Hormones and Behavior 134 (2021) 105019

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

FireMaster® 550 (FM 550) exposure during the perinatal period impacts partner preference behavior and nucleus accumbens core medium spiny neuron electrophysiology in adult male and female prairie voles, *Microtus ochrogaster*

Amanda A. Krentzel [a], Laney C. Kimble [a], David M. Dorris [a], Brian M. Horman [a], John Meitzen [a,b,c,*], Heather B. Patisaul [a,b]

[a] Department of Biological Sciences, North Carolina State University, Raleigh, NC 27695, USA
[b] Center for Human Health and the Environment, North Carolina State University, Raleigh, NC 27695, USA
[c] Comparative Medicine Institute, North Carolina State University, Raleigh, NC 27695, USA

ARTICLE INFO

Keywords:
Vole
Electrophysiology
Nucleus accumbens
Flame retardants
Endocrine disruptors
Neurodevelopment
Endocrine disrupting chemical

ABSTRACT

One of the most widely used flame retardant (FR) mixtures in household products is Firemaster 550 (FM 550). FM 550 leaches from items such as foam-based furniture and infant products, resulting in contamination of the household environment and biota. Previous studies indicate sex-specific behavioral deficits in rodents and zebrafish in response to developmental FM 550 exposure. These deficits include impacts on social and attachment behaviors in a prosocial rodent: the prairie vole (*Microtus ochrogaster*). The prairie vole is a laboratory-acclimated rodent that exhibits spontaneous attachment behaviors including pair bonding. Here we extend previous work by addressing how developmental exposure to FM 550 impacts pair bonding strength via an extended-time partner preference test, as well as neuron electrophysiological properties in a region implicated in pair bond behavior, the nucleus accumbens (NAcc) core. Dams were exposed to vehicle or 1000 μg of FM 550 via subcutaneous injections throughout gestation, and female and male pups were directly exposed beginning the day after birth until weaning. Pair bond behavior of adult female and male offspring was assessed using a three hour-long partner preference test. Afterwards, acute brain slices of the NAcc core were produced and medium spiny neuron electrophysiological attributes recorded via whole cell patch-clamp. Behavioral impacts were sex-specific. Partner preference behavior was increased in exposed females but decreased in exposed males. Electrophysiological impacts were similar between sexes and specific to attributes related to input resistance. Input resistance was decreased in neurons recorded from both sexes exposed to FM 550 compared to vehicle. This study supports the hypothesis that developmental exposure to FM 550 impacts attachment behaviors and demonstrates a novel FM 550 effect on neural electrophysiology.

1. Introduction

Developmental exposure to environmental contaminants is widely regarded as a potential contributing factor for rising rates of neurodevelopmental disorders with social and attachment behavior deficits (Grandjean and Landrigan, 2014; Kalkbrenner et al., 2014; Ye et al., 2017). Many chemicals have been identified as candidates of concern, including multiple classes of flame retardants (FRs)(Blum et al., 2019). Firemaster 550 (FM 550) is one of the most widely used FR mixtures in household and commercial products including foam-based furniture, infant products, electronics, and construction materials (Stapleton et al., 2011; Stapleton et al., 2012). FM 550 constituents are also used in other ways, including as plasticizers, and are present in numerous consumer products including nail polish (Mendelsohn et al., 2016). Like other chemical FRs, FM 550 readily leaches from many products thereby contaminating household dust, air, food, and, ultimately, the environment. Thus FM 550 and its components are common pollutants in both indoor and outdoor environments (Stapleton et al., 2008; Roze et al., 2009; Dodson et al., 2012; Ma et al., 2012; van der Veen and de Boer, 2012). A growing body of work from a number of laboratories, including

* Corresponding author at: Dept. of Biological Sciences, NC State University, 144 David Clark Labs, Campus Box 7617, Raleigh, NC 27695-7617, USA.
E-mail address: jemeitze@ncsu.edu (J. Meitzen).

https://doi.org/10.1016/j.yhbeh.2021.105019
Received 6 April 2021; Received in revised form 27 May 2021; Accepted 11 June 2021
Available online 25 June 2021

our own, has identified FM 550 and its constituent chemical components as endocrine disrupting and disruptive of the developing nervous system (McGee et al., 2013; Saunders et al., 2013; Belcher et al., 2014; Hoffman et al., 2014; Saunders et al., 2015; Phillips et al., 2016; Baldwin et al., 2017; Peng et al., 2017; Tung et al., 2017; Oliveri et al., 2018; Gillera et al., 2020; Witchey et al., 2020). Given this potential neurological impact, environmental prevalence, nearly ubiquitous human exposure, and FM 550's links to deficits in social and attachment behaviors in rodents and humans (Castorina et al., 2017a; Gillera et al., 2020), we sought to assess the impact of developmental FM 550 exposure on attachment behavior and the electrophysiological function of a relevant neuron population.

To accomplish this goal, we employed a prosocial rodent species, the prairie vole (*Microtus ochrogaster)*, building upon previous experiments in our laboratories (Willett et al., 2018; Gillera et al., 2020). Prairie voles are a valuable animal model for assessing social and attachment behaviors because they are able to form strong, lasting pair bonds that persist across breeding cycles (Winslow et al., 1993; Young et al., 2011). This attachment behavior, encompassing preference for the bonded partner animal and thus characterized as social monogamy, is present in approximately 5% to 15% of mammals and even fewer laboratory rodent models, including mice and rats (Kleiman, 1977; Lukas and Clutton-Brock, 2013). We focused on affiliation and attachment because it is identified as a critical construct of the Systems for Social Processes Domain of the NIMH Research Domain Criteria Initiative (RDoC)(https://www.nimh.nih.gov/research/research-funded-by-nimh/rdoc/index.shtml). Clinical manifestations of deficits in the Affiliation and Attachment Domain include social withdrawal, social indifference and anhedonia, as well as over-attachment. A remarkable body of research employing the prairie vole has generated paradigm-shifting advances in the understanding of the neural and genetic mechanisms underlying prosocial and attachment behaviors (Carter et al., 1995; Aragona et al., 2006; Bosch et al., 2018; Ford and Young, 2020; López-Gutiérrez et al., 2021). Notably, and of relevance for human behavior, individual prairie voles can display variation in these social and attachment behaviors; the underlying genetic and neuroendocrine mechanisms for which are reasonably well understood (Hammock and Young, 2005; Barrett et al., 2013; Perkeybile et al., 2013; Vogel et al., 2018; Willett et al., 2018; Gillera et al., 2020).

Partner preference formation and maintenance are regulated by specific brain networks that include specific regions such as the nucleus accumbens (NAcc) (Aragona et al., 2003; Aragona et al., 2006). The NAcc is a nexus region that links limbic and other inputs to the premotor system, and plays a prominent role in modulating social, reward, and motivated behaviors and relevant disorders (Salgado and Kaplitt, 2015; Yager et al., 2015; Yoest et al., 2018). Of special relevance for this study, the NAcc is necessary for pair bond formation and maintenance (Aragona et al., 2003, Aragona et al., 2006). In female voles, oxytocin receptor density in the NAcc strongly associates with individual vole and species differences in alloparental and other attachment behaviors (Insel and Shapiro, 1992; Olazábal and Young, 2006; Ross et al., 2009). The NAcc has functional subdivisions, including the NAcc core and shell. Here we targeted the NAcc core, given prior work demonstrating links between pair bond strength and the electrophysiological properties of the region's output neurons, the medium spiny neurons (Amadei et al., 2017; Willett et al., 2018). Alterations in medium spiny neuron electrophysiological properties can correlate with or directly influence changes in behavior in rodents (Grillner et al., 2005; Mu et al., 2010; Tan et al., 2013), including in voles (Amadei et al., 2017, Willett et al., 2018).

The present study builds on our prior work showing FM 550 exposure across gestation and lactation at environmentally-relevant doses (500, 1000, or 2000 μg /day) impacts a range of socioemotional behaviors in adult prairie voles. Developmental FM 550 exposure disrupted sociality in both sexes but dose-responsively heightened social and generalized anxiety in females. Of specific relevance to the present study, sex-specific effects of developmental FM 550 exposure on pair bonding were observed, with females displaying a heightened partner preference and exposed males failing to show a preference. However, the employed partner preference test (PPT) was only 10 min long because it was one component of a broader testing battery. Longer testing paradigms are more typically employed (Young et al., 2011; Blocker and Ophir, 2016). Thus, here we sought to confirm that developmental exposure to FM 550 disrupts attachment behavior by using the more conventional, longer test. We also assessed the electrophysiological properties of medium spiny neurons in the NAcc core, including those previously linked to pair bonding behavior (Willett et al., 2018). Specifically, we hypothesized that FM 550 exposed male voles would display decreased attachment behavior, while exposed female voles would display increased attachment, and that FM 550 exposure would disrupt neuron excitability in both sexes.

To test these hypotheses, vole dams were exposed to vehicle or 1000 μg of FM 550 via subcutaneous (sc) injections throughout gestation, and pups were then directly exposed beginning the day after birth until weaning. Although human FM 550 exposure is typically oral or via inhalation, sc injection was used here to model the prior study, as almost nothing is known about how prairie voles metabolize FM 550, other than what we published in our prior study (Gillera et al., 2020). Pair bond behavior of adult female and male offspring was assessed using a three hour-long PPT. Importantly, both sexes were assessed given previous work demonstrating sex-specific impacts of FM 550 and other endocrine disrupting compounds in many species on a variety of behavioral endpoints (Palanza et al., 1999; Schantz and Widholm, 2001; Palanza et al., 2008; Patisaul and Adewale, 2009; Rebuli et al., 2016; Baldwin et al., 2017; Gillera et al., 2020; Witchey et al., 2020). After behavioral testing, acute brain slices of the NAcc core were produced and medium spiny neuron electrophysiological attributes recorded via whole cell patch-clamp. These studies contribute to our understanding of how FR exposures may heighten risk of autism spectrum disorder (ASD) and other neurodevelopmental disorders with social and attachment deficits.

2. Methods and materials

The ARRIVE (Animal Research: Reporting of In Vivo Experiments) Guidelines Checklist for Reporting Animal Research was used in the construction of this manuscript (Kilkenny et al., 2010). The ARRIVE guidelines were developed in consultation with the scientific community as part of an NC3Rs (National Centre for the Replacement Refinement and Reduction of Animals in Research) initiative to improve the standard of reporting of research using animals.

2.1. Prairie voles

All animal procedures were approved by the North Carolina State University Institutional Animal Care and Use Committee and the resident veterinarian. Prairie voles (*Microtus ochrogaster*) were obtained from a colony housed in the North Carolina State University Biological Resources Facility originally derived from founder prairie voles generously donated by Dr. Bruce S. Cushing at the University of Texas El Paso and bred to maximize genetic diversity. As in our prior studies (Patisaul and Adewale, 2009; Patisaul et al., 2013) and in accordance with recommended practices for endocrine disruption research (Hunt et al., 2009; Schug et al., 2016), all prairie voles were housed in conditions specifically designed to minimize unintended toxicological exposures including use of glass water bottles with metal sippers, woodchip bedding, and thoroughly washed polysulfone caging. Rooms were temperature, humidity, and light controlled (22 °C, 30% humidity, 12:12-h light-dark cycle. 6 AM–6 PM lights on) appropriately for this diurnal species. Food (High Fiber Rabbit Diet, 5326, LabDiet) and water (filtered by reverse osmosis) were provided ad libitum. The diet was not a low phytoestrogen diet, which can be a confound for endocrine disruptor studies, but phytoestrogens are required to maximize health and

fertility of this herbivorous species (National Research Council (U.S.). Subcommittee on Laboratory Animal Nutrition., 1995).

2.2. Dose preparation

FM 550 comprises two brominated compounds, 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) and bis(2-ethylhexyl) 2,3,4,5-tetrabromophthalate (BEH-TEBP), previously abbreviated TBPH), and the organophosphates triphenyl phosphate (TPHP, previously abbreviated TPP) and a suite of isopropylated triarylphosphate esters (ITPs) (van der Veen and de Boer, 2012; Stapleton et al., 2014; Phillips et al., 2017). EH-TBB and BEH-TEBP are brominated FRs (BFRs) while TPHP and the ITPs are organophosphate FRs (OPFRs). The mixture contains approximately 50% brominated compounds and 50% organophosphates. As we have done previously (Patisaul et al., 2013; Baldwin et al., 2018; Gillera et al., 2020), the sesame oil-based dosing solutions were prepared and coded at the Stapleton laboratory at Duke University, and then transferred to North Carolina State University to ensure accurate dose preparation and experimental blinding. A 1000 μg/μL mixture of FM 550 (Great Lakes Chemical, West Lafayette, IN, USA) in sesame oil (Sigma) was prepared via stirring for 6 h, and then stored in amber bottles at 4 °C until use. As previously described (Gillera et al., 2020), a representative aliquot of FM 550 was analyzed by gas chromatography mass spectrometry in the Stapleton laboratory to confirm dose accuracy. The single FM 550 dose was selected based upon prior work in voles and rats and was the lowest dose to induce consistent behavioral differences in our prior vole study (Gillera et al., 2020). The employed dose (approx. 15–20 mg/kg bw/day) is below the previously purported NOAEL of 50 mg/kg bw/day for the BFRs in the mixture, as described in (Baldwin et al., 2017). As discussed in our previous study (Gillera et al., 2020), the employed FM 550 dose is estimated to be marginally above current maximum human exposure. Notably, human exposure continues to rise, occupational exposure can be considerably higher, and most risk assessment studies encompass a 100-fold safety factor for interspecies comparison.

