ACS Chemical Neuroscience

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Potentiated Striatal Dopamine Release Leads to Hyperdopaminergia in Female Brain-Derived Neurotrophic Factor Heterozygous Mice

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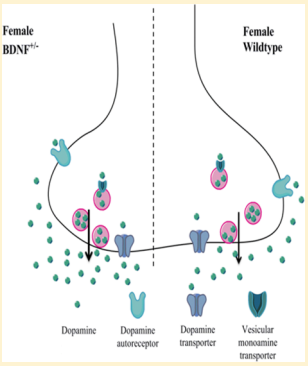
ABSTRACT: The goal of this study was to determine whether a reduction in brain-derived neurotrophic factor (BDNF) levels in female mice leads to dopaminergic system dysregulation. Through a series of in vivo brain microdialysis and slice voltammetry experiments, we discerned that female BDNF heterozygous ($BDNF^{+/-}$) mice are hyperdopaminergic, similar to their male $BDNF^{+/-}$ counterparts. Zero-net flux microdialysis results showed that female $BDNF^{+/-}$ mice had increased striatal extracellular dopamine levels, while stimulated regional release by high potassium concentrations potentiated dopamine release through vesicular-mediated depolarization. Using the complementary technique of fast scan cyclic voltammetry, electrical stimulation evoked greater dopamine release in the female $BDNF^{+/-}$ mice, whereas dopamine uptake remained unchanged relative to that of female wildtype mice. Following psychostimulant methamphetamine administration, female $BDNF^{+/-}$ mice showed potentiated dopamine release compared to their wildtype counterparts. Taken together, these dopamine release impairments in female mice appear to result in a hyperdopaminergic phenotype without concomitant alterations in dopamine uptake.



KEYWORDS: *Caudate-putamen, microdialysis, fast-scan cyclic voltammetry, zero-net flux, methamphetamine, high potassium*

The risk of developing neurologic diseases and disorders differs between men and women. For example, men are approximately 1.5 times more likely than women to develop Parkinson's disease, which is characterized by dopamine (DA) neuron degeneration in the striatum.[1] Furthermore, women are twice as likely to develop depressive disorders compared to men.[2] To better understand sex differences in these neurologic conditions, research has focused on the molecules implicated in these conditions, such as the neurotransmitters DA and serotonin, as well as the neurotrophic factors involved in the growth, differentiation, plasticity, and protection of neurons, like brain-derived neurotrophic factor (BDNF).[3−5]

To better understand sex differences in disease risk and progression, gonadal hormones such as estrogen and testosterone have also been investigated. In ovariectomized rats, a physiologic dose of estrogen increases striatal extracellular DA concentrations and tyrosine hydroxylase activity.[6] Furthermore, estrogen stimulates similar downstream second messengers, ERK1 and ERK2, in a manner analogous to BDNF in cortical explant cultures.[7] Additionally, female rats show increased depression vulnerability relative to their male counterparts when their BDNF levels are at their lowest, which correlates with their maximum estrogen levels.[8−10]

Sex differences are also observed in neurologic disorders such as addiction, with women and men responding differently to drugs of abuse. For example, in clinical studies, women are more likely to start using illicit drugs sooner and in greater quantities than men,[11,12] whereas men are more likely to die from an overdose than women.[13] In animal models, males are more susceptible to the neurotoxic effects of methamphetamine (METH) than females, as shown by reduced striatal DA and DA transporter (DAT) binding.[14−16] These observations in animal and clinical studies, along with others using METH and incorporating estrogen and testosterone, have suggested that females may be protected from the harmful effects of drugs because of their naturally higher of estrogen levels.[15,17,18]

Numerous studies have elucidated the importance of BDNF in maintaining the function and survival of striatal DA neurons.[19−22] To better understand the endogenous role of BDNF throughout the brain and body, we used genetically modified heterozygous ($BDNF^{+/-}$) mice developed by Ernfors and colleagues.[23] Male $BDNF^{+/-}$ mice have increased extracellular and intracellular DA levels compared to their male wildtype littermates,[21,24,25] which are not associated with alterations in DAT. The objective of this study was to determine if these striatal DA alterations are present in female $BDNF^{+/-}$ mice. Using the complementary techniques of in vivo microdialysis and slice fast scan cyclic voltammetry (FSCV), we investigated DA dynamics in female wildtype and $BDNF^{+/-}$ mice.

