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Unraveling Sex Differences in Affect Processing: Unique Oscillatory Signaling Dynamics in the Infralimbic Cortex and Nucleus Accumbens Shell

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ABSTRACT

BACKGROUND: Negative affect is prevalent in psychiatric diseases such as depression and addiction. Projections from the infralimbic cortex (IL) to the nucleus accumbens shell (NAcSh) are causally linked to learned negative affect as 20 Hz optogenetic stimulation of this circuit reduces conditioned taste aversion (CTA) in male but not female rats. However, the prior study did not provide insight into how innate versus learned negative affect are processed in these areas across sex.

METHODS: To address this issue, local field potential activity was simultaneously recorded in the IL and NAcSh in response to intraoral infusion of rewarding (saccharin) and aversive (quinine) tastants and following induction of a CTA in male and female Sprague Dawley rats.

RESULTS: Local field potential oscillatory activity within each brain region to saccharin varied across sex. In males, CTA increased IL resting-state power, which was correlated with the strength of the learned aversion, and reduced beta power and IL-NAcSh coherence. In females, CTA increased gamma power in the NAcSh. Similar effects were observed in males and females after CTA in theta-low gamma phase-amplitude coupling. Finally, while quinine produced similar effects in oscillatory power across sex, females showed differences in phase-amplitude coupling within the NAcSh that may be linked to aversion resistance.

CONCLUSIONS: We revealed sex-specific hedonic processing in the IL and NAcSh and how oscillatory signaling is disrupted in learned negative affect, revealing translationally relevant insight into potential treatment strategies that can help to reduce the deleterious effects of learned negative affect in psychiatric illnesses.

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The ability to feel normal pleasure is essential to healthy physiological and psychological functions (1). A disruption in hedonic processing is prevalent in psychiatric illnesses such as depression and substance use disorders, in which negative affect during withdrawal can promote relapse or drive aversive emotional states that can result in suicide (1–4). Given the current "deaths of despair" health crisis in the United States that is linked to excessive drug and alcohol use (5–7), it is critical to understand how affective states are encoded in different brain regions to help develop treatment strategies to restore normal hedonic processing in psychiatric illnesses.

Affective states can be studied in rat models by using taste reactivity (TR), in which rewarding and aversive stereotypic oromotor responses are elicited in rats when tastants are intraorally infused (8). Moreover, learned changes in affective states can also be studied using TR and conditioned taste aversion (CTA) (9). By pairing a rewarding solution (e.g., sucrose, saccharin) with the malaise-producing agent lithium chloride (LiCl), we can study how the activity of different brain regions changes with the development of a conditioned aversive state that causes a shift in the perceived palatability of an otherwise rewarding solution (10–12).

In humans, the ventromedial prefrontal cortex (PFC) has been associated with affective processing (13,14) as ventromedial PFC activation is correlated with self-reported negative affect (15), psychological stress (16,17), the modulation of negative mood (18), suppressing negative emotions (19), and retention of fear extinction learning (20). The rat infralimbic cortex (IL) is the homolog of the human ventromedial PFC and has been shown to be involved in promoting the extinction of conditioned fear (21) and CTA (22–24). The IL sends efferent projections to the nucleus accumbens shell (NAcSh), which has also been critically implicated in affective processing (25–30). Indeed, our laboratory has shown that NAc neurons exhibit opposite activity patterns in response to rewarding and aversive tastants (29), and the development of CTA shifts the response pattern of NAc neurons from appetitive to aversive (30).

Importantly, our lab has also shown that the IL projections to the rostral NAcSh are causally linked to CTA. Specifically, 20 Hz optogenetic stimulation of this circuit reduced aversive responses to LiCl-paired intraorally delivered sucrose after CTA, but not to quinine, showing a functional involvement of the IL-NAcSh pathway in the expression of learned but not innate aversion (31). Critically, this study also showed sex-

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specific differences as stimulation of the IL-NAcSh pathway decreased aversive behavior after CTA in male but not in female rats (31). While our optogenetic findings clearly implicate a crucial role of the IL-NAcSh pathway in learned aversive behavior in male but not female rats, it does not provide insight into how reward and aversion (both learned and innate) are processed in these areas under normal conditions across sex, information that can then be used to help develop unique sex-specific treatment strategies for aberrant affective processing in psychiatric disorders.

To address these issues, here we used in vivo electrophysiology to simultaneously record local field potential (LFP) activity in the IL and NAcSh in response to rewarding and aversive (innate and learned) stimuli in male and female rats. We sought to determine whether oscillatory signaling dynamics in this circuit differ in males and females during innate and learned negative affect and, if so, how. We examined different LFP parameters (power, coherence, phase-amplitude coupling [PAC]) to determine the contribution of each brain region to innate (i.e., quinine) and learned (i.e., CTA) negative affect, as well as functional connectivity between regions, and dissected the involvement of different frequency bands in affective processing. Remarkably, we found that while male and female rats showed similar behavioral profiles in response to innate and learned aversion, the IL and NAcSh exhibited distinct features of neural processing across sex.

METHODS AND MATERIALS

Detailed methods are described in the Supplement.