2.3. FM 500 exposure

Vole dams were randomly assigned to either a vehicle or FM 550 exposure group. Dosing was via subcutaneous (sc) injection of 20 μl of vehicle (sesame oil) or FM 550 (1000 μg/20 μl of vehicle). Dam exposure began the day after parturition of the previous litter (gestational day (GD) 1 for the experimental prairie voles, postnatal day (PND) 1 for the extant litter) through the day of experimental offspring birth. No dosing occurred on the day of birth. Beginning the day after parturition (PND 1), pups were directly dosed via sc injection until weaning, PND 21. Dosing started at 1 PM for both dams and pups. The sc injection paradigm was employed as in our previous study given the poorly characterized pharmacokinetics of FM 550 in prairie voles (Gillera et al., 2020) and to ensure both gestational and post-natal exposure (lactational transfer of all chemicals in the mixture is uncertain) (Phillips et al., 2016). To assess internal levels following dosing, for our prior study a separate set of sexually naïve male ($n = 4$) and female ($n = 5$) adult prairie voles were exposed to 2000 μg FM 550 for 5 days via sc injection and their blood collected 4 h post-injection. Serum levels of EH-TBB were between 15 and 25 ng/ml and TPHP and several of the ITP isomers ranged from 0.2 to 20 ng/ml. Although BEH-TEBP accounts for ~14% of FM 550, it was not detected in serum which is likely due to its rapid body clearance (Knudsen et al., 2017). Given the dose used herein was half, internal levels are anticipated to be lower. For comparison, human blood levels are generally in the low ng/ml range (Blum et al., 2019) but exposure can be much higher for carpet installers, nail salon workers, fire fighters, and other FR-intensive occupations (Estill et al., 2020).

After weaning, vole offspring were housed with 2 to 3 same sex littermates or similarly aged conspecifics of the same treatment group. Litters were randomly segregated into experimental groups, with the goal of obtaining $n = 10$ pups for each group but that ultimately was not possible due to skewed sex ratios and small litter size. Final prairie vole numbers were as follows: 14 FM 550 females from 6 litters, 7 FM 550 males from 4 litters, 10 vehicle females from 4 litters, and 7 vehicle males from 5 litters. Individual pups constituted the statistical unit for this study to maximize power. Although for traditional rodent toxicology studies the litter is the statistical unit, that was not feasible. The overall study design is depicted in Fig. 1.

2.4. Behavioral testing: Partner Preference Test (PPT)

Behavioral testing for partner preference was adapted from established protocols (Slob et al., 1987; Ahern et al., 2009; Willett et al., 2018; Gillera et al., 2020). A PPT enables assessment of the strength of pair bond formation, as this species exhibits spontaneous social monogamy (Williams et al., 1992; Modi and Young, 2012). All partner preference testing was conducted in a single room at the North Carolina State University Biological Resources Facility solely dedicated to this purpose. All tests were video recorded by a camera suspended above the arena for later analysis completed using TopScan version 3.0 (Cleversys Inc., Reston, VA). The tests were conducted in a 3-chamber arena made out of plexiglass with a total length of 198.12 cm, 30.48 cm deep, and 30.48 cm wide and divided into three chambers roughly equal in size with small compartments (17.78 × 30.48 × 30.48 cm) on either side. The openings between the center and two adjacent chambers were 9 cm. Wired cups 9 cm in diameter were used to restrain prairie voles (Product 60,451, Stoelting, Wood Dale, IL), as in Gillera et al., 2020. Cups were placed adjacent to the wall furthest from each chamber opening. The use of cups limits direct physical contact, allowing the prairie voles to interact but not harm each other. This limitation is particularly pertinent for interactions with "stranger" prairie voles. We note that the use of cups does prohibit the prairie voles from laying together in direct whole body contact for extended periods of time, unlike the methods used in the other studies formative for the current employed PPT protocol (Slob et al., 1987; Ahern et al., 2009; Willett et al., 2018). All equipment was thoroughly cleaned with 70% ethanol between testing, and only one behavioral assessment was conducted at a time to facilitate brain removal and slice preparation for electrophysiology. Experimenters were blind to exposure group both at the time of testing and partner preference index calculations.

Between P60-P100, 24 sexually naive females and 14 sexually naive males were individually partnered with an adult prairie vole of the opposite sex for 24 h to allow for pair-bond formation. One prairie vole in the FM 550 exposed female group was accidentally partnered for 48 h, and this data point was retained in the experiment as it did not qualify as a statistical outlier. The "partner" prairie vole was sexually naïve, not closely related, of the opposite sex, had no prior interaction with the test

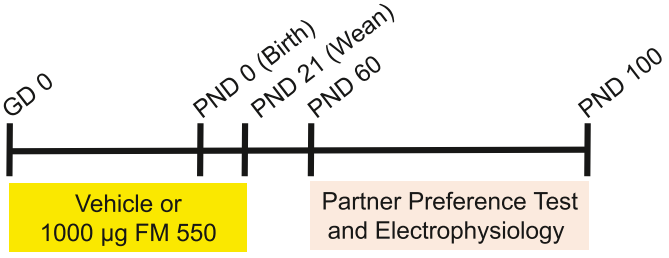

Fig. 1. Study design. Dams were exposed to vehicle or 1000 μg of FM 550 via subcutaneous injections throughout gestation, and female and male pups were directly exposed beginning the day after birth until weaning. Pair bond behavior of adult female and male offspring was assessed using a three hour-long partner preference test. After the partner preference test, nucleus accumbens core medium spiny neuron electrophysiological properties were assessed. Acronyms: GD, gestational day; PND, postnatal day.

vole, and was of reproductive age (aged 58 to 122 days). 24 h is sufficient time to induce pair bond formation, whether or not actual mating occurs (Williams et al., 1992). After 24 h, the PPT was performed. Two cups were placed on opposite ends of the 3-chamber arena. One cup held the partner vole, while the other held a "stranger" vole. The stranger vole was an unrelated, novel, sexually naïve prairie vole of the opposite sex. The test prairie vole was placed in the middle chamber and given 3 h to explore, following Ahern et al., 2009 and Willett et al., 2018. The test prairie vole was placed into the chamber at approximately 8:00 AM and removed at approximately 11 AM. One test prairie vole was assessed on a given day, given that the electrophysiological technique employed was low throughput, albeit high resolution. The duration in time spent in proximity to the stranger cup or partner cup was recorded, as assessed by 80% of the test prairie vole's body being proximate (within 6 cm) to a respective cup. The number of entries was also recorded, as defined by the number of times 80% of the test prairie vole's body entered the stranger or partner area of the arena through the respective opening. A partner preference index (PPI) was calculated by subtracting the time spent with the stranger prairie vole from the time spent with the partner prairie vole, divided by the total time spent with both.

2.5. Electrophysiology: acute brain slice preparation

Methods for acute brain slice preparation and electrophysiological recording of vole NAcc core neurons followed previously published procedures (Willett et al., 2018). Briefly, acute brain slice preparation occurred approximately 1 h after the PPT, at approximately noon. Prairie voles were deeply anesthetized with isoflurane gas and killed by decapitation. The brain was dissected rapidly into ice-cold, oxygenated (95% O_2, 5% CO_2) sucrose artificial CSF (s-ACSF) containing (in mM): 75 sucrose, 1.25 NaH_2PO_4, 3 $MgCl_2$, 0.5 $CaCl_2$, 2.4 Na pyruvate, 1.3 ascorbic acid from Sigma-Aldrich, St. Louis, MO, and 75 NaCl, 25 $NaHCO_3$, 15 dextrose, 2 KCl from Fisher, Pittsburg, PA; osmolarity 295–305 mOsm, pH 7.2–7.4. Serial 300 μm coronal brain slices containing the NAcc were prepared using a vibratome and incubated in regular ACSF containing (in mM): 126 NaCl, 26 $NaHCO_3$, 10 dextrose, 3 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 295–305 mOsm, pH 7.2–7.4, for 30 min at 35 °C, and at least 30 min at room temperature (21–23 °C). Slices were stored submerged in room temperature, oxygenated (95% O_2, 5% CO_2) ACSF for up to 5 h after sectioning in a large volume bath holder.

2.6. Electrophysiology: whole cell patch-clamp recording

After resting for ≥1 h after sectioning, slices were placed in a Zeiss Axioscope equipped with IR-DIC optics, a Dage IR-1000 video camera, and 10× and 40× lenses with optical zoom. Slices were superfused with oxygenated (95% O_2, 5% CO_2) ACSF heated to 28 ± 1 °C. Whole-cell patch-clamp recordings were made from medium spiny neurons (MSNs) in the NAcc core (Fig. 2). The NAcc was identified using the lateral ventricle and anterior commissure as landmarks. Recordings were made using glass electrodes containing (in mM): 115 K D-gluconate, 8 NaCl, 2 EGTA, 2 $MgCl_2$, 2 MgATP, 0.3 NaGTP, 10 phosphocreatine from Sigma-Aldrich and 10 HEPES from Fisher, 285 mOsm, pH 7.2–7.4). Signals were amplified, filtered (2 kHz), and digitized (10 kHz) with a MultiClamp 700B amplifier attached to a Digidata 1550a system and a personal computer using pClamp 10 software. Membrane potentials were corrected for a calculated liquid junction potential of −13.5 mV following previous studies (Dorris et al., 2015; Cao et al., 2016; Willett et al., 2016). The following metrics are reported using adjustment for liquid junction potential: resting membrane potential and action potential threshold. Recordings were made initially in current clamp to assess neuronal electrophysiological properties. MSNs were identified by their medium-sized somas, lack of spontaneous action potential generation, and the presence of at least 1 of the following characteristics: the presence of a slow-ramping subthreshold depolarization in response to low-magnitude positive current injections, a resting potential equaling or more hyperpolarized than −65 mV, and/or inward rectification (O'Donnell and Grace, 1993; Belleau and Warren, 2000; Cao et al., 2016). In a subset of recordings, oxygenated ACSF containing the $GABA_A$ receptor antagonist Picrotoxin (150 μM; Fisher) and the voltage-gated sodium channel blocker tetrodotoxin (TTX, 1 μm, Abcam Biochemicals) was applied to the bath to abolish action potentials and inhibitory post-synaptic current events. Once depolarizing current injection no longer elicited an action potential, MSNs were voltage-clamped at −70 mV and miniature excitatory post-synaptic current events (mEPSCs) were recorded for at least 5 min. These settings specifically target AMPA receptor-mediated mEPSCs (Proano et al., 2018). Input and series resistance was monitored for changes. mEPSCs were assessed so that any detected difference in synapse property could be linked to pre- and post-synaptic mechanisms.

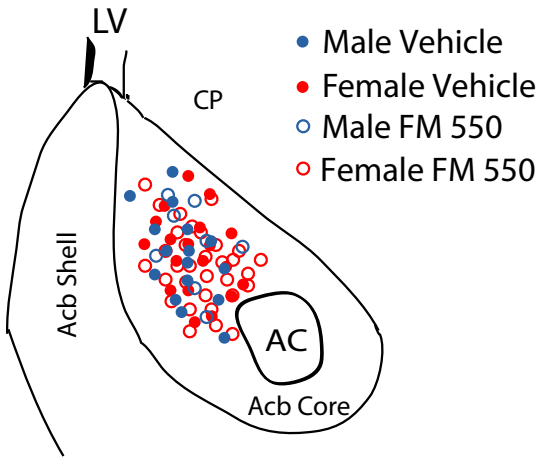

Fig. 2. Location of whole-cell patch clamped medium spiny neurons (MSNs) sorted by experimental group in the nucleus accumbens core of female and male voles. Acronyms: Acb, nucleus accumbens; AC, anterior commissure; LV, lateral ventricle; 3 V, third ventricle; CP, caudate-putamen.

