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Females are less sensitive than males to the motivational- and dopamine-suppressing effects of kappa opioid receptor activation

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Abstract

The neuropeptide dynorphin (DYN) activates kappa opioid receptors (KORs) in the brain to produce depressive-like states and decrease motivation. KOR-mediated suppression of dopamine release in the nucleus accumbens (NAc) is considered one underlying mechanism. We previously showed that, regardless of estrous cycle stage, female rats are less sensitive than males to KOR agonist-mediated decreases in motivation to respond for brain stimulation reward, measured with intracranial self-stimulation (ICSS). However, the explicit roles of KORs, circulating gonadal hormones, and their interaction with dopamine signaling in motivated behavior are not known. As such, we measured the effects of the KOR agonist U50,488 on ICSS stimulation thresholds before and after gonadectomy (or sham surgery). We found that ovariectomized females remained less sensitive than sham or castrated males to KOR-mediated decreases in brain stimulation reward, indicating that circulating gonadal hormones do not play a role. We used qRT-PCR to examine whether sex differences in gene expression in limbic brain regions are associated with behavioral sex differences. We found no sex differences in *Pdyn* or *Oprk1* mRNA in the NAc and ventral tegmental area (VTA), but tyrosine hydroxylase (*Th*) mRNA was significantly higher in female compared to male VTA. To further explore sex-differences in KOR-mediated suppression of dopamine, we used fast scan cyclic voltammetry (FSCV) and demonstrated that U50,488 was less effective in suppressing evoked NAc dopamine release in females compared to males. These data raise the possibility that females are protected from KOR-mediated decreases in motivation by an increased capacity to produce and release dopamine.

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The authors declare no conflict of interest

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found here.

Graphical Abstract

Keywords

Intracranial self-stimulation; fast scan cyclic voltammetry; U50,488; rat; tyrosine hydroxylase; dynorphin

1. INTRODUCTION

Dynorphins (DYN) are a class of opioid peptide that act as endogenous ligands at the kappa opioid receptor (KOR; (Chavkin et al., 1982). KORs are expressed throughout brain regions involved in affect, cognition, and motivated behavior, including the ventral tegmental area (VTA), nucleus accumbens (NAc), dorsal striatum, prefrontal cortex, bed nucleus of the stria terminalis (BNST), and hypothalamic regions such as the paraventricular nucleus of the hypothalamus (PVN) (Mansour et al., 1995; Mansour et al., 1994; Svingos et al., 1999). Activation of pre- or post-synaptic KORs typically inhibits neuronal release or neural activation, respectively (for review, see (Simmons and Chavkin, 1996).

To date, the bulk of functional studies have been conducted in males, where it has been shown that activation of KORs produces anxiogenic and (Knoll and Carlezon, 2010), depressive-like behaviors including anhedonia (Ebner et al., 2010; Todtenkopf et al., 2004), which is defined as a decrease in the ability to experience reward that is often measured in rodents using intracranial self-stimulation (ICSS), an operant behavior sensitive to increases and decreases in the reinforcing efficacy of rewarding brain stimulation (Carlezon and Chartoff, 2007). KORs have also been shown to encode the dysphoric component of stress (Abraham et al., 2018; Land et al., 2008), and promote drug-seeking behavior (Negus, 2004; Schindler et al., 2012). Only a handful of studies have examined the effects of KOR activation on motivation- and reward-related behavior in females (Abraham et al., 2018; Laman-Maharg et al., 2017; Robles et al., 2014; Russell et al., 2014; Serksen et al., 1998;

Wang et al., 2011). Recently, our group showed that female rats are less sensitive to KOR agonist-induced decreases in motivation to work for brain stimulation reward as measured with intracranial self-stimulation (ICSS), regardless of estrous cycle stage or castration in males (Russell et al., 2014). Combined with the finding that plasma and brain levels of U50,488 are similar between males and females (Russell et al., 2014), this study suggested that neither circulating gonadal hormones nor pharmacokinetics comprise mechanisms underlying the behavioral sex difference. However, complete removal of circulating female gonadal hormones was not tested, leaving open the possibility that these could interact with KORs to modulate reward-related behavior. As such, the neurobiological mechanisms mediating the decreased sensitivity of females to the motivation-suppressing effects of KOR activation remain unknown.

The distinct neural circuits responsible for the different aspects of negative affect described above are still being parsed (Bruchas et al., 2010; Knoll and Carlezon, 2010). The mesolimbic dopamine system has been implicated in most aspects of KOR-mediated negative affective states, consistent with the ability of intra-NAc KOR agonists to inhibit tonic and phasic dopamine release (Britt and McGehee, 2008). For example, direct infusion of KOR agonists into the NAc mediates conditioned place aversion (Bals-Kubik et al., 1989) and decreased brain stimulation reward (Muschamp et al., 2011), while selective genetic deletion of KORs in dopamine neurons reduces anxiety-like behavior (Van't Veer et al., 2013), and local antagonism of KOR in the NAc prevents depressive-like behaviors including learned helplessness (Shirayama et al., 2004). In addition to the mesolimbic dopamine pathway, numerous other brain regions interact to regulate stress and modulate affective state. Indeed, we found significant increases in U50,488-induced c-Fos expression in female BNST and PVN (Russell et al., 2014), suggesting a sex-dependent role for the BNST and PVN in KOR-mediated behavioral effects.

Dopamine within the mesolimbic system is necessary for motivated behavior (for a recent review, see (Berke, 2018). As such, we hypothesized that sex differences in the ability of the KOR agonist U50,488 to decrease motivated behavior as measured by ICSS, would be associated with sex differences in dopamine release. Previously we and others have shown that KOR activation profoundly decreases dopamine release in the male NAc (Ebner et al., 2010; Ehrich et al., 2014) in a manner temporally consistent with KOR-mediated increases in ICSS stimulation thresholds, which reflect a decrease in the reinforcing value of stimulation frequencies (Ebner et al., 2010). It has been shown that the ability of the KOR agonist spiradoline to reduce stimulated dopamine release in the striatum is decreased in female, compared to male, mice (Sershen et al., 1998), raising the possibility that enhanced dopamine signaling serves to protect females from KOR-mediated decreases in motivated behavior.