Behavioral Task

Male and female Sprague Dawley rats were surgically implanted with intraoral (IO) cannulas and microwire electrode arrays (NB Labs) in the IL and the NAcSh as described previously (29). After recovering from surgery, rats were water deprived (15 mL/day) and habituated to the experimental chamber and IO infusions for 2 days. Chambers had a plexiglass floor and a mirror located underneath at a 45° angle to record the behavior of the rat. Figure 1A shows the experimental timeline. During habituation (2 days), rats were placed in the chamber and received 30 IO infusions of water. After habituation, the experiment consisted of 3 trials, naïve, CTA and quinine, and each trial consisted of 2 phases, baseline and IO. During the start of the naïve trial (baseline phase), rats were placed in the chamber, and resting baseline electrophysiological activity was recorded for 15 minutes. Then, during the IO phase, rats received 30 IO infusions (200 μL over 3.5/infusion on a variable-time 30-second schedule) of 0.15% saccharin, and behavior was recorded using a GoPro for future video analysis of TR. At the end of the IO phase in the first trial (naïve), rats received an intraperitoneal injection of LiCl (127 mg/kg) to induce CTA. Following 2 days of recovery, rats were placed back in the chamber, and a second 15-minute baseline recording was completed followed by a CTA test during which they were re-exposed to the saccharin IO infusion. Finally, following 4 extinction days (saccharin infusions only), rats experienced a final 15-minute baseline recording in the chamber followed by a test in which quinine (1 mM) was given IO instead of saccharin to assess innate aversion (quinine trial). Water deprivation lasted throughout the experiment.

Electrophysiology (LFPs)

Network activity within and between regions was assessed using LFPs. LFP isolation was performed using commercially available neurophysiological systems (OmniPlex system; Plexon) described previously (29). Briefly, raw electrical

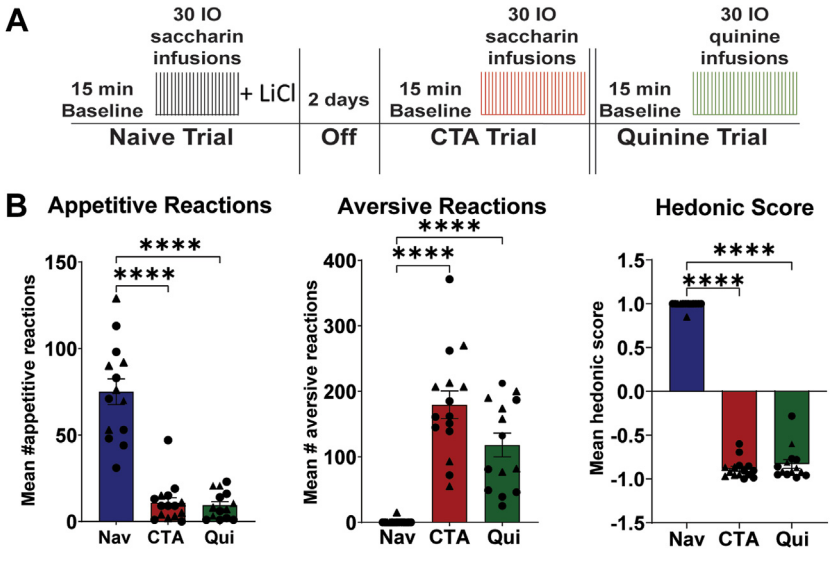

Figure 1. **(A)** Timeline of experiment. On the first day, naïve rats received 30 IO infusions (200 μL over 3.5/infusion on a variable-time 30-second schedule) of 0.15% saccharin followed by a LiCl injection (127 mg/kg intraperitoneally) to induce CTA. After 2 days of recovery, CTA was tested by infusing the rats with 0.15% saccharin. Following 5 extinction days (indicated by double line), innate aversion was tested by infusing the rats with 1 mM quinine (30–200 μL over 3.5/infusion on a variable-time 30-second schedule). Fifteen-minute baseline recordings were conducted at the start of each trial. **(B)** Taste reactivity behaviors across trials. In the naïve state, rats showed significantly higher appetitive responses than in the CTA and quinine trials (left panel), supported by a significant mixed-effects RM ANOVA followed by post hoc analyses ($F_{1.32,17.20}$ = 66.97, $p < .0001$; post hoc naïve vs. CTA: $p < .0001$, naïve vs. Qui: $p < .0001$). After conditioning, in the CTA and quinine trials, rats showed a higher number of aversive responses relative to naïve (middle panel), confirmed by a mixed-effects RM ANOVA followed by post hoc analyses ($F_{1.80,23.44}$ = 35.38, $p < .0001$; post hoc naïve vs. CTA: $p < .0001$, naïve vs. Qui: $p < .0001$, CTA vs. Qui: $p = .065$). When comparing hedonic scores, a shift from a rewarding-like to an aversive-like profile, similar to that observed by quinine, was observed between naïve and CTA trials (right panel); a mixed-effects RM ANOVA revealed significant differences between naïve and the other trials ($F_{1.49,18.67}$ = 941.1, $p < .0001$; post hoc, naïve vs. CTA: $p < .0001$, naïve vs. Qui: $p < .0001$). Behaviors in males indicated by closed circles, behaviors in females indicated by closed triangles. ****$p < .0001$. CTA, conditioned taste aversion; IO, intraoral; LiCl, lithium chloride; Nav, naïve; Qui, quinine; RM ANOVA, repeated measures analysis of variance.

outputs from each region were low-pass filtered (200 Hz) from individual wires implanted within either the IL or NAcSh. Recordings from one wire in the IL and another wire in the NAcSh (based on histological verification of electrode placement and low noise levels) were examined relative to baseline and task events for each rat. Power spectral density (PSD) log at the peak of each frequency band (delta: 0.5–4 Hz, theta: 4–12 Hz, beta: 12–30 Hz, low gamma: 30–58 Hz and high gamma: 63–100 Hz) was used to analyze baseline activity within each region. Perievent spectrograms were built using a z score ($\frac{power\ in\ bin - average\ baseline}{SD\ baseline}$) of event-related spectral perturbations (0.2-ms bins) relative to IO infusion and normalized to baseline (2 seconds prior to IO delivery). Average z scores across the first 5 seconds after IO delivery were graphed for each frequency. For visualization, maximum and minimum values were set at 2 standard deviations from baseline ($z = \pm 2$). LFP power spectra were computed using fast Fourier transform, and coherence values were exported from NeuroExplorer. Comodulograms were obtained from PAC analyses performed with Brainstorm (32), which is documented and freely available for download online under the GNU general public license (http://neuroimage.usc.edu/brainstorm).

