Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

A Comprehensive Voltammetric Characterization of *In Vivo* Histamine Dynamics Reveals Critical Modulatory Roles of This Elusive Messenger

Shane Berger
University of South Carolina
Beatrice Baumberger
Imperial College London
S Samaranayake
University of South Carolina
M Hersey
University of South Carolina
Sergio Mena
Imperial College London
Ian Bain
University of South Carolina
W Duncan
Montana State University
M C Reed
Duke University
F Nijhout
Duke University
J Best
Ohio State University
Parastoo Hashemi (phashemi@imperial.ac.uk)
Imperial College London

Research Article

Keywords: inflammation, neuroprotection, antihistamine

Posted Date: March 21st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1462510/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Histamine is well known for mediating peripheral inflammation, however, histamine is found in high concentrations in the brain where amongst other roles, this amine is a neuromodulator. Histamine modulation is critical for brain function, yet dynamic histamine chemistry is very difficult to measure and thus several fundamental aspects of the mechanisms that control the extracellular and modulatory behavior of this messenger remain undefined. In this work we undertake an in-depth characterization of *in vivo* histamine dynamics using fast-scan cyclic voltammetry at carbon fiber microelectrodes. We measure electrically evoked histamine in the mouse hypothalamus and find that histamine release is sensitive to pharmacological manipulation of synthesis, packaging, autoreceptor control of release, and metabolism. We find two breakthrough aspects of histamine modulation. First, there are differences in H_3 receptor regulation of histamine between sexes showing that histamine release in female mice is more much tightly regulated than in male mice under H_3 or inflammatory drug challenge. We hypothesize that this finding may contribute to hormone-mediated neuroprotection mechanisms in female mice. Second, we find that a high dose of a commonly available antihistamine, the H_1 receptor antagonist diphenhydramine, rapidly decreased serotonin levels. This high dose is considered overdose; however, this finding highlights the sheer significance of better consideration of the modulatory nuances of histamine on serotonin when studying/designing histamine targeting pharmaceuticals. We, thus, present the first in depth *in vivo* characterization of fast histamine dynamics and presents two breakthrough regulatory aspects of this elusive modulator.

Introduction

Histamine is a biological amine best known for its roles in mediating peripheral inflammation (Samaranayake et al., 2015). Bodily histamine is synthesized in immune cells including mast cells (Ennis et al., 1981; Metcalfe, Baram and Mekori, 1997) and T-cells (Jutel et al., 2001). Histamine is also found in the brain in microglia(Rocha et al., 2014) and neurons (Panula, Yang and Costa, 1984). The histaminergic cell bodies reside in the tuberomammillary nucleus (TMN) and innervate the entire brain (Watanabe et al., 1983; Panula et al., 1989; Giannoni et al., 2009), where the primary action of this messenger is thought to be neuromodulatory (Panula et al., 1989; Haas, Sergeeva and Selbach, 2008; Rocha et al., 2014). Histamine has been shown to modulate serotonin, dopamine, acetylcholine, glutamate and GABA (Panula, Yang and Costa, 1984; Panula et al., 1989; Haas, Sergeeva and Selbach, 2008).

An elegant body of work has revealed the critical importance of histamine modulation on the brain's processes (Panula, Yang and Costa, 1984; Haas, Sergeeva and Selbach, 2008; Passani and Blandina, 2011; Rocha et al., 2014; Carthy and Ellender, 2021; Manz et al., 2021). However, because dynamic histamine chemistry is very difficult to measure, several fundamental aspects of the mechanisms that control the extracellular and modulatory behavior of this messenger remain undefined. CNS histamine has been previously studied using brain homogenates (Green and Erickson, 1964; Taylor and Snyder, 1972), *in vivo* microdialysis coupled to high-performance liquid chromatography(Itoh et al., 1991; Flik et al., 2015), and electrophysiology (Ferretti et al., 1998; Dupré et al., 2010).

We recently developed a voltammetric method that can measure histamine in real time *in vivo*. The method, fast-scan cyclic voltammetry (FSCV), facilitates sub-second evoked histamine measurements in the TMN from implanted devices with minimal spatial footprint, carbon fiber microelectrodes (CFMs). We further used this method to measure histamine and serotonin simultaneously *in vivo* and discovered that histamine instantaneously inhibits serotonin release *via* (inhibitory) histamine 3 (H_3) heteroreceptors (Samaranayake et al., 2015, 2016). In follow up work, we showed that acute peripheral inflammation in mice increases brain histamine and showed real-time evidence for active histamine reuptake *via* organic cation transporters (OCTs)(Hersey et al., 2021).

Here, we extend on this work by taking a detailed voltammetric approach to characterizing *in vivo* histamine dynamics in male and female mice by pharmacologically targeting histamine synthesis, packaging, autoreceptor control of release, reuptake, and metabolism. We find that our histamine signal is sensitive to pharmacological compounds that target histamine 1 (H_1) and H_3 receptors, vesicular monoamine transporters (VMATs) and histamine synthesis and metabolic pathways. We found that even though histamine release and reuptake dynamics do not differ in control male and female models, H_3 pharmacology statistically differs between the sexes, leading us to explore the possibility of cycling hormones playing a key role in the female mouse's ability to mitigate immunologic signaling. An additional finding of importance is that an agent that inhibits the H_1 receptors (diphenhydramine (DPH)) prolonged histamine's lifetime in the synapse, leading us to hypothesize that this increased temporal influence may negatively modulate extracellular serotonin. Indeed, we found that high dose DPH administration decreased ambient hippocampal serotonin levels.

In sum, this work constitutes the first in depth characterization of fast histamine dynamics *in vivo* and presents two meaningful, breakthrough aspects of histamine modulation; the differences in H_3 regulation between the sexes and H_1 modulation of serotonin dynamics.

Materials And Methods

Chemicals and Reagents

All chemicals were used as received from the supplier. Diphenhydramine hydrochloride (20 mg kg^{-1}; Sigma Aldrich, St. Louis, MO, USA), zolantidine dimaleate (10 mg kg^{-1}; Tocris, Minneapolis, MN, USA), immepip dihydrobromide (5 mg kg^{-1}; Sigma Aldrich), thioperamide maleate (20 mg kg^{-1} or 50 mg kg^{-1}; Sigma Aldrich and Tocris), tacrine hydrochloride (2 mg kg^{-1}; Tocris), and α-fluoromethylhistidine (20 mg kg^{-1}; Toronto Research Chemicals, North York, ON, CAN) were all dissolved in sterile saline (0.9% NaCl solution, Mountainside Medical Equipment, NY, USA) at 5 mL kg^{-1}. Reserpine (10 mg kg^{-1}; Sigma Aldrich) was dissolved in 0.1% acetic acid (Sigma Aldrich) in sterile saline at 5 mL kg^{-1}. Tetrabenazine (Sigma Aldrich) was dissolved in 10% DMSO (Sigma Aldrich) in sterile saline with 1 M HCl (10 µL mL^{-1} injection volume). All solutions were made fresh at the time of injection and all injections were given *via*

intraperitoneal (*i.p.*) injection. Urethane (Sigma Aldrich) was dissolved in sterile saline as a 25% w/v solution and administered at 7 μL kg^{-1}.

Electrode Fabrication

All electrodes were made in house. A single carbon fiber was aspirated into a borosilicate capillary (0.6 mm x 0.4 mm x 10 cm; OD x ID x L) (A-M Systems, Sequim WA, USA) and sealed under gravity and heat by a vertical pipette puller (Narishige, Amityville, NY, USA) to create two separate electrodes. The protruding fiber was trimmed under a light microscope to ~ 150 μm by scalpel. An electrical connection was forged with the fiber through a stainless-steel connecting wire (Kauffman Engineering, Cornelius, OR, USA) and silver epoxy. Finally, a thin layer of Nafion (LQ-1105, Ion Power, New Castle, DE, USA) was electrodeposited onto the fiber surface at 1 V for 30 s; the coated fiber was dried for 10 min at 70°C. (Hashemi et al., 2009)

Data Collection and Analysis

Fast-scan cyclic voltammetry was performed on anesthetized mice using a Chem-Clamp potentiostat (Dagan Corporation, Minneapolis, MN, USA), custom built hardware interfaced with PCIe 6341 & PCI 6221 DAC/ADC cards (National Instruments, Austin, TX), and a Pine Research headstage (Pine Research Instruments, Durham, NC, USA). WCCV 3.06 software (Knowmad Technologies LLC, Tucson, AZ, USA) was used for data analysis. The histamine waveform (-0.5 to -0.7 to + 1.1 to -0.5 V at 600 V s^{-1}) was applied at 60 Hz for 10 min, then at 10 Hz for 10 min prior to data collection at 10 Hz. Histamine was evoked *via* biphasic stimulation applied through a linear constant current stimulus isolator (NL800A Neurolog, Digitimer North America LLC, Fort Lauderdale, FL, USA) with stimulations at 60 Hz, 360 μA, 2 ms in width, and 2 s in length.

Data were collected and filtered on WCCV software (Butterworth, 3 kHz low pass filter). Four control evoked files 10 min apart were averaged for the control evoked histamine signal after which a compound was administered and data were collected every 10 min for 2 h. For immepip-thioperamide experiments, data were collected for 60 min followed by administration of thioperamide immediately after the 60 min file was collected. Files were then collected for an additional 60 min. Current was converted to concentration through calibration factors for both histamine (2.8 μM nA^{-1}) and serotonin (11 μM nA^{-1}). (Samaranayake et al., 2015, 2016) A large voltage (~ 10 V; ~2 min) was used to lesion the tissue surrounding the CFM. Mice were euthanized, brains rapidly harvested and stored in 4% paraformaldehyde. Brains were transferred to 30% sucrose solution for 24 h, rapidly frozen and sectioned into 25 μm slices (Thermo Scientific Cryotome FSE, Thermo Scientific, Waltham, MA, USA) to confirm electrode placement.