2.7. Electrophysiology: data analysis

Basic electrophysiological properties and action potential characteristics were analyzed using pClamp 10. After break-in, the resting membrane potential was first allowed to stabilize for ~1–2 min, following (Mu et al., 2010). Three series of 600 ms depolarizing and hyperpolarizing current injections were applied to elicit basic neurophysiological properties. Current injections were stepwise increased or decreased, respectively, to sample a broad spectrum of neurophysiological responsiveness. All assessed properties followed the definitions of (Krentzel et al., 2019; Proaño and Meitzen, 2020), which were formulated from previous work by Perkel and colleagues (Farries and Perkel, 2000, 2002; Farries et al., 2005; Meitzen et al., 2009). The experimental unit for electrophysiological experiments was the individual neuron, following standard convention. Neuron experimental numbers were as follow. FM 550 female: 33 neurons from 6 litters, distributed per litter as: 3 neurons from 1 prairie vole, 1 neuron from 1 prairie vole, 4 neurons from 2 prairie voles, 10 neurons from 5 prairie voles, 3 neurons from 1 prairie vole, and 12 neurons recorded from 5 prairie voles. FM 550 male: 9 neurons from 4 litters, distributed per litter as: 4 neurons from 2 prairie voles, 1 neuron from 1 prairie voles, 3 neurons from 2 prairie voles, and 1 neuron recorded from 1 prairie vole. Vehicle female: 20 neurons from 4 litters, distributed per litter as 5 neurons from 2 prairie voles, 8 neurons from 3 prairie voles, 4 neurons from 3 prairie voles, and 3 neurons from 3 prairie voles recorded per litter. Vehicle male: 16 neurons from 5 litters, distributed per litter as: 5 neurons from 2 prairie voles, 4 neurons from 2 prairie voles, 1 neurons

from 1 prairie, and 6 neurons from 2 prairie voles recorded per litter. 1–3 neurons were recorded from each individual prairie vole. Litter number for the electrophysiological experiment does not match that of the behavioral experiment as recordings were unable to be obtained from some prairie voles. Briefly, for each neuron, measurements were made of at least three action potentials generated from minimal current injections. These measurements were then averaged to generate the reported action potential measurement for that neuron. Action potential threshold was defined as the first point of sustained positive acceleration of voltage ($\delta^2V/\delta t^2$) that was also more than three times the SD of membrane noise before the detected threshold (Baufreton et al., 2005). Action potential amplitude was calculated as the difference between threshold and the maximum mV value reached at action potential peak. The short afterhyperpolarization peak amplitude was calculated as the difference between the threshold value after the action potential peak and the lowest point after the action potential peak. The slope of the linear range of the evoked firing rate to positive current curve (FI slope) was calculated from the first current stimulus that evoked an action potential to the first current stimulus that generated an evoked firing rate that persisted for at least two consecutive current stimuli. Input resistance in the linear, non-rectified range was calculated from the steady-state membrane potential in response to −0.01 nA hyperpolarizing pulses. Measures of input resistance in the rectified range (rectified range input resistance, and percent inward rectification) were calculated from the steady-state membrane potential in response to the most hyperpolarizing current pulse injected into the neuron, following (Belleau and Warren, 2000). Percent inward rectification was defined as the rectified range input resistance divided by the linear range input resistance multiplied by 100. A neuron with no rectification will have a percent inward rectification of 100%. A neuron exhibiting rectification will have a small percent inward rectification score. The membrane time constant was calculated by fitting a single exponential curve to the membrane potential change in response to −0.02 nA hyperpolarizing pulses. Membrane capacitance was calculated using the following equation: capacitance = membrane time constant/input resistance. mEPSCs frequency, amplitude, and decay were analyzed using Mini Analysis (Synaptosoft, http://www.synaptosoft.com/MiniAnalysis/), following (Cao et al., 2016). Recordings were filtered (1 kHz), and mEPSC threshold was set at a minimum value of 5 pA. Accurate event detection was validated by visual inspection.

2.8. Statistics

Experiments were analyzed using two-tailed *t*-tests or 2-way repeated measures ANOVA with Fisher's LSD post-hoc tests (Prism version 6.0; GraphPad Software, La Jolla, CA). *P* values <0.05 were considered a priori as significant. Data are presented as mean ± SEM. Effect size for F tests are reported as partial eta squared (η^2p) and for t-tests as Cohen's d (d).

3. Results

3.1. Partner Preference Test (PPT)

PPI was first calculated for the entire 3 h, and compared to equal preference (0) using a one-sample t-test (Fig. 3A). As expected, vehicle males displayed a strong partner preference ($t_6 = 3.1$, $p < 0.03$, d = 1.17). By contrast, vehicle females did not display a preference ($t_9 = 0.6$, $p = 0.56$, d = 0.19). Males exposed to FM 550 did not display a preference ($t_6 = 1.9$, $p = 0.11$, d = 0.72). Females exposed to FM 550 displayed a strong partner preference ($t_{13} = 2.5$, $p < 0.03$, d = 0.66). To assess whether PPI was stable across time (Fig. 3B), PPI was calculated for one-hour bins, and found to have a significant interaction between exposure group and time (Interaction: $F_{6,68} = 2.6$, $p < 0.03$, $\eta^2p = 0.19$; Exposure: $F_{3,34} = 0.5$, $p > 0.05$, $\eta^2p = 0.04$; Time: $F_{2,68} = 1.2$, $p > 0.05$, $\eta^2p = 0.03$). Vehicle males displayed a strong partner preference in all 3 h. Males exposed to FM 550 displayed a weak partner preference in hour 1, which significantly increased by hour 3 ($t_{68} = 2.6$, $p < 0.02$, d = 0.94). Vehicle females began with a positive PPI in hour 1, which significantly decreased by hour 3 ($t_{68} = 2.3$, $p < 0.02$, d = 0.31). In contrast, females exposed to FM 550 displayed a stable positive PPI for all 3 h.

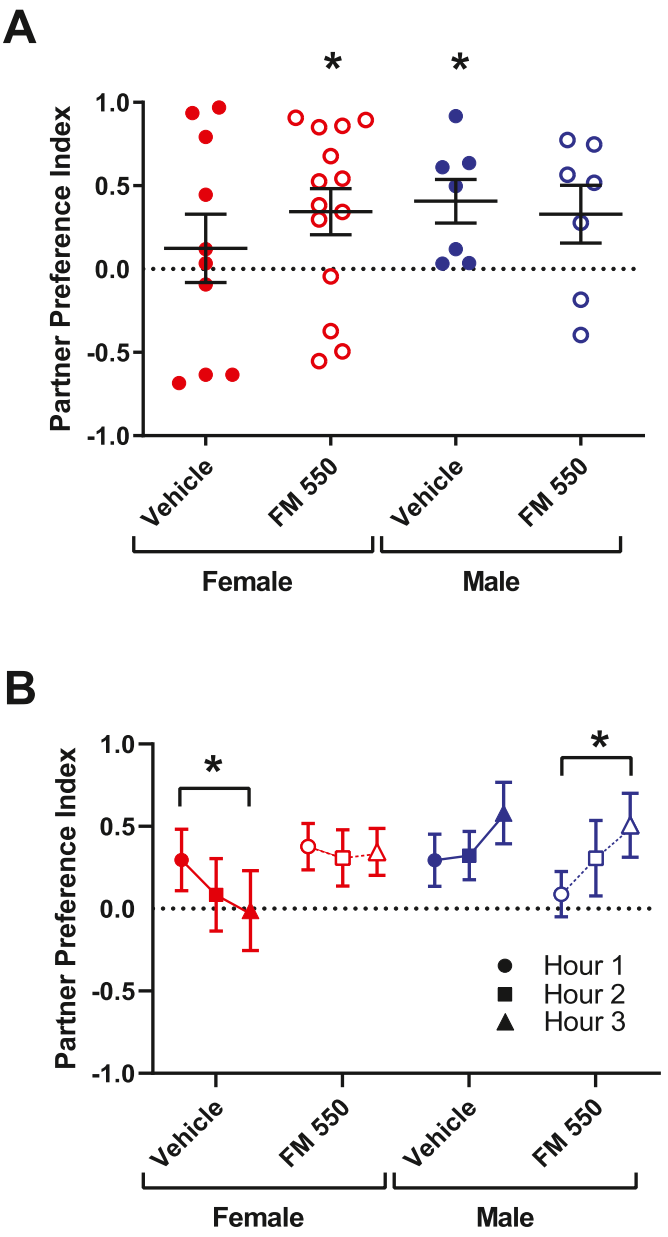

Fig. 3. Partner preference index (PPI). A) PPI calculated for the entire three hour period of the partner preference test. Vehicle females did not display a significant partner preference, in contrast to females exposed to FM 550. Vehicle males also displayed a significant partner preference, unlike males exposed to FM 550. B) PPI disaggregated by hour. Vehicle females displayed a steadily decreasing partner preference. Females exposed to FM 550 displayed a strong partner preference across the entirety of the test. Vehicle males displayed a relatively stable partner preference. Males exposed to FM 550 displayed an initial lack of partner preference, and then steadily increase partner preference behavior over time. A PPI of 1.0 indicates maximal preference for partner while an index of−1.0 indicates maximal preference for the stranger. Acronyms: *, $p < 0.05$.

Activity was binned into 30-minute intervals to quantify and compare via *t*-test the time spent investigating the partner and the stranger vole. Vehicle females exhibited a significant preference in the first 30 min bin (Fig. 4A; $t_{18} = 2.3$, $p < 0.04$, d = 1.01), and thereafter

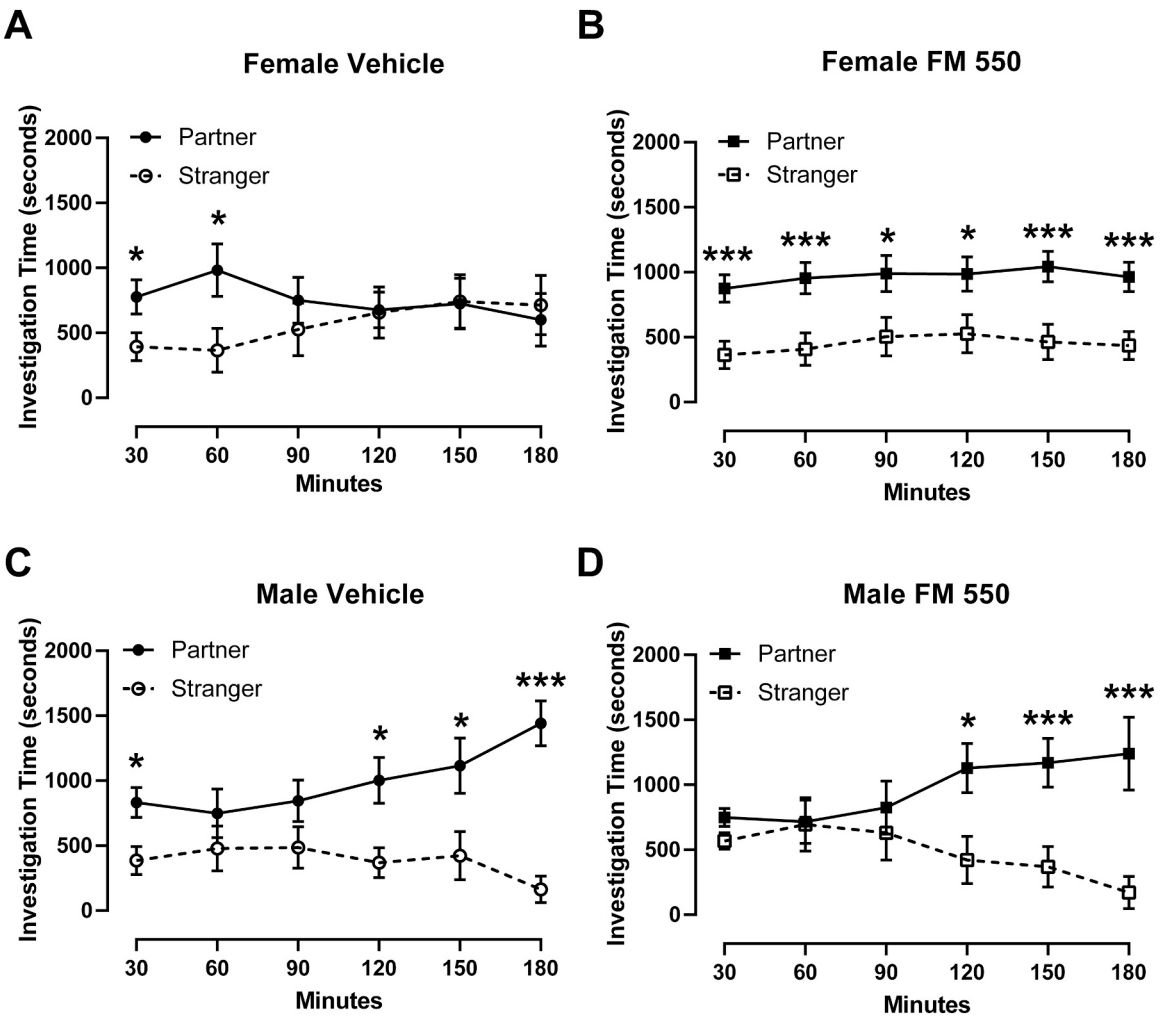

Fig. 4. Investigation time. A) Vehicle females. Investigation time with the partner (solid line) and stranger (dotted line) disaggregated by 30 min intervals across the entire partner preference test period indicate decreasing partner preference across time. B) Females exposed to FM 550 display strong preference for the partner versus the stranger across the entirety of the test. C) Vehicle males display a preference for the partner versus the stranger for the majority of the testing period. D) Males exposed to FM 550 display an initial lack of partner preference, and then steadily increase partner preference over time. Investigation time is defined at the amount of time spent in immediate proximity to the stranger or partner. Acronyms: *, $p < 0.05$; ***, $p < 0.001$.

only in the 60 min bin ($t_{18} = 2.3$, $p < 0.04$, $d = 1.04$). In contrast, females exposed to FM 550 exhibited a sustained partner preference, beginning with the 30 min bin and all bins thereafter (Fig. 4B; $t_{26} = 3.4$, $p < 0.003$, $d = 1.29$). Vehicle males exhibited a significant preference beginning in the first 30 min bin (Fig. 4C; $t_{12} = 2.8$, $p < 0.02$, $d = 1.51$), and thereafter starting in the 120 min bin ($t_{12} = 3.0$, $p < 0.02$, $d = 1.61$). Males exposed to FM 550 did not exhibit a significant preference until the 120 min bin and thereafter (Fig. 4D; $t_{12} = 2.7$, $p < 0.02$, $d = 1.44$).

