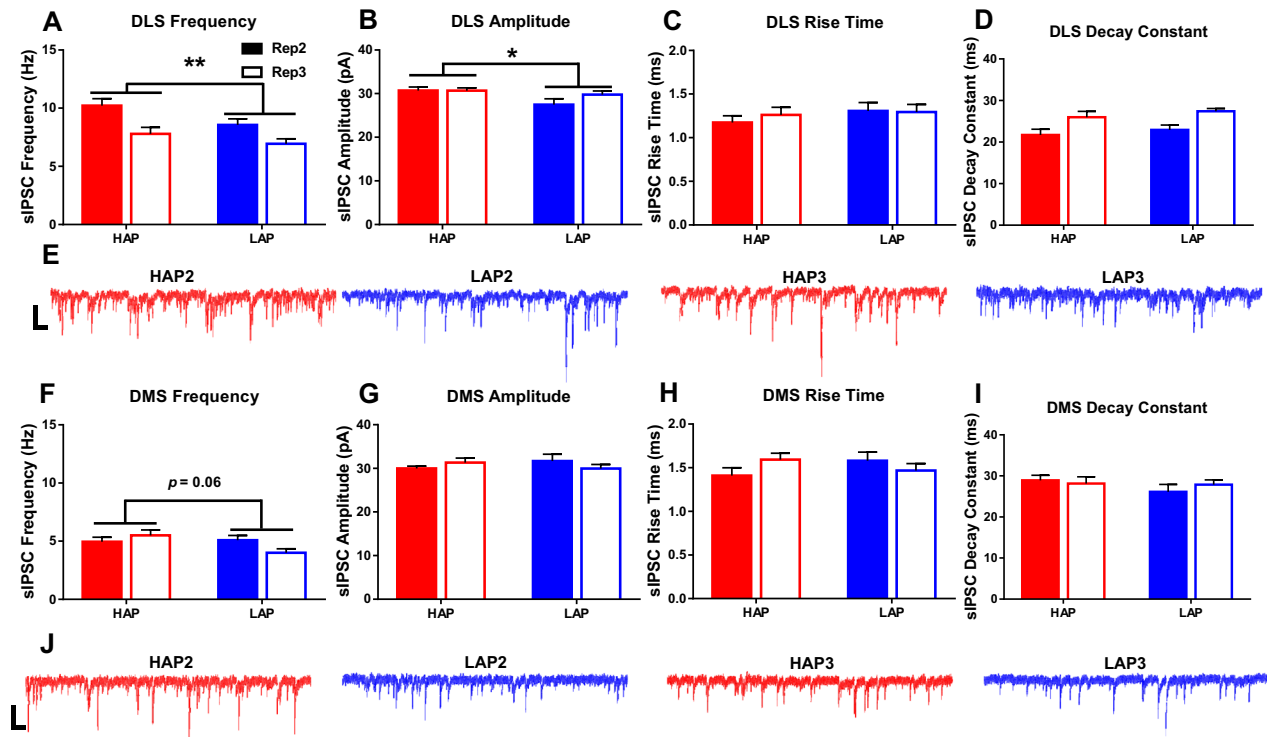
Within the DMS, a trend toward greater sIPSC frequency within HAP versus LAP was observed,  $F(1, 84) = 3.555$ ,  $p = 0.06$ ; Fig. 5F. Analyses of postsynaptic measure of amplitude, rise time, and decay (Fig. 5G–I), however, yielded no significant main effects or interactions ( $p$ 's  $> 0.05$ ).

### DISCUSSION

The above experiments demonstrated that genetic predisposition for high or low alcohol consumption produced sub-region-specific differences in dorsal striatal neurotransmission (summary of electrophysiology results in Table 1). Specifically within the DLS, HAP MSNs were more excitable than LAP MSNs. Glutamate release was also elevated in HAP DLS and DMS; however, this was accompanied by a complementary increase in GABA transmission. Additionally, lower observed AMPA/NMDA ratios in both subregions of HAP mice, another facet of glutamate transmission, suggest line differences in postsynaptic glutamate receptor characteristics. Collectively, these data indicate that the basal functional state of HAP DLS and DMS is substantially and significantly different from LAP DLS and DMS.