The goals of the present study were to 1) determine whether circulating male or female gonadal hormones are necessary for KOR agonist-induced suppression of motivated behavior as measured with ICSS and 2) determine whether KOR-related gene expression and/or KOR-mediated reduction in MFB-evoked dopamine release is sex-dependent. These studies identify putative sex differences in neurobiological mechanisms that underlie negative affective states, highlighting the importance of understanding sex differences and

incorporating this understanding into development of pharmacotherapies for stress and mood disorders.

2. MATERIALS AND METHODS

2.1 Animals

For ICSS experiments with sham and gonadectomized rats and for quantitative RT-PCR (qRT-PCR) experiments performed at McLean Hospital, age-matched, sexually mature female ($n = 20$) and male ($n = 20$) Sprague-Dawley rats (Charles River Laboratories) between 75 and 85 days old and weighing 300 – 325 g (female) and 380 – 410 g (male) at the start of experiments were used. Upon arrival at the facility, rats were group housed (4 rats/cage) and segregated by sex. All rats were acclimatized for one week in a 12 h/12 h light/dark cycle (lights on at 0700) with free access to food and water. Rats were housed using Alpha Chip Heat Treated Pine Softwood bedding (Nepco) and fed with Rat Diet 5012 (LabDiet). After ICSS electrode surgery or after the one-week habituation period in the colony, all rats were singly housed. All experiments were conducted during the light phase. Rats were treated according to the guidelines recommended by the Animal Care and Use Committee of McLean Hospital and by the National Institutes of Health guide for the care and use of Laboratory animals.

For FSCV experiments performed at University of Illinois, Chicago, sexually mature male ($n=7$) and female ($n=5$) Long-Evans rats (bred in-house) 75–90 days old and weighing 290–325 g (female) and 380–440 g (male) at the start of the experiments were used. Rats were group housed (2 rats/cage) with the same sex in plastic cages until reaching sufficient weight (250–425 g) for surgery. Thereafter, all animals were singly housed on a 12:12 light:dark cycle (lights on at 0700). Throughout, rats had free access to food and water. Rats were housed using Envigo 7090A Aspen Chips bedding and fed with Envigo Teklad 2018 Diet. Animal care and use was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

2.2 Surgery

2.2.1 ICSS surgery—ICSS is an operant paradigm in which rodents self-administer rewarding electrical stimulation through electrodes implanted in the medial forebrain bundle (MFB; (Carlezon and Chartoff, 2007)). Rats ($n = 14$ female; $n = 14$ male) were anesthetized with sodium pentobarbital (65 mg/kg, IP; Abbott Laboratories, North Chicago, IL) supplemented with subcutaneous atropine (0.25 mg/kg) to minimize bronchial secretions and implanted with stainless steel monopolar electrodes (0.25 mm diameter; Plastics One, Roanoke, VA) aimed at the MFB at the level of the lateral hypothalamus (2.8 mm posterior to bregma, 1.7 mm lateral to midline, 7.8 mm below dura), as described in (Ebner et al., 2010; Russell et al., 2014). Note that 1 male and 1 female rat died during surgery, resulting in a total of 13 rats per sex for the ICSS studies. After one week of recovery from surgery, rats began ICSS training (see below).

2.2.2 Gonadectomy surgeries—For both castration and ovariectomy surgeries, rats were anesthetized with a mixture of ketamine plus xylazine (80 mg/kg plus 12 mg/kg, i.p.; Sigma-Aldrich). An isoflurane-oxygen vapor mixture was administered via nosecone as needed (e.g. if twitching was observed) during the procedure. For castration surgeries, a ventral transverse incision (~2.0 cm) was made at the midline above the scrotum and the subcutaneous tissues were pulled back. A small cut was made with a scalpel in the muscle surrounding the testes and surgical forceps were inserted and opened and closed to spread out the muscle and tissue in order to expose the testes. The testes were externalized, tied off with suture, and excised. For ovariectomy surgeries, a single, small, vertical incision was made to expose the abdominal cavity. The horns of the uterus were located and lifted out of the body cavity. Absorbable surgical sutures (Ethicon polydioxanone suture, clear monofilament) were tied below each uterine horn and each ovary was subsequently removed with surgical scissors. The uterine horns were then replaced in the abdominal cavity. For both males and females, sham surgeries were done exactly as described above, except in each case the testes or the ovaries were merely externalized from the body cavity and then replace, unharmed. For all surgeries, a 2% lidocaine solution was applied to the incision area to minimize pain, and the incision wounds were closed with sterile non-absorbable sutures, and surgical staples were used to help protect the sutures. All wounds were treated with antibiotic ointment, and rats were treated with ketoprofen (5.0 mg/kg, s.c.) for post-operative pain. Rats recovered for 4 days before ICSS behavior began again, but a total of 3 weeks was allowed before subsequent U50,488 testing to allow for testosterone, estradiol, and other hormones to be eliminated and/or stabilize.

2.2.3 Fast scan cyclic voltammetry surgery—Rats were anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg) via intraperitoneal (IP) injection, placed in a stereotaxic instrument (Kopf, Inc), and prepared for fast-scan cyclic voltammetry (FSCV) recording as previously described (Fortin et al., 2015). All target coordinates were measured relative to bregma using a rat brain atlas (Paxinos and Watson, 2007). A FSCV guide cannula (Bioanalytical Systems, West Lafayette, IN) was implanted above the NAc core using the following coordinates: 1.3 mm anterior to bregma, 1.5 mm lateral to midline, and 2.5 mm below the skull surface. A glass-insulated carbon fiber microelectrode housed in a custom micromanipulator (UIC Research Resource Center, Chicago, IL) was advanced into the NAc core (same dorsoventral placement used in all rats) through the guide cannula.

A chlorinated silver reference electrode was implanted in the contralateral cortex. Lastly, a bipolar stimulating electrode was implanted in the MFB at the level of the lateral hypothalamus (2.8 mm posterior to bregma, 1.7 mm lateral to midline, and between 7.8—8.8 mm below the skull surface). Dorsoventral placement variability in the MFB, while minimal, was a result of individually optimizing for maximal evoked phasic dopamine release during surgery by slowly lowering the stimulating electrode in 0.2-mm increments and using FSCV to record dopamine release in the NAc resulting from electrical stimulation (20 monophasic pulses, 4ms/pulse, 60 Hz, 200 μ A). All implants were secured with skull screws and dental cement. Following surgery, rats were given subcutaneous (SC) meloxicam

(1.0 mg/kg) and monitored in their home cage. Experiments began once rats reached preoperative weight (5–7 days).