Mathematical Modeling of the Experimental Data

For all of our modeling experiments, we incorporated data on the H3 receptor ((Prast et al., 1996; Brown, Stevens and Haas, 2001; Suvarna, Maity and Shivamurthy, 2016) into the model of histamine synthesis, release, reuptake and control by H3 autoreceptor that we have previously published (Prast et al., 1996;

Brown, Stevens and Haas, 2001; Suvarna, Maity and Shivamurthy, 2016; Best et al., 2017). In all experiments, the 0 on the y-axis represents the baseline concentration of histamine in the extracellular space. All experimental curves are plotted relative to baseline. Similarly, in the mathematical model we adjusted parameters so that the baseline concentration of extracellular histamine is 5.32 μM in all simulations except female-thioperamide. In that case, the extracellular concentration descends approximately 8 μM below baseline so the baseline concentration must be higher than 8 μM. We adjusted the reuptake parameter so that the baseline concentration was 14.62 μM. It makes sense that the baseline concentration rises after thioperamide administration because thioperamide is an H3 antagonist. The results of simulation are plotted in each case relative to baseline.

Calculations of solution curves were performed in MATLAB using the differential equations solver ODE15s. The complete model is described in (Best et al., 2017) and the code is available from the authors by request. For all experiments we first made small adjustments in parameters so that the model solution matched the experimental control curve. We then investigated what change of parameters made the solution curve match the experimental post-drug curve. We discuss these parameter changes and their significance in the text close to Figs. 3 and 4.

Statistical Analyses

Average control response was generated from four current vs time traces per animal and averaged to create an overall group average. To determine the $t_{1/2}$, a code was custom written in Excel to fit the reuptake component of the curve and calculate the time taken to reach half the maximum amplitude. Exclusion criteria were based on outliers (*via* Grubbs test) and animals that did not survive the experimental paradigm. Standard error of the mean (SEM) was calculated using the average response of each animal (n = # animals). Significance between two points was determined by 2-tailed paired t-test and taken as $p < 0.05$. For non-normally distributed data (*via* Shapiro Wilk test), the Kruskal-Wallis H test was used to determine significance and taken as $p < 0.05$. All error bars represent the standard error of the mean (SEM).

Animals and Surgical Procedure

All animal procedures were in accordance with the regulations of the Institutional Animal Care and Use Committee (IACUC) at the University of South Carolina, accredited through the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Male and female C57BL/6J mice aged 6–12 weeks were used. Animals were group housed with *ad libitum* access to food and water and were kept on a 12 h light/dark cycle (0700/1900, on/off).

Stereotaxic surgery (David Kopf Instruments, Tujunga, CA, USA) followed induction of deep and sustained anesthesia from an intraperitoneal (*i.p*) injection of urethane (above). Mouse body temperature was maintained by thermal heating pad (Braintree Scientific, Braintree, MA, USA). All surgical coordinates were taken in reference to bregma (Paxinos and Franklin, 2019). A CFM was lowered into the posterior hypothalamus (AP: -2.45, ML: 0.50, DV: -5.45 to -5.55) and a stimulating electrode (insulated stainless-steel, d = 0.2 mm, untwisted; Plastics One, Roanoke, VA, USA) was placed into the medial forebrain bundle

(AP: -1.07, ML: +1.10, DV: -5.00).(Samaranayake et al., 2016) The Ag/AgCl reference electrode, created by chloridizing a polished silver wire (15 s in 1M HCl at 5 V), was placed in the contralateral hemisphere. All agents were given *via i.p.* injection at doses determined suitable from previous studies. Vehicle solutions were administered (5 mL kg^{-1}) to 30 min between control and drug files to determine impact. Here, vehicle injections did not significantly change the evoked release in males or females. Some agents were not soluble in saline. Reserpine was dissolved in 0.1% acetic acid (AcOH) in saline, tetrabenazine required 10% DMSO in saline with 1 M HCl (10 µL mL^{-1}). The post-drug files show the maximal effect within 1 hour per drug.

To analyze sex and estrous cycle, control histamine and serotonin data were pooled. Due to the sensitivity of the measurements being made, we are unable to determine the estrous cycle stage prior to the experiment as we have observed in previous animals that doing so influences release and reuptake characteristics. For cycle determination, vaginal lavage was performed following the conclusion of data collection. Briefly, approximately 10 µL of sterile saline was administered and quickly removed from the vagina and then visualized under low power light microscope to determine estrous cycle stage *via* cytological examination (Caligioni, 2009).

Post Experimental Histological Analysis

The tissue immediately around the CFM was lesioned by applying 10V to the CFM for 20 seconds. Lesioned brains (previously stored in 4% PFA and transferred to 30% sucrose 24 hr prior to analysis) were flash frozen and mounted onto a cutting slide. 25 μm tissue slices were collected and visualized under light microscope. The tissue lesion coordinates (A/P and D/V) were recorded in reference to bregma to determine brain region (Paxinos and Franklin, 2019) and are shown in **S.I. Figure 1**.

Results

Evoked Histamine in Male and Female Mice

We first developed a method to measure histamine *in vivo* with voltammetry in 2015, (Samaranayake et al., 2015) and utilized male mice as proof of principle. In follow up work we showed simultaneous inhibition of serotonin, *via* the H_3 receptors, when histamine was electrically evoked (Samaranayake et al., 2016). Here we repeated those same experiments, where the MFB was stimulated and the resultant, evoked histamine was measured in the posterior hypothalamus in both male and female mouse cohorts (Fig. 1**)**. Figure 1**A** is a representative color plot with a CV inset in the top right corner. Interpretation of these color plots are described in detail elsewhere,(Michael, Travis and Wightman, 1998), briefly the FSCV potential ramp is on the y axis, the time of acquisition on the x axis and the current is in a color blind-friendly false color scale.

Figure 1: (A) representative color plot shows in the mouse TMN upon MFB stimulation. Inset in the top right corner is the characteristic CV with peaks occurring around 0.2 V for histamine and 0.7 V for serotonin oxidation. The concentration vs time traces for the release of histamine and inhibition of

serotonin is shown for **(B)** male (blue) and female (red) mice. Electrical stimulation (2 s) is represented by the grey bars. **(C)** Evoked histamine release and serotonin inhibition for female mice in estrous (n = 23), metestrus (n = 16), diestrus (n = 10), and proestrus (n = 10). The shaded grey bar represents the 2 s electrical stimulation.

The averaged concentration vs. time traces are shown in **Fig. 1B** where the stimulated histamine release in male mice was 7.54 ± 1.20 μM and 7.11 ± 1.10 μM in females. The magnitude of serotonin inhibition was 42.53 ± 4.74 nM in male mice and 45.33 ± 4.72 nM in females. Tabulated in **S.I. Table 1** are the histamine release and serotonin inhibition amplitudes, the rate of decay of the stimulated histamine release ($t_{1/2}$: male: 3.1 ± 0.4 s; female: 3.9 ± 0.7 s; p = 0.34) and the ratio of peak histamine release to peak serotonin inhibition. The sample size was equal for male and female mice at n = 20. There were no statistical differences in any of these metrics between male and female mice.

We next evaluated the effect of estrous cycle stage on the signal in female mice. Figure 1C shows averaged signals in female mice throughout estrus, metestrus, diestrus, and proestrus stages (verified *via* vaginal lavage). We found no significant difference in the evoked histamine amplitude (Amp_{max}: estrus (blue; n = 23): 6.58 ± 0.56 μM; metestrus (orange; n = 16): 6.82 ± 0.74 μM; diestrus (yellow; n = 10): 7.86 ± 1.33 μM; proestrus (green; n = 10): 5.80 ± 1.83 μM; p = 0.84 Kruskal-Wallis H-test) or $t_{1/2}$ (estrus: 3.9 ± 0.7 s; metestrus: 5.4 ± 1.1 s; diestrus: 3.8 ± 0.9 s; proestrus: 4.3 ± 1.1 s; p = 0.79 Kruskal-Wallis H-test) of reuptake curve across estrous stages. The peak serotonin inhibition was also not significantly different and shown across cycle stages (Amp_{max}: estrus (blue; n = 23): 44.70 ± 4.04 nM; metestrus (orange; n = 16): 40.76 ± 5.93 nM; diestrus (yellow; n = 10): 37.04 ± 4.92 nM; proestrus (green; n = 10): 31.74 ± 5.17 nM; p = 0.27 Kruskal-Wallis H test). These data are tabulated, with the addition of the HA/5HT amplitude ratio in **S.I. Table 2**. There were no significant differences in any of these metrics.

Targeting Histamine Packaging, Synthesis and Metabolism

Next, we investigated some of the mechanisms that control extracellular histamine levels *in vivo*. Figure 2 shows average evoked histamine (normalized to maximum control level) before (control, blue) and after (green) drug manipulation. We studied histamine packaging using two VMAT inhibitors with different affinities for VMAT1 and VMAT2 (reserpine: 10 mg kg^{-1} and tetrabenazine (TBZ): 10 mg kg^{-1}). 60 min following reserpine, a significant decrease in evoked histamine was observed in both male (vehicle: 5.43 ± 0.64 μM; reserpine: 2.72 ± 0.47 μM; p = 0.009) and female mice (vehicle: 9.17 ± 1.22 μM; reserpine: 6.53 ± 1.01 μM; p = 0.016).