**Fig. 4.** Dorsal striatal AMPAR-to-NMDAR current ratios are lower in HAP mice relative to LAP mice. The ratio of AMPA receptor-driven current ( $-80$  mV holding potential) to that of NMDA receptor-driven current ( $+60$  mV holding potential + NBQX treatment) in **A, C** DLS and **B, D** DMS. Representative AMPA and NMDA receptor-mediated current traces are from HAP (red) and LAP (blue) mouse MSNs. Scale bars = 500 ms, 200 pA. Bar graphs represent  $\pm$  SEM. \* $p < 0.05$  and \*\* $p < 0.01$  for main effect of line. Statistical analyses of sex, replicate, and interactions may be found in the main body of the text.  $n = 7$  to 10 cells per line/replicate/sex combination from 3 to 5 animals per each combination. AMPAR,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; NMDAR,  $N$ -methyl-D-aspartate receptor.



**Fig. 5.** Basal inhibitory transmission is greater in HAP mice relative to LAP mice within the dorsolateral striatum. Measures of spontaneous inhibitory postsynaptic current (sIPSC): **A, F**) frequency, **B, G**) amplitude, **C, H**) rise time, and **D, I**) decay constant. **E, J**) Representative sIPSC recording traces from HAP (red) and LAP (blue) MSNs. Scale bars = 500 ms, 50 pA. Bar graphs represent mean  $\pm$  SEM. \* $p < 0.05$  and \*\* $p < 0.01$  for main effect of line. Statistical analyses of sex, replicate, and interactions may be found in the main body of the text.  $n = 8$  to 14 cells per line/replicate/sex combination from 3 to 5 animals per each combination.

Assessment of the intrinsic physiological properties of neurons provides information about their excitability, or the likelihood of AP firing. These intrinsic properties are influenced by a variety of factors including conductance through various ion channels and/or the activity of ion pumps (Llinás, 1988). Genetic line effects on these intrinsic parameters may reflect differences in the quantity, subtype, localization, or post-translational functional modifications of ion channels or other proteins (Curran and Mohler, 2015). Protracted alcohol exposure increases neuronal excitability in numerous brain regions, including the striatum (Marty and Spigelman, 2012). Within the DLS, primary genetic line differences were found for input resistance and AP threshold potential (Fig. 1). These cells also fired more frequent APs in response to current injection. Strikingly, no line differences were observed within the DMS, thus indicating a subregion-specific effect of genetic background. Some possible mechanisms for this functional difference are that HAP MSNs may have lower conductance through particular  $K^+$  channels and/or increased conductance through voltage-gated  $Na^+$  channels at lower membrane potentials (Johnston et al., 2010; Kruger and Isom, 2016). Ultimately, this experiment determined that HAP MSNs within the DLS fired more APs in response to escalating current injection. This increased intrinsic excitability likely translates to larger DLS output compared to the output of LAP DLS, although this will require deeper exploration in the future. Prior studies have shown that the DLS governs

habitual alcohol seeking (Corbit et al., 2012, 2014). Therefore, increased excitability may be a predisposing phenotype influencing excessive alcohol consumption in HAP mice.

**Table 1.** Summary of Significant Genetically “Correlated” Physiological Parameters in HAP and LAP Mice Using the Methodology of Crabbe and colleagues (1990)

Parameter	Line difference	Strength of correlation
<b>Excitability</b>		
DLS	HAP > LAP	+++
DMS	-	-
<b>sEPSC frequency</b>		
DLS	HAP > LAP	+
DMS	HAP > LAP	++
<b>sEPSC amplitude</b>		
DLS	-	-
DMS	HAP < LAP	++
<b>sEPSC rise-time</b>		
DLS	-	-
DMS	HAP < LAP	+
<b>sIPSC frequency</b>		
DLS	HAP > LAP	++
DMS	HAP > LAP	+
<b>sIPSC amplitude</b>		
DLS	HAP > LAP	++
DMS	-	-
<b>AMPA/NMDA ratio</b>		
DLS	HAP < LAP	+++
DMS	HAP < LAP	++

AMPA,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; DMS, dorsomedial; DLS, dorsolateral; NMDA, *N*-methyl-D-aspartate.