Data Analysis

TR was assessed as described previously (8) by an experimenter who was blind to treatment conditions. Specifically, rhythmic tongue protrusions and lateral tongue protrusions were scored as appetitive TR. Gaping behavior was scored as aversive TR. Hedonic scores were calculated as $\frac{Appetitive\ TR - Aversive\ TR}{Appetitive\ TR + Aversive\ TR}$. Statistical analyses are described in the figure legends and further explained in the Supplement. Linear regression analysis was used to correlate logPSD at each individual frequency bin (1 Hz) with appetitive and aversive behaviors. Correlations were considered significant when more than 50% of a frequency band (consisting of at least 4 consecutive significant 1 Hz bins) showed a significant correlation with behavior and were confirmed by Pearson's correlations between the average logPSD of the entire frequency band and behavior. Statistical analyses of spectrograms were performed on raw z scores, where a difference of $\geq$2 SDs between trials was observed. PAC data were extracted from Brainstorm and analyzed using a one-way repeated measures analysis of variance.

RESULTS

All rats displayed clear CTA behavior, as illustrated in Figure 1B. During IO saccharin infusion in the naïve state, both male and female rats exhibited a high number of appetitive responses and a low number of aversive responses. After LiCl pairing, the number of appetitive responses was reduced to a level similar to that elicited by quinine. Additionally, after conditioning, rats showed a high number of aversive responses. Conditioning also produced a shift in the hedonic score from appetitive-like to aversive-like. There were no significant sex differences in the hedonic score ($F_{1,36} = 0.93$, $p = .34$), aversive TR ($F_{1,13} = 0.473$, $p = .5$) or appetitive TR ($F_{1,13} = 0.64$, $p = .4$) in any of the trials.

We first studied resting-state oscillatory power (logPSD) within each brain region during the 15-minute baseline period prior to IO infusion in both males and female rats to assess whether the development of CTA affected basal activity in the IL and/or NAcSh in a similar manner across sex. In males, CTA produced an overall (across all frequencies) increase in baseline oscillatory activity in the IL but not the NAcSh (Figure 2A). Specifically, most rats showed an increased resting-state logPSD in the IL after LiCl treatment compared with the naïve trial (Figure 2A, inset). In contrast, in females, no differences in baseline power were observed between naïve and CTA baseline trials in either brain region (Figure 2B). These findings indicate that despite similar behavioral profiles across sex, baseline oscillatory power was exclusively altered by conditioning in the IL of male rats.

Next, we investigated whether oscillatory power during baseline on the CTA trial was correlated with subsequent aversive and appetitive TR during the IO infusions on the CTA trial by performing a linear regression between each individual frequency point and the behavior for each rat, followed by Pearson's correlation of the entire frequency bands that showed significance and the behavior. In males, delta ($r = 0.78$, $p = .013$), theta ($r = 0.7$, $p = .035$), and beta ($r = 0.89$, $p = .0012$) frequency bands in the IL showed a significant positive correlation with aversive behaviors (Figure 2C, top left; Figure S1) while no significant correlations were observed in the NAcSh (Figure 2C, top right). In females, no significant correlation was observed between oscillatory power in the IL or the NAcSh and aversive or appetitive behaviors at any frequency point (Figure 2D, left). Collectively, these findings suggest that the development of negative affect changes overall baseline activity in the IL of male but not female rats, and activity at low-frequency bands can predict the strength of aversiveness elicited in male rats in response to the LiCl-paired saccharin solution.

Then, we performed time-frequency analyses to assess how conditioning affected neural oscillations in response to the tastant in the IL and NAcSh and whether innate and learned aversion produced similar effects across sex. Time-frequency analyses are depicted as perievent spectrograms (Figure 3A, B) for each of the 3 trial types (naïve, CTA, and quinine) relative to IO infusions using the 2 seconds prior to infusion as baseline for z score calculation. The bar graphs below the perievent spectrograms (Figure 3C, D) summarize significant differences in IL and NAcSh oscillatory signaling between trial types (naïve, CTA, quinine) within specific frequency bands. In males (left), under normal (naïve) conditions, rewarding saccharin produced an increase in beta frequency oscillations in both the IL and the NAcSh. Interestingly, this frequency-specific increase in beta activity during saccharin infusion disappeared after CTA and tended to disappear during quinine infusion in both regions. At the low-theta frequency band, both learned (during saccharin IO after CTA) and innate (quinine) aversive tastants elevated activity in the NAcSh, although this finding was only significant during CTA (Figure 3C). Additionally, in males, quinine produced a significant activation in the high-gamma band (75–80 Hz) relative to the naïve state in the NAcSh and relative to both the naïve condition and CTA in the IL (Figure 3C). In females (Figure 3B, D), conditioned (i.e., CTA) and innate (quinine) changes in activity occurred only at higher frequencies. In normal (naïve) conditions, rewarding saccharin reduced activation in both the IL and NAcSh within high