There was no change in the rate of reuptake of histamine for either sex (male: $t_{1/2}$: control: 2.4 ± 0.7 s; reserpine: 2.8 ± 0.7 s; p = 0.51) (female: $t_{1/2}$: control: 6.3 ± 2.5 s; reserpine: 4.4 ± 1.6 s; p = 0.23). 60 minutes after mice received TBZ, a significant decrease in evoked histamine was observed for both sexes (male vehicle: 7.08 ± 1.42 μM; TBZ: 5.49 ± 1.44 μM; p = 0.023) (female vehicle: 7.56 ± 075 μM; TBZ: 3.83 ± 0.40 μM; p = 0.008). We next targeted synthesis and metabolism by using tacrine, an *N*-methyltransferase inhibitor, and α-fluoromethylhistidine (FMH), an L-histidine decarboxylase inhibitor. Administration of

tacrine to male mice resulted in no change in histamine amplitude (control: 9.56 ± 0.89 μM; tacrine: 9.41 ± 1.22 μM; p = 0.92) but slowed clearance of histamine from the extracellular space ($t_{1/2}$: control: 2.8 ± 0.8 s; tacrine: 6.0 ± 0.7 s; p = 0.025). In female mice there was also no amplitude change following tacrine (control: 7.01 ± 1.79 μM; tacrine: 7.74 ± 1.85 μM; p = 0.22) and and a similar reuptake, but non-significant reuptake change ($t_{1/2}$: control: 4.8 ± 1.4 s; tacrine: 4.9 ± 2.4 s; p = 0.095).

FMH availability was severely limited, thus we combined the male and female responses into one grouping for the next experiment. 60 min following this inhibition of histamine synthesis, a significant decrease in stimulated histamine was observed (control: 8.59 ± 1.86 μM; post-FMH: 5.83 ± 1.24 μM; p = 0.038). There was no change in histamine clearance (($t_{1/2}$: control: 2.4 ± 0.2 s; FMH: 2.3 ± 0.4 s; p = 0.82).

In none of these experiments were the male and female responses substantially different from each other.

H_3R Autoreceptor Control in Male and Female Mice

Next, we investigated autoreceptor control of histamine release in male and female mice *via* an H_3R agonist, immepip (5 mg kg^{-1}), and an H_3R antagonist, thioperamide (20 mg kg^{-1}) in Fig. 3A-C.

Male and female mice respond similarly to H_3R agonism with an overall decrease in max amplitude (male control: 7.72 ± 1.55 μM; post-drug: 4.77 ± 1.56 μM; p = 0.024; female control: 6.20 ± 0.86 μM; post-drug: 3.58 ± 0.51 μM; p = 0.005) and no change in histamine clearance (male $t_{1/2}$: control: 4.5 ± 1.7 s; post-drug: 2.9 ± 0.6 s; p = 0.4; female $t_{1/2}$: control: 5.6 ± 1.8 s; post-drug: 5.6 ± 2.6 s; p = 1 paired t-test).

We mathematically treated these responses with a previously developed model (see methods) and show the fits to experimental data in Fig. 3A-B. The major model parameter that was changed to fit the post-immepip data in both male and female mice was the strength of the H3 autoreceptor. For the male and female control curves, this parameter was set to 0.9. We obtained the post-drug curve by increasing the strength of the autoreceptor to 1.9 in both males and females.

Thioperamide administration caused a significant increase in evoked hypothalamic histamine in male mice (control: 8.83 ± 1.35 μM; post-drug: 12.10 ± 1.75 μM; p = 0.046) and no significant change in histamine clearance (control: 4.5 ± 1.7 s; post-drug: 9.9 ± 2.2 s; p = 0.051). However, in the female mice, no change in amplitude (control: 7.37 ± 1.40 μM; post-drug: 7.01 ± 1.65 μM; p = 0.39) or rate of reuptake ($t_{1/2}$: control: 4.2 ± 1.6 s; post-drug: 3.9 ± 1.3 s; p = 0.48) was observed following the same dose of thioperamide. When we modeled these responses, in male mice we obtained the post-drug curve by decreasing the strength of the autoreceptor from 0.9 to 0. In female mice however, thioperamide did not change the response, thus the autoreceptor strength in the model remained constant.

To test whether this significant finding between male and female mice was a consequence of female mice simply not responding to thioperamide, we did a further experiment where we first agonized the H_3

receptor (*via* immepip (5 mg kg^{-1}) 60 min), and then antagonized the H_3 receptor in the same, female mice with thioperamide (20 mg kg^{-1}). Figure 3C shows the histamine vs. time profiles of control (blue), 60 min post-immepip (orange), and 40 min post-thioperamide (green). Following a significant decrease from control with immepip, we found that thioperamide restored stimulated histamine only to control levels (immepip: 3.58 ± 0.51 μM; post-immepip-thioperamide: 5.89 ± 1.13 μM; p = 0.13); the evoked histamine in female mice did not increase above control values in this (control: 6.20 ± 0.86 μM; post-immepip-thioperamide: 5.89 ± 1.13 μM; p = 0.83 paired t-test), or any of the other experiments in this work.

In previous work, we showed that lipopolysaccharide (LPS) injection in male mice increased histamine release levels, likely as a consequence of inflammation (Hersey et al., 2021). Here we repeated this finding in male mice, represented by the blue bars in **Figure D.** This chart shows % difference (w.r.t to control) in evoked histamine release, averaged between animals, with time after LPS injection (*i.p*). In male mice, LPS injection increased histamine release rapidly after 5 minutes (*post-hoc* t-test, 61.58 ± 12.14% increase, p = 0.0017). We performed the same experiments in female mice where LPS failed to significantly increase evoked histamine 5 min (post-hoc t-test, 0.52 ± 4.55% increase, p = 1.0000) or 10 min after injection (post-hoc t-test, 6.61 ± 1.35% increase, p = 0.3955) and trends for a decrease in histamine amplitude 60 min after injection (post-hoc t-test, -21.07 ± 5.79% increase, p = 0.1816). Importantly, again in female mice, evoked histamine did not substantially increase above a control threshold. A two-way ANOVA analysis (with effects of sex and time after injection) showed that there was a statistically significant effect of the interference of the two factors (F = 3.2606, p = 0.0050) showing that LPS injection differently affects mice of different sexes. A Tukey-Kramer post-hoc analysis of the individual groups was then performed.

Histamine Post-Synaptic Receptor Pharmacology

We next investigated post-synaptic H_1 and H_2 receptors. Figure 4A shows response of male and female mice to zolantidine (10 mg kg^{-1}), an H_2 antagonist. No significant change in evoked histamine release was seen in male and female mice given zolantidine (male control: 5.84 ± 0.55 μM; post-drug: 4.90 ± 0.60 μM; p = 0.33 paired t-test, female control: 5.40 ± 0.65 μM; post-drug: 5.45 ± 0.87μM; p = 0.96 paired t-test). Clearance profile was also not significantly different (male $t_{1/2}$: control: 4.2 ± 1.3 s; post-drug: 3.2 ± 1.0 s; p = 0.46 paired t-test, female $t_{1/2}$: control: 2.7 ± 0.7 s; post-drug: 3.8 ± 1.6 s; p = 0.59 paired t-test).

Figure 4A shows the response to diphenhydramine (DPH) (20 mg kg^{-1}), an H_1 antagonist. DPH did not significantly change the release amplitude in either sex (male control: 9.22 ± 3.06 μM; post-drug: 9.38 ± 2.66 μM; p = 0.83 paired t-test, female control: 6.78 ± 0.16 μM; post-drug: 5.34 ± 0.56 μM; p = 0.077 paired t-test). In both sexes a significant decrease in clearance occurred between 50–60 minutes post DHP (male $t_{1/2}$ control: 2.7 ± 0.4 s; post-drug: 7.2 ± 1.1 s; p = 0.014 paired t-test, female $t_{1/2}$: control: 3.3 ± 0.8 s; post-drug: 12.0 ± 2.6 s; p = 0.042 paired t-test). To understand the mechanism of this decreased clearance, we modeled the data in male mice. To fit the data, the model required incorporation of a strong inhibition of histamine release from t = 9 seconds to t = 15 seconds (control curve), indicating an ongoing inhibitory stimulus on the presynaptic cell in response to H1 activation. DPH blocks the H1 receptors, so in the

presence of DPH the histamine signal to the post-synaptic cell is reduced, we thus assumed that in the presence of DPH that this inhibition of HA release was removed. When we did this, we obtained the post-drug curve in Fig. 4A, lending strong support to the hypothesis.

This slowed clearance is a finding of interest since DPH is a common over the counter antihistamine drug and we know that histamine inhibits serotonin. (Threlfell et al., 2004; Samaranayake et al., 2016). We therefore studied the histaminergic inhibition of serotonin in these same mice, shown in Fig. 4A. The inhibition profile of serotonin was not statistically significant in either case, however there seemed to be a trend towards increased inhibition after DPH. Therefore, we formally tested how a large DPH dose may affect extracellular serotonin levels. We measured extracellular ambient serotonin levels in the CA2 region of the hippocampus once a minute (Fig. 4B) and saw a significant decrease in extracellular serotonin levels after a large DPH dose (50 mg kg-1). To certify this drop-in serotonin levels, a two-way ANOVA (with effects of treatment and mice) was performed on the basal data. A significant effect of the treatment was found, ($F = 4.85$, $p < 0.0001$). Tukey-Kramer multiple comparisons t-test identified that the first significant decrease in serotonin respect to the control state was 42 min after DPH injection (60.68 ± 1.25 vs. 50.31 ± 8.42, $p = 0.0308$). An ANCOVA analysis was then performed to compare the slopes of the average control state and after DPH injection ($F = 31.88$, $p < 0.0001$). The multiple comparisons t-test showed a significant decrease in the slope of the curve after DPH injection (0.05 nM/min vs. -0.21 nM/min, $p < 0.0001$).