RESULTS AND DISCUSSION

Basal Striatal DA Levels Are Elevated in Female $BDNF^{+/-}$ Mice. We used female $BDNF^{+/-}$ mice (aged 3−5 months) to determine the effect of reduced BDNF protein and mRNA on the striatal dopaminergic system compared to female wildtype littermates. Microdialysis results showed that the uncorrected extracellular DA levels (averages of 3−4 baseline samples per mouse) in the caudate putamen (CPu) of wildtype

Received: August 16, 2013
Revised: February 3, 2014
Published: February 11, 2014

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and BDNF$^{+/-}$ mice showed no difference between genotypes (wildtype mice: 3.3 ± 0.6 nM, n = 7; BDNF$^{+/-}$ mice: 3.8 ± 0.5 nM, n = 10; P = 0.44; Figure 1 inset). We conducted an in-depth evaluation of extracellular DA levels using the in vivo zero-net flux microdialysis method, in which basal extracellular DA ([DA]$_{ext}$) levels and the extraction fraction (E_d) were determined for each genotype (Figure 1). We observed an approximately 2-fold increase in [DA]$_{ext}$ in the female BDNF$^{+/-}$ mice compared to their wildtype littermates (wildtype mice: 8.2 ± 1.6 nM, n = 7; BDNF$^{+/-}$ mice: 15.0 ± 1.8 nM, n = 10; P < 0.05; Figure 1). Basal DA levels in female BDNF$^{+/-}$ mice were similar to those of male BDNF$^{+/-}$ mice of the same age, with both sexes showing an approximate 2-fold increase in striatal DA levels compared to their wildtype littermates.[24] To determine if this difference in basal DA levels was due to alterations in DAT, we calculated E_d (slope of the line). E_d, a measure of DAT-mediated uptake,[26] did not differ between female BDNF$^{+/-}$ (0.30 ± 0.02, n = 10) and female wildtype (0.31 ± 0.04, n = 7, P = 0.47) mice (Figure 1 inset). This finding is in agreement with others showing that striatal DAT density and activity is unchanged in BDNF$^{+/-}$ mice,[21,27] as well as the zero-net flux data from Bosse and colleagues, where no difference was observed in E_d values between male wildtype and BDNF$^{+/-}$ mice.[24] Furthermore, when DA neuron markers were evaluated in the striatum and substantia nigra in wildtype and BDNF$^{+/-}$ mice, there was no difference in DA immunochemistry as measured by tyrosine hydroxylase, DAT, or the vesicular monoamine transporter (VMAT) between the genotypes.[21,27−29] Although female BDNF$^{+/-}$ mice have elevated extracellular DA levels, our zero-net flux results suggest this is not a result of striatal DAT alterations, which is further supported by previous studies demonstrating that DA neuronal expression and DAT density remain uncompromised in BDNF$^{+/-}$ mice compared to their wildtype littermates.

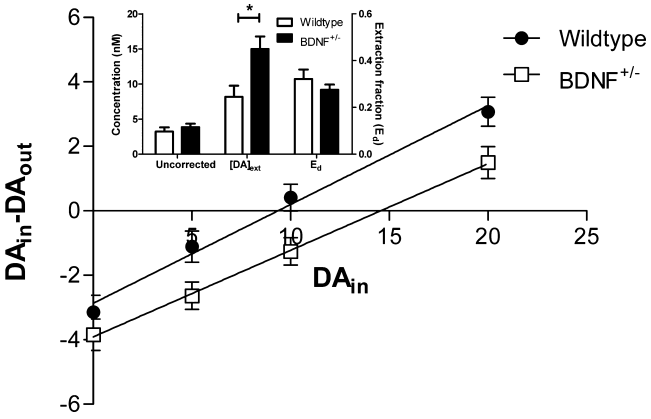

Figure 1. Extracellular dopamine (DA) concentration measured by zero-net flux microdialysis. The inset shows uncorrected DA extracellular levels, [DA]$_{ext}$, and the extraction fraction, E_d. The [DA]$_{ext}$ differed significantly between wildtype and brain-derived neurotrophic factor heterozygous (BDNF$^{+/-}$) mice (widltype: 8.2 ± 1.6 nM; BDNF$^{+/-}$: 15.0 ± 1.8 nM, *P < 0.05). No difference was observed in uncorrected DA levels or E_d. n = 7−10 mice per genotype.