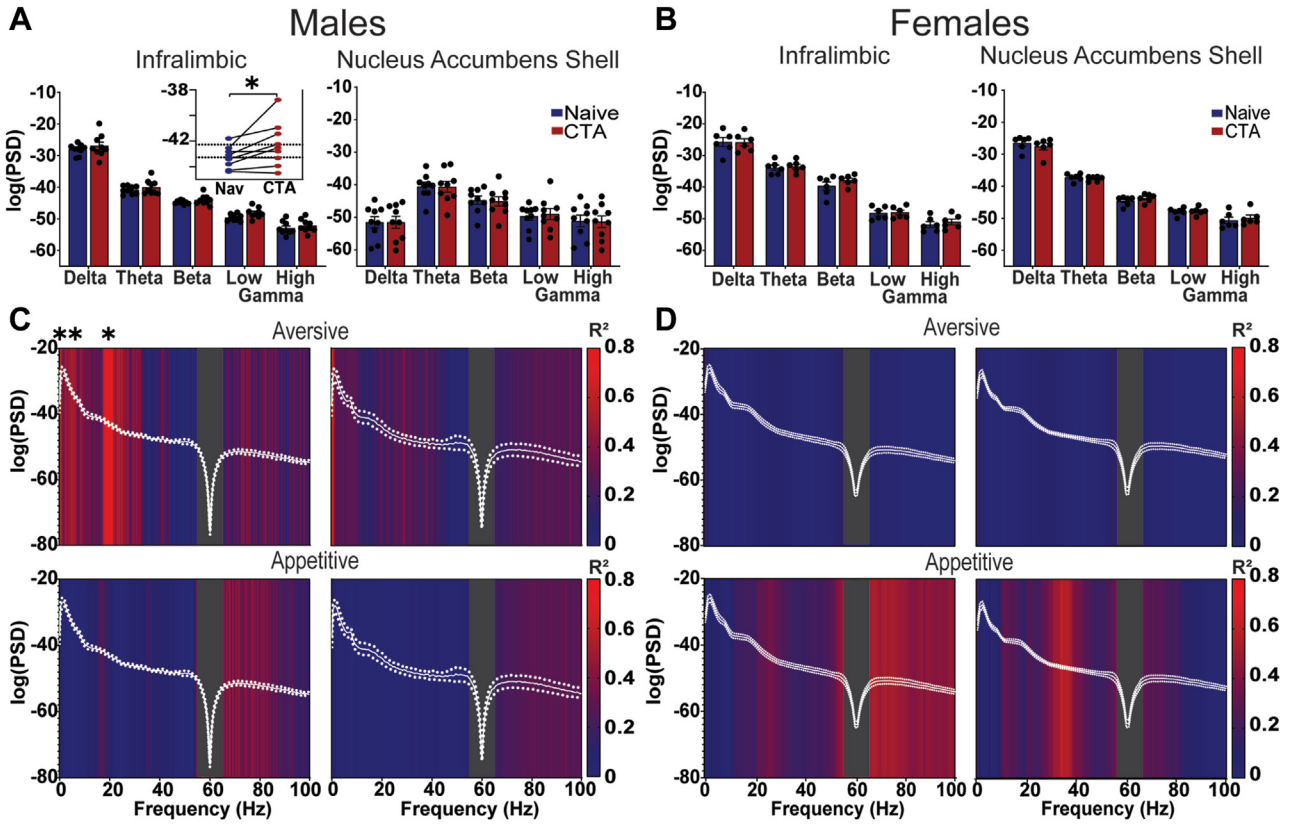

Figure 2. Overall baseline oscillatory power in the IL is altered by CTA and predicts subsequent TR behavior after CTA in males but not in females. **(A)** Mean ± SEM logPSD during baseline across peak frequency bands in the IL (left) and the NAcSh (right) between naïve (blue) and CTA (red) trials for male rats. Two-way repeated measures analysis of variance revealed a main frequency effect in both the IL ($F_{4,32}$ = 423.6, $p < .0001$) and NAcSh ($F_{4,32}$ = 60.70, $p < .0001$), a main effect of trial ($F_{1,8}$ = 5.936, p = .04) in the IL but not NAcSh ($F_{1,8}$ = 0.0004368, p = .9838), and no frequency × trial interaction (IL: $F_{4,32}$ = 0.6524, p = .6294; NAcSh: $F_{4,32}$ = 0.6792, p = .6114). Inset: Plot of trial effect in the IL: shift in average power of each rat between trials. **(B)** Mean ± SEM log PSD during baseline across peak frequency bands in the IL (left) and the NAcSh (right) between naïve (blue) and CTA (red) trials for female rats. Two-way repeated measures analysis of variance showed a main frequency effect in both the IL ($F_{4,20}$ = 577.4, $p < .0001$) and NAcSh ($F_{4,20}$ = 330.0, $p < .0001$), no main trial effect in either region (IL: $F_{1,5}$ = 2.171, p = .2006; NAcSh: $F_{1,5}$ = 1.578, p = .2646), no significant frequency × trial interaction in the IL ($F_{4,20}$ = 2.638, p = .0643) and a signification interaction in the NAcSh ($F_{4,20}$ = 4.648, p = .0081; although subsequent post hoc analysis did not reveal any significant differences). **(C)** Linear regression between logPSD at each frequency bin during the baseline phase of the CTA trial (white line) and aversive TR (top) and appetitive TR (bottom) during the intraoral phase for IL (left) and NAcSh (right) for male rats. **(D)** Linear regression between logPSD at each frequency bin during the baseline phase (white line) and aversive TR (top) and appetitive TR (bottom) during the intraoral phase for IL (left) and NAcSh (right) for female rats. R^2 values for each of the correlations are shown in the overlay. *$p < .05$. A correlation was considered significant when more than 50% of a frequency band (consisting of at least 4 consecutive significant 1 Hz bins) showed significant correlation with gapes. CTA, conditioned taste aversion; IL, infralimbic cortex; NAcSh, nucleus accumbens shell; Nav, naïve; PSD, power spectral density; TR, taste reactivity.