2.3 ICSS behavior

After recovery from surgery, rats were trained to respond for brain stimulation using a continuous reinforcement schedule (FR1) at 141 Hz, where each lever press earned a 500-ms train of square wave cathodal pulses (100 μ s per pulse), as described (Carlezon and Chartoff, 2007). The delivery of the stimulation was accompanied by illumination of a 2-watt house light. The stimulation current was adjusted (final range: 110 – 250 μ A, with no significant difference between males and females, Figure 2B) for each rat to the lowest value that would sustain a reliable rate of responding (at least 40 responses per 50 s). After the minimal effective current was found for each rat, it was kept constant throughout training and testing.

Rats were then trained using the rate-frequency method, in which a series of 15 descending stimulation frequencies (141 – 28 Hz, in 0.05 $\log_{10}$ Hz increments) is presented to the rat, as described previously (Carlezon and Chartoff, 2007; Russell et al., 2014). To characterize the functions relating response strength to reward magnitude (rate-frequency function), a least-squares line of best fit was plotted across the frequencies that sustained responding at 20, 30, 40, 50, and 60% of the maximum rate using customized analysis software. The stimulation frequency at which the line intersected the X-axis (theta 0) was defined as the ICSS threshold (see (Carlezon and Chartoff, 2007)). This is considered the theoretical point at which the stimulation becomes reinforcing. Rats were trained for an average of 3–4 weeks until mean ICSS thresholds remained stable ($\pm 10\%$ for 4 consecutive days).

To determine the effects of gonadectomy (or sham surgery) on KOR-mediated decreases in brain stimulation reward using ICSS, a within-subjects design was used in which male and female rats were treated with varying doses of ($\pm$)-trans-U-50,488 methanesulfonate salt (U-50488, Sigma-Aldrich, St. Louis, Missouri) both before (pre-gonadectomy or pre-sham) and 3 weeks after gonadectomy (or sham surgery). To determine if gonadectomy itself impacted ICSS thresholds, ICSS baseline measurements, comprised of daily (M-F) 4 rate-frequency curves (60 min) were taken during the 3 weeks after gonadectomy (or sham surgery; Fig 2C, D). For both pre- and post-gonadectomy U50,488 treatments, drug was administered intraperitoneally (IP) in random order at doses of 0.0, 2.5, 5.0, and 10 mg/kg dissolved in water, based on weight of the salt. U50,488 treatment days were separated by 2 or 3 nondrug days during which baseline ICSS thresholds were maintained. For each drug treatment day, rats first performed 3 rate-frequency curves (45 min) to establish pre-drug baseline thresholds. Immediately following this, one dose of U50,488 was administered and 6 more rate-frequency curves (90 min) were measured, as in (Russell et al., 2014). Data are presented as percent change from the average of the latter 2 of the 3 pre-drug baseline thresholds. ICSS boxes were used exclusively for either males or females and were cleaned with isopropyl alcohol wipes between rats.

2.4 FSCV recordings

Rats were connected to the FSCV head stage, and a carbon fiber recording microelectrode was lowered into the NAc core, as in (Chartoff et al., 2016; Ebner et al., 2010). We chose to

focus on dopamine release in the NAc core because KOR-mediated inhibition of dopamine release in the NAc core is greater than in the shell (Ebner et al., 2010), and because this region is more associated with operant, motivated behavioral output, reward encoding and learning as well as in locomotor and approach behaviors (for review, see (Gale et al., 2014). A triangular waveform was applied to the carbon-fiber, from a negative resting potential of -400mV to $+1300\text{mV}$ to -400mV , relative to the reference electrode (400V/s). This waveform was originally applied at 60Hz for 20min to accelerate electrode equilibration and stabilize background current after which it was switched to 10Hz for 15min . A train of current pulses was then delivered to the MFB (20 monophasic pulses, 4ms/pulse , 60Hz , $200\mu\text{A}$) to evoke a phasic rise in NAc dopamine concentration measured as current changes resulting from the oxidation of dopamine and detected using background subtracted cyclic voltammograms (Fortin et al., 2015). Peak evoked dopamine was confirmed stable over 3 stimulations (2min inter-stimulation interval; $<10\%$ variability), and baseline data acquisition began 20min later. Stimulations occurred once every 2min with varying frequencies delivered in descending order ($60, 40, 20, 10, 5\text{Hz}$). After the baseline frequency sweep, rats received an IP injection of U50,488 (5mg/kg) or vehicle (water). The same stimulation frequency sweeps were performed 20 and 50min later and peak evoked dopamine by each MFB stimulation train was measured. Data collected post-treatment were normalized to baseline values and expressed as '% change from baseline'. All rats received both treatments in a counterbalanced fashion with test sessions separated by at least 2 days (to allow for drug washout). At the end of the experiment, placements of the microelectrode and MFB stimulating electrode were histologically verified (Supplementary Figure 1S).

2.5 Histological Verification

For anatomical verification of FSCV carbon fiber microelectrode placements, recording sites were verified by lowering a polyimide-insulated stainless steel electrode (A-M Systems, Carlsborg, WA) to the same DV depth as that of the carbon fiber during FSCV recordings and passing current to produce an electrolytic lesion in rats deeply anesthetized with sodium pentobarbital (100mg/kg ; Sigma-Aldrich, St. Louis, MO). Brains were removed, stored in formalin for 24 hours and then 30% sucrose in 0.1M phosphate buffer. A cryostat was used to cut $40\mu\text{m}$ brain sections through the NAc and the MFB, which were then mounted on microscope slides. Lesion locations in the NAc and stimulation electrode tracks in the MFB were determined using light microscopy.