Discussion

Control Evoked Histamine Does Not Vary Between Sexes

In this study we set out to investigate the machinery of the central histaminergic systems in mice. Under control conditions, electrically stimulated histamine release and reuptake was measured with FSCV in the TMN. We did not find any significant differences in the release and reuptake of hypothalamic histamine between male and female mice. This finding agrees with our own previous work that compared hippocampal serotonin between sexes and found no statistical differences. (Saylor et al., 2019). Our results differ from some literature reports that suggested histamine turnover and histamine cerebrospinal fluid concentration are higher in females (Prell and Green, 1994) or show lowered histamine release from tissue slice preparations in females.(Ferretti et al., 1998) These previous studies were *ex-vivo* and *in vitro*, which by their nature involve observing the extrinsic system. Our *in vivo* model keeps innate brain circuitry intact, thus these previous studies may indicate that sex differences can be in found subtler, circuit or molecular levels (*vide infra*). Additionally, our data are not normalized, highlighting the high level of conservation in neurochemical regulatory mechanisms across individual mice.

There is an intrinsic belief that there may be neurochemical differences between the different stages of the estrous cycle that has limited the use of females in research.(Beery and Zucker, 2011) Due to histamine's potential role in neuroinflammation, this notion is important to explore; the extent of immune reactivity has been thought to depend on the different stages of the estrous cycle.(Krzych et al., 1978;

Munõz-Cruz et al., 2015) Therefore, we compared evoked histamine in female mice during different stages of the estrous cycle and found that histamine was not significantly different throughout. This finding is not surprising given our prior experience with measuring neurotransmitters with FSCV where we have had to employ aggressive pharmacological means to affect a significant but small change from homeostasis.(Samaranayake et al., 2016; Saylor et al., 2019) Additionally, we saw no difference in serotonin releases in female mice during different stages of the estrous cycle. (Saylor et al., 2019)

Our histamine measurements are in a specifically targeted region of the posterior hypothalamus where we detect both evoked histamine and the resulting inhibition of serotonin. This has been shown to be an H_3R mediated process by our lab and others (Schlicker, Betz and Göthert, 1988; Threlfell et al., 2004; Rocha et al., 2014; Samaranayake et al., 2016). After confirming no statistical differences in evoked histamine between male and female mice and throughout the estrous cycle, we analyzed the level of serotonin inhibition resulting from histamine release in the same mice. Here also, the inhibition of serotonin is not different between males and females, and the ratio of maximum release to peak inhibition does not differ (Fig. 1B-C). Throughout the estrous cycle serotonin inhibition did not vary significantly.

Thus, from these data it is clear that under control conditions, histamine signaling is conserved between male and female mice. We next asked whether histamine release was dependent on the mechanics of histamine synthesis, packaging and metabolism.

Histamine Release is Sensitive to Packaging, Synthesis and Metabolism

We first questioned histamine packaging with tetrabenazine, which is selective for VMAT2 (responsible for packaging in neurons). Tetrabenazine caused significant decreases in overall evoked histamine in both males and females highlighting the major role that VMAT2 plays in packaging neuronal histamine. This finding is in line with prior reports in zebrafish(Eriksson et al., 1998; Puttonen et al., 2017) An additional agent with affinity for VMAT2 inhibition is reserpine. This agent also has affinity for VMAT1, but because VMAT1 is exclusively located in endocrine cells 60 utilizing this agent allows us to test VMAT2 inhibition in the brain. Reserpine administration similarly decreased histamine release in both male and female mice. (Muroi, Oishi and Saeki, 1991)

We next tested if histamine synthesis affected evocable histamine. Other groups have used FMH to successfully lower histamine(Garbarg et al., 1980; Maeyama et al., 1982; Takehiko et al., 1990) and here we utilized the same compound at doses previously described. Due to the extremely limited amount of the compound available to us (this compound is not currently commercially available and was custom synthesized for this study), we combined the sexes' responses for FMH (20 mg kg-1) thus we are not able to comment on sex specific effects for this drug. However, our data are in good agreement with prior reports showing that FMH decreases histamine.

Finally, we targeted histamine metabolism. CNS histamine is metabolized exclusively by HA N-methyltransferase which is located intracellularly; we utilized tacrine to inhibit this enzyme. In male and female mice, tacrine caused a significant slowing of histamine reuptake (Cumming and Vincent, 1992; Taraschenko et al., 2005). Because N-methyltransferase is located intracellularly, inhibition of the enzyme results in higher systolic histamine levels, which manifests in the electrochemical signal as a slowed reuptake rate. This is because of the now higher concentration gradient that the transporters have to work against to clear histamine. Tacrine has additional affinity for blocking acetylcholine esterase which we acknowledge may confound our interpretations(Eagger, Levy and Sahakian, 1991; Farlow et al., 1992).

These data confirm that histamine has similar packaging and synthesis/metabolism mechanisms to other common neurotransmitters such as dopamine and serotonin. Next, we studied histamine receptor pharmacology.

H_3R Autoreceptors Differentiate Histaminergic Response in Male and Female Mice

There is a substantial body of literature documenting the autoregulatory role of the H_3 receptor (Schlicker, Betz and Göthert, 1988; Threlfell et al., 2004; Haas, Sergeeva and Selbach, 2008; Rocha et al., 2014; Saylor et al., 2019). To test this in our system, we administered immepip, an H_3R agonist, to cohorts of male and female mice. In both cases, we found that H_3R agonism resulted in a significant decrease in evoked histamine (Fig. 3A-B), and modeling these data mathematically confirmed autoregulatory action *via* H3Rs. Next we turned to H_3R antagonism; we previously utilized the antagonist thioperamide when developing histamine FSCV(Samaranayake et al., 2015, 2016) and found dose dependent increases in evoked histamine release in male mice(Samaranayake et al., 2016). Here we repeat that finding (Fig. 3A-B) and our models again confirm H3R autoregulation. However, critically, an equivalent dose of thioperamide to female mice did not alter evoked histamine and our model did not require a change in autoregulation after this drug (Fig. 3B). This effect is likely not due to differences in receptor expression, (Schlicker, Betz and Göthert, 1988) since comparable H3 expression is found in male and female rats, or function, as evidenced by the experiment in Fig. 3C. Here in a cohort of female mice, the effects of agonism could be reversed by thioperamide. Importantly evoked histamine levels were restored to control, but no higher, by thioperamide, not only validating the functionality of the receptors but also showing that a threshold level of evoked histamine cannot be surpassed in female mice by thioperamide. Another important aspect of this agonism/antagonism experiment is verification of thioperamide's effects on H_3Rs in this context since this agent also has affinity for H_4Rs.

Therefore, a clear, intrinsic control is present in female mice that strictly regulates the levels of evoked histamine in the hypothalamus. This increased control may have evolutionary underpinnings as it is often thought that female animals exhibit more homeostatic control and that female hormones, estrogens and progesterones, have neuroprotective functions (Roof and HALL, 2000; McEwen et al., 2001). While histamine's roles in bodily inflammation are well-established, less is known about histamine's inflammatory functions in the brain.

We recently published work that showed rapid (5–10 minutes) increases in evoked hypothalamic histamine in male mice upon systemic lipopolysaccharide (LPS) injection, that we attributed to an inflammatory response (Hersey et al., 2021). Here, we repeated this experiment in a separate cohort of male mice. In Fig. 3D the average difference from baseline of evoked histamine release is presented with time after systemic LPS injection in male mice (blue). Here we also observe an initial spike in evoked histamine from 5–20 minutes. Interestingly, from 20–30 minutes the evoked histamine begins to decline and then presents a bimodal increase until the end of the experiment. While initially we attributed the rapid spike to inflammation alone (Hersey et al., 2021) we add here the hypothesis that the response may also mediate pain. LPS induced inflammatory pain is well-described (Verri et al., 2006; Chiu et al., 2013; Calil et al., 2014; Meseguer et al., 2014; Ruiz-Miyazawa et al., 2015). In this model it is thought that LPS triggers the synthesis of proinflammatory cytokines and activation of tissue resident macrophages and neutrophils that release inflammatory mediators to regulate pain perception (Ruiz-Miyazawa et al., 2015). This process if well studied; however, it cannot account for the immediate (within minutes) effects seen with LPS (Watkins et al., 1994). An elegant study from Viania and colleagues showed that LPS caused a rapid, membrane delimited, excitation *via* transient receptor potential cation channels (TRPA1)(Meseguer et al., 2014). TRPA1 links external irritant stimuli with nociceptor (pain receptor) activity. Here we propose that histamine may be part of this signaling cascade (*via* the first histamine peak in Fig. 3D), while the second peak is ongoing inflammation due to more inflammatory processes being recruited. Histamine has been thought to play a role in pain previously (Mobarakeh et al., 2000; Obara et al., 2020) and rapid physiological responses, while not processed due to anesthesia, remain intact (Hua et al., 2020).