gamma. Relative to the naïve condition, CTA significantly increased oscillatory activity in the NAcSh at the 70 Hz high-gamma frequency band (Figure 3D). In contrast, quinine significantly increased oscillatory amplitude relative to naïve at 2 portions of the high-gamma band in the IL (~70 Hz and 90–100 Hz) and at 90 to 100 Hz band in the NAcSh (Figure 3D). Collectively, these results suggest that while both IL and NAcSh appear to be involved in encoding learned aversion in males, the IL appears to not be particularly linked to this behavior in females. Additionally, while beta frequency appears to be key in both regions in males, higher frequencies in the NAcSh appear to be more relevant to learned aversion in females.

We also examined whether functional connectivity between the IL and NAcSh was altered by CTA. This was assessed by analyzing IL-NAcSh coherence across frequencies in males (Figure 3E) and females (Figure 3F) during saccharin IO in the naïve state, after CTA, and during quinine infusions. In males, relative to naïve infusion of saccharin, CTA produced a significant reduction in coherence (Figure 3E, left panel) while no effect was observed during quinine infusion (Figure 3E, middle panel). CTA also tended to produce a greater reduction in coherence than quinine, but this difference was not statistically significant (Figure 3E, right panel). In females (Figure 3F), no difference in overall coherence was observed between any of the trial types. These results suggest an important role of the IL-NAcSh pathway in encoding rewarding stimuli that is disrupted by learned aversion in male but not in female rats.

To determine interactions between oscillations of different frequency bands within the same brain regions and how they