DA Metabolites Do Not Different Across Genotypes. Inactivation of extracellular DA is regulated through the enzymatic breakdown of DA by the enzyme monoamine oxidase (MAO) to form 3,4-dihydroxypheylacetic acid (DOPAC) and catechol-o-methyl transferase (COMT) to form the metabolite 3-methyoxytyramine (3-MT), and these respective metabolites can be further be metabolized by the other's enzyme to form homovanillic acid (HVA). To evaluate DOPAC and HVA, we collected and analyzed three to four microdialysis samples. Both female wildtype and BDNF$^{+/-}$ mice showed similarities in their extracellular metabolite concentrations of DOPAC (wildtype mice: 378 ± 69 nM, n = 8; BDNF$^{+/-}$ mice: 421 ± 110 nM, n = 7, P = 0.73, Figure 2) and HVA (wildtype mice: 610 ± 52 nM, n = 7, and BDNF$^{+/-}$ mice: 672 ± 120 nM, n = 5; P = 0.61; Figure 2). Metabolite levels were not different between the genotypes, suggesting that the increase in extracellular DA is not caused by alterations in DA metabolism. Our DA metabolite data in the female wildtype and BDNF$^{+/-}$ mice are consistent with those reported previously in male wildtype and BDNF$^{+/-}$ mice.[24]

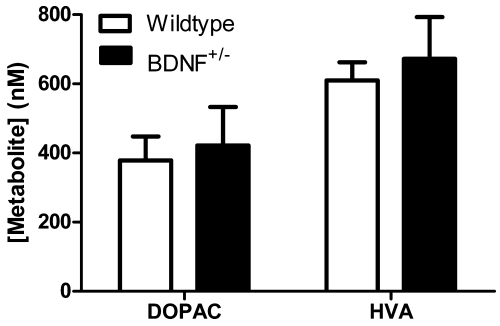

Figure 2. Extracellular dopamine (DA) metabolite levels as measured by microdialysis. No difference was observed between the genotypes for metabolites 3,4-dihyroxypheylacetic acid (DOPAC) or homovanillic acid (HVA) metabolites. Data represented the mean ± SEM; n = 5−9 mice per genotype.

DOPAC and HVA are two of the major DA metabolites resulting from enzymatic breakdown of DA by MAO and COMT, respectively. Typically, these metabolites are measured by tissue content analysis, which primarily reflects intraneuronal metabolism, whereas microdialysis mainly measures extracellular levels. Striatal tissue content studies have demonstrated an increase in both DA and DOPAC levels in young BDNF$^{+/-}$ mice compared to wildtype controls.[12,25,30] Although an increase in both DA and DOPAC was observed, there was no difference in the CPu DOPAC/DA ratio,[12,25,30] suggesting that turnover is not different between the two genotypes. Our microdialysis results of uncorrected extracellular DA, DOPAC, and HVA levels also suggest that extracellular turnover rates do not different, as these concentrations were similar across genotypes. However, when corrected DA levels were evaluated with zero-net flux, an increase in extracellular DA was observed in the female BDNF$^{+/-}$ mice compared to their wildtype littermates. Furthermore, combining the corrected extracellular DA levels with the uncorrected DOPAC and HVA levels suggest an approximately 2-fold decrease in turnover rates in female BDNF$^{+/-}$ mice compared to wildtype controls. Since extracellular neurotransmitter and metabolite levels reflect the balance between DA release, uptake, metabolism, and diffusion, it is possible that the functionality or expression of MAO and COMT are compromised in female BDNF$^{+/-}$ mice compared to controls. Turnover rates are not normally examined using microdialysis or by combining zero-net flux data with uncorrected metabolite levels; however, doing so may indicate that BDNF's influence on the DA system is much further reaching than initially observed. To better understand whether BDNF can influence DA metabolism, future experiments