Ferretti *et al.* suggested that stressor-induced increases in histamine release may be lower in females than males (Ferretti et al., 1998), given that we found tighter H_3R control of histamine release in female mice (Fig. 3C), we repeated the LPS inflammation experiment in a cohort of female mice. In Fig. 3D, the LPS histamine response in female mice is heavily blunted, if not, totally compensated for. A small increase upon injection (10 minutes) may signal pain as we hypothesized above, however this response is significantly lower than in the male mice. There is long standing evidence that male and female mice process pain differently (Mogil et al., 2003; Wiesenfeld-Hallin, 2005). More recently, Mogli and colleagues have revealed that pain hypersensitivity mechanisms in female mice are dramatically different than in male mice and involve adaptive immune cells such as T-lymphocytes(Sorge et al., 2015). Thus, the differences in our histamine signal between male and female mice shortly after LPS injection are not surprising, and very informative of completely different signaling mechanisms that mediate pain perception between the sexes. Importantly, after this initial response, the histamine levels do not go above baseline, and in fact decrease. A large body of work has established that estradiol is a potent neuroprotective factor with roles at both the level of mitigating onset of disease/injury and reducing the pathological consequences of the disease/injury.(Wise et al., 2001) This hormone has been shown to be an important regulator in the ventromedial nucleus (VMN) of the hypothalamus (Lund et al., 2005; Musatov et al., 2007; Dupré et al., 2010). H_1R and estrogen receptor alpha (ERα) mRNA are co-expressed in histaminergic neurons (Dupré et al., 2010; Mori et al., 2014) and ERβ are expressed in the TMN (Gotoh et al., 2009). The localization of estrogen receptors on histamine projections highlights the potential role

estrogen plays in regulating immune response. Indeed, estrogen and progesterone have been shown to mitigate the acute inflammatory response to lipopolysaccharide exposure (Deshpande et al., 1997; Vegeto et al., 2001; Baker, Brautigam and Watters, 2004; Lei et al., 2014).

In sum, we find that evoked histamine in female mice is strictly governed, such that neither thioperamide nor LPS is able to significantly elevate evoked histamine in female mice. These findings may provide an interesting new avenue to explore in studies that investigate neuroprotection mechanism in females.

H1 Receptor Antagonist Modulates Serotonin Levels

We next looked at postsynaptic H1 and H2 receptor pharmacology. H1 receptor antagonists are clinically available as antihistamines(Paton and Webster, 1985), and H2 receptor antagonists are used clinically to reduce stomach acid production for chronic reflux (Bell and Hunt, 1992), both are based on the notion of reducing the histamine signaling cascades that result in unwanted inflammatory responses.

Zolantidine (H2 receptor antagonist) administration did not significantly affect histamine release or reuptake, however an H1 receptor antagonist (DPH), in both male and female mice, resulted in histamine's lifetime in the synapse to be prolonged. This effect may be due to slowed uptake by transporters (we and others recently showed that histamine is reuptaken by organic cation transporters) or prolonged histamine release by the presynaptic cell. Either scenario necessitates crosstalk between H1 receptors and the presynaptic cell's transporters (Oishi, Adachi and Saeki, 1993) or activity; there is precedence for this since similar effects have been observed with D2 receptor inhibition (Benoit-Marand, Borrelli and Gonon, 2001; Garris et al., 2003; Bamford, Wightman and Sulzer, 2018). Our modeling pointed us towards crosstalk of activity of the presynaptic cell by the post-synaptic cell upon H1 activation. In an elegant 2000 review by Haas and colleagues (Brown, Stevens and Haas, 2001), evidence was presented for H1 receptor modulation of presynaptic transmitter release *via* retrograde messengers such as arachidonic acid (AA) and nitric oxide (NO) (Prast et al., 1996; Brown, Stevens and Haas, 2001; Suvarna, Maity and Shivamurthy, 2016; Best et al., 2017). In the review, the authors put forth this hypothesis of retrograde messengers, pointing out the proposed mechanism is yet to be experimentally demonstrated. We believe we have now provided compelling evidence for Haas's hypothesis.

We thus present evidence for modulation of activity of histamine release *via* H1 receptors. The effect of this phenomenon (increased histamine lifetime in the synapse) is perhaps contrary to the intended mode of action of such a drug (classic antihistamine designed to stop histamine signaling). Because the H1 receptor is antagonized, these increased histamine levels do not contribute to increased histamine signaling (as per the pharmacological intention). However, in the context of serotonin/histamine modulation, these findings may present another nuance; since we know that histamine inhibits serotonin release, what happens to serotonin if histamine signaling is prolonged?

We thus looked at the inhibition of serotonin by histamine before and after DPH and found a non-significant trend towards increased inhibition after DPH. We therefore, formally tested the notion that DPH affects serotonin by measuring ambient (minute to minute) serotonin levels in the CA2 region of the

hippocampus in a separate cohort of mice and found that after a large, acute dose of DPH, serotonin levels rapidly, significantly fall. It is worth noting that this large DPH dose is clinically considered as 'overdose', (indeed DPH overdose symptoms are consistent with serotonin depletion (depression, anxiety, increased sleepiness) (FDA, 2020; Hughes, Lin and Hendrickson, 2021) and that acute, clinical doses of DPH are unlikely to have significant effects on serotonin. However, this finding does highlight the fact that one should consider serotonin when investigating/designing pharmaceuticals for histamine.

To sum our work, we performed an in-depth characterization of fast chemical histamine dynamics by pharmacologically targeting histamine synthesis, packaging, autoreceptor control of release, reuptake and metabolism. We presented 2 particularly meaningful, breakthrough aspects of histamine modulation. First, we found the differences in H3 regulation between the sexes, showing that in female mice evoked histamine could not be increased *via* H3 inhibition or an inflammatory stimulus. This led us to hypothesize that this histamine may underlie neuroprotective mechanisms in female mice. Second, we found that high dose DPH rapidly decreased serotonin levels. While such high doses are considered overdose, this finding highlights the sheer significance of better considering the modulatory nuances of neurotransmitters when studying/designing pharmaceuticals.

References

1. Baker, A. E., Brautigam, V. M. and Watters, J. J. (2004) 'Estrogen modulates microglial inflammatory mediator production via interactions with estrogen receptor beta.', Endocrinology, 145(11), pp. 5021–5032. doi: 10.1210/en.2004-0619.
2. Bamford, N. S., Wightman, R. M. and Sulzer, D. (2018) 'Dopamine's Effects on Corticostriatal Synapses during Reward-Based Behaviors', Neuron, 97(3), pp. 494–510. doi: 10.1016/j.neuron.2018.01.006.
3. Beery, A. K. and Zucker, I. (2011) 'Sex bias in neuroscience and biomedical research', Neuroscience and Biobehavioral Reviews, 35(3), pp. 565–572. doi: 10.1016/j.neubiorev.2010.07.002.
4. Bell, N. J. and Hunt, R. H. (1992) 'Role of gastric acid suppression in the treatment of gastro-oesophageal reflux disease.', *Gut*, 33(1), pp. 118 LP – 124. doi: 10.1136/gut.33.1.118.
5. Benoit-Marand, M., Borrelli, E. and Gonon, F. (2001) 'Inhibition of dopamine release via presynaptic D2 receptors: Time course and functional characteristics in vivo', Journal of Neuroscience, 21(23), pp. 9134–9141. doi: 10.1523/jneurosci.21-23-09134.2001.
6. Best, J. *et al.* (2017) 'A mathematical model for histamine synthesis, release, and control in varicosities', Theoretical Biology and Medical Modelling, 14(1), pp. 1–19. doi: 10.1186/s12976-017-0070-9.
7. Brown, R. E., Stevens, D. R. and Haas, H. L. (2001) 'The physiology of brain histamine', Progress in Neurobiology, 63(6), pp. 637–672. doi: 10.1016/S0301-0082(00)00039-3.
8. Caligioni, C. S. (2009) 'Assessing Reproductive Status/Stages in Mice', Current Protocols in Neuroscience, 48(1), p. A.4I.1-A.4I.8. doi: https://doi.org/10.1002/0471142301.nsa04is48.

9. Calil, I. L. *et al.* (2014) 'Lipopolysaccharide induces inflammatory hyperalgesia triggering a TLR4/MyD88-dependent cytokine cascade in the mice paw', PLoS ONE, 9(3), pp. 2–9. doi: 10.1371/journal.pone.0090013.
10. Carthy, E. and Ellender, T. (2021) 'Histamine, Neuroinflammation and Neurodevelopment: A Review', Frontiers in Neuroscience, 15(July), pp. 1–26. doi: 10.3389/fnins.2021.680214.
11. Chiu, I. M. *et al.* (2013) 'Bacteria activate sensory neurons that modulate pain and inflammation', Nature, 501(7465), pp. 52–57. doi: 10.1038/nature12479.
12. Cumming, P. and Vincent, S. R. (1992) 'Inhibition of histamine-N-methyltransferase (HNMT) by fragments of 9-amino-1,2,3,4-tetrahydroacridine (tacrine) and by β-carbolines', Biochemical Pharmacology, 44(5), pp. 989–992. doi: 10.1016/0006-2952(92)90133-4.
13. Deshpande, R. *et al.* (1997) 'Estradiol down-regulates LPS-induced cytokine production and NFkB activation in murine macrophages', American Journal of Reproductive Immunology, 38(1), pp. 46–54. doi: 10.1111/j.1600-0897.1997.tb00275.x.
14. Dupré, C. *et al.* (2010) 'Histaminergic responses by hypothalamic neurons that regulate lordosis and their modulation by estradiol', Proceedings of the National Academy of Sciences of the United States of America, 107(27), pp. 12311–12316. doi: 10.1073/pnas.1006049107.
15. Eagger, S. A., Levy, R. and Sahakian, B. J. (1991) 'Tacrine in Alzheimer's disease', The Lancet, 337(8748), pp. 989–992. doi: https://doi.org/10.1016/0140-6736(91)92656-M.
16. Ennis, M. *et al.* (1981) 'Inhibition of histamine secretion from mast cells', Nature, 289(5794), pp. 186–187. doi: 10.1038/289186a0.
17. Eriksson, K. S. *et al.* (1998) 'Development of the histaminergic neurons and expression of histidine decarboxylase mRNA in the zebrafish brain in the absence of all peripheral histaminergic systems', European Journal of Neuroscience, 10(12), pp. 3799–3812. doi: 10.1046/j.1460-9568.1998.00394.x.
18. Farlow, M. *et al.* (1992) 'A controlled trial of tacrine in Alzheimer's disease. The Tacrine Study Group.', JAMA, 268(18), pp. 2523–2529.
19. FDA (2020) 'FDA Warns about Serious Problems with High Doses of the Allergy Medicine Diphenhydramine (Benadryl)', *Fda*. Available at: https://www.fda.gov/drugs/drug-safety-and-availability/fda-warns-about-serious-problems-high-doses-allergy-medicine-diphenhydramine-benadryl.
20. Ferretti, C. *et al.* (1998) 'Hypothalamic histamine release in normal and stressed rats is affected by sex and aging', Pharmacology Biochemistry and Behavior, 59(1), pp. 255–260. doi: 10.1016/S0091-3057(97)00395-X.
21. Flik, G. *et al.* (2015) 'Interaction Between Brain Histamine and Serotonin, Norepinephrine, and Dopamine Systems: In Vivo Microdialysis and Electrophysiology Study', Journal of Molecular Neuroscience, 56(2), pp. 320–328. doi: 10.1007/s12031-015-0536-3.
22. Garbarg, M. *et al.* (1980) 'Inhibition of histamine synthesis in brain by α-fluoromethylhistidine, a new irreversible inhibitor: in vitro and in vivo studies', Journal of neurochemistry, 35(5), pp. 1045–1052.