The number of chamber entries was also quantified to determine whether the test vole was actively examining both the partner and stranger chambers. As above, the number of entries was binned into 30-minute intervals and *t*-test was employed to assess the binned data at each point. Vehicle females did not exhibit a significantly different number of entries between the partner and stranger chambers (Fig. 5A; $t_{18} = 0.6$, $p = 0.57$, $d = 0.26$). Females exposed to FM 550 exhibited an increased number of entries into the partner chamber compared to the stranger chamber, reaching significance in the first 30 min bin (Fig. 5B; $t_{26} = 2.1$, $p < 0.05$, $d = 0.80$). The total number of entries per chamber decreased over time. Vehicle males did not exhibit a significantly different number of entries between the partner and stranger chamber (Fig. 5C; $t_{12} = 1.9$, $p = 0.08$, $d = 1.01$). The total number of entries per chamber decreased over time. Males exposed to FM 550 did not exhibit a significantly different number of entries between the partner and stranger chambers (Fig. 5D; $t_{12} = 0.3$, $p = 0.75$, $d = 0.18$). The total number of entries per chamber decreased over time. Collectively, these data suggest that the test vole actively entered both the partner and stranger chambers, and that this behavior decreased over time in three of the four experimental groups: FM 550 females, vehicle males, and FM 550 males.

3.2. Electrophysiology

After assessing partner preference strength we tested whether FM 550 exposure impacted NAcc core medium spiny neuron fundamental electrophysiological properties (Table 1). Complete ANOVA statistical information as well as documentation of all assessed electrophysiological properties are in Table 1. Electrophysiological properties were assessed via a series of positive and negative current injections (Fig. 6A). No differences were detected in evoked action potential firing rates across groups (Fig. 6B). However, the steady-state voltage deflection evoked by injected hyperpolarizing current curve differed between groups (Fig. 6C). This finding suggests that differences between groups exist in input resistance in the linear and rectified ranges, as well as inward rectification properties (Table 1). Consistent with this conclusion, input resistance in the linear range in response to hyperpolarizing current injection was decreased in medium spiny neurons recorded from

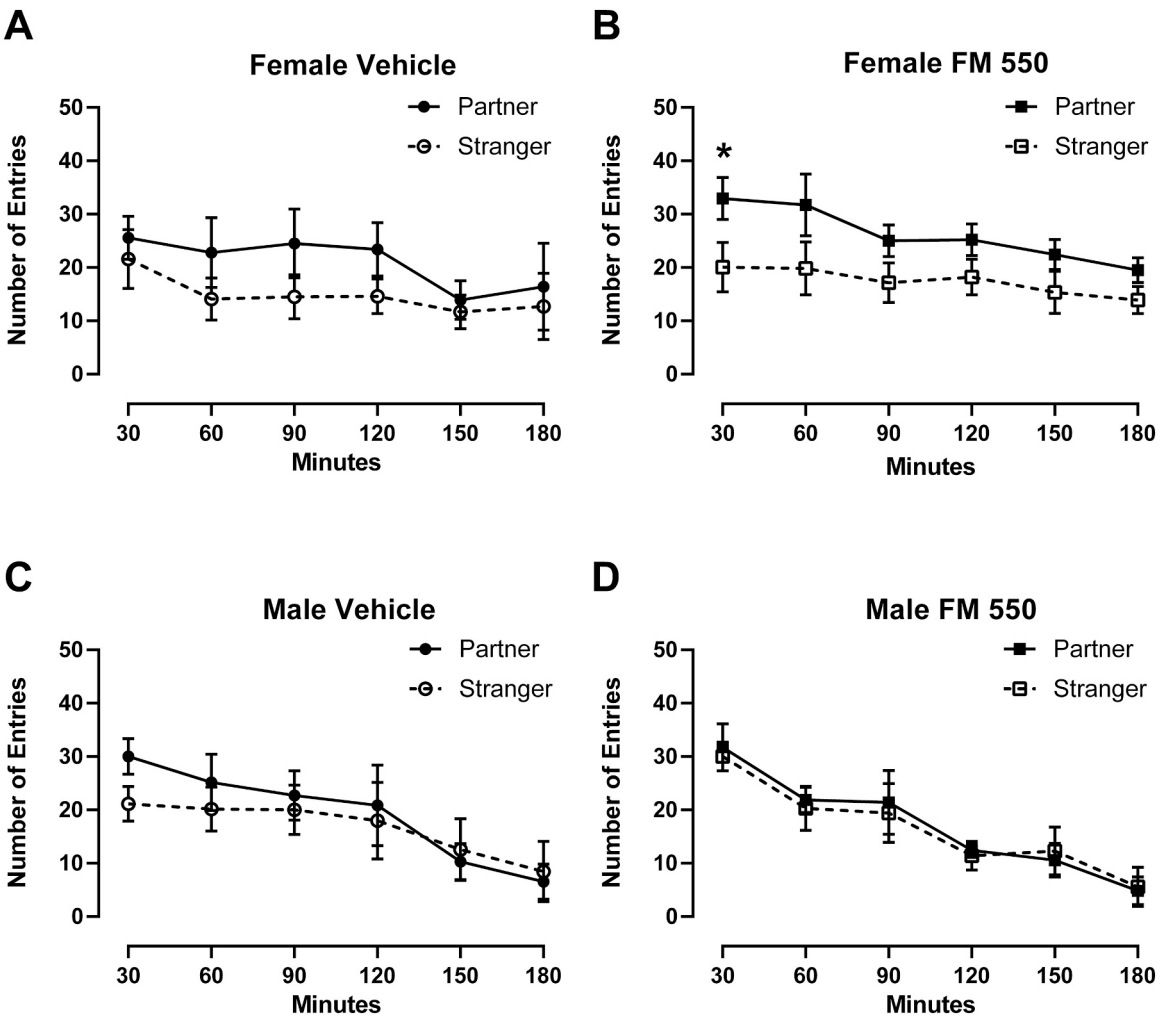

Fig. 5. Number of entries. A) Vehicle females. Number of entries into the partner room (solid line) and stranger room (dotted line) disaggregated by 30 min intervals across the entire partner preference test period display no differences across time. B) Females exposed to FM 550 display an increased number of entries into the partner room versus the stranger room. C) Vehicle males display no differences in number of entries across time. D) Males exposed to FM 550 display no differences in number of entries across time. Number of entries is defined as 80% of the vole's body entering the partner or stranger room. Acronyms: *, $p < 0.05$.

female and male voles exposed to FM 550 (Fig. 7A). Likewise, input resistance in the rectified range in response to hyperpolarizing current injection was decreased in medium spiny neurons recorded from female and male voles exposed to FM 550 (Fig. 7B). The decrease in input resistance in response to hyperpolarizing currents was accompanied by differences between groups in inward rectification properties, a canonical electrophysiological signature of medium spiny neurons when hyperpolarized (Mermelstein et al., 1998). The absolute magnitude of inward rectification in female medium spiny neurons exposed to vehicle differed from those recorded from females exposed to FM 550, and males exposed to vehicle and FM 550 (Fig. 7C). Percent inward rectification differed between female medium spiny neurons exposed to vehicle compared to male medium spiny neurons exposed to vehicle (Fig. 7D). Importantly, the steady-state voltage deflection evoked by injected depolarizing current curve did not differ between groups (Fig. 8). Values in response to depolarizing currents greater than +0.06 nA were not calculated due to the increasing presence of action potentials across medium spiny neurons, which prohibits accurate steady state deflection assessment. This lack of difference in response to positive current injection, as well as the robust difference in absolute inward rectification is consistent with the conclusion that FM 550 exposure particularly impacts input resistance in the linear and rectified ranges in response to hyperpolarizing currents in both males and females.

4. Discussion

Developmental FM 550 exposure enhanced partner preference behavior in females and weakened partner preference in males, an outcome that replicates and extends our previous study in voles (Gillera et al., 2020). Additionally, input resistance across the linear and rectified ranges was decreased in neurons recorded from females and males exposed to FM 550 compared to vehicle controls. This exposure-related decrease indicates a specific impact of exposure on input resistance and inward rectification values in response to negative current injections. Overall, this study further solidifies the conclusion that FM 550 affects attachment behavior in both sexes and generated novel evidence that FM 550 exposure alters fundamental electrophysiological properties in a key region critical for the coordination of prosocial behaviors.

As anticipated based on our prior work (Gillera et al., 2020), exposure-related effects on partner preference behavior were sex specific. Exposed females displayed a consistently strong PPI and greater time with the partner for the entire duration of the test. By contrast, exposed males did not display a positive PPI until the second half of the test, with partner investigation time not significantly greater than stranger investigation time until 120 min into the three hour test. The number of entries did not differ across groups with the exception of the exposed females. Females exposed to FM 550 made significantly more entries into the partner chamber compared to the stranger chamber in

Table 1
Nucleus accumbens core medium spiny neuron electrophysiological properties from female and male voles exposed to vehicle or FM 550.

Property	Vehicle	FM 550	Statistics (F, P, η^2p)
Resting membrane potential (mV)	F: −82.8 ± 1.5 (20) M: −83.9 ± 1.2 (16)	−82.3 ± 1.4 (33) −84.5 ± 0.8 (9)	0.09, 0.76, 0.001 0.93, 0.34, 0.01 0.00, 0.97, 0.00002
Linear range input resistance (MΩ)	**F: 245.4 ± 23.5 (20)** **M: 201.8 ± 20.9 (16)**	**181.9 ± 12.4 (33)** **165.7 ± 16.9 (9)**	**0.44, 0.51, 0.006** **2.10, 0.15, 0.03** **5.82, 0.02, 0.08**
Rectified range input resistance (MΩ)	**F: 211.7 ± 22.5 (20)** **M: 190.5 ± 20.1 (16)**	**163.8 ± 11.2 (33)** **149.6 ± 15.1 (9)**	**0.03, 0.86, 0.0004** **0.83, 0.36, 0.01** **5.24, 0.03, 0.07**
Inward rectification (MΩ)	**F: 33.7 ± 6.9 (20)** **M: 11.3 ± 2.6 (16)**	**18.1 ± 2.9 (33)** **16.1 ± 7.4 (9)**	**3.77, 0.06, 0.05** **5.33, 0.02, 0.07** **1.05, 0.31, 0.01**
% inward rectification (%)	**F: 86.1 ± 2.3 (20)** **M: 93.6 ± 1.2 (16)**	**90.2 ± 1.2 (33)** **90.6 ± 3.3 (9)**	**3.31, 0.07, 0.04** **4.06, 0.05, 0.05** **0.08, 0.77, 0.001**
Time constant of the membrane (ms)	F: 16.5 ± 1.5 (20) M: 16.6 ± 2.3 (16)	13.8 ± 1.0 (33) 15.6 ± 2.6 (9)	0.27, 0.61, 0.003 0.29, 0.59, 0.004 1.13, 0.29, 0.02
Capacitance (pF)	F: 74.4 ± 5.7 (20) M: 97.9 ± 16.9 (16)	83.5 ± 7.0 (33) 99.6 ± 15.8 (9)	0.11, 0.75, 0.001 3.04, 0.09, 0.04 0.23, 0.64, 0.003
Rheobase (pA)	F: 112.5 ± 16.3 (20) M: 137.8 ± 13.6 (16)	153.0 ± 11.5 (33) 152.0 ± 25.0 (9)	0.61, 0.44, 0.008 0.51, 0.48, 0.006 2.62, 0.11, 0.03
Delay to first AP (ms)	F: 449.7 ± 19.3 (13) M: 453.4 ± 11.6 (11)	462.2 ± 12.2 (29) 426.7 ± 19.4 (8)	1.25, 0.27, 0.02 0.82, 0.37, 0.01 0.16, 0.69, 0.002
AP threshold (mV)	F: −48.0 ± 1.9 (20) M: −44.0 ± 3.1 (16)	−44.4 ± 2.1 (33) −47.1 ± 3.5 (9)	1.39, 0.24, 0.02 0.05, 0.82, 0.0006 0.008, 0.93, 0.0001
AP width at half-peak amplitude (ms)	F: 2.3 ± 0.2 (20) M: 2.0 ± 0.1 (16)	2.1 ± 0.1 (33) 1.9 ± 0.1 (9)	0.01, 0.92, 0.0001 1.41, 0.24, 0.02 0.96, 0.33, 0.01
AP amplitude (mV)	F: 43.0 ± 2.8 (20) M: 36.5 ± 3.6 (16)	39.5 ± 2.9 (33) 41.6 ± 4.2 (9)	1.40, 0.24, 0.02 0.36, 0.55, 0.005 0.05, 0.83, 0.0006
AHP peak amplitude (mV)	F: −5.5 ± 0.6 (19) M: −5.3 ± 0.8 (16)	F: −5.5 ± 0.4 (33) M: −6.7 ± 0.7 (9)	1.16, 0.28, 0.02 0.67, 0.42, 0.009 1.18, 0.28, 0.02
AHP time to peak (ms)	F: 24.4 ± 2.9 (19) M: 30.8 ± 6.7 (16)	F: 25.7 ± 1.4 (33) M: 22.0 ± 2.3 (9)	1.82, 0.18, 0.02 0.14, 0.71, 0.002 0.96, 0.33, 0.01
FI slope (Hz/nA)	F: 328.1 ± 42.3 (20) M: 376.8 ± 65.4 (16)	F: 341.5 ± 36.6 (33) M: 241.4 ± 16.3 (9)	2.06, 1.16, 0.03 0.24, 0.62, 0.003 1.38, 0.24, 0.02
mEPSC frequency (Hz)	F: 2.4 ± 0.6 (16) M: 3.6 ± 0.7 (14)	F: 3.7 ± 0.7 (25) M: 4.0 ± 1.0 (6)	0.28, 0.60, 0.005 0.80, 0.37, 0.01 1.0, 0.31, 0.02
mEPSC amplitude (pA)	F: 14.3 ± 0.7 (16) M: 14.7 ± 0.7 (14)	F: 14.9 ± 0.4 (25) M: 15.6 ± 1.6 (6)	0.06, 0.81, 0.001 0.45, 0.51, 0.007 1.0, 0.32, 0.02
mEPSC decay (ms)	F: 1.8 ± 0.2 (16) M: 1.7 ± 0.2 (14)	F: 1.9 ± 0.1 (25) M: 2.2 ± 0.3 (6)	0.79, 0.38, 0.01 0.22, 0.64, 0.004 2.10, 0.15, 0.04

Values are mean ± SEM. Numbers in parentheses indicate the number of neurons in each group (experimental "n"). Statistics column lists F,P, and η2p values for interaction, sex, and exposure for a two-way ANOVA analysis. Bold font indicates statistical significance regarding that attribute. Input resistance values are in response to hyperpolarizing current injections (please see Methods). F, female; M, male; AP, action potential; AHP, afterhyperpolarization; FI, evoked firing rate-to-positive current curve; mEPSC, miniature excitatory post-synaptic current.

the first hour, an outcome that further supports the conclusion that they showed a particularly strong partner preference reminiscent of hyper-attachment. Notably, unexposed females demonstrated relatively weak partner preference behavior, and here we found that it decreased over the three hour period. By the end of the test period, vehicle females spent equivalent time investigating the partner and stranger vole. This is not entirely unexpected because partner preference behavior can be highly variable in this species, with not all displaying social monogamy (Ophir et al., 2012; Vogel et al., 2018; Willett et al., 2018), including in our prior study involving FM 550 (Gillera et al., 2020).