As the primary excitatory neurotransmitter in the central nervous system, alterations in glutamate transmission can have wide-reaching effects on the activity of neurons and neural circuits. Elevated glutamate transmission is also a hallmark of AUD in humans (Quednow and Herdener, 2016). Prior work in nonhuman primates demonstrated that chronic alcohol exposure (up to 30 months) increased glutamate transmission within the putamen, a region analogous to the rodent DLS (Cuzon Carlson et al., 2011). Lesser alcohol intake (6 weeks) in a mouse model of binge-like drinking, however, observed no change in glutamate transmission in either the DMS or DLS of inbred C57BL/6J mice (Wilcox et al., 2014). Data from other alcohol-preferring selectively bred rodent lines suggest that glutamate transmission is an important factor in their high-drinking phenotype. Although not directly assessed within the dorsal striatum subregions, 8 weeks of alcohol consumption increased extracellular glutamate levels in mesolimbic regions of alcohol-preferring P rats by as much as 100% (Ding et al., 2013). Additionally, pharmacological manipulation of various metabotropic glutamate receptors significantly influences alcohol seeking and relapse behavior in P rats (Besheer et al., 2010; Czachowski et al., 2012; Rodd et al., 2006). The current study demonstrated an increase in sEPSC frequency in HAP compared to LAP cells in both DLS and DMS (Fig. 3). sEPSC event frequency is an index of neurotransmitter release from presynaptic terminals. Therefore, excitatory upstream cortical, thalamic, or amygdalar projections to dorsal striatum in HAP mice may be more excitable or have more efficient vesicle release mechanisms than LAP mice. It is possible that more frequent spontaneous glutamate release could explain the greater excitability observed in HAP DLS MSNs; however, the coinciding greater frequency of GABA-mediated sIPSCs in DLS (Fig. 5) suggests that excitation/inhibition balance mechanisms may be in effect (discussed further below). Additionally, the postsynaptic parameters of sEPSC amplitude and rise time were both found to be decreased in HAP DMS (Fig. 3). Reduced amplitude may indicate reduced AMPA receptor surface expression. However, faster ion channel kinetics, as determined by a shorter sEPSC rise time, may reflect functional compensation. Our data are consistent with the above previous observations of elevated glutamate transmission in animal models of chronic alcohol consumption. It is important to bear in mind, however, that the mice used in the current study were alcohol-naïve. As such, our experiments probed the effect of genetic background, not direct alcohol exposure. Nevertheless, a family history of high alcohol intake produced significantly higher basal glutamate transmission in both the DLS and DMS relative to a history of alcohol avoidance.

An assessment of the ratio of postsynaptic AMPA to NMDA receptor-driven current can reveal other important functional differences in excitatory transmission. An increase in the AMPA/NMDA current ratio can be produced by repeated drug exposure in certain brain regions (Thomas et al., 2008) and is often interpreted as increased excitatory

transmission, likely reflecting higher numbers of functional, membrane-bound AMPA receptors. However, an increase in NMDA receptor function would decrease this ratio, even though it too reflects an increase in excitatory transmission. Indeed, repeated alcohol exposure is known to up-regulate particular NMDA receptor subunits in the brain (Chen et al., 2018; Nagy, 2004). This adaptation may be tied to the hyperexcitability observed in the CNS during alcohol withdrawal, an effect which may drive relapse behavior (Krystal et al., 2003). Repeated alcohol exposure increases NMDA receptor function in the DMS of rats (Wang et al., 2010). A variety of NMDA receptor antagonists also reduced alcohol consumption of selectively bred alcohol-preferring rats (Malpass et al., 2010; McMillen et al., 2004). Interestingly, the current study found substantially lower AMPA/NMDA ratios in both the DLS and DMS of HAP mice compared to LAP mice (Fig. 4). Considering no line difference was observed for sEPSC amplitude in the DLS, the lower ratio in this region might reflect an increase in NMDA receptor function rather than a decrease in AMPA receptor function or number. Within the DMS, however, a slightly reduced sEPSC amplitude in HAP mice suggests lower numbers of AMPA receptors could explain the smaller ratio in this region. Future work will more deeply probe the molecular dynamics that underlie the differing AMPA-to-NMDA current ratios between these selected lines.