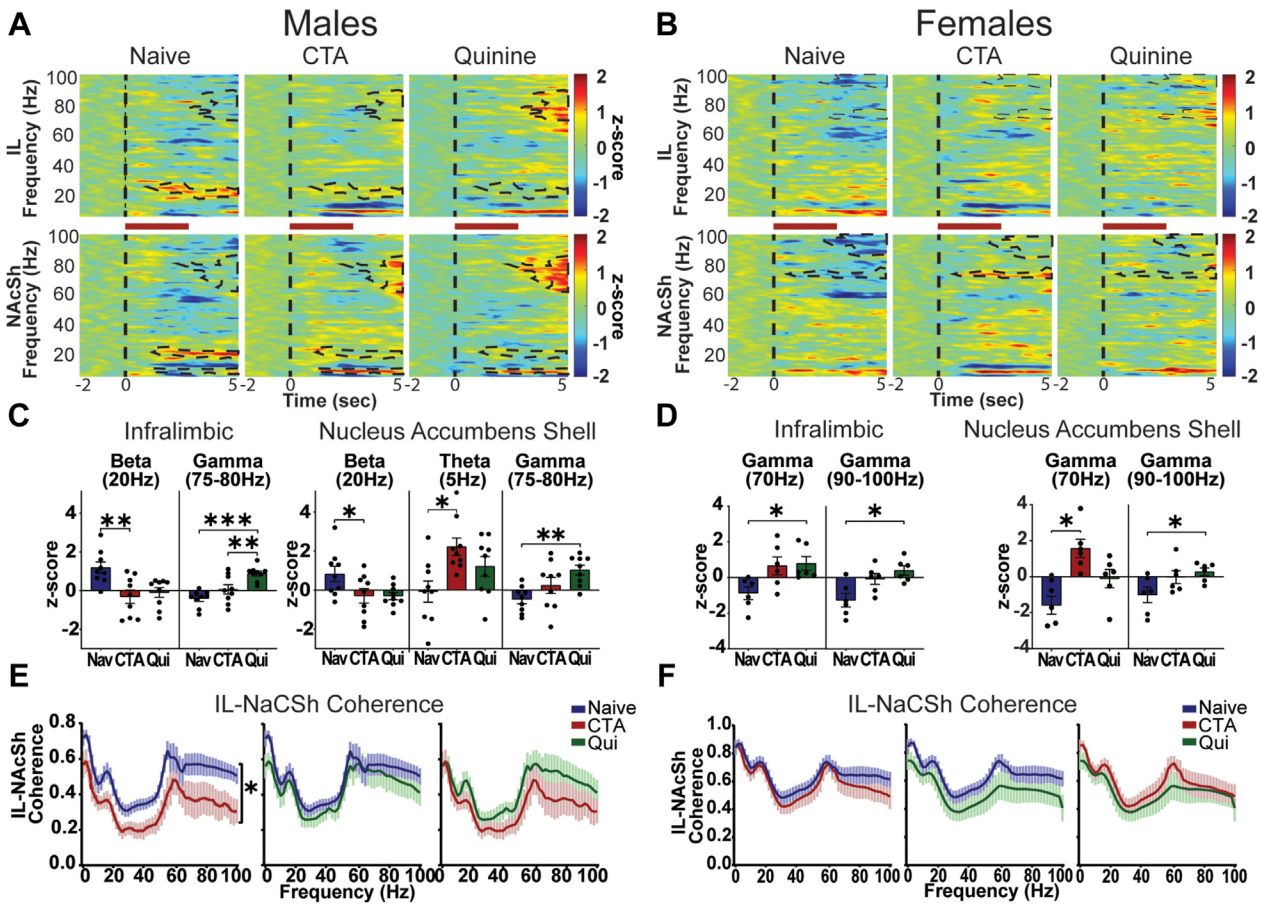

Figure 3. Effects of conditioned and innate aversive stimuli on oscillatory activity in the IL and NAcSh and their coherence. **(A)** Average perievent spectrograms (*z*-normalized to baseline) during naïve (left), CTA (middle), and quinine (right) (infusion onsets at dashed vertical lines) in the IL (top) and the NAcSh (bottom) for male rats. **(B)** Average perievent spectrograms (*z*-normalized to baseline) during naïve (left), CTA (middle), and quinine (right) in the IL (top) and the NAcSh (bottom) for female rats. *z* score values are shown in overlay (right). Red lines indicate the infusion duration. Dashed areas indicate where significant differences were observed. **(C)** Bar graphs showing main differences between trials for IL (left) and NAcSh (right) for male rats. One-way RM ANOVA followed by post hoc analyses revealed significant differences between naïve and CTA at beta frequency in both IL ($F_{1.662,11.64} = 6.827$, $p = .0136$; post hoc naïve vs. CTA: $p = .008$) and NAcSh ($F_{1.791,14.32} = 6.057$, $p = .0143$; post hoc naïve vs. CTA: $p = .0045$); between quinine and both trials at high gamma in the IL ($F_{1.724,13.79} = 24.51$, $p < .0001$; post hoc: naïve vs. Qui: $p = .0002$; CTA vs. Qui: $p = .0026$) and between quinine and naïve in the NAcSh ($F_{1.637,13.10} = 7.952$, $p = .0074$; post hoc: naïve vs. Qui: $p = .0015$) and between CTA and naïve at theta in the NAcSh ($F_{1.410,9.871} = 7.398$, $p = .0158$; post hoc: naïve vs. CTA: $p = .0482$). **(D)** Bar graphs showing main differences between trials for IL (left) and NAcSh (right) for female rats. One-way RM ANOVA and subsequent post hoc analyses revealed significant differences between naïve and quinine at high gamma (70 Hz and 90–100 Hz) in the IL (70 Hz: $F_{1.731,8.654} = 4.845$, $p = .0423$; post hoc: naïve vs. Qui, $p = .038$; 90–100 Hz: $F_{1.909,9.544} = 7.279$, $p = .0126$; post hoc: naïve vs. Qui: $p = .0438$) and at high gamma (900–100 Hz) in the NAcSh ($F_{1.679,8.396} = 4.807$, $p = .0449$; post hoc: naïve vs. Qui: $p = .0457$) and between CTA and naïve at high gamma 70 Hz in the NAcSh ($F_{1.591,7.955} = 8.709$, $p = .0123$; post hoc: naïve vs. CTA: $p = .0434$). **(E)** IL-NAcSh coherence across frequencies and trial types for male rats. Two-way RM ANOVA showed a significant main effect of trial ($F_{1,8} = 5.474$, $p = .043$) between naïve and CTA (left); and a significant frequency × trial interaction between CTA and quinine, although not confirmed by post hoc analyses (*p*s > .05). No effects were observed between naïve and quinine trials. **(F)** IL-NAcSh coherence across frequencies and trial types for female rats. No significant effects were observed between any of the trials after two-way RM ANOVA. *$p < .05$, **$p < .001$, ***$p < .0001$. CTA, conditioned taste aversion; IL, infralimbic cortex; NAcSh, nucleus accumbens shell; Nav, naïve; Qui, quinine; RM ANOVA, repeated measures analysis of variance.

were affected by learned and innate aversion, we conducted PAC analysis in both the IL and the NAcSh in male and female rats. In males, comodulograms showed strong coupling between theta phase and beta and low-gamma amplitude in the IL when processing rewarding saccharin (Figure 4A). Interestingly, such coupling was reduced after CTA but was not affected during quinine infusion (Figure 4B). In addition, as was observed in males, the IL of female rats showed strong coupling between theta phase and low-gamma amplitude that was altered by CTA but not by quinine (Figure 4D, top panel). Finally, the NAcSh of female rats showed most changes on interfrequency oscillation coupling, mainly during innate aversion. Specifically, quinine elicited stronger coupling between theta phase and beta and high-gamma amplitudes than naïve and CTA trials. Additionally, quinine produced a reduction in theta-low gamma coupling when compared with the naïve trial. These results indicate a modulation of high-frequency oscillations by lower frequency oscillations in the IL during reward