A critical difference between the present study and our previous FM 550 study in voles (Gillera et al., 2020) is the length of the employed test. Here we specifically focused on attachment behavior, enabling us to employ an extended three hour partner preference test, which is more typical. The prior study employed only a ten minute test because it was just one test out of several in a comprehensive battery specifically designed to assess a broad range of socioemotional and locomotor behaviors across three doses of FM 550. Significantly, those tests revealed a pattern of higher anxiety in exposed females and impaired sociality in both sexes. Exposed females were aversive to novel situations and individuals but displayed heightened attachment for familiar prairie voles including a cage mate or partner. Presumably, this hyper-affiliative behavior is a social buffering strategy to help cope with an otherwise stressful situation. Heightened anxiety has also been reported in developmentally exposed Wistar rats but most often in males (Patisaul et al.,

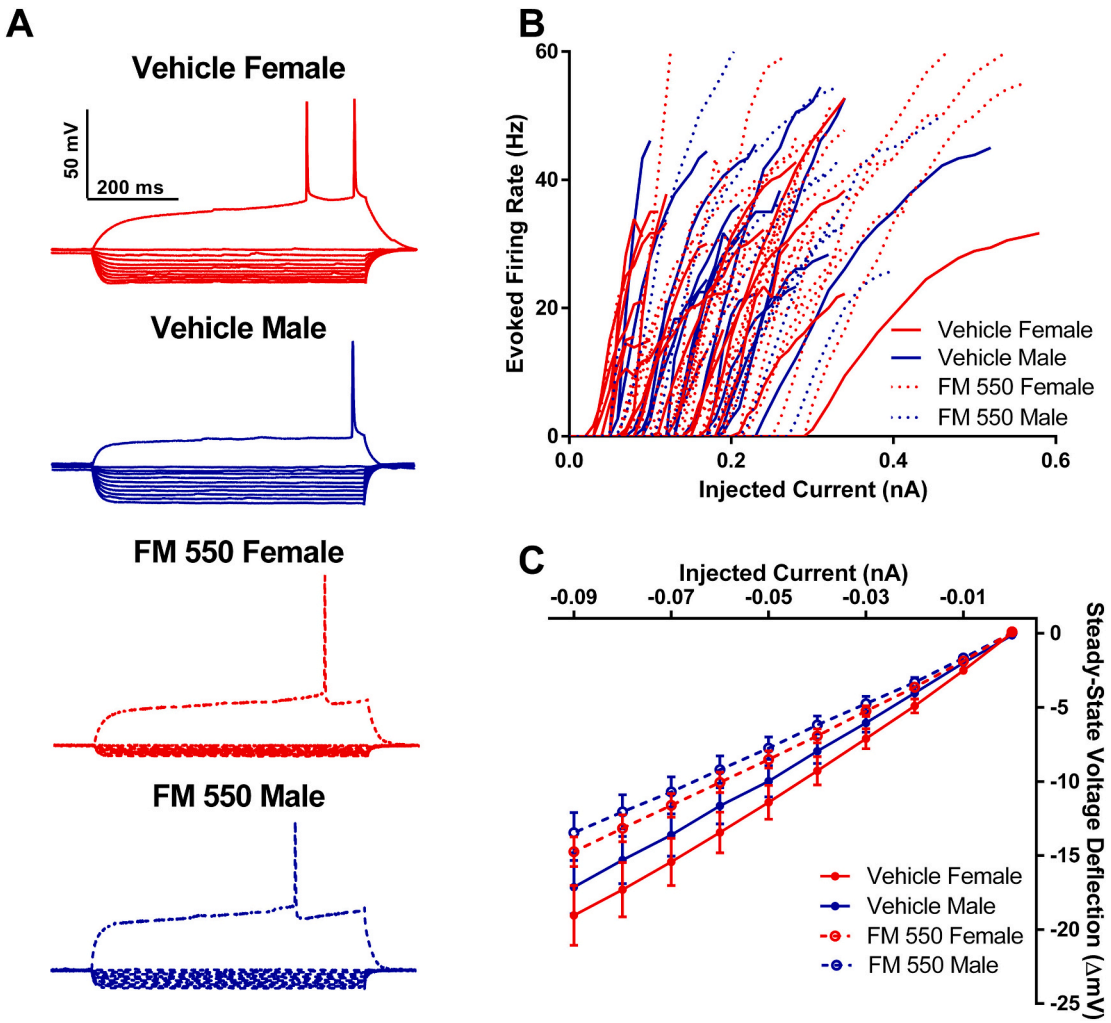

Fig. 6. Medium spiny neuron electrophysiological properties. A) Voltage responses of medium spiny neurons from vole NAcc core to a series of depolarizing and hyperpolarizing current injections. Depicted hyperpolarizing current injections ranged from −0.01 to −0.09 nA in −0.01 nA increments for all neurons. Depicted depolarizing current injections were chosen to illustrate one or two action potentials and are as follows: +0.05 nA (vehicle female), +0.06 nA (vehicle male), +0.16 nA (FM 550 female), and +0.18 nA (FM 550 male). B) Action potential firing rates evoked by depolarizing current injection. C) Injected negative current to steady-stage voltage deflection curve (IV curve). Table 1 contains statistical information.

2013; Baldwin et al., 2017). Species differences in sex-specific phenotypes is not unexpected given that, in addition to profound differences in prosocial behaviors, the hormone-dependent mechanisms by which the brains of prairie voles masculinize differs from rats and mice (Lonstein et al., 2005; De Vries and Panzica, 2006).

It has long been recognized that some chemical FRs have neurotoxic and endocrine disrupting activities that may contribute to increased risk of neurodevelopmental disorders. Polybrominated diphenyl ethers (PBDEs), a once prevalent class of brominated FRs, were phased out of use in the early 2000s for this reason and then replaced with OPFRs and newer BFRs, including those found in FM 550. Considered collectively with our prior data in prairie voles and rats, our data show that early life exposure compromises aspects of socioemotional behavior consistent with impairments in the Systems for Social Processing Domain of the RDoC; a framework developed by NIMH to improve interrogation of the basic dimensions of human behavior. These domains emphasize quantified measures of behavior and cognition, including those that can be measured in animal models (Simmons and Quinn, 2014; Mittal and Wakschlag, 2017). Key strengths of this approach are improving translational power and causality. Use of the prairie vole further strengthens the translational value of our data because their affiliative and attachment behaviors are more aligned with human social traits than traditional lab rats or mice, including those considered models for ASD and other psychosocial disorders. The adverse outcomes on affiliative behaviors reported herein are also consistent with the small but growing pool of human studies on FM 550 FRs. Exposure to OPFRs, for example, has been associated with poorer social skills in pre-school age children (Lipscomb et al., 2017). Similarly, maternal exposure to OPFRs, including TPHP, is associated with higher behavioral and externalizing problems at 36 months including withdrawal, attention problems, depression and aggression (Doherty et al., 2019). Urinary metabolites of the FM 550 component TPHP were also dose-dependently associated with decrements in working memory and aspects of intelligence and IQ in 7 year old children (Castorina et al., 2017a; Castorina et al., 2017b). In a 2014 study, FM 550 was detected in 100% of dust samples collected in California early childhood education facilities (Bradman et al., 2014), with many subsequent studies finding similar across the globe (McGrath et al., 2018; Li et al., 2019; Zuiderveen et al., 2020), demonstrating their broad prevalence, and the unavoidable risk of early life exposure.

While these behavioral impacts are increasingly well characterized, the mechanisms by which FM 550 affects socioemotional behavior are not known for either sex. Guiding us in this initial investigation, it is well established that neuromodulators such as oxytocin, vasopressin, and dopamine contribute to pair bonding, affiliation, and other prosocial behaviors (Young et al., 2011; Bosch and Young, 2018) Differences in social behaviors such as attachment between prairie voles and other

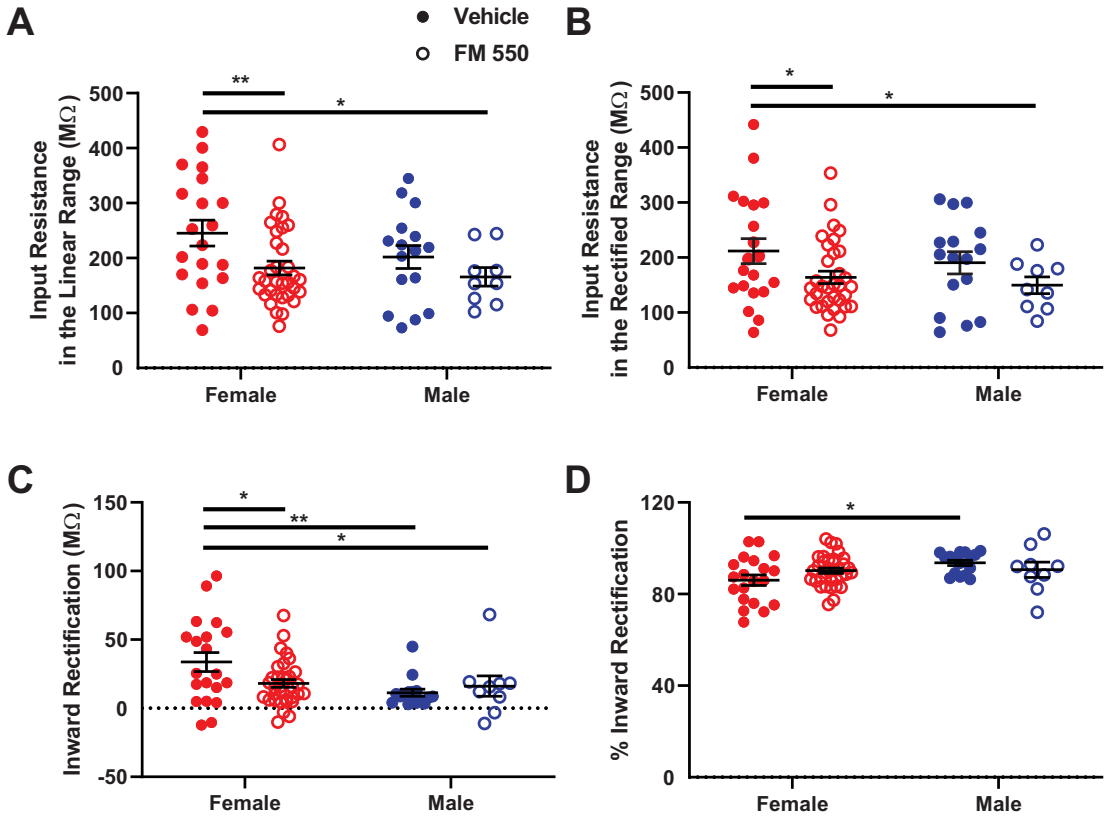

Fig. 7. Input resistance in response to negative current injection varies by FM 550 or vehicle exposure. A) Input resistance in the linear range in response to negative current injection is decreased in medium spiny neurons recorded from females and males exposed to FM 550 compared to vehicle. B) Input resistance in the rectified range in response to negative current injection is decreased in medium spiny neurons recorded from females and males exposed to FM 550 compared to vehicle. C) The absolute magnitude of inward rectification in female medium spiny neurons exposed to vehicle differed from those recorded from females exposed to FM 550, and males exposed to vehicle and FM 550. D) Percent inward rectification differed between female medium spiny neurons exposed to vehicle compared to male medium spiny neurons exposed to vehicle. Decreased percent inward rectification values typically correspond with increased absolute magnitude inward rectification values. Acronyms: *: $P < 0.05$; **: $P < 0.01$. Table 1 contains statistical information.