23. Garris, P. A. *et al.* (2003) 'A role for presynaptic mechanisms in the actions of nomifensine and haloperidol', Neuroscience, 118(3), pp. 819–829. doi: 10.1016/S0306-4522(03)00005-8.
24. Giannoni, P. *et al.* (2009) 'Heterogeneity of histaminergic neurons in the tuberomammillary nucleus of the rat', European Journal of Neuroscience, 29(12), pp. 2363–2374. doi: 10.1111/j.1460-9568.2009.06765.x.
25. Gotoh, K. *et al.* (2009) 'Hypothalamic neuronal histamine signaling in the estrogen deficiency-induced obesity', Journal of Neurochemistry, 110(6), pp. 1796–1805. doi: 10.1111/j.1471-4159.2009.06272.x.
26. Green, H. and Erickson, R. W. (1964) 'Effect of some drugs upon rat brain histamine content', International Journal of Neuropharmacology, 3(3), pp. 315–320. doi: https://doi.org/10.1016/0028-3908(64)90023-1.
27. Haas, H. L., Sergeeva, O. A. and Selbach, O. (2008) 'Histamine in the nervous system', Physiological Reviews, 88(3), pp. 1183–1241. doi: 10.1152/physrev.00043.2007.
28. Hashemi, P. *et al.* (2009) 'Voltammetric detection of 5-hydroxytryptamine release in the rat brain', Analytical Chemistry, 81(22), pp. 9462–9471. doi: 10.1021/ac9018846.
29. Hersey, M. *et al.* (2021) 'Inflammation-Induced Histamine Impairs the Capacity of Escitalopram to Increase Hippocampal Extracellular Serotonin', The Journal of Neuroscience, 41(30), pp. 6564–6577. doi: 10.1523/jneurosci.2618-20.2021.
30. Hua, T. *et al.* (2020) 'General anesthetics activate a potent central pain-suppression circuit in the amygdala', Nature Neuroscience, 23(7), pp. 854–868. doi: 10.1038/s41593-020-0632-8.
31. Hughes, A. R., Lin, A. and Hendrickson, R. G. (2021) 'Clinical and patient characteristics associated with severe outcome in diphenhydramine toxicity', Clinical Toxicology, 59(10), pp. 918–925. doi: 10.1080/15563650.2021.1891244.
32. Itoh, Y. *et al.* (1991) 'Characterization of Histamine Release from the Rat Hypothalamus as Measured by In Vivo Microdialysis', Journal of Neurochemistry, 56(3), pp. 769–774. doi: 10.1111/j.1471-4159.1991.tb01990.x.
33. Jutel, M. *et al.* (2001) 'Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors', Nature, 413(6854), pp. 420–425. doi: 10.1038/35096564.
34. Krzych, U. *et al.* (1978) 'Quantitative differences in immune responses during the various stages of the estrous cycle in female BALB/c mice', Journal of Immunology, 121(4), pp. 1603–1605.
35. Lei, B. *et al.* (2014) 'Anti-inflammatory effects of progesterone in lipopolysaccharide-stimulated BV-2 microglia', PLoS ONE, 9(7), pp. 3–9. doi: 10.1371/journal.pone.0103969.
36. Lund, T. D. *et al.* (2005) 'Novel actions of estrogen receptor-β on anxiety-related behaviors', Endocrinology, 146(2), pp. 797–807. doi: 10.1210/en.2004-1158.
37. Maeyama, K. *et al.* (1982) 'Effect of α-fluoromethylhistidine, a suicide inhibitor of histidine decarboxylase, on histamine levels in mouse tissues', Biochemical Pharmacology, 31(14), pp. 2367–2370. doi: 10.1016/0006-2952(82)90531-7.

38. Manz, K. M. *et al.* (2021) 'Accumbal Histamine Signaling Engages Discrete Interneuron Microcircuits', Biological Psychiatry, 1, pp. 1–12. doi: 10.1016/j.biopsych.2021.10.004.
39. McEwen, B. *et al.* (2001) 'Tracking the estrogen receptor in neurons: Implications for estrogen-induced synapse formation', Proceedings of the National Academy of Sciences of the United States of America, 98(13), pp. 7093–7100. doi: 10.1073/pnas.121146898.
40. Meseguer, V. *et al.* (2014) 'TRPA1 channels mediate acute neurogenic inflammation and pain produced by bacterial endotoxins', Nature Communications, 5. doi: 10.1038/ncomms4125.
41. Metcalfe, D. D., Baram, D. and Mekori, Y. A. (1997) 'Mast cells', Physiological Reviews, 77(4), pp. 1033–1079. doi: 10.1152/physrev.1997.77.4.1033.
42. Michael, D., Travis, E. R. and Wightman, R. M. (1998) 'Color Images for Fast-Scan CV', Analytical Chemistry, 70(17). doi: 10.1002/chin.199848350.
43. Mobarakeh, J. I. *et al.* (2000) 'Role of histamine H1 receptor in pain perception: A study of the receptor gene knockout mice', European Journal of Pharmacology, 391(1–2), pp. 81–89. doi: 10.1016/S0014-2999(00)00060-1.
44. Mogil, J. S. *et al.* (2003) 'The melanocortin-1 receptor gene mediates female-specific mechanisms of analgesia in mice and humans', Proceedings of the National Academy of Sciences of the United States of America, 100(8), pp. 4867–4872. doi: 10.1073/pnas.0730053100.
45. Mori, H. *et al.* (2014) 'Estrogenic regulation of histamine receptor subtype H1 expression in the ventromedial nucleus of the hypothalamus in female rats', PLoS ONE, 9(5). doi: 10.1371/journal.pone.0096232.
46. Munõz-Cruz, S. *et al.* (2015) 'Gender-related effects of sex steroids on histamine release and Fc ε RI expression in rat peritoneal mast cells', *Journal of Immunology Research*, 2015. doi: 10.1155/2015/351829.
47. Muroi, N., Oishi, R. and Saeki, K. (1991) 'Effect of reserpine on histamine metabolism in the mouse brain.', *Journal of Pharmacology and Experimental Therapeutics*, 256(3), pp. 967 LP – 972. Available at: http://jpet.aspetjournals.org/content/256/3/967.abstract.
48. Musatov, S. *et al.* (2007) 'Silencing of estrogen receptor α in the ventromedial nucleus of hypothalamus leads to metabolic syndrome', Proceedings of the National Academy of Sciences of the United States of America, 104(7), pp. 2501–2506. doi: 10.1073/pnas.0610787104.
49. Obara, I. *et al.* (2020) 'Histamine, histamine receptors, and neuropathic pain relief', British Journal of Pharmacology, 177(3), pp. 580–599. doi: 10.1111/bph.14696.
50. Oishi, R., Adachi, N. and Saeki, K. (1993) 'Nα-Methylhistamine inhibits intestinal transit in mice by central histamine H1 receptor activation', *European Journal of Pharmacology*, 237(2–3), pp. 155–159. doi: 10.1016/0014-2999(93)90263-H.
51. Panula, P. *et al.* (1989) 'Histamine-immunoreactive nerve fibers in the rat brain', Neuroscience, 28(3), pp. 585–610. doi: 10.1016/0306-4522(89)90007-9.
52. Panula, P., Yang, H. Y. T. and Costa, E. (1984) 'Histamine-containing neurons in the rat hypothalamus', Proceedings of the National Academy of Sciences of the United States of America, 81(8 I), pp. 2572–

2576. doi: 10.1073/pnas.81.8.2572.