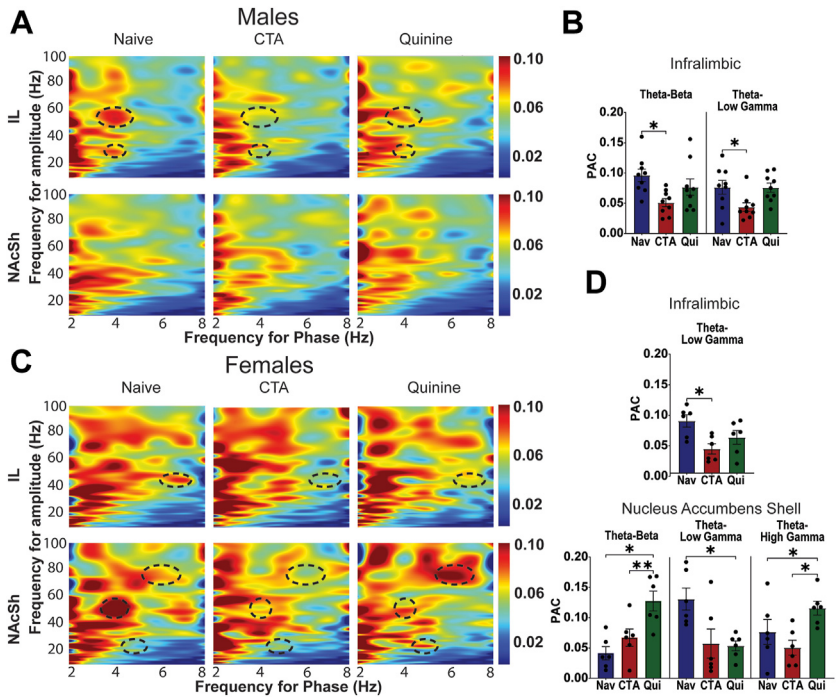

Figure 4. Effects of aversive stimuli on PAC in the IL and NAcSh in male and female rats. **(A)** Comodulogram of PAC between delta/theta phases and beta/gamma amplitudes for naïve (left), CTA (middle), and quinine (right) in the IL (top) and NAcSh (bottom) for male rats. PAC values are displayed in the overlay (right). Dashed circles indicate where significant differences were observed. **(B)** Bar graphs showing main differences in PAC values between trials for IL for male rats. One-way repeated measures analysis of variance and subsequent post hoc analyses revealed significant reduction in theta-beta ($F_{1.809,14.47}$ = 5.103, p = .0234; post hoc: naïve vs. CTA: p = .0227) and theta-low gamma ($F_{1.823,14.58}$ = 4.442, p = .0339; post hoc: naïve vs. CTA: p = .0209) coupling between naïve and CTA trials in the IL. No changes in PAC values were observed in the NAcSh. **(C)** Comodulogram of PAC between delta/theta phases and beta/gamma amplitudes for naïve (left), CTA (middle), and quinine (right) in the IL (top) and NAcSh (bottom) for female rats. PAC values are displayed in the overlay (right). Dashed circles indicate where significant differences were observed. **(D)** Bar graphs showing main differences in PAC values between trials for IL (top) and NAcSh (bottom) for female rats. One-way repeated measures analysis of variance and subsequent post hoc analyses revealed significant reduction in theta-low gamma ($F_{1.992,9.959}$ = 6.694, p = .0144; post hoc: naïve vs. CTA: p = .0365) coupling between naïve and CTA trials in the IL, in theta-low gamma coupling between naïve and quinine in the NAcSh ($F_{1.538,7.692}$ = 8.382, p = .0147; post hoc: naïve vs. Qui, p = .0108), and an increase in theta-beta ($F_{1.458,7.291}$ = 15.12, p = .0036; post hoc: naïve vs. Qui, p = .0178; CTA vs. Qui, p = .0079) and theta-high gamma ($F_{1.117,5.586}$ = 6.137, p = .0490; post hoc: naïve vs. Qui, p = .0306; CTA vs. Qui, p = .0292) coupling between quinine and other trials in the NAcSh. $^{*}p < .05$, $^{**}p < .01$. CTA, conditioned taste aversion; IL, infralimbic cortex; NAcSh, nucleus accumbens shell; Nav, naïve; PAC, phase-amplitude coupling; Qui, quinine.

encoding that is disrupted by CTA and that is common in both males and females, particularly between theta and low-gamma bands. Additionally, theta modulation of higher-frequency oscillations appears to be important in the NAcSh of female rats during innate aversion but irrelevant in male rats.

DISCUSSION

Disruption of affective processing is prevalent in several psychiatric disorders, and both the IL and NAcSh have been implicated in processing such information. Optogenetic work indicates that projections from the IL to the NAcSh have been causally linked to CTA expression in male but not female rats (31). Inspired by these findings, here we sought to determine whether oscillatory signaling dynamics in the IL-NAcSh circuit differ in males and females during innate and learned negative affect. We observed 4 key findings in negative affect processing across sex. First, we found that CTA affected overall resting-state oscillatory activity only in the IL of male rats, and this was correlated with gaping behavior. Second, LFP oscillatory activity in response to saccharin was different for male and female rats, and CTA produced sex-dependent differences. Third, while there were some sex-dependent differences in PAC, similar effects after CTA in theta-low gamma PAC were observed. Finally, while quinine produced some similar effects in oscillatory power, females showed differences in PAC within the NAcSh that may be linked to the aversive resistance observed in females. Each of these key findings is considered in detail below.

First, we studied the effects of CTA on the IL and NAcSh by analyzing changes in resting-state power before and after LiCl treatment. We found that the development of CTA only affected overall (non–frequency-specific) resting-state power in the IL of male rats. Neither the NAcSh of males nor both regions in female rats were affected. Additionally, altered resting-state activity in the IL of male rats at delta, theta, and beta frequency bands was positively correlated with subsequent gaping behavior in response to the LiCl-paired saccharin solution, showing that rats with higher resting-state activation developed a stronger CTA. These individual differences in IL activation associated with the strength of the aversion developed may serve as biomarkers that predict susceptibility to negative affect in male rats. Alternatively, changes in baseline activity may reflect differences in learning such that rats with greater neural activity and higher gaping may exhibit more robust learning. However, all rats showed hedonic scores near −1 on the CTA trial, indicating similar degrees of learning. Regardless, these results indicate that the IL is differentially engaged during learned aversion in male rats compared with females. Additionally, they are consistent with human studies showing increased medial PFC resting-state activity in patients with illnesses associated with negative affect such as patients with trauma, including posttraumatic stress disorder (33), and in different kinds of depression, including major depressive disorder (34,35). Although no sex differences were observed in

those studies, data were not always separated in individual regions within the PFC. Based on our results, it will be of great value for future studies in human subjects to dissect the data from the PFC into individual regions to more thoroughly assess whether similar sex-dependent differences are observed.