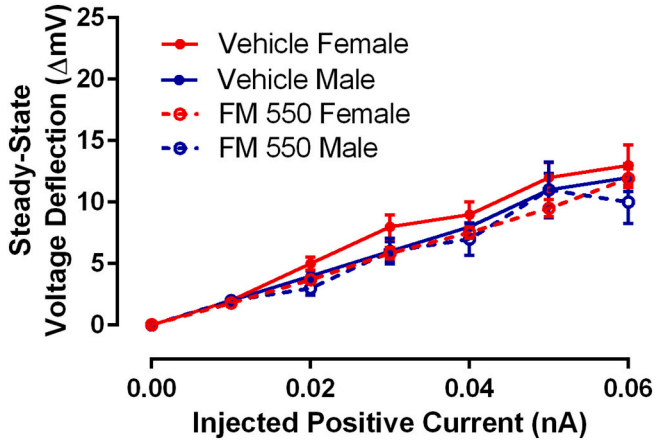

Fig. 8. Injected positive current to steady-stage voltage deflection curve (IV curve). No differences are noted in voltage deflections in response to positive currents, unlike in response to negative currents.

rodent species (including polygynous vole species such as the montane vole) is largely attributed to the distribution of oxytocin, vasopressin, and other relevant receptors, and their interactions with the mesolimbic dopamine system. To exert a meaningful effect, these modulators and relevant genetic mechanisms must in some way alter the electrophysiological function of neurons in key brain regions, including the NAcc. The NAcc acts as a nexus region between the limbic and premotor systems and is key for regulating motivated and reward-related behaviors, including those involving attachment. Indeed, injection of oxytocin into the NAcc and blockade of oxytocin receptors in the NAcc induces or blocks attachment behavior, respectively (Young et al., 2001; Liu and Wang, 2003). A series of elegant experiments have demonstrated that dopamine receptor activation in the NAcc is likewise instrumental in pair bond formation (Young et al., 2011). Prior work has also demonstrated links between pair bond strength and the electrophysiological properties of the NAcc output neurons, the medium spiny neurons (Amadei et al., 2017; Willett et al., 2018). Given this preponderance of evidence, for this study we targeted assessing the electrophysiological properties of medium spiny neurons.

Interestingly, FM 550 exposure decreased input resistance across the linear and rectified ranges in response to negative current injection in medium spiny neurons recorded from both females and males. Input resistance is a fundamental neuronal electrophysiological property that quantifies how, in this case, neuronal membrane voltage responds to an injected current. Typically input resistance is determined by the subunit allocation, modulation, and/or expression of leak potassium and sodium ion channels, and in select neuron types inwardly rectifying potassium channels. Some neuron types exhibit a linear input resistance that does not change with hyperpolarizing or depolarizing membrane potentials. In contrast, medium spiny neuron input resistance is notorious for changing with membrane potential, especially with increasing hyperpolarization (Nisenbaum and Wilson, 1995; Farries and Perkel, 2000). In medium spiny neurons, as the membrane potential hyperpolarizes, the

input resistance value typically decreases, including in voles (Willett et al., 2018). This phenomenon is primarily due to the additional presence of inwardly rectifying potassium ion channels (Karschin et al., 1996; Mermelstein et al., 1998). These inwardly rectifying currents are a primary influence on medium spiny neuron electrical activity in the NAcc (Uchimura et al., 1989, 1990; Uchimura and North, 1990; Wilson, 1993), and are crucial players in determining input resistance, resting membrane potential, and synaptic input integration (Wilson, 1993; Hille, 2001). Thus, in the present study, input resistance in response to negative and positive current injection, as well as multiple assessments of inward rectification were performed. Collectively, all of these metrics indicate that FM 550 exposure specifically decreases input resistance across both the linear and rectified range, as well as inward rectification. Importantly, no differences were detected in response to positive current injections. Collectively, this evidence points to several potential mechanisms. One is cell autonomous, in that FM 550 exposure may directly impact the expression and/or subunit composition of leak sodium and potassium channels or inwardly rectifying potassium channels in medium spiny neurons. We currently favor inwardly rectifying potassium channels as the most likely candidate, given the demonstrated impact in changes in steady-state voltage deflection in response to negative but not positive current injections. In other models, environmental contaminants have been shown to impact select sodium and potassium ion channel function and/or expression (Magby et al., 2011; Soriano et al., 2019), however, FM 550 has not yet been investigated. Other potential mechanisms include a combination of cell autonomous and non-autonomous actions. For example, OPFRs have been shown to not only affect the intrinsic electrophysiological properties of arcuate nucleus neurons, including decreasing a hyperpolarizing potassium M-current, but also to modulate the neuronal sensitivity to the neuromodulator ghrelin (Vail and Roepke, 2020). Thus, it is also possible that FM 550 exposure may change the medium spiny neuron's sensitivity to an important neuromodulator or neurotransmitter, including but not limited to oxytocin, dopamine, acetylcholine, glutamate, or serotonin. For example, dopamine has long been known as a potential modulator of inwardly rectifying potassium currents (Dong et al., 2004). Of note, FM 550 exposure has been shown to impact the synthesis and transport of serotonin into the fetal rat brain (Rock et al., 2020), and the responsiveness to dopamine antagonists in zebrafish larvae (Oliveri et al., 2018). Future studies should address these potential cell autonomous and non-autonomous mechanisms.

While the behavioral impacts of FM 550 exposure were sex-specific, the electrophysiological impacts does not follow this directionality. At first consideration, the lack of a sex specific effect on electrophysiological properties seems puzzling, however, it is possible that modulation of the same electrophysiological endpoint in both females and males could exert differential, sex-specific effects in behavior. Indeed, multiple types of sex differences exist, and it has been previously documented that a sex difference at the level of an individual neuron property could exert a differential effect at the level of the circuit and behavioral output (De Vries, 2004). This differential effect at the level of behavior induced by a similar effect at the level of the neuron could occur via multiple mechanisms, including sex differences or impacts in other portions of the relevant circuit, other brain regions that control attachment behavior, and/or to modulatory factors, such as oxytocin, dopamine, and steroid sex hormones such as estradiol. Sex-specific rapid modulation of NAcc medium spiny neuron electrophysiological properties such as glutamatergic synapse properties (Krentzel et al., 2019) and L-type calcium channel currents (Mermelstein et al., 1996) have been previously reported in rats. Medium spiny neurons express nuclear estrogen receptors in early development and membrane estrogen receptors in adulthood (Almey et al., 2012; Almey et al., 2015, 2016; Krentzel et al., 2021; Quigley and Becker, 2021). The dopaminergic neuromodulator system feeding into the NAcc has long been documented to show sex differences and sensitivity to steroid sex hormone action in other rodents such as rats and mice (Di Paolo, 1994; Becker and Chartoff, 2019), and may be sensitive to endocrine disruption by FM 550 and potentially the perinatal actions of steroid hormones (Cao et al., 2016; Bonansco et al., 2018; Harp et al., 2020). Also, the neuromodulator oxytocin is heavily implicated in social reward and partner preference behavior (Young et al., 2011; Modi and Young, 2012). Acute oxytocin exposure has been reported to modulate glutamatergic synapse properties in rodent brain regions, including mEPSC properties in male mouse NAcc (Ninan, 2011; Dölen et al., 2013; Zheng et al., 2014).

One pertinent caveat to the interpretation that FM 550 exposure impacts both females and males in an equivalent fashion is the relatively smaller number of medium spiny neuron recordings made from males exposed to FM 550. While the median and means of the data groups support the conservative interpretation that the data do not differ by sex, it is always possible that the addition of more neuronal recordings may reveal a more subtle impact on medium spiny neuron electrophysiological phenotype. Similarly, the relatively smaller number of FM 550 males impacts interpretation of the PPT data, in particular the 3-hour total PPI comparison. In this comparison, FM 550 females and FM 550 males exhibit similar means and effect sizes, however, the FM 550 males do not reach a statistically significant PPI. We believe that the time binned analyses more accurately represent the results of the PPT test. These time binned analyses indicate stark differences in the pattern of partner preference changes across time between FM 550 exposed males and females. Another potential limitation to this study is the use of subcutaneous injection to deliver FM 550, because this is not the typical route of human exposure and it bypasses first-pass metabolism in the liver. Injection was necessary because little is known regarding FM 550 metabolism in voles other than what we previously published. This unique species requires a high fiber diet and has a urogenital system that scavenges and preserves water, thus metabolism similar to other rodent species cannot be assumed. To address this potential limitation, in our previous study the internal levels of FM 550 components were assessed in a subset of experimental prairie voles to evaluate internal dose levels (Gillera et al., 2020). Serum levels of the parent compounds and their primary metabolites were in the low to undetectable ng/ml range. We also note that experiments in rats exposed to FM 550 via oral consumption (Witchey et al., 2020) largely replicate the other non-attachment behavioral outcomes reported in injected voles (Gillera et al., 2020). Establishing the pharmacokinetics of FM 550 in prairie voles remains an important goal of future experiments to further the utility of this important animal model.

In conclusion, here we showed, in a unique species displaying prosocial affiliative traits including pair bonding, that early life exposure to the FR mixture FM 550 sex-specifically compromises aspects of attachment behavior. Attachment was weaker in exposed males, with evidence of partner preference not readily evident or displayed until well into a three hour PPT. By contrast, exposed females quickly displayed evidence of hyper-attachment, behavior that persisted across the duration of the test and is likely a consequence of heightened anxiety. These outcomes are consistent with our prior study using a shorter PPT, and this reproducibility heightens confidence in the conclusion that FRs may be contributing to higher rates of neurodevelopmental disorders with social components, including ASD. Paired with these behavioral findings is the novel finding that neurons in a brain region key for attachment behavior displayed reduced input resistance in prairie voles exposed to FM 550, demonstrating that early life exposure to this FR mixture, or one of its components, impacts fundamental neuronal input/output computation. Available epidemiology data along with behavioral and mechanistic data emerging from a variety of animal models implicates the OPFRs over the BFRs, but this requires further interrogation. Our findings are consistent with well-established work showing a critical role for the mesolimbic dopamine system for coordinating prosocial behaviors and reveal it may be vulnerable to endocrine disruption. Future work should also focus on other components of this system including interactions with the oxytocin and vasopressin system.

Acknowledgements

The authors thank Dr. Heather Stapleton and her laboratory for preparing the FM 550 dosing solutions. We also thank Sagi Gillera and Dr. Jinyan Cao for technical support, and the staff at the Biological Resource Facility for their exceptional animal care support. This work was supported by the following funding sources: National Institutes of Health P30 ES-025128 (Center for Human Health and the Environment); NC State University Center for Human Health and the Environment Pilot Grant (J.M., H.B.P.); National Institutes of Health (NIH) Grant R01 MH-109471 (J.M.); NC State University Faculty Research and Professional Development Award (J.M.); NC State University Provost Professional Experience Program (L.C.K.); NC State University Office of Undergraduate Research Award (L.C.K.); Department of Defense Autism Research Program, Idea Development Award AR160055 (H.B.P.)