2.6 Quantitative real-time PCR (qRT-PCR)

Naïve male ($n=6$) and female ($n=6$) Sprague Dawley rats were killed by decapitation and brains removed and immediately frozen in isopentane kept over dry ice. Frozen brains were coronally sectioned on a cryostat ($-20\text{ }^{\circ}\text{C}$) until reaching the anterior NAc. Bilateral 1-mm^3 punches of the NAc were made. Brains were sectioned further until reaching the anterior BNST (dorsal portion), followed by the basolateral amygdala (BLA), the PVN, and finally the VTA. At each brain region, bilateral 1-mm^3 punches were taken. RNA extraction, cDNA synthesis, and qRT-PCR were performed as described (Chartoff et al., 2016). Briefly, RNA was extracted using PureLink RNA Mini Kit (Invitrogen). RNA quality and quantity were assessed using an RNA 6000 Nano Chip (Agilent, Santa Clara, CA) on an Agilent Bioanalyzer 2100. One microgram of total RNA was used to synthesize cDNA using iScript

cDNA Synthesis Kit (Bio-Rad) in a ThermoHybaid iCycler (Thermo Scientific). Primers (Integrated DNA Technologies, Coralville, IA) were: *Pdyn* (Forward: CGCAAATACCCCAAGAGGAG; Reverse: GCAGGAAGCCCCCATAGC), *Oprk1* (Forward: CTCCAGTGCTTGCCTACTC; Reverse: AGATGTTGGTTGCGGTCTTC), and *Th* (Forward: TGAAGGCTTATGGTGCAGGGC; Reverse: AATGGGCGCTGGATACGAGAGG), *Beta-actin* (Forward: AGGGAAATCGTGCGTGACAT; Reverse: AAGGAAGGCTGGAAGAGAGC), *GtfIIb* (Forward: TCGGATAGCTTCTGCTTGTC; Reverse: TCAGATCCACGCTCGTCTC), and *Gfap* (Forward: GCAGTGGCCACCAGTAACATGC; Reverse: TCCTCCTGTTGCGCATTTGCC) as described and used in (Chartoff et al., 2016).

The qRT-PCR reactions were carried out on a MyiQ Single Color Real-Time PCR Detection System (Bio-Rad) using iQ SybrGreen Supermix (Bio-Rad) in a volume of 20 µl, using identical conditions described in (Chartoff et al., 2016). For each brain region, standard dilution curves were generated for each primer set by serially diluting (1.00-, 0.25-, 0.0625-, and 0.0156-fold) a master cDNA stock comprising an equal mix of cDNA from each brain region of males and females. MyiQ Optical System Software (Bio-Rad) was used to plot the log10 of the dilution values against the threshold cycle values to obtain standard curves for each primer set. Reported values were normalized to the average values of the internal standards *Beta-actin*, *GtfIIb*, and *Gfap* for each sample. Data are expressed as mean relative levels of [gene of interest/internal standard average] ± SEM.

2.7 Statistical Analysis

Effects of U50,488 on ICSS behavior pre-gonadectomy were analyzed using two-way ANOVAs [Sex × Dose (or Time)] with repeated measures on Dose (or Time). Effects of gonadectomy of gonadal hormone levels were analyzed with unpaired t-tests and on minimal current required for ICSS with a two-way ANOVA (Sex × Surgery). Effects of gonadectomy on ICSS thresholds over time (3-weeks) were analyzed with two-way repeated ANOVAs (Sex × Time) with repeated measures on Time. Post-gonadectomy (or sham) surgeries, the effects of U50,488 on ICSS behavior were analyzed using three-way ANOVAs (Sex × Surgery × Dose) with repeated measures on Dose or (Surgery × Dose × Time) with repeated measures on Dose and Time. Effects of sex on gene expression were analyzed with two-way ANOVAs (Sex × Gene) with repeated measures on Gene, followed by Fisher's unprotected t-tests in the event of interactions. For FSCV experiments, data were analyzed with two-way ANOVAs (Sex × Frequency) with repeated measures on Frequency. In all analyses (except where noted), significant interactions were followed by Bonferroni multiple comparison *post hoc* tests, or main effects of individual factors were reported.

3. RESULTS

3.1 Pre-gonadectomy, females are less sensitive than males to the reward-decreasing, but not the rate-decreasing, effects of U50,488

To assess the effect of KOR activation on reward sensitivity in male and female rats prior to gonadectomy or sham surgery, ICSS thresholds and maximum rates of responding were measured for 90 min after U50,488 treatment and compared to pre-U50,488 baseline values.

For reward-related ICSS thresholds, there was a significant Sex $\times$ Dose interaction ($F_{3,72} = 5.29$, $p < 0.01$), and *post hoc* Bonferroni tests showed that the U50,488-induced increase in ICSS thresholds was significantly less in females compared to males at doses of 5.0 and 10.0 mg/kg (Figure 1A). In contrast, there was no interaction between Sex and Dose on maximum rates of responding, although there was a significant main effect of Dose ($F_{3,72} = 12.45$, $p < 0.0001$; Figure 1B), with the 10 mg/kg dose of U50,488 significantly decreasing rates of responding compared to vehicle treatment in both males and females. These results are similar to those found previously (Russell et al., 2014). Time course analysis of the effects of U50,488 (5.0 mg/kg) on ICSS thresholds shows a sex difference for the entire 90-min testing period: main effect of Sex ($F_{1,24} = 13.29$, $p < 0.01$; Figure 1C).

3.2 Gonadectomy has no effect on baseline ICSS behavior

To confirm successful sham and gonadectomy surgeries, trunk blood was collected from all rats at the very end of the experiment and testosterone or estradiol levels measured in plasma using ELISAs (mouse/rat estradiol and testosterone ELISAs, Calbiotech, Inc), as performed in (Russell et al., 2014). Figure 2A shows that gonadectomy, but not sham, surgery resulted in almost absolute elimination of circulating testosterone levels ($p < 0.0001$) and a significant reduction of estradiol ($p < 0.01$). There is evidence that subcutaneous abdominal adipose and liver tissues contribute to extragonadal aromatization that promotes circulating estradiol levels in ovariectomized rats over time (Zhao et al., 2005), which might explain the presence of low levels (~4 pg/ml) of estradiol in the ovariectomized group. Similar to what was shown in Russell *et al* (2014), both sham and gonadectomized male and female rats responded similarly for MFB electrical stimulation as shown by almost identical optimal currents (~150 μ A) of stimulation required for stable baseline determinations during post-surgical training (Figure 2B). During the 3 weeks post-gonadectomy (or sham) surgeries during which circulating gonadal hormones were being eliminated, we measured daily ICSS thresholds and found no difference between sham and gonadectomized rats and no change from pre-surgery baseline (Figure 2C, D).