Repeated alcohol exposure has also been shown to alter dorsal striatal GABA transmission. Rodents and nonhuman primates exhibit a reduction in miniature IPSC frequency and amplitude following long periods of repeated alcohol consumption, thus indicating both pre- and postsynaptic changes (Cuzon Carlson et al., 2011, 2018; Wilcox et al., 2014). Chronic alcohol exposure significantly reduces GABA<sub>A</sub> receptor numbers and alters subunit expression (Kumar et al., 2009), an effect that is also seen specifically in selectively bred alcohol-preferring rats (McClintick et al., 2015). This reduction in GABA transmission could translate to a disinhibition of MSNs, thus increasing the likelihood of MSN output. However, in the current study, HAP mice were observed to have more frequent presynaptic GABA input relative to LAP mice in both DLS and DMS (Fig. 4). Additionally, the postsynaptic parameter of amplitude was larger in HAP DLS. As previously mentioned, this was also met with significantly more frequent glutamate transmission in HAP dorsal striatum. This could be reflective of excitation/inhibition balance, wherein increases/decreases in excitatory transmission are met with compensatory inhibition to achieve a state of balance. In disease states such as addiction, this balance can be disrupted and particular neurocircuits can be pathologically over- or underactive (Yizhar et al., 2011). Within striatal circuitry, it is important to distinguish between the “direct” and “indirect” pathways that either promote or inhibit behavioral action patterns, respectively. Indeed, previous work has demonstrated that excessive alcohol intake in mice resulted in increased glutamatergic input onto direct pathway MSNs as well as increased GABA input onto indirect pathway MSNs (Cheng et al., 2017), thus



resulting in much higher ratio of direct pathway output compared to indirect pathway output. This pathway imbalance may reflect an overall reduced ability to inhibit highly active alcohol-seeking behavior. In the absence of alcohol exposure in the current study, no clear evidence of excitation/inhibition imbalance was observed in either line. However, given the previous findings mentioned above, distinguishing GABA and glutamate transmission onto pathway-specific MSNs will be an important future step toward clarifying how net MSN input/output is affected by genetic selection for alcohol preference. It will also be important to evaluate how alcohol consumption in HAP mice influences these parameters in the future.

In conclusion, the current study demonstrated that family histories of high or low alcohol consumption can significantly affect basal neurotransmission within the dorsal striatum. These different functional states may interact with alcohol exposure in a way that promotes/inhibits further alcohol consumption. It is important to note that the origin of the selected lines was from a bidirectional selection, and thus, genetic and physiological differences could alternatively underlie a predisposition for alcohol avoidance in LAP mice rather than an increased predisposition for alcohol preference in HAP mice. Unfortunately, the genetically heterogeneous HS/Ibg stock, the one from which HAP and LAP mice were originally selected, that would serve as a wild-type control line no longer exists. Nevertheless, there are clear functional differences produced by this genetic divergence that indicate that dorsal striatal neurotransmission is indeed related to relative AUD risk. The strongest evidence for genetic correlation (Table 1) points to increased excitability of DLS MSNs in individuals with a positive family history of excessive alcohol intake relative to those with a family history of low alcohol intake. This provides an attractive hypothesis that elevated excitability/activity of DLS MSNs may be a predisposing phenotype for future problematic alcohol seeking and/or consumption. Future work will evaluate how these basal electrophysiological measures in the HAP and LAP selected lines are altered by alcohol exposure. In addition, examination of line differences in dorsal striatal synaptic plasticity may offer valuable insight concerning their capacity for behavioral flexibility (Gremel and Costa, 2013), a phenomenon which is associated with addictive potential. Furthermore, in the future it will be important to define the molecular mechanisms underlying these neurophysiological differences we measured here. Once these are determined, we may begin to elucidate the complex relationship between differential gene expression, neuronal function, and the endophenotypes of these selected mouse lines that may offer novel insights into the development of alcohol abuse in those with a genetic predisposition for AUDs.

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#### AUTHOR CONTRIBUTIONS

BMF and BKA were responsible for study concept and design. BMF and BM collected electrophysiology data. BMF conducted statistical analysis for electrophysiology data and drafted the manuscript. BM and BKA provided critical revision of the manuscript for intellectual content. All authors critically reviewed the content and approved the final version of the manuscript for publication.

#### CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Summary of replicate and sex main effects and interactions for electrophysiology data.