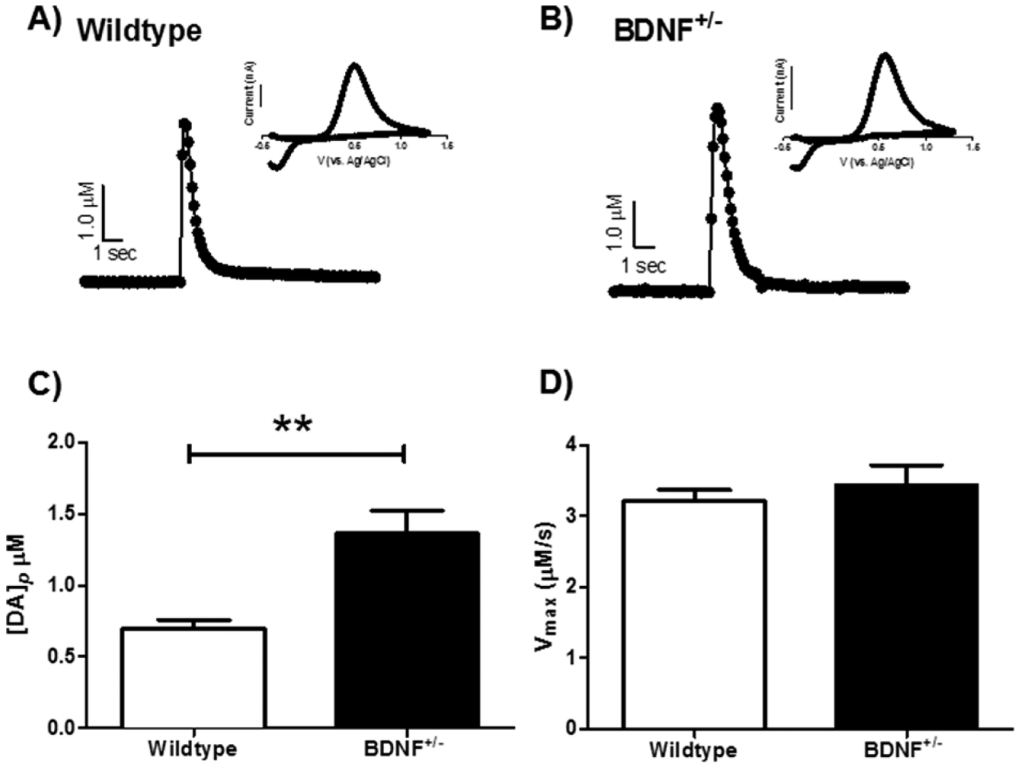

Figure 3. Dopamine (DA) release and uptake in the CPu of female mice measured by slice fast scan cyclic voltammetry. Release and uptake profiles and CV for (A) wildtype and (B) brain-derived neurotrophic factor heterozygous ($BDNF^{+/-}$) mice. (C) Mean DA release per pulse ($[DA]_p$ in μM), and (D) mean DA uptake rates (V_{max} in μM/s). Data represent the mean ± standard error of the mean; n = 6–8 mice per genotype, $^{**}P < 0.01$.

should evaluate this relationship between BDNF and the enzymes that mediate DA break down.

Slice FSCV: DA Release Is Elevated in the Female $BDNF^{+/-}$ Mice. We examined presynaptic DA release ($[DA]_p$) and DA reuptake (V_{max}) to determine if these parameters are altered in female $BDNF^{+/-}$ mice compared to their wildtype littermates, which may contribute to the elevation of extracellular striatal DA levels. Using 400 μm thick brain slices containing the CPu of the mouse, we used a bipolar stimulating electrode to electrically stimulated DA release, which we subsequently measured using a carbon-fiber microelectrode. Female $BDNF^{+/-}$ mice exhibited increased DA release per pulse ($[DA]_p$ = 1.4 ± 0.2 μM, n = 8) compared to their wildtype counterparts ($[DA]_p$ = 0.70 ± 0.6 μM, n = 6, $P < 0.05$; Figure 3C), but there was no difference between the genotypes in the rate of DA uptake (V_{max} values wildtype: 3.2 ± 0.2 μM/s, n = 6; $BDNF^{+/-}$: 3.4 ± 0.3 μM/s, n = 8; P = 0.54; Figure 3D).

The increase in stimulated DA release corroborates our zero-net flux findings, in which higher basal DA levels in the striatum of female $BDNF^{+/-}$ mice were observed compared to the wildtype female mice. Interestingly, when the slice FSCV results were compared between sexes, evoked DA release from female $BDNF^{+/-}$ mice was potentiated compared to male $BDNF^{+/-}$ mice.[24] Unlike female $BDNF^{+/-}$ mice, male $BDNF^{+/-}$ mice show a decrease in both DA release and uptake compared to their wildtype littermates.[24] Thus, low endogenous BDNF levels appear to have a greater impact on stimulated DA release in female $BDNF^{+/-}$ mice compared to their male counterparts.