References

Ahern, T.H., Modi, M.E., Burkett, J.P., Young, L.J., 2009. Evaluation of two automated metrics for analyzing partner preference tests. J. Neurosci. Methods 182, 180–188.
Almey, A., Filardo, E.J., Milner, T.A., Brake, W.G., 2012. Estrogen Receptors Are Found in Glia and at Extranuclear Neuronal Sites in the Dorsal Striatum of Female Rats: Evidence for Cholinergic But Not Dopaminergic Colocalization. Endocrinology 153, 5373–5383.
Almey, A., Milner, T.A., Brake, W.G., 2015. Estrogen receptors in the central nervous system and their implication for dopamine-dependent cognition in females. Horm. Behav. 74, 125–138.
Almey, A., Milner, T.A., Brake, W.G., 2016. Estrogen receptor alpha and G-protein coupled estrogen receptor 1 are localized to GABAergic neurons in the dorsal striatum. Neurosci. Lett. 622, 118–123.
Amadei, E.A., Johnson, Z.V., Jun Kwon, Y., Shpiner, A.C., Saravanan, V., Mays, W.D., Ryan, S.J., Walum, H., Rainnie, D.G., Young, L.J., Liu, R.C., 2017. Dynamic corticostriatal activity biases social bonding in monogamous female prairie voles. Nature 546, 297–301.
Aragona, B.J., Liu, Y., Curtis, J.T., Stephan, F.K., Wang, Z., 2003. A critical role for nucleus accumbens dopamine in partner-preference formation in male prairie voles. J. Neurosci. 23, 3483–3490.
Aragona, B.J., Liu, Y., Yu, Y.J., Curtis, J.T., Detwiler, J.M., Insel, T.R., Wang, Z., 2006. Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. Nat. Neurosci. 9, 133–139.
Baldwin, K.R., Phillips, A.L., Horman, B., Arambula, S.E., Rebuli, M.E., Stapleton, H.M., Patisaul, H.B., 2017. Sex Specific Placental Accumulation and Behavioral Effects of Developmental Firemaster 550 Exposure in Wistar Rats. Sci. Rep. 7, 7118.
Baldwin, K.R., Horman, B., Phillips, A.L., McRitchie, S.L., Watson, S., Deese-Spruill, J., Jima, D., Sumner, S., Stapleton, H., Patisaul, H., 2018. EDC IMPACT: Molecular effects of developmental FM 550 exposure in Wistar rat placenta and fetal forebrain. Endocr Connect.
Barrett, C.E., Keebaugh, A.C., Ahern, T.H., Bass, C.E., Terwilliger, E.F., Young, L.J., 2013. Variation in vasopressin receptor (Avpr1a) expression creates diversity in behaviors related to monogamy in prairie voles. Horm. Behav. 63, 518–526.
Baufreton, J., Atherton, J.F., Surmeier, D.J., Bevan, M.D., 2005. Enhancement of excitatory synaptic integration by GABAergic inhibition in the subthalamic nucleus. J. Neurosci. 25, 8505–8517.
Becker, J.B., Chartoff, E., 2019. Sex differences in neural mechanisms mediating reward and addiction. Neuropsychopharmacology 44, 166–183.
Belcher, S.M., Cookman, C.J., Patisaul, H.B., Stapleton, H.M., 2014. In vitro assessment of human nuclear hormone receptor activity and cytotoxicity of the flame retardant mixture FM 550 and its triarylphosphate and brominated components. Toxicol. Lett. 228, 93–102.
Belleau, M.L., Warren, R.A., 2000. Postnatal development of electrophysiological properties of nucleus accumbens neurons. J. Neurophysiol. 84, 2204–2216.
Blocker, T.D., Ophir, A.G., 2016. A preference to bond? Male prairie voles form pair bonds even in the presence of multiple receptive females. Anim. Behav. 122, 89–97.
Blum, A., Behl, M., Birnbaum, L., Diamond, M.L., Phillips, A., Singla, V., Sipes, N.S., Stapleton, H.M., Venier, M., 2019. Organophosphate ester flame retardants: are they a regrettable substitution for polybrominated diphenyl ethers? Environ. Sci. Technol. Lett. 6, 638–649.
Bonansco, C., Martínez-Pinto, J., Silva, R.A., Velásquez, V.B., Martorell, A., Selva, M.V., Espinosa, P., Moya, P.R., Cruz, G., Andrés, M.E., Sotomayor-Zárate, R., 2018. Neonatal exposure to oestradiol increases dopaminergic transmission in nucleus accumbens and morphine-induced conditioned place preference in adult female rats. J. Neuroendocrinol. 30, e12574.
Bosch, O.J., Young, L.J., 2018. Oxytocin and social relationships: from attachment to bond disruption. Curr. Top. Behav. Neurosci. 35, 97–117.
Bosch, O.J., Pohl, T.T., Neumann, I.D., Young, L.J., 2018. Abandoned prairie vole mothers show normal maternal care but altered emotionality: potential influence of the brain corticotropin-releasing factor system. Behav. Brain Res. 341, 114–121.
Bradman, A., Castorina, R., Gaspar, F., Nishioka, M., Colon, M., Weathers, W., Egeghy, P. P., Maddalena, R., Williams, J., Jenkins, P.L., McKone, T.E., 2014. Flame retardant exposures in California early childhood education environments. Chemosphere 116, 61–66.
Cao, J., Dorris, D.M., Meitzen, J., 2016. Neonatal masculinization blocks increased excitatory synaptic input in female rat nucleus accumbens core. Endocrinology 157, 3181–3196.
Carter, C.S., DeVries, A.C., Getz, L.L., 1995. Physiological substrates of mammalian monogamy: the prairie vole model. Neurosci. Biobehav. Rev. 19, 303–314.
Castorina, R., Bradman, A., Stapleton, H.M., Butt, C., Avery, D., Harley, K.G., Gunier, R. B., Holland, N., Eskenazi, B., 2017a. Current-use flame retardants: Maternal exposure and neurodevelopment in children of the CHAMACOS cohort. Chemosphere 189, 574–580.
Castorina, R., Butt, C., Stapleton, H.M., Avery, D., Harley, K.G., Holland, N., Eskenazi, B., Bradman, A., 2017b. Flame retardants and their metabolites in the homes and urine of pregnant women residing in California (the CHAMACOS cohort). Chemosphere 179, 159–166.
De Vries, G.J., 2004. Minireview: sex differences in adult and developing brains: compensation, compensation, compensation. Endocrinology 145, 1063–1068.
De Vries, G.J., Panzica, G.C., 2006. Sexual differentiation of central vasopressin and vasotocin systems in vertebrates: different mechanisms, similar endpoints. Neuroscience 138, 947–955.
Di Paolo, T., 1994. Modulation of brain dopamine transmission by sex steroids. Rev. Neurosci. 5, 27–41.
Dodson, R.E., Perovich, L.J., Covaci, A., Van den Eede, N., Ionas, A.C., Dirtu, A.C., Brody, J.G., Rudel, R.A., 2012. After the PBDE phase-out: a broad suite of flame retardants in repeat house dust samples from California. Environ. Sci. Technol. 46, 13056–13066.
Doherty, B.T., Hammel, S.C., Daniels, J.L., Stapleton, H.M., Hoffman, K., 2019. Organophosphate esters: are these flame retardants and plasticizers affecting children's health? Curr. Environ. Health Rep. 6, 201–213.
Dölen, G., Darvishzadeh, A., Huang, K.W., Malenka, R.C., 2013. Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. Nature 501, 179–184.
Dong, Y., Cooper, D., Nasif, F., Hu, X.T., White, F.J., 2004. Dopamine modulates inwardly rectifying potassium currents in medial prefrontal cortex pyramidal neurons. J. Neurosci. 24, 3077–3085.
Dorris, D.M., Cao, J., Willett, J.A., Hauser, C.A., Meitzen, J., 2015. Intrinsic excitability varies by sex in pre-pubertal striatal medium spiny neurons. J. Neurophysiol. 113, 720–729.
Estill, C.F., Slone, J., Mayer, A., Chen, I.C., La Guardia, M.J., 2020. Worker exposure to flame retardants in manufacturing, construction and service industries. Environ. Int. 135, 105349.
Farries, M.A., Perkel, D.J., 2000. Electrophysiological properties of avian basal ganglia neurons recorded in vitro. J. Neurophysiol. 84, 2502–2513.
Farries, M.A., Perkel, D.J., 2002. A telencephalic nucleus essential for song learning contains neurons with physiological characteristics of both striatum and globus pallidus. J. Neurosci. Off. J. Soc. Neurosci. 22, 3776–3787.
Farries, M.A., Meitzen, J., Perkel, D.J., 2005. Electrophysiological properties of neurons in the basal ganglia of the domestic chick: conservation and divergence in the evolution of the avian basal ganglia. J. Neurophysiol. 94, 454–467.
Ford, C.L., Young, L.J., 2020. Translational opportunities for circuit-based social neuroscience: advancing 21st century psychiatry. Curr. Opin. Neurobiol. 68, 1–8.
Gillera, S.E.A., Marinello, W.P., Horman, B.M., Phillips, A.L., Ruis, M.T., Stapleton, H.M., Reif, D.M., Patisaul, H.B., 2020. Sex-specific effects of perinatal FireMaster(R) 550 (FM 550) exposure on socioemotional behavior in prairie voles. Neurotoxicol. Teratol. 79, 106840.
Grandjean, P., Landrigan, P.J., 2014. Neurobehavioural effects of developmental toxicity. Lancet Neurol. 13, 330–338.
Grillner, S., Hellgren, J., Menard, A., Saitoh, K., Wikstrom, M.A., 2005. Mechanisms for selection of basic motor programs–roles for the striatum and pallidum. Trends Neurosci. 28, 364–370.
Hammock EA, Young LJ (2005) Microsatellite instability generates diversity in brain and sociobehavioral traits. Science (New York, NY) 308:1630-1634.
Harp SJ, Martini M, Lynch WJ, Rissman EF (2020) Sexual Differentiation and Substance Use: A Mini-Review. Endocrinology 161:bqaa129.
Hille B (2001) Ion Channels of Excitable Membranes.
Hoffman, K., Fang, M., Horman, B., Patisaul, H.B., Garantziotis, S., Birnbaum, L.S., Stapleton, H.M., 2014. Urinary tetrabromobenzoic acid (TBBA) as a biomarker of exposure to the flame retardant mixture Firemaster® 550. Environ. Health Perspect. 122, 963–969.
Hunt, P.A., Susiarjo, M., Rubio, C., Hassold, T.J., 2009. The bisphenol A experience: a primer for the analysis of environmental effects on mammalian reproduction. Biol. Reprod. 81, 807–813.
Insel, T.R., Shapiro, L.E., 1992. Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. Proc. Natl. Acad. Sci. U. S. A. 89, 5981–5985.
Kalkbrenner, A.E., Schmidt, R.J., Penlesky, A.C., 2014. Environmental chemical exposures and autism spectrum disorders: a review of the epidemiological evidence. Curr. Probl. Pediatr. Adolesc. Health Care 44, 277–318.
Karschin, C., Dissmann, E., Stühmer, W., Karschin, A., 1996. IRK(1-3) and GIRK(1-4) inwardly rectifying K+ channel mRNAs are differentially expressed in the adult rat brain. J. Neurosci. 16, 3559–3570.
Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M., Altman, D.G., 2010. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. J. Pharmacol. Pharmacother. 1, 94–99.
Kleiman, D.G., 1977. Monogamy in mammals. Q. Rev. Biol. 52, 39–69.
Knudsen, G.A., Sanders, J.M., Birnbaum, L.S., 2017. Disposition of the emerging brominated flame retardant, bis(2-ethylhexyl) tetrabromophthalate, in female