53. Passani, M. B. and Blandina, P. (2011) 'Histamine receptors in the CNS as targets for therapeutic intervention', Trends in Pharmacological Sciences, 32(4), pp. 242–249. doi: 10.1016/j.tips.2011.01.003.
54. Paton, D. M. and Webster, D. R. (1985) 'Clinical Pharmacokinetics of H1-Receptor Antagonists (The Antihistamines)', Clinical Pharmacokinetics, 10(6), pp. 477–497. doi: 10.2165/00003088-198510060-00002.
55. Paxinos, G. and Franklin, K. B. J. (2019) *Paxinos and Franklin's the mouse brain in stereotaxic coordinates*. Academic press.
56. Prast, H. *et al.* (1996) 'Nitric oxide influences the release of histamine and glutamate in the rat hypothalamus', Naunyn-Schmiedeberg's Archives of Pharmacology, 354(6), pp. 731–735. doi: 10.1007/BF00166899.
57. Prell, G. D. and Green, J. P. (1994) 'Measurement of histamine metabolites in brain and cerebrospinal fluid provides insights into histaminergic activity', Agents and Actions, 41(1 Supplement), pp. 5–8. doi: 10.1007/BF02007743.
58. Puttonen, H. A. J. *et al.* (2017) 'Storage of neural histamine and histaminergic neurotransmission is VMAT2 dependent in the zebrafish', Scientific Reports, 7(1), pp. 1–12. doi: 10.1038/s41598-017-02981-w.
59. Rocha, S. M. *et al.* (2014) 'Histamine: A new immunomodulatory player in the neuron-glia crosstalk', *Frontiers in Cellular Neuroscience*, 8(1 APR), pp. 1–7. doi: 10.3389/fncel.2014.00120.
60. Roof, R. L. and HALL, E. D. (2000) 'Gender differences in acute CNS trauma and stroke: neuroprotective effects of estrogen and progesterone', Journal of neurotrauma, 17(5), pp. 367–388.
61. Ruiz-Miyazawa, K. W. *et al.* (2015) 'Vinpocetine reduces lipopolysaccharide-induced inflammatory pain and neutrophil recruitment in mice by targeting oxidative stress, cytokines and NF-κB', Chemico-Biological Interactions, 237, pp. 9–17. doi: 10.1016/j.cbi.2015.05.007.
62. Samaranayake, S. *et al.* (2015) 'In vivo histamine voltammetry in the mouse premammillary nucleus', Analyst, 140(11), pp. 3759–3765. doi: 10.1039/c5an00313j.
63. Samaranayake, S. *et al.* (2016) 'A voltammetric and mathematical analysis of histaminergic modulation of serotonin in the mouse hypothalamus', Journal of Neurochemistry, pp. 374–383. doi: 10.1111/jnc.13659.
64. Saylor, R. A. *et al.* (2019) 'In vivo hippocampal serotonin dynamics in male and female mice: Determining effects of acute escitalopram using fast scan cyclic voltammetry', Frontiers in Neuroscience, 13(APR), pp. 1–13. doi: 10.3389/fnins.2019.00362.
65. Schlicker, E., Betz, R. and Göthert, M. (1988) 'Histamine H3 receptor-mediated inhibition of serotonin release in the rat brain cortex', Naunyn-Schmiedeberg's Archives of Pharmacology, 337(5), pp. 588–590. doi: 10.1007/BF00182737.
66. Sorge, R. E. *et al.* (2015) 'Different immune cells mediate mechanical pain hypersensitivity in male and female mice', Nature Neuroscience, 18(8), pp. 1081–1083. doi: 10.1038/nn.4053.

67. Suvarna, Y., Maity, N. and Shivamurthy, M. C. (2016) ‘Emerging Trends in Retrograde Signaling’, Molecular Neurobiology, 53(4), pp. 2572–2578. doi: 10.1007/s12035-015-9280-5.
68. Takehiko, W. *et al.* (1990) ‘Pharmacology of α fluoromethylhistidine, a specific inhibitor of histidine decarboxylase’, Trends in Pharmacological Sciences, 11(9), pp. 363–367. doi: 10.1016/0165-6147(90)90181-7.
69. Taraschenko, O. D. *et al.* (2005) ‘Actions of tacrine and galanthamine on histamine-N-methyltransferase.’, Methods and findings in experimental and clinical pharmacology, 27(3), pp. 161–165. doi: 10.1358/mf.2005.27.3.890872.
70. Taylor, K. M. and Snyder, S. H. (1972) ‘Isotopic Microassay of Histamine, Histidine, Histidine Decarboxylase and Histamine Methyltransferase in Brain Tissue’, Journal of Neurochemistry, 19(5), pp. 1343–1358. doi: 10.1111/j.1471-4159.1972.tb01459.x.
71. Threlfell, S. *et al.* (2004) ‘Histamine H3 receptors inhibit serotonin release in substantia nigra pars reticulata’, Journal of Neuroscience, 24(40), pp. 8704–8710. doi: 10.1523/JNEUROSCI.2690-04.2004.
72. Vegeto, E. *et al.* (2001) ‘Estrogen prevents the lipopolysaccharide-induced inflammatory response in microglia’, Journal of Neuroscience, 21(6), pp. 1809–1818. doi: 10.1523/jneurosci.21-06-01809.2001.
73. Verri, W. A. *et al.* (2006) ‘Hypernociceptive role of cytokines and chemokines: Targets for analgesic drug development?’, Pharmacology and Therapeutics, 112(1), pp. 116–138. doi: 10.1016/j.pharmthera.2006.04.001.
74. Watanabe, T. *et al.* (1983) ‘Evidence for the presence of a histaminergic neuron system in the rat brain: An immunohistochemical analysis’, Neuroscience Letters, 39(3), pp. 249–254. doi: 10.1016/0304-3940(83)90308-7.
75. Watkins, L. R. *et al.* (1994) ‘Neurocircuitry of illness-induced hyperalgesia’, Brain Research, 639(2), pp. 283–299. doi: 10.1016/0006-8993(94)91742-6.
76. Wiesenfeld-Hallin, Z. (2005) ‘Sex differences in pain perception’, Gender Medicine, 2(3), pp. 137–145. doi: https://doi.org/10.1016/S1550-8579(05)80042-7.
77. Wise, P. M. *et al.* (2001) ‘Minireview: Neuroprotective effects of estrogen - New insights into mechanisms of action’, Endocrinology, 142(3), pp. 969–973. doi: 10.1210/endo.142.3.8033.

Declarations

Competing interests: The authors declare no competing interests.

Figures

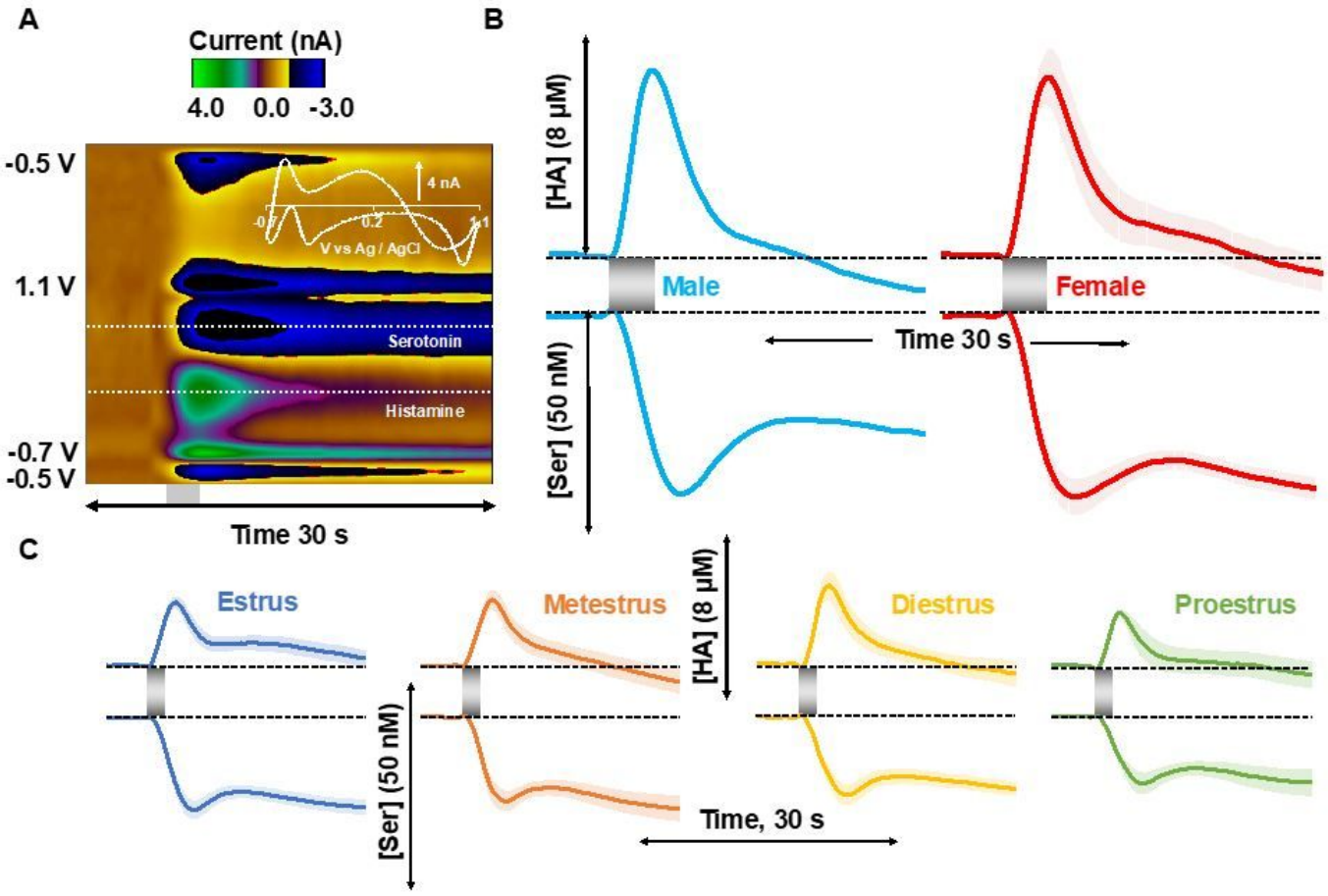

Figure 1

(A) representative color plot shows in the mouse TMN upon MFB stimulation. Inset in the top right corner is the characteristic CV with peaks occurring around 0.2 V for histamine and 0.7 V for serotonin oxidation. The concentration vs time traces for the release of histamine and inhibition of serotonin is shown for **(B)** male (blue) and female (red) mice. Electrical stimulation (2 s) is represented by the grey bars. **(C)** Evoked histamine release and serotonin inhibition for female mice in estrous (n=23), metestrus (n=16), diestrus (n=10), and proestrus (n=10). The shaded grey bar represents the 2 s electrical stimulation.