Microdialysis Stimulated DA release is Potentiated in Female $BDNF^{+/-}$ Mice. To assess vesicular DA release, we used in vivo microdialysis to infuse a high concentration of potassium (K^+) to stimulate the striatal neuronal terminals. In these experiments, while perfusing artificial cerebrospinal spinal fluid (aCSF), we collected three baseline samples. After collection of the third baseline, we perfused high-K^+ (60 mM KCl) aCSF through the microdialysis probe during one 20 min collection period, followed by perfusion of aCSF (3.5 mM KCl) for the remainder of the experiment (Figure 4).

The 60 mM K^+ aCSF elevated extracellular DA levels by at least 5-fold in both genotypes, but striatal extracellular DA levels were not different between the genotypes as shown by two-way analysis of variance (ANOVA; $F_{(1,72)}$ = 3.326, P = 0.1015). Posthoc analysis revealed a significant potentiation in

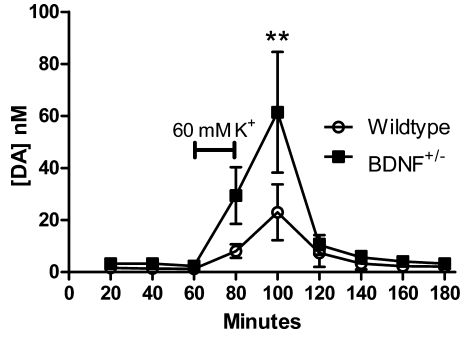

Figure 4. High potassium (K^+)-stimulated dopamine (DA) release from the CPu. Three 20 min baseline samples were collected, after which one 20 min perfusion of 60 mM K^+ artificial cerebrospinal fluid was administered through the dialysis probe directly into the CPu. Data represent the mean ± standard error of the mean, showing change in extracellular DA concentration upon K^+ infusion. n = 6 mice per genotype; $^{**}P < 0.01$ (Bonferroni post-test).

dx.doi.org/10.1021/cn400157b | ACS Chem. Neurosci. 2014, 5, 275–281

DA levels in female BDNF$^{+/-}$ mice 20 min after high-K$^+$ aCSF perfusion versus wildtype mice (Bonferroni posttest, $P < 0.01$).

Overall, both in vivo microdialysis and slice FSCV demonstrated that stimulated DA release in the CPu of female BDNF$^{+/-}$ mice was potentiated compared to female wildtype mice. Compared to their male counterparts, BDNF$^{+/-}$ mice exhibited a divergence in their response to high-K$^+$ stimulation with 60 mM KCl aCSF, as no effect on DA release in was observed in male BDNF$^{+/-}$ mice compared to their wildtype littermates.[24,27] We cannot make a comparison to the 120 mM KCl-aCSF perfusion because we did not evaluate this concentration in female mice. This potentiation in stimulated DA release in females could be supported by the presence of estrogen, since estrogen pretreatment on the striatum significantly enhances DA extracellular levels, whereas testosterone treatment has no effect.[31−33] However, to clearly delineate the roles of these sex hormones on striatal DA release dynamics, it is imperative that future studies evaluate these interactions to increase our understanding of estrogen's complex effects on neurotransmitter systems. Overall, both microdialysis and slice FSCV data suggest that female BDNF$^{+/-}$ mice release more DA when the system is stimulated either via high K$^+$ concentrations or electrical stimulation compared to their wildtype counterparts. If the striatal DA system is hyperresponsive, then these results could possibly explain why female BDNF$^{+/-}$ mice show elevated extracellular DA levels without a difference in DAT activity.

Methamphetamine-Stimulated DA Release via Microdialysis Is Potentiated in the Female BDNF$^{+/-}$ Mice. METH, a DAT substrate, causes a conformational change primarily due to DAT phosphorylation, which causes DAT internalization.[34−36] Subsequently, METH-induced DA release is hypothesized to be primarily released from its transporter instead of being reuptaken.[34−36] Furthermore, METH disrupts the VMAT-proton pump, causing reduced DA uptake into vesicles, and redistributes the VMAT throughout the nerve terminals.[36−38] In clinical and animal models, high or repeated doses of METH in a single day are neurotoxic to the DA system via, perturbation of central DA signaling mechanisms.[39−42] METH-induced elevations in nonsequestered intracellular DA levels likely lead to an environment in which there is a greater probability of DA oxidation, leading to the eventual formation of reactive oxygen species in the cytosol and ultimately nerve terminal damage.[36,43] However, low doses of METH, as used in this study, can be locomotor activating, and increases in locomotor activity can indicate increases in extracellular DA levels.[44]