Sprague Dawley rats: effects of dose, route and repeated administration. Xenobiotica; the fate of foreign compounds in biological systems 47, 245–254.
Krentzel, A.A., Barrett, L.R., Meitzen, J., 2019. Estradiol rapidly modulates excitatory synapse properties in a sex- and region-specific manner in rat nucleus accumbens core and caudate-putamen. J. Neurophysiol. 122, 1213–1225.
Krentzel, A.A., Willett, J.A., Johnson, A.G., Meitzen, J., 2021. Estrogen receptor alpha, G-protein coupled estrogen receptor 1, and aromatase: Developmental, sex, and region-specific differences across the rat caudate-putamen, nucleus accumbens core and shell. J. Comp. Neurol. 529, 786–801.
Li, J., Zhao, L., Letcher, R.J., Zhang, Y., Jian, K., Zhang, J., Su, G., 2019. A review on organophosphate Ester (OPE) flame retardants and plasticizers in foodstuffs: levels, distribution, human dietary exposure, and future directions. Environ. Int. 127, 35–51.
Lipscomb, S.T., McClelland, M.M., MacDonald, M., Cardenas, A., Anderson, K.A., Kile, M. L., 2017. Cross-sectional study of social behaviors in preschool children and exposure to flame retardants. Environ. Health 16, 23.
Liu, Y., Wang, Z.X., 2003. Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. Neuroscience 121, 537–544.
Lonstein, J.S., Rood, B.D., De Vries, G.J., 2005. Unexpected effects of perinatal gonadal hormone manipulations on sexual differentiation of the extrahypothalamic arginine-vasopressin system in prairie voles. Endocrinology 146, 1559–1567.
López-Gutiérrez, M.F., Gracia-Tabuenca, Z., Ortiz, J.J., Camacho, F.J., Young, L.J., Paredes, R.G., Díaz, N.F., Portillo, W., Alcauter, S., 2021. Brain functional networks associated with social bonding in monogamous voles. Elife 10, e55081.
Lukas D, Clutton-Brock TH (2013) The evolution of social monogamy in mammals. Science (New York, NY) 341:526-530.
Ma, Y., Venier, M., Hites, R.A., 2012. 2-Ethylhexyl tetrabromobenzoate and bis(2-ethylhexyl) tetrabromophthalate flame retardants in the Great Lakes atmosphere. Environ. Sci. Technol. 46, 204–208.
Magby, J.P., Neal, A.P., Atchison, W.D., Pessah, I.P., Shafer, T.J., 2011. Channelopathies: summary of the hot topic keynotes session. Neurotoxicology 32, 661–665.
McGee, S.P., Konstantinov, A., Stapleton, H.M., Volz, D.C., 2013. Aryl phosphate esters within a major PentaBDE replacement product induce cardiotoxicity in developing zebrafish embryos: potential role of the aryl hydrocarbon receptor. Toxicol. Sci. 133, 144–156.
McGrath, T.J., Morrison, P.D., Ball, A.S., Clarke, B.O., 2018. Concentrations of legacy and novel brominated flame retardants in indoor dust in Melbourne, Australia: an assessment of human exposure. Environ. Int. 113, 191–201.
Meitzen, J., Weaver, A.L., Brenowitz, E.A., Perkel, D.J., 2009. Plastic and stable electrophysiological properties of adult avian forebrain song-control neurons across changing breeding conditions. J. Neurosci. 29, 6558–6567.
Mendelsohn, E., Hagopian, A., Hoffman, K., Butt, C.M., Lorenzo, A., Congleton, J., Webster, T.F., Stapleton, H.M., 2016. Nail polish as a source of exposure to triphenyl phosphate. Environ. Int. 86, 45–51.
Mermelstein, P.G., Becker, J.B., Surmeier, D.J., 1996. Estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor. J. Neurosci. 16, 595–604.
Mermelstein, P.G., Song, W.J., Tkatch, T., Yan, Z., Surmeier, D.J., 1998. Inwardly rectifying potassium (IRK) currents are correlated with IRK subunit expression in rat nucleus accumbens medium spiny neurons. J. Neurosci. Off. J. Soc. Neurosci. 18, 6650–6661.
Mittal, V.A., Wakschlag, L.S., 2017. Research domain criteria (RDoC) grows up: Strengthening neurodevelopment investigation within the RDoC framework. J. Affect. Disord. 216, 30–35.
Modi, M.E., Young, L.J., 2012. The oxytocin system in drug discovery for autism: animal models and novel therapeutic strategies. Horm. Behav. 61, 340–350.
Mu, P., Moyer, J.T., Ishikawa, M., Zhang, Y., Panksepp, J., Sorg, B.A., Schluter, O.M., Dong, Y., 2010. Exposure to cocaine dynamically regulates the intrinsic membrane excitability of nucleus accumbens neurons. J. Neurosci. 30, 3689–3699.
National Research Council (U.S.). Subcommittee on Laboratory Animal Nutrition, 1995. Nutrient requirements of laboratory animals. National Academy of Sciences, Washington, D.C.
Ninan, I., 2011. Oxytocin suppresses basal glutamatergic transmission but facilitates activity-dependent synaptic potentiation in the medial prefrontal cortex. J. Neurochem. 119, 324–331.
Nisenbaum, E.S., Wilson, C.J., 1995. Potassium currents responsible for inward and outward rectification in rat neostriatal spiny projection neurons. J. Neurosci. Off. J. Soc. Neurosci. 15, 4449–4463.
O'Donnell, P., Grace, A.A., 1993. Physiological and morphological properties of accumbens core and shell neurons recorded in vitro. Synapse 13, 135–160.
Olazábal, D.E., Young, L.J., 2006. Species and individual differences in juvenile female alloparental care are associated with oxytocin receptor density in the striatum and the lateral septum. Horm. Behav. 49, 681–687.
Oliveri, A.N., Ortiz, E., Levin, E.D., 2018. Developmental exposure to an organophosphate flame retardant alters later behavioral responses to dopamine antagonism in zebrafish larvae. Neurotoxicol. Teratol. 67, 25–30.
Ophir, A.G., Gessel, A., Zheng, D.J., Phelps, S.M., 2012. Oxytocin receptor density is associated with male mating tactics and social monogamy. Horm. Behav. 61, 445–453.
Palanza P, Morellini F, Parmigiani S, vom Saal FS (1999) Prenatal exposure to endocrine disrupting chemicals: effects on behavioral development. Neurosci. Biobehav. Rev. 23:1011-1027.
Palanza, P., Gioiosa, L., vom Saal, F.S., Parmigiani, S., 2008. Effects of developmental exposure to bisphenol A on brain and behavior in mice. Environ. Res. 108, 150–157.
Patisaul, H.B., Adewale, H.B., 2009. Long-term effects of environmental endocrine disruptors on reproductive physiology and behavior. Front. Behav. Neurosci. 3, 10.
Patisaul, H.B., Roberts, S.C., Mabrey, N., McCaffrey, K.A., Gear, R.B., Braun, J., Belcher, S.M., Stapleton, H.M., 2013. Accumulation and endocrine disrupting effects of the flame retardant mixture firemaster((R)) 550 in rats: an exploratory assessment. J. Biochem. Mol. Toxicol. 27, 124–136.
Peng H, Sun J, Saunders DMV, Codling G, Wiseman S, Jones PD, Giesy JP (2017) Hydroxylated 2-Ethylhexyl tetrabromobenzoate isomers in house dust and their agonistic potencies with several nuclear receptors. Environmental pollution (Barking, Essex: 1987) 227:578-586.
Perkeybile, A.M., Griffin, L.L., Bales, K.L., 2013. Natural variation in early parental care correlates with social behaviors in adolescent prairie voles (Microtus ochrogaster). Front. Behav. Neurosci. 7, 21.
Phillips, A.L., Chen, A., Rock, K.D., Horman, B., Patisaul, H.B., Stapleton, H.M., 2016. Editor's highlight: transplacental and lactational transfer of Firemaster® 550 components in dosed wistar rats. Toxicol. Sci. 153, 246–257.
Phillips, A.L., Hammel, S.C., Konstantinov, A., Stapleton, H.M., 2017. Characterization of individual isopropylated and tert-butylated triarylphosphate (ITP and TBPP) isomers in several commercial flame retardant mixtures and house dust standard reference material SRM 2585. Environ. Sci. Technol. 51, 13443–13449.
Proaño, S.B., Meitzen, J., 2020. Estradiol decreases medium spiny neuron excitability in female rat nucleus accumbens core. J. Neurophysiol. 123, 2465–2475.
Proano, S.B., Morris, H.J., Kunz, L.M., Dorris, D.M., Meitzen, J., 2018. Estrous cycle-induced sex differences in medium spiny neuron excitatory synaptic transmission and intrinsic excitability in adult rat nucleus accumbens core. J. Neurophysiol. 120, 1356–1373.
Quigley, J.A., Becker, J.B., 2021. Activation of G-protein coupled estradiol receptor 1 in the dorsolateral striatum attenuates preference for cocaine and saccharin in male but not female rats. Horm. Behav. 130, 104949.
Rebuli, M.E., Gibson, P., Rhodes, C.L., Cushing, B.S., Patisaul, H.B., 2016. Sex differences in microglial colonization and vulnerabilities to endocrine disruption in the social brain. Gen. Comp. Endocrinol. 238, 39–46.
Rock, K.D., St Armour, G., Horman, B., Phillips, A., Ruis, M., Stewart, A.K., Jima, D., Muddiman, D.C., Stapleton, H.M., Patisaul, H.B., 2020. Effects of prenatal exposure to a mixture of organophosphate flame retardants on placental gene expression and serotonergic innervation in the fetal rat brain. Toxicol. Sci. 176, 203–223.
Ross, H.E., Cole, C.D., Smith, Y., Neumann, I.D., Landgraf, R., Murphy, A.Z., Young, L.J., 2009. Characterization of the oxytocin system regulating affiliative behavior in female prairie voles. Neuroscience 162, 892–903.
Roze, E., Meijer, L., Bakker, A., Van Braeckel, K.N.J.A., Sauer, P.J.J., Bos, A.F., 2009. Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age. Environ. Health Perspect. 117, 1953–1958.
Salgado, S., Kaplitt, M.G., 2015. The nucleus accumbens: a comprehensive review. Stereotact. Funct. Neurosurg. 93, 75–93.
Saunders, D.M., Higley, E.B., Hecker, M., Mankidy, R., Giesy, J.P., 2013. In vitro endocrine disruption and TCDD-like effects of three novel brominated flame retardants: TBPH, TBB, & TBCO. Toxicol. Lett. 223, 252–259.
Saunders, D.M., Podaima, M., Codling, G., Giesy, J.P., Wiseman, S., 2015. A mixture of the novel brominated flame retardants TBPH and TBB affects fecundity and transcript profiles of the HPGL-axis in Japanese medaka. Aquat. Toxicol. 158, 14–21.
Schantz, S.L., Widholm, J.J., 2001. Cognitive effects of endocrine-disrupting chemicals in animals. Environ. Health Perspect. 109, 1197–1206.
Schug, T.T., Johnson, A.F., Birnbaum, L.S., Colborn, T., Guillette Jr., L.J., Crews, D.P., Collins, T., Soto, A.M., Vom Saal, F.S., McLachlan, J.A., Sonnenschein, C., Heindel, J. J., 2016. Minireview: endocrine disruptors: past lessons and future directions. Mol. Endocrinol. 30, 833–847.
Simmons, J.M., Quinn, K.J., 2014. The NIMH Research Domain Criteria (RDoC) project: implications for genetics research. Mamm. Genome 25, 23–31.
Slob, A.K., de Klerk, L.W., Brand, T., 1987. Homosexual and heterosexual partner preference in ovariectomized female rats: effects of testosterone, estradiol and mating experience. Physiol. Behav. 41, 571–576.
Soriano, S., Gil-Rivera, M., Marroqui, L., Alonso-Magdalena, P., Fuentes, E., Gustafsson, J.A., Nadal, A., Martinez-Pinna, J., 2019. Bisphenol A regulates sodium ramp currents in mouse dorsal root ganglion neurons and increases nociception. Sci. Rep. 9, 10306.
Stapleton, H.M., Allen, J.G., Kelly, S.M., Konstantinov, A., Klosterhaus, S., Watkins, D., McClean, M.D., Webster, T.F., 2008. Alternate and new brominated flame retardants detected in U.S. house dust. Environ. Sci. Technol. 42, 6910–6916.
Stapleton, H.M., Klosterhaus, S., Keller, A., Ferguson, P.L., van Bergen, S., Cooper, E., Webster, T.F., Blum, A., 2011. Identification of flame retardants in polyurethane foam collected from baby products. Environ. Sci. Technol. 45, 5323–5331.
Stapleton, H.M., Sharma, S., Getzinger, G., Ferguson, P.L., Gabriel, M., Webster, T.F., Blum, A., 2012. Novel and high volume use flame retardants in US couches reflective of the 2005 PentaBDE phase out. Environ. Sci. Technol. 46, 13432–13439.
Stapleton, H.M., Misenheimer, J., Hoffman, K., Webster, T.F., 2014. Flame retardant associations between children's handwipes and house dust. Chemosphere 116, 54–60.
Tan CL, Plotkin JL, Veno MT, von Schimmelmann M, Feinberg P, Mann S, Handler A, Kjems J, Surmeier DJ, O'Carroll D, Greengard P, Schaefer A (2013) MicroRNA-128 governs neuronal excitability and motor behavior in mice. Science (New York, NY) 342:1254-1258.
Tung, E.W.Y., Ahmed, S., Peshdary, V., Atlas, E., 2017. Firemaster® 550 and its components isopropylated triphenyl phosphate and triphenyl phosphate enhance adipogenesis and transcriptional activity of peroxisome proliferator activated receptor (Pparγ) on the adipocyte protein 2 (aP2) promoter. PLoS One 12, e0175855.
Uchimura, N., North, R.A., 1990. Muscarine reduces inwardly rectifying potassium conductance in rat nucleus accumbens neurones. J. Physiol. 422, 369–380.

Uchimura, N., Cherubini, E., North, R.A., 1989. Inward rectification in rat nucleus accumbens neurons. J. Neurophysiol. 62, 1280–1286.
Uchimura, N., Cherubini, E., North, R.A., 1990. Cation current activated by hyperpolarization in a subset of rat nucleus accumbens neurons. J. Neurophysiol. 64, 1847–1850.
Vail GM, Roepke TA (2020) Organophosphate flame retardants excite arcuate melanocortin circuitry and increase neuronal sensitivity to ghrelin in adult mice. Endocrinology 161:bqaa168.
van der Veen, I., de Boer, J., 2012. Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis. Chemosphere 88, 1119–1153.
Vogel, A.R., Patisaul, H.B., Arambula, S.E., Tiezzi, F., McGraw, L.A., 2018. Individual Variation in Social Behaviours of Male Lab-reared Prairie voles (Microtus ochrogaster) is Non-heritable and Weakly Associated with V1aR Density. Sci. Rep. 8, 1396.
Willett JA, Will T, Hauser CA, Dorris DM, Cao J, Meitzen J (2016) No evidence for sex differences in the electrophysiological properties and excitatory synaptic input onto nucleus accumbens shell medium spiny neurons. eNeuro 3: pii: ENEURO.0147-0115.2016. .
Willett, J.A., Johnson, A.G., Vogel, A.R., Patisaul, H.B., McGraw, L.A., Meitzen, J., 2018. Nucleus accumbens core medium spiny neuron electrophysiological properties and partner preference behavior in the adult male prairie vole, Microtus ochrogaster. J. Neurophysiol. 119, 1576–1588.
Williams, J.R., Carter, C.S., Insel, T., 1992. Partner preference development in female prairie voles is facilitated by mating or the central infusion of oxytocin. Ann. N. Y. Acad. Sci. 652, 487–489.
Wilson, C.J., 1993. The generation of natural firing patterns in neostriatal neurons. Prog. Brain Res. 99, 277–297.
Winslow, J., Hastings, N., Carter, C., Harbaugh, C., Insel, T., 1993. A role for central vasopressin in pair bonding in monogamous prairie voles. Nature 365, 545–547.
Witchey, S.K., Al Samara, L., Horman, B.M., Stapleton, H.M., Patisaul, H.B., 2020. Perinatal exposure to FireMaster(R) 550 (FM550), brominated or organophosphate flame retardants produces sex and compound specific effects on adult Wistar rat socioemotional behavior. Horm. Behav. 126, 104853.
Yager, L.M., Garcia, A.F., Wunsch, A.M., Ferguson, S.M., 2015. The ins and outs of the striatum: role in drug addiction. Neuroscience 301, 529–541.
Ye BS, Leung AOW, Wong MH (2017) The association of environmental toxicants and autism spectrum disorders in children. Environmental pollution (Barking, Essex: 1987) 227:234-242.
Yoest, K.E., Quigley, J.A., Becker, J.B., 2018. Rapid effects of ovarian hormones in dorsal striatum and nucleus accumbens. Horm. Behav. 104, 119–129.
Young, K.A., Gobrogge, K.L., Liu, Y., Wang, Z., 2011. The neurobiology of pair bonding: insights from a socially monogamous rodent. Front. Neuroendocrinol. 32, 53–69.
Young, L.J., Lim, M.M., Gingrich, B., Insel, T.R., 2001. Cellular mechanisms of social attachment. Horm. Behav. 40, 133–138.
Zheng, J.J., Li, S.J., Zhang, X.D., Miao, W.Y., Zhang, D., Yao, H., Yu, X., 2014. Oxytocin mediates early experience-dependent cross-modal plasticity in the sensory cortices. Nat. Neurosci. 17, 391–399.
Zuiderveen, E.A.R., Slootweg, J.C., de Boer, J., 2020. Novel brominated flame retardants - a review of their occurrence in indoor air, dust, consumer goods and food. Chemosphere 255, 126816.