3.3 Ovariectomized and sham control females are less sensitive than castrated and sham control males to the reward-decreasing, but not rate-decreasing, effects of U50,488

To assess the effect of KOR activation on reward sensitivity in the same male and female rats as above, ICSS thresholds and maximum rates of responding were measured for 90 min after U50,488 treatment and compared to pre-U50,488 baseline values determined post-surgery (sham, gonadectomy). Effects of U50,488 on ICSS thresholds (Figure 3A) and maximum rates of responding (Figure 3B) over the 90-minute test period were analyzed with three-way ANOVAs (Sex $\times$ Surgery $\times$ U50,488 Dose, with repeated measures on Dose). U50,488 dose-dependently increased ICSS thresholds in both males and females, regardless of surgical history (main effect of Dose: $F_{2,7,59,2} = 36.07$, $p < 0.0001$), indicating that KOR activation produces anhedonia in both sexes. However; similar to previous findings (Russell et al., 2014), females were less sensitive to the threshold-increasing (i.e. anhedonic) effects of U50,488 compared to males, regardless of surgical history (Dose $\times$ Sex interaction: $F_{3,66} = 2.76$, $p < 0.05$). Both sham control and castrated male rats showed significantly increased ICSS thresholds in response to 5.0 and 10.0 mg/kg U50,488 compared to sham control and ovariectomized female rats ($p < 0.05$). In contrast to the sex-specific effects of U50,488 on

brain stimulation reward, U50,488 dose-, but not sex-, dependently decreased maximum rates of responding, with 5.0 and 10.0 mg/kg U50,488 significantly decreasing lever press rates compared to vehicle, regardless of sex or surgical history (main effect of Dose: $F_{2,45} = 19.12$, $p < 0.001$).

To further analyze the effects of sex and gonadectomy on KOR-mediated anhedonia, the time course of U50,488 (5.0 mg/kg) on ICSS thresholds (% post-surgery baseline) is plotted for males (Figure 3C) and females (Figure 3D). A three-way ANOVA (Surgery $\times$ Drug $\times$ Time, with repeated measures on Drug and Time) in males revealed that ICSS thresholds increased over time (main effect of Time: $F_{5,55} = 6.12$, $p < 0.001$) and that U50,488 significantly increased ICSS thresholds compared to vehicle in rats regardless of their being castrated or sham surgical controls (main effect of Treatment: $F_{0.72,7.9} = 21.46$, $p < 0.01$). In females, ICSS thresholds did not significantly differ over the 90-min test period, but U50,488 significantly increased thresholds compared to vehicle in rats regardless of their being ovariectomized or sham surgical controls (main effect of Treatment: $F_{0.52,5.7} = 9.10$, $p < 0.05$). In both males and females, the time course for U50,488 to produce visible increases in ICSS stimulation thresholds was around 30–50 minutes, suggesting that it took about that long for anhedonia to be apparent.

3.4 KOR- and dopamine-related gene expression in the mesolimbic system of males and females

Given the above results that surgical removal of gonads and subsequent depletion of circulating gonadal hormones in either males or females do not account for females being less sensitive than males to the motivation-suppressing effects of KOR activation, we hypothesized that sex-dependent expression of KOR- and dopamine-related genes might underlie the behavioral sex difference. In tissue from the NAc, there were no sex differences in expression of either *Oprk1* or *Pdyn* mRNAs (Figure 4A). However, in the VTA, females had significantly more *Th* mRNA compared to males, with no sex differences in either *Oprk1* or *Pdyn* mRNA (Sex $\times$ Gene interaction, with repeated measures on Gene: $F_{2,20} = 5.39$, $p < 0.05$; Figure 4B). We also measured *Oprk1* and *Pdyn* mRNA in the basolateral amygdala (BLA), BNST, and PVN, regions modulated by KORs and important for processing information related to fear, anxiety, and stress—all of which can contribute to anhedonia. *Pdyn* mRNA was similar between males and females in each region except for the PVN, in which it was significantly higher in females (Sex $\times$ Gene interaction: $F_{3,30} = 4.82$, $p < 0.01$; Figure 4C). *Oprk1* mRNA was similar between males and females in each region except for the BNST, in which it was significantly lower in females (Sex $\times$ Gene interaction: $F_{3,30} = 3.96$, $p < 0.05$; Figure 4D).

3.5 The effects of U50,488 on evoked dopamine release are reduced in females compared to males

Given the sex specific effects of U50,488 on MFB ICSS threshold, higher *Th* mRNA in female compared to male VTA, and that MFB stimulation evokes phasic increases in NAc dopamine in awake and behaving rats (Owesson-White et al., 2008), we hypothesized that an increased ability to synthesize dopamine might result in reduced KOR-mediated dopamine release suppression in females and hence a protection against U50,488-mediated

decreases in brain stimulation reward. To approximate ICSS, separate male and female rats were implanted with stimulating electrodes in the MFB and carbon fiber FSCV recording electrodes were implanted into the NAc core. Prior to any drug treatment, the MFB was stimulated across a range of descending frequencies (60, 40, 20, 10, 5 Hz), which evokes phasic and stereotypical increases in dopamine concentrations in the NAc core that rapidly rise and then exponentially decay to pre-stimulation levels. Average peak evoked dopamine concentration in male and female rats at baseline across the full frequency range is shown in Figure 5A. Although dopamine concentration increased significantly with frequency (main effect of Frequency: $F_{4,50} = 17.59$; $p < 0.0001$), there was no effect of Sex ($F_{1,50} = 1.67$; $p > 0.05$) or interaction between the two factors: $F_{4,50} = 0.13$; $p > 0.05$.

Peak oxidative current of dopamine evoked by each MFB stimulation train was measured across the full frequency range at 3 distinct timepoints: baseline (pre-treatment), 20 min post-treatment (a time when motivation-suppressing effects of U50,488 are beginning to appear in the ICSS test), and 50 min post-treatment (peak time of motivation-suppressing effects of U50,488 in ICSS). Data collected post-treatment are represented as both dopamine concentration (nM) and normalized to baseline values, expressed as ‘% change from baseline’. Group differences post-treatment were restricted to the higher stimulation frequencies (20, 40, 60 Hz) due to the fact that low stimulation frequencies (<20 Hz) are insufficient to drive reliable increases in dopamine concentrations, and U50,488’s suppressive effects on these low baseline levels of evoked dopamine eliminated our ability to detect any measurable amount. Furthermore, these higher frequencies are within the range of frequencies for which rats typically perform ICSS behavior (Carlezon and Chartoff, 2007). Figure 5B shows representative background-subtracted color plots and traces of NAc dopamine concentration as a function of time that are extracted from the color plot in response to 40 Hz stimulation before and 50 min after systemic treatment of U50,488.