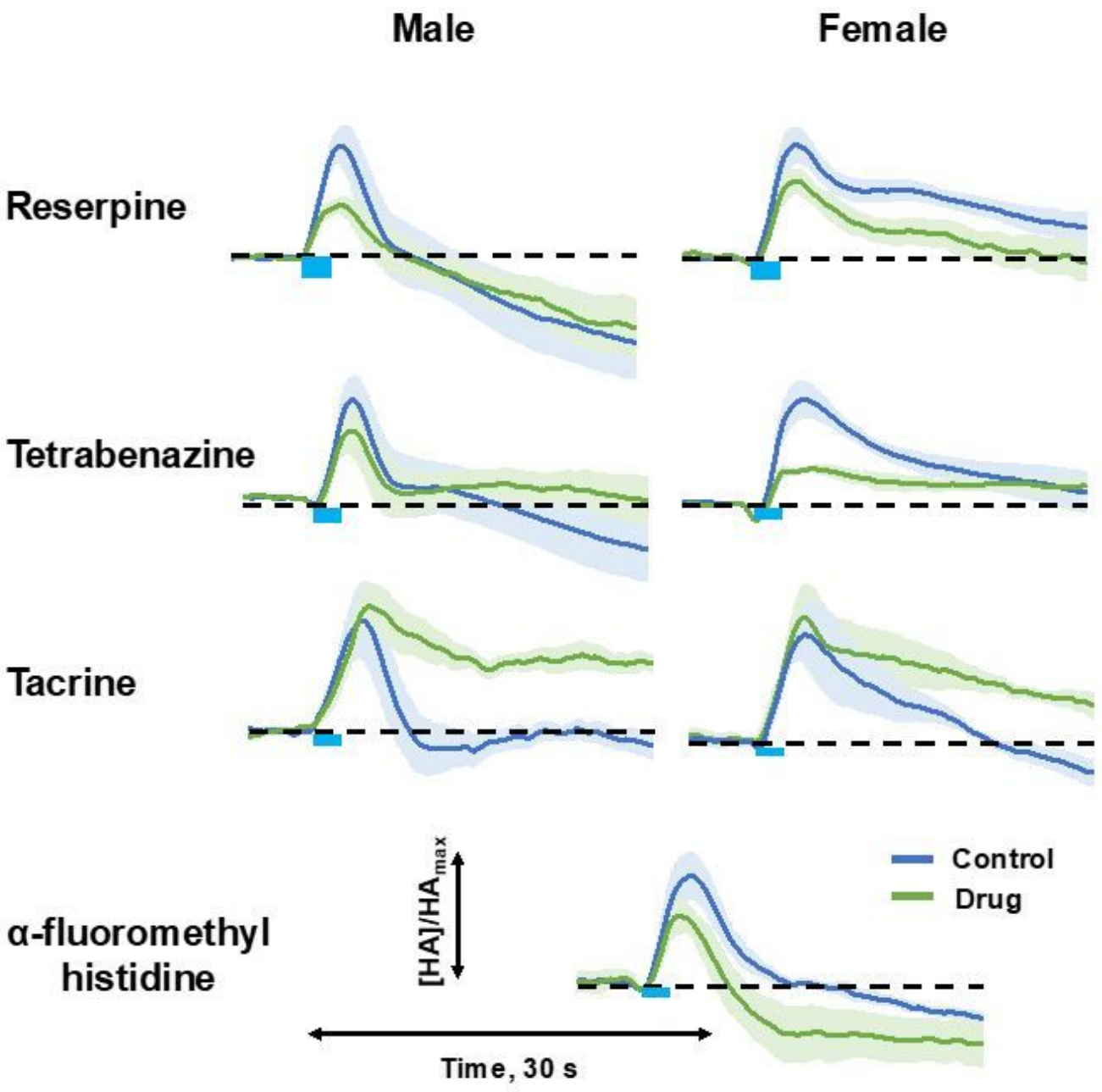

Figure 2

Averaged concentration versus time traces for control (blue) and post-drug (green) evoked histamine. The drug and mouse's sex are listed. For α-fluoromethylhistidine male and female traces were averaged.

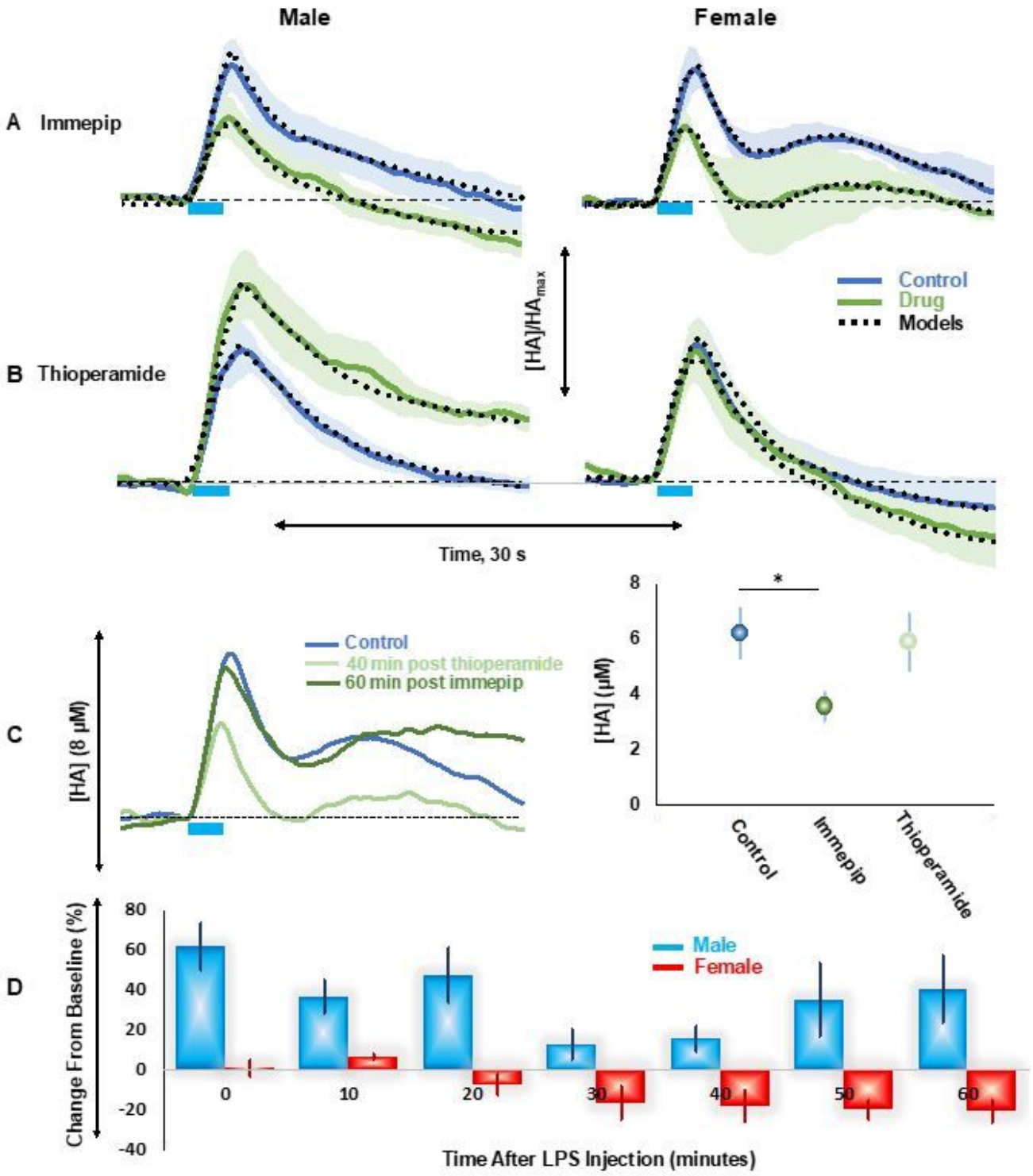

Figure 3

(A-B) The drug and mouse's sex are listed. Concentration versus time traces for control (blue) and post-drug (green) evoked histamine. The mathematical model results are in black dotted lines. **(C)** [HA] vs time profiles of evoked histamine for control (n=5, blue), 60 min following immepip (n=5, orange), and 40 min following thioperamide after 60 min immepip (n=4, green). Max amplitude of evoked histamine for control (blue), 60 min immepip (orange), and 40 min following thioperamide after initial 60 min immepip

(green). Significance between two points was taken as $p < 0.05$ **(D)** Male vs female % difference (*vs.* control) in evoked histamine release with time after LPS injection.

Figure 4

(A) The drug and mouse's sex are listed. Concentration versus time traces for control (blue) and post-drug (green) evoked histamine, and control (purple) and post-drug (orange) inhibited serotonin. The light blue bar and the shaded grey bar represent the 2 s electrical stimulation. The mathematical model results are in black dotted lines. **(B)** Extracellular ambient serotonin levels in the CA2 region of the hippocampus measured once a minute after a large DPH dose (gray bar)

Figure 5

A comic strip illustration of DPH effects on histamine and serotonin.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SIHApaper.pdf