Title: Intranasal oxytocin drives coordinated social approach

Patrick K. Monari[1†], Nathaniel S. Rieger[1,2], Juliette Schefelker[1], Catherine A. Marler[1]

[1]Department of Psychology, University of Wisconsin-Madison, Madison, WI, USA
[2]Department of Psychology and Neuroscience, Boston College, Chestnut Hill, MA, USA
[†] Correspondence to: Patrick Monari, monari@wisc.edu

Abstract

Coordinated responses to challenge are essential to survival for bonded monogamous animals and may depend on behavioral compatibility. Oxytocin (OT) context-dependently regulates social affiliation and vocal communication, but its role in pair members' decision to jointly respond to challenge is unclear. To test for OT effects, California mouse females received an intranasal dose of OT (IN-OT) or saline after bonding with males either matched or in their approach response to an aggressive vocal challenge. Pair mates were re-tested jointly for approach response, time spent together, and vocalizations. Females and males converged in their approach after pairing, but mismatched pairs with females given a single dose of IN-OT displayed a greater convergence that resulted from behavioral changes by both pair members. Unpaired females given IN-OT did not change their approach, indicating a social partner was necessary for effects to emerge. Moreover, IN-OT increased time spent approaching together, suggesting behavioral coordination beyond a further increase in bonding. This OT-induced increase in joint approach was associated with a decrease in the proportion of sustained vocalizations, a type of vocalization that can be associated with intra-pair conflict. Our results expand OT's effects on behavioral coordination and underscore the importance of emergent social context.

Keywords: Oxytocin; intranasal oxytocin; pair bonding; coordination; approach; ultrasonic vocalizations; *Peromyscus californicus*; California mouse

Introduction

Coordinated behavior is essential to compatibility and survival for species that are biparental and develop mating pair bonds. Examples across vertebrate taxa illustrate environmental and social challenges that require joint strategies by bonded partners [1–3], and for monogamous and territorial species one such challenge is the introduction of an unfamiliar conspecific. Pairs with an established bond must appropriately recognize and confront outgroup individuals, as their presence threatens territorial infringement [4], resource theft [5], and infanticide [6], all of which can hamper pair fitness [7–9]. Some species, such as cichlid fish [10] and Kirk's dik-dik [11] respond with rigid, sex-specific behavioral roles such that one sex invariably defends territories while the other cares for offspring. Other pair bond-forming species, however, display more flexible behavior between sexes. For example, prairie vole [12,13] and California mouse [14–16] pair mates closely overlap in their behavior, and both sexes can complete any necessary task besides nursing. Compatible responses between pair-bonded individuals to an extrapair challenge are therefore uniquely important to coordination in these species.

The neuropeptide oxytocin (OT) context-dependently regulates social affiliation [17] and vocal communication [18], and by extension may impact response compatibility, but its role in pair members' decision to jointly react to challenge is unclear. OT signaling facilitates prosocial behaviors in some cases, while it promotes social vigilance [19], social anxiety [20], and agonistic behaviors [21,22] in others. The effects of OT on investigation and aggression are therefore complex and rely on social context [23] and may modulate behavior and emotion by enhancing an individual's attunement to its social landscape [24–26]. For example, in marmosets, intranasal OT (IN-OT) increased approach towards an unfamiliar conspecific when a mate was also present but decreased approach when the mate was absent [27]. In humans, IN-OT reduced xenophobic

aggression when paired with injunctive prosocial norms of peers, but not when administered without them [28]. Taken together, this evidence supports the notion that OT signaling influences how social affiliations moderate responses to the outgroup.

An outstanding question is whether, and under what circumstances, OT facilitates coordination of approach toward an unfamiliar conspecific. California mice (*Peromyscus californicus*) are uniquely positioned to answer this question as biparental, highly territorial mammals that flexibly cooperate within the pair bond to accomplish goals such as intruder investigation [16,29]. In the wild and the laboratory, adults form lifelong female-male dyads and are well documented to be highly aggressive toward other same-sex or opposite-sex individuals [14,16,30–36], restricting the ecological relevance of cooperative behavior to mating pairs and possibly family units. Paired California mice can show both synchronous or divided defense in response to intrusion, with varying degrees of individual and joint approach by pair members, suggesting variability in pair coordination and division of labor [37]. Flexible application of coordinated strategies may depend on the type and degree of challenge; in some cases, joint action may help to efficiently remove a threat, while in other cases it might be advantageous to divide location and simultaneously defend resources or pups while addressing an intruder. We recently found that, prior to pup birth and following pair-bonding, individuals of both sexes adjusted to match their partner's level of approach in a compatibility-dependent manner when exposed to a low level of challenge; paired individuals that were mismatched (one individual higher, one lower) in their approach before pairing were more likely to change their behavior to become similar after pairing, while matched pairs (similar levels of approach between individuals