The peak % baseline dopamine evoked by MFB stimulation 20 min after vehicle treatment was similar between males and females, and there were no main effects of sex or frequency, nor was there a Sex $\times$ Frequency interaction ($F_{2,30} = 0.22$, *ns*; data not shown). In contrast, U50,488 (5.0 mg/kg) caused a moderate reduction in the peak % baseline evoked dopamine at each frequency, but again there were no significant sex differences at 20 minutes post-U50,488: no main effects of either stimulation frequency or sex [frequency: ($F_{2,30} = 0.044$, *ns*; data not shown) or sex: ($F_{1,30} = 0.49$, *ns*; data not shown)]. The peak dopamine concentration (Figure 5C) and % baseline dopamine (Figure 5D) evoked by MFB stimulation 50 min after vehicle treatment was similar in males and females. In contrast, U50,488 (5.0 mg/kg) caused a notable reduction in the peak evoked dopamine concentration (Figure 5E), with a corresponding negative % change from baseline (Figure 5F), 50 min after treatment in both males and females (peak time of motivation-suppressing effects of U50,488 in ICSS, Figure 3C, D). Importantly, the magnitude of U50,488’s suppressive effects on evoked dopamine was significantly blunted in female compared to male rats. Concentrations of dopamine (nM) increased in both sexes with frequency after U50,488 (main effect of Frequency: $F_{1,10.45} = 10.86$, $p < 0.01$; Figure 5E), but absolute concentrations were significantly higher in female compared to male NAc 50 min after U50,488 treatment (main effect of Sex: $F_{1,10} = 5.01$, $p < 0.05$; Figure 5E). Similarly, Figure 5F illustrates that the average % change in peak dopamine release 50 min post-U50,488

relative to baseline at 20, 40, and 60 Hz stimulation was significantly blunted in females compared to males (main effect of Sex: $[F_{1,30} = 5.53; p < 0.05]$).

Another factor that regulates the temporal and overall impact of dopamine signaling in the brain is dopamine reuptake mediated by the dopamine transporter (DAT). FSCV is sensitive to DAT properties by allowing for the measurement of the latency to 50% peak dopamine concentration after initial vesicular dopamine release - used to index uptake mechanisms (Yorgason et al., 2011). We measured dopamine reuptake using this method in all our treatment groups at both 20- and 50-minutes post-Veh or U50,488 treatment. There were no significant effects of sex or stimulation frequency on dopamine reuptake measures in the NAc core in either vehicle- or U50,488-treated rats (No main effect of sex for U50,488-treated rats at 20 min: $[F_{1,10} = 0.61, ns]$; no main effect of sex for U50,488-treated rats at 50 min: $[F_{1,10} = 3.03, ns]$; data not shown)

4. DISCUSSION

This study demonstrates that circulating male and female gonadal hormones are not necessary for the decreased sensitivity of females to KOR-mediated suppression of motivated behavior. This finding is consistent with our prior work (Russell et al., 2014) and supported by the current data in which U50,488-induced increases in ICSS thresholds are reduced in both intact, ovariectomized, and sham surgery females compared to intact, castrated and sham surgery males. This study also demonstrates that *Th* mRNA levels in the VTA are twice as high in females compared to males, whereas *Oprk1* and *Pdyn* mRNA levels in the NAc and VTA are similar between the sexes. To determine if sex differences in dopamine signaling could contribute to functional consequences, consistent with (Wightman et al., 1988), which showed that exogenous L-DOPA—the synthetic product of TH—increased evoked dopamine release, we used FSCV to show that KOR-mediated suppression of MFB-stimulated dopamine release in the NAc is less effective in females compared to males, suggesting that increased *Th* in females allows for increased dopamine synthesis and subsequently protects against KOR-mediated suppression of dopamine release and anhedonia.

4.1 Activational effects of circulating gonadal hormones do not contribute to sex differences in anhedonic effects of KOR activation in ICSS.

ICSS is sensitive to shifts in reward sensitivity as well as motor capacity. Manipulations that increase reward sensitivity shift thresholds such that lower frequencies that did not sustain responding at baseline now reinforce lever pressing. Likewise, manipulations that decrease reward sensitivity [e.g. KOR activation, social defeat, drug withdrawal (Chartoff et al., 2012; Donahue et al., 2014; Ebner et al., 2010)] shift baseline thresholds such that higher frequencies are required to reinforce lever pressing, an operational measure of anhedonia (Carlezon and Chartoff, 2007). Increases or decreases in the rate of lever pressing can identify changes in motor capacity that are separable from shifts in motivation (Carlezon and Chartoff, 2007). We show that FSCV has been used to demonstrate that ICSS evokes dopamine release in the NAc (Owesson-White et al., 2008), and manipulations in the NAc have robust effects on ICSS reward-related behavior (Carlezon and Wise, 1996; Muschamp

et al., 2011), confirming the importance of the mesolimbic dopamine circuit in brain stimulation reward.

These studies confirm and extend our prior findings that females are less sensitive than males to KOR-mediated suppression of motivated behavior (ICSS), regardless of estrous cycle stage (Russell et al., 2014). This was accomplished by demonstrating that complete removal of the gonads during adulthood (thus removing the activational effects of circulating gonadal hormones) did not alter the decreased sensitivity of females to the anhedonic effects of KOR agonism in the ICSS test. However, it has been shown that both male and female mice form similar conditioned place aversions to a KOR agonist (Abraham et al., 2018), with naïve female mice forming aversions to a lower dose of U50,488 than males (Laman-Maharg et al., 2017). These findings underscore the importance of behavioral construct (brain stimulation reward versus learned aversion), species (mouse versus rat) and anatomical site of relevant KOR activation. Furthermore, prior stress ablates KOR-mediated place aversions in females but not males (Laman-Maharg et al., 2017), suggesting that endogenous KOR systems are more reactive to stress in females, thus occluding behavioral responses to KOR agonists.