To investigate the sex-specific role of BDNF in pharmacologic DAT manipulation, we injected female wildtype and BDNF$^{+/-}$ mice with a low dose of METH (1 mg/kg intraperitoneally (i.p.)) known to activate locomotor activity.[45,46] Microdialysis samples were collected in 20 min fractions for 3 h after injection (Figure 5). Two-way ANOVA analysis revealed a main effect of genotype ($F_{1,178} = 17.7$, $P < 0.001$), and treatment ($F_{11,178} = 20.7$, $P < 0.001$), with a significant interaction effect ($F_{11,178} = 1.91$, $P < 0.05$), demonstrating that METH induced genotype-dependent elevations in extracellular DA levels. The maximal METH-induced response for both genotypes occurred 40 min after injection. A posthoc test indicated that stimulated DA release was significantly potentiated at 100 and 120 min ($P < 0.01$ and $P < 0.05$, respectively) in female BDNF$^{+/-}$ mice compared to wildtype controls (Figure 5).

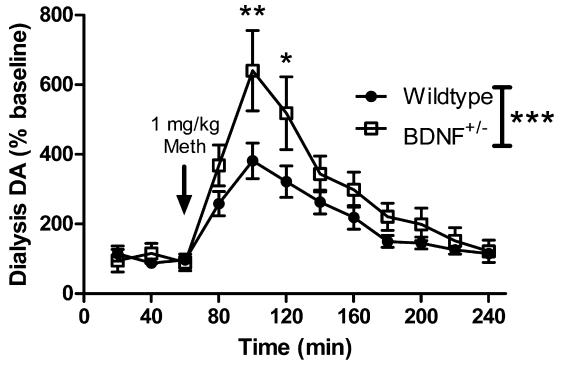

Figure 5. Methamphetamine (METH)-stimulated striatal dopamine (DA) release. METH (1 mg/kg) was administered intraperitoneally at the end of the third baseline sample and DA levels were measured for the next 180 min. Data represent the percent baseline of extracellular DA. $n = 7 - 10$ mice per genotype. Two-way ANOVA indicates main effect of time and genotype, ***$P < 0.001$. *$P < 0.05$ and **$P < 0.01$, Bonferroni post-test.

Sex differences cannot be attributed to a single neurotransmitter, hormone, gene, or protein, but rather involves the convergence of numerous biochemical processes. The key unanswered question is: do low levels of BDNF influence sex differences, or do sex hormones influence BDNF levels? Furthermore, the relationship between sex hormones and BDNF could be more complicated than one neuromodulator or hormone influencing another, which could lead to susceptibility to numerous neurological diseases/disorders in men and women. Empirical observations from individuals abusing illicit drugs suggest that they experiment with drugs of abuse to self-medicate stress, anxiety, and depression.[47] Women are more susceptible to depression and anxiety disorders, and a leading hypothesis is that BDNF plays a critical role in regulating mood via neurotransmission.[8,48] Although our FSCV DA uptake results show no difference in reuptake between female genotypes, Dluzen's work suggests that BDNF$^{+/-}$ mice might have decreased uptake indicating that METH's effects are not as potent in heterozygous mice.[15] These METH results further suggest that the combination of neuromodulators such as BDNF and sex hormones have a powerful impact on striatal DA dynamics: female BDNF$^{+/-}$ mice show alterations only in DA release, whereas male BDNF$^{+/-}$ mice exhibit alterations in both release and uptake. These sex differences in neurotransmission may be of particular interest in the context of administration of pharmacological agents meant to relieve anxiety or depression.

■ CONCLUSIONS

The increase in extracellular DA as measured by zero-net flux microdialysis suggests that female BDNF$^{+/-}$ mice are hyperdopaminergic, similar to their male counterparts. However, this hyperdopaminergia is not a result of alterations in DAT functionality or DA metabolism, as both were consistent between the genotypes. Instead, there appears to be a difference in how the sexes reach a hyperdopaminergic state. Female BDNF$^{+/-}$ mice show potentiated DA release compared to their wildtype littermates as measured in three independent experiments: slice FSCV, high-K$^+$ microdialysis, and microdialysis following a low-dose of the psychostimulant METH. Overall, these DA release findings suggest that a complex relationship exists between BDNF, sex hormones, and how they

influence striatal DA release together. The potentiation of extracellular DA levels in female BDNF$^{+/-}$ mice appears to be due to increased DA release (as shown by slice voltammetry, stimulation with high-K^+, and METH), while the elevated extracellular DA levels in male BDNF$^{+/-}$ mice are hypothesized to be a result of compensatory mechanism between their release and uptake (shown by slice voltammetry and high-K^+ stimulation).[24] Estrogen may be one component responsible for these DA changes, as it has been hypothesized that estrogen acts directly on DA terminals to increase DA release via downregulation of DA D_2 receptors.[49] Taken together, when comparing these current findings with regard to sex differences in DA dynamics in BDNF$^{+/-}$ mice,[24] it appears that an intricate relationship is at work between BDNF and sex hormones.

METHODS

Mice. Female C57/Bl6J (wildtype) and BDNF$^{+/-}$ (heterozygous) mice aged 3−5 months were used in this study. Mice were bred in-house from breeder pairs of female wildtype and male BDNF$^{+/-}$ mice obtained from Jackson Laboratories (Bar Harbor, ME). Mice were weaned 21 days after birth, tail clipped, and ear punched for identification purposes. Genotyping was performed by PCR reaction using DNA from the tail clippings to identify the wildtype mice from the heterozygotes, as no phenotypic differences are observed in these mice.[24] Mice were housed in groups of three to six animals per cage. All procedures and experiments were designed to minimize any pain or discomfort to the animals and were conducted in accordance with the National Institute of Health Animal guidelines and approved by the Wayne State University Institutional Animal Care and Use Committee.

Microdialysis: Surgery and Experimentation. Female mice were used for all experiments and were not examined for their estrous cycle phase when microdialysis and voltammetry experiments were conducted. Walker and Yu showed that DA release and uptake is independent of the estrous state and does not change in C57/Bl6J mice; therefore, the estrous cycle was not taken into account for any experimental conditions.[16,50,51] Mice were anesthetized using isoflurane, and a burr hole was drilled from coordinates relative to the bregma (anterior: +0.8, lateral: −1.3, ventral: −2.5).[24,52] A CMA/7 guide cannula was inserted into the burr hole that was drilled into the skull targeting the CPu. The guide cannula was affixed to the skull using dental cement and the mice were allowed to recover for 3−4 h after surgery before a microdialysis probe (2 mm membrane length, 0.24 mm membrane diameter, cuprophane, 6 kDa cutoff) was inserted through the guide cannula. Next, aCSF (composition [mM]: 145 NaCl, 3.5 KCl, 2 Na_2HPO_4, 1.0 $CaCl_2$, 1.2 $MgCl_2$; pH 7.4) was perfused at a flow rate of 0.4 μL/min overnight. The next morning, the flow rate was increased to 1.1 μL/min and equilibrated for 1 h before experimentation began. Dialysate samples were collected in 20 min fractions for a total sample volume of 22 μL from the freely moving mice.

To determine basal extracellular DA levels, the method of zero-net flux was employed as described previously.[24,53,54] Four 20 min baseline samples were collected, and aCSF perfusate containing 5, 10, and 20 nM DA was perfused into the striatum using a CMA/402 programmable gradient infusion pump. Collected dialysate samples were stored in a −80 °C freezer until analysis.[55]

For the zero-net flux experiments, the plotted x-axis represented the DA concentration perfused into the probe, DA_{in} (determined by in vitro analysis), and the y-axis was plotted as the difference in the concentration perfused in from the concentration of DA collected from the probe (DA_{out}). The point at which this linear regression line crosses the x-axis is known at the DA_{ext}, which corresponds to the basal extracellular DA concentration DA_{in}.[53] The slope of the regression line, E_d, was used to determine in vivo recovery of DA.[26]

DA vesicular release by neuron depolarization, a method that requires perfusion of high-K^+ aCSF at 60 mM (in [mM]: 60 KCl, 90.5 NaCl, 2.0 Na_2HPO_4, 1.2 $MgCl_2$, 1.0 $CaCl_2$; pH 7.4) was used. In this method, three baseline samples were collected with standard aCSF, and following the third collected sample, high-K^+ aCSF was perfused through the probe for 20 min. After the 20 min perfusion of the high-K^+ aCSF was complete, the pump was switched so that only standard aCSF was perfused for the last five subsequent dialysis fractions collected.

Pharmacologic release of extracellular DA was achieved using METH. Mice were weighed before analysis to calculate proper doses for i.p. injection. Three baseline samples were collected before mice were injected with a 1 mg/kg dose of METH, and samples were collected every 20 min for another 3 h after injection.