Next, we studied how IL and NAcSh oscillatory activity in male and female rats responded to saccharin before and after LiCl pairing. Here, we also observed differential activation between male and female rats in response to the tastants. Specifically, rewarding saccharin produced an activation in both the IL and NAcSh of male rats at beta frequency. In female rats, beta frequency was not altered, and rewarding saccharin mainly produced an inactivation of higher-frequency bands (high gamma). CTA development, on the other hand, prevented the activation at beta frequency band in both the IL and NAcSh of male rats. These results are consistent with human studies showing diminished corticolimbic response to pleasant and aversive stimuli in patients with posttraumatic stress disorder (36). Conversely, in female rats, the development of CTA prevented the inactivation at high gamma in the IL and significantly increased activity in a portion of the high-gamma band (70 Hz) in the NAcSh. Additionally, the fact that decreased IL-NAcSh coherence was observed only in male rats indicates that CTA differentially affected functional connectivity as well.

As mentioned, the IL-NAcSh circuit has already been causally linked to CTA in male but not in female rats (31). Although the current study did not examine causal links between oscillatory signaling and learned negative affect, our findings are consistent with the optogenetic study in that CTA prevented 20 Hz activity during saccharin infusion and reduced functional connectivity between the IL and NAcSh only in male rats. Overall, we observed more differences than similarities in the processing of saccharin and LiCl-paired saccharin between males and females, suggesting that at least the affective component of the stimulus is processed differently across sexes.

Within each brain region, frequency bands are hierarchically organized, and the phase of low oscillatory frequency bands (e.g., delta, theta) can synchronize networks over long distances and modulate the amplitude of fast oscillations (e.g., beta, low gamma, and high gamma) that are thought to synchronize ensembles within short distances (37,38). Hence, we also sought to analyze how oscillations at different frequency bands interact within each brain region by examining PAC. Interestingly, we observed heterogeneous findings with respect to PAC across sex. CTA specifically affected theta/beta coupling in the IL of males, perhaps reflecting a unique role of lower frequency activity in the IL of males in learned negative affect, consistent with our previous optogenetic findings (31). In contrast, the effects of CTA on theta/low gamma coupling in the IL was generalized across sexes. The latter finding indicates that lower frequency modulation of high-frequency (gamma) oscillations in the IL during reward encoding is disrupted by CTA, common in both males and females.

Finally, similarities between sexes were observed in the processing of the innate aversive stimulus (quinine) in the IL and NAcSh. Quinine produced an increase in power at gamma frequency relative to the naïve trial in both the IL and NAcSh of male and female rats. IL-NAcSh functional connectivity has already been shown to not be critical for innate aversion in either sex, at least when optogenetically stimulated at 20 Hz (31), and this was corroborated in this study because quinine did not significantly alter IL-NAcSh coherence in either sex. However, differences in LFP signaling were observed when analyzing PAC. Specifically, increased theta/beta and theta/high gamma and lower theta/low gamma phase-amplitude coupling were observed in the NAcSh of female rats after quinine infusion, while no PAC differences were evident in male rats. The sex-specific changes in PAC in females are consistent with previous research showing the involvement of theta-gamma PAC on affect processing such as decreased theta-high gamma coupling in the NAc during appetitive conditioning (39) and enhanced theta-high gamma coupling in the basolateral amygdala during periods of fear (40). Likewise, female rats exhibit less anxiety/fear in the elevated gradient of aversion test (41), less contextual fear when freezing behavior is measured after foot shock (42), and greater aversion resistance in experiments using quinine-adulterated alcohol (43,44), although differential processing in the IL-NAcSh circuit of those types of aversive-related behaviors across sex remains to be examined.

Conclusions

In closing, the current study revealed unique oscillatory signaling dynamics in the IL-NAcSh circuit during innate versus learned negative affect that differed in male and female rats. While our previous optogenetic work implicated a role of this circuit in learned negative affect only in male rats (31), future studies using other techniques such as closed-loop signal processing and optogenetics are needed to determine whether the specific oscillatory signaling properties that were observed here are indeed causally linked to innate and/or learned negative affect (45–49). Nevertheless, our findings clearly show that while innate and learned affective behaviors do not differ across sex, signaling dynamics in the IL-NAcSh are not engaged in the same manner in male and female rats. Importantly, such information can provide much needed insight into the use of noninvasive brain stimulation such as transcranial alternating-current stimulation to treat psychiatric illnesses including depression and substance use disorders. Specifically, transcranial alternating-current stimulation is used to apply weak, exogenous electric fields to the scalp to modulate disrupted cortical oscillations evident in psychiatric illnesses (50–54), although the optimal stimulation parameters needed remain unclear (55). As such, disentangling sex-specific oscillatory signaling dynamics within the IL-NAcSh circuit, as we achieved in the current study, provides critical information that can ultimately inform optimal noninvasive brain stimulation parameters to effectively treat psychiatric illnesses in humans.

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RMC designed the experiments. JED ran the experiments, performed surgeries, and analyzed the data. RMC and JED interpreted the data and wrote the manuscript.

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ARTICLE INFORMATION

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