4.2 Females are less sensitive than males to the NAc dopamine release-suppressing effects of U50,488

Focusing on the NAc and VTA, we did not observe any sex differences in basal levels of *Oprk1* or *Pdyn* mRNA, consistent with (Abraham et al., 2018), but we did measure substantially higher *Th* levels in female VTA. TH, considered the rate-limiting step in dopamine synthesis, converts tyrosine to L-DOPA. Early FSCV work showed that administration of L-DOPA itself increased phasic dopamine release (Wightman et al., 1988). Taken together, we pursued the hypothesis that females are less sensitive to KOR-mediated suppression of dopamine release, in part, because they have an increased ability to produce dopamine, which could serve a protective role. There is mounting data supporting sex differences in basal, stimulated, and KOR-modulated dopamine synthesis and release in the striatum (for reviews see (Becker and Chartoff, 2018; Yoest et al., 2018). For example, basal extracellular dopamine levels are lower in ovariectomized females compared to castrated males (Cummings et al., 2014), and yet putative dopamine neuron firing in the VTA of anesthetized female mice during periods of high estrogen are higher than in male mice (Calipari et al., 2017). FSCV, which measures phasic dopamine release in real time, has shown that electrical stimulation of the MFB at different frequencies results in higher dopamine release in the dorsal striatum of females in one study (Walker et al., 2000) but no baseline difference between the sexes in a more recent study (Walker et al., 2006), although cocaine-induced dopamine release is greater in the female, compared to the male, striatum—regardless of estrous cycle stage (Walker et al., 2006). This is consistent with our finding that females have increased *Th* expression and thus an increased capacity to synthesize dopamine. However, it is critical to note that the Walker (2000) study tested dopamine release in the dorsal striatum whereas we are measuring dopamine release in the NAc core. As such, we found that U50,488-mediated inhibition of dopamine release in the NAc core was muted in females compared to males. This effect was not due to changes in the rate of

dopamine reuptake, as there were no significant sex or treatment differences in the latency (seconds) to 50% peak dopamine concentration (Yorgason et al., 2011).

Dopamine is considered to exist in two distinct pools (Ewing et al., 1983): a readily releasable pool in synaptic vesicles docked at or close to sites of release, and a “reserve” pool that can be mobilized under certain circumstances of high demand (Yavich and MacDonald, 2000). Although speculative, it is possible that increased TH expression in females allows for a greater sum total of dopamine in these two pools. Combined MFB stimulation (stimulating dopamine release) with U50,488 treatment (suppressing dopamine release) could engage the packaging and release of the reserve dopamine pool resulting in females overcoming KOR-mediated suppression of release upon MFB stimulation to a greater degree. Other evidence supporting a decreased ability of KOR activation to fully suppress dopamine release in females is that pretreatment of mice with the KOR agonist spiradoline potentiates cocaine-induced locomotor activity in males but not females (Sershen et al., 1998), which was attributed to a reduced ability of spiradoline to decrease stimulated dopamine release in the striatum of female mice (Sershen et al., 1998). These findings are consistent with (Chartoff et al., 2016), which demonstrated that the KOR agonist salvinorin A potentiates the locomotor stimulant effects of cocaine (in males) by increasing the delta in stimulated dopamine release: the total change (increase) in dopamine release is greater in rats treated with salvinorin A and cocaine than cocaine alone.

Although our study did not investigate the molecular mechanisms by which males and females show differential KOR-mediated suppression of dopamine release in the NAc, a recent study showed that G-protein receptor kinase 2 (GRK2) can inhibit KOR-mediated G-protein signaling by sequestering GPCR $\beta\gamma$ subunits, an effect enhanced in females by estrogen-induced phosphorylation and activation of GRK2 (Abraham et al., 2018). Indeed, similar to our findings, the Abraham *et al* (2018) study demonstrated that KOR-mediated suppression of dopamine release in the NAc was decreased in female compared to male mice. Furthermore, this sex difference could be ablated by pretreatment of females with a GRK2 antagonist. Although this paper suggests that cycling levels of estrogen result in estrous cycle-dependent effects on KOR-modulation of dopamine release, it is important to note that females always have more circulating estrogen than males, consistent with the idea that GRK2-mediated sequestration of $\beta\gamma$ signaling would be consistently greater in females and hence reduce the ability of KOR activation to inhibit dopamine release throughout the estrous cycle.

4.3 Sex differences in KOR and DYN gene expression are evident in the extended amygdala

In this study and in Russell *et al* (2014), we report sex differences in KOR-mediated signaling in the PVN and BNST, which are critical for integrating stress and affective states (Herman et al., 2002). Here we show that, compared to males, DYN mRNA is increased in the PVN and KOR mRNA is increased in the BNST of females. Previously we showed that U50,488 induced the greatest number of Fos-positive cells in the oval nucleus of the BNST (BNSTov) of females during estrous cycle stages associated with high estrogen levels and in the PVN of females regardless of estrous cycle stage (Russell et al., 2014). Taken together, it

is likely that the PVN and BNST contribute to KOR-mediated negative affective states, although the exact mechanisms are unclear. It is known that neuronal populations within the BNST nuclei project to the VTA (Dabrowska et al., 2016; Rodaros et al., 2007). Of particular interest, corticotropin releasing factor (CRF)-containing neurons of the BNSTov send substantial projections to the VTA (Rodaros et al., 2007), raising the possibility that KOR activation of c-Fos in the BNSTov (Russell et al., 2014) modulates dopamine firing and release in the NAc. Furthermore, there is evidence that a small percentage of dynorphin- and vasopressin-containing neurons of the PVN project to the VTA (Rodaros et al., 2007), thus allowing another indirect means for these extended amygdala regions to influence dopaminergic signaling involved in negative affective states such as anhedonia. Sex-dependent KOR signaling within this stress-related circuit is relatively unexplored and requires additional study.

4.4 Conclusions

We have shown that KOR-mediated anhedonia is more robust in males compared to females and does not depend on circulating gonadal hormones in either sex. This behavioral sex difference is associated with elevated VTA *Th* expression and a reduced ability of the KOR agonist U50,488 to suppress NAc dopamine release in females. These findings suggest that increased capacity for dopamine synthesis and release can protect females against stimuli that suppress dopamine (e.g. KOR activation) but can also make females more vulnerable to the positive reinforcing effects of drugs of abuse (Becker and Chartoff, 2018; Greenfield et al., 2010). Future studies are needed to determine if and what distinct mechanisms underlie stress-induced anhedonia in females, as this information is critical for development of comprehensively effective treatments for stress-related disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Kappa opioid receptor (KOR) activation decreases motivated behavior in ICSS.
- KOR-mediated decreases in motivated behavior are greater in males than females.
- Gonadectomy does not change sex differences in KOR effects on motivated behavior.
- Females have more tyrosine hydroxylase mRNA expression in the VTA than males.
- KOR activation is less effective at suppressing NAc dopamine release in females.

Figure 1.