Slice Fast Scan Cyclic Voltammetry. Slice FSCV experiments were conducted as described previously.[24,56] Briefly, female mice were asphyxiated using CO_2 and immediately sacrificed, and then their brains were removed and placed into preoxygenated (95% O_2/5% CO_2) cold high-sucrose aCSF buffer (in mM: 180 sucrose, 30 NaCl, 4.5 KCl, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4, and 10 D-glucose; pH 7.4) for 10 min. The brains were sectioned into 400 μm thick coronal slices, and the CPu containing slices were placed into an oxygenating aCSF (in mM: 108 NaCl, 5 KCl, 2 $CaCl_2$, 8.2 $MgCl_2$, 4 $NaHCO_3$, 1 NaH_2PO_4, 11 D-glucose, 0.4 ascorbic acid; pH = 7.4) chamber at room temperature. After a 1 h equilibration period, the slices were placed onto a custom-made submersion chamber kept maintained at 32 °C, and the oxygenated aCSF was perfused over the brain slices at 1 mL/min for the remainder of the experiment.

Carbon fiber microelectrodes (50−200 μm in length) were made in-house for FSCV analysis of DA as described elsewhere.[56,57] The microelectrode was placed in the CPu approximately 75 μm into the tissue slice. The stimulating electrode was placed 100−200 μm away from the carbon microelectrode. A triangle waveform was used to detect DA from the surface of the electrode by applying a potential starting at −0.4 V versus an Ag/AgCl reference electrode, ramping it up to +1.2 V, then bringing it back down to −0.4 V at a frequency of 10 Hz and scan rate of 400 V/s.[24,56,57] Stimulation was applied every 5 min and subsequent DA release and uptake were recorded until three stable baseline readings were achieved. All the electrode and stimulation parameters (1 pulse monophasic, 350 μA, 4 ms pulse width) were controlled by TH software (Thermo Scientific, Chelmsford, MA). Postcalibration of electrodes were completed after each experiment using a 3 μM DA solution so that peak oxidation could be converted to concentration. Current versus time plots were fitted by nonlinear regression as described by John and Jones, using LabVIEW National Instrument software.[58] DA release per pulse ($[DA]_p$) and uptake (V_{max}) were determined using Michaelis−Menten-based kinetics by fitting the DA concentration versus time traces.[57,59]

Statistical Analysis. All analyses for microdialysis experiments were performed using GraphPad Prism software (La Jolla, CA). Values are reported as mean ± standard error of the mean with statistical significance set at $P < 0.05$. Student's t tests were used to determine significance between genotypes with respect to uncorrected DA, DA_{ext}, and metabolites, whereas genotypic analysis for high K^+ and METH stimulation were compared using two-way ANOVA analysis.

FSCV results of DA release and uptake were analyzed using the Michaelis−Menten kinetic model, which measures the change in $[DA]_p$ and V_{max}.[58,60,61] Student's t tests were used to determine changes in electrically stimulated DA release and uptake rates between genotypes.

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Author Contributions

J.A.B. and T.A.M. were responsible for the study concept and design. J.A.B. and M.K. contributed to the acquisition of the

animal experiments and performed the data analysis. J.A.B. performed all microdialysis experiments and wrote the document. M.K. performed all slice voltammetry experiments. J.A.B., M.K., and T.A.M. interpreted the findings. The manuscript was drafted by J.A.B., and T.A.M. provided critical revision of the manuscript for important intellectual content. All authors critically reviewed and approved the final version for submission.

Funding

Funding for this work provided by the National Institute on Alcohol Abuse and Alcoholism (NIAAA; AA-016967 T.A.M.), Wayne State University Summer Dissertation fellowship (J.A.B. and M.K.), and start-up funds from Wayne State University (T.A.M.).

Notes

The content is solely the responsibility of the authors and does not represent the official views of NIAAA or National Institutes of Health.

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We would like to acknowledge Brooke Newman, Michelle Colombo, and Stephanie Godden for their technical genotyping support.

■ ABBREVIATIONS

BDNF, brain-derived neurotrophic factor; $BDNF^{+/-}$, brain-derived neurotrophic factor heterozygous; DA, dopamine; FSCV, fast scan cyclic voltammetry; K^+, potassium; aCSF, artificial cerebrospinal fluid; DOPAC, 3,4-dihydroxypheylacetic acid; HVA, homovanillic acid; 3-MT, 3-methyoxytyramine; MAO, monoamine oxidase; COMT, catechol-*o*-methyltransferase; DAT, dopamine transporter; CPu, caudate-putamen; METH, methamphetamine; VMAT, vesicular monoamine oxidase

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