Prior to gonadectomy, intact female rats are less sensitive than intact males to the anhedonic effects of the KOR agonist, U50,488. (A) U50,488 dose-dependently increases % pre-U50,488 baseline thresholds in males (U50,488: 5.0, 10.0 mg/kg) and females (U50,488: 10.0 mg/kg) over the 90-minute test period. The increase in % baseline thresholds is greater in males compared to females (U50,488: 5.0, 10.0 mg/kg). (B) Regardless of sex, the highest dose of U50,488 (10 mg/kg) significantly reduced % baseline maximum rate of responding over the 90-minute test period. (C) Time course of changes in % pre-U50,488 baseline thresholds in males and females after injection of U50,488 (5.0 mg/kg). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to U50,488 (0.0 mg/kg) of the corresponding sex [or irrespective of sex, as in (B)]; # $p < 0.05$, ## $p < 0.01$ comparing groups indicated by brackets. (n = 13/sex). Pre-Cast, precastration; Pre-Ovx, pre-ovariectomy.

Figure 2.

Gonadectomy does not alter baseline ICSS behavior. (A) At the very end of ICSS testing, testosterone (left) or estradiol (right) levels were measured by ELISA from plasma isolated from trunk blood. Both castration and ovariectomy significantly reduced testosterone and estradiol levels, respectively, compared to sham surgery controls of each sex. (B) The average minimum currents required to sustain approximately 50–60 lever presses per minute in ICSS did not differ among sham surgery, castrated males, or ovariectomized females. One week after gonadectomy, ICSS was run for 1-h/d (M-F) for 3 weeks and thresholds compared to pre-surgery baselines. Neither castration (C) nor ovariectomy (D) altered % pre-surgery baseline thresholds or differed from sham surgery controls. ** $p < 0.01$ comparing sham and gonadectomized groups. (n=6/sham group; 7/gonadectomized group). Cast, castrated; Ovx, ovariectomized; Gdx, gonadectomized.

Figure 3.

After gonadectomy, ovariectomized or sham control female rats are less sensitive than castrated or sham control males to the motivation-suppressing effects of U50,488. (A) U50,488 dose-dependently increases % pre-U50,488 baseline thresholds in both sham and gonadectomized males (U50,488: 5.0, 10.0 mg/kg) and females (U50,488: 5.0, 10.0 mg/kg) over the 90-minute test period, compared to the U50,488 (0.0 mg/kg) dose. The increase in % baseline thresholds is greater in males compared to females (U50,488: 5.0, 10.0 mg/kg), regardless of surgical history. (B) Regardless of sex or surgical history, U50,488 (5.0, 10.0 mg/kg) significantly reduced % baseline maximum rate of responding over the 90-minute test period compared to U50,488 (0.0 mg/kg). Time course of changes in % pre-U50,488 baseline thresholds in sham and gonadectomized males (C) and females (D) after injection of U50,488 (5.0 mg/kg). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to U50,488 (0.0 mg/kg) of the corresponding sex and surgical history [or irrespective of sex/surgical history, as in

(b)]; # $p < 0.05$, ## $p < 0.01$ comparing groups indicated by brackets. (n=6/sham group; 7/gonadectomized group). Cast, castrated; Ovx, ovariectomized.

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Figure 4.

Brain region-specific sex differences in *Oprk1*, *Pdyn*, and *Th* mRNA. (A) In the NAc, *Oprk1* and *Pdyn* mRNA levels are the same between males and females. (B) In the VTA, *Oprk1* and *Pdyn* mRNA levels are the same between males and females, but *TH* mRNA levels are significantly increased in females compared to males. (C) In the BLA and BNST, *Pdyn* mRNA levels are similar between males and females, but in the PVN, *Pdyn* mRNA is significantly higher in females compared to males. (D) In the BLA and PVN, *Oprk1* mRNA levels are similar between males and females, but in the BNST, *Oprk1* mRNA is significantly lower in females compared to males. Data are expressed as fold induction of the gene of interest (normalized to the average of the three reference genes, *Beta-actin*, *GtfIib*, and *Gfap*) to males for each brain region. * $p < 0.05$, ** $p < 0.01$ compared to the indicated gene product in males. (n = 6/sex). BLA, basolateral amygdala; BNST, bed

nucleus of the stria terminalis (dorsal region); *Pdyn*, dynorphin; *Oprk1*, kappa opioid receptor; PVN, paraventricular nucleus of the hypothalamus; *Th*, tyrosine hydroxylase.

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Figure 5.

U50,488-mediated suppression of MFB-stimulated dopamine release in the NAc is reduced in females compared to males. (A) The average peak evoked dopamine concentration in the NAc of untreated male and female rats increases with the frequency of MFB stimulation (5 – 60 Hz). (B) Representative examples of electrochemical data acquired in response to electrical stimulation of the MFB. Colorplots depict changes in current (color) as a function of applied electrode potential (E_{app} ; y-axis) and time (s; x-axis). ‘Stim’ denotes the time at which a train of current pulses at 40 Hz was delivered to the MFB. In all colorplots, dopamine can be observed based on its oxidation (green feature at $\sim +0.65$ V) and reduction (light yellow feature at ~ -0.2 V) currents just after the onset of electrical stimulation. Dopamine concentration over time was extracted from the colorplots by conversion of current from the oxidation of dopamine based on post-recording electrode calibration. Examples were taken just before (top plots and black concentration traces) and 50 min after (bottom plots and blue or red concentration traces) systemic U50,488 (5.0 mg/kg) treatment in awake male (left plots) and female (right plots) rats. (C) The peak dopamine concentration (nM) evoked by MFB stimulation was similar in males and females after vehicle treatment. (D) When data in (C) are represented as % change from baseline at the relevant frequency of stimulation (A), vehicle does not change MFB-stimulated dopamine release. (E) The peak dopamine concentration (nM) evoked by MFB stimulation was significantly higher in females compared to males after U50,488 (5.0 mg/kg) treatment. (F)

When data in (E) are represented as % change from baseline at the relevant frequency of stimulation (A), U50,488 significantly decreases MFB-stimulated dopamine release, with females showing reduced suppression compared to males. For C-F, data are plotted for 20, 40, and 60 Hz, 50-min post-injection. ** $p < 0.01$ main effect of frequency; # $p < 0.05$ main effect of sex. (n=5 females, 7 males). DA, dopamine; U50, U50,488; Veh, vehicle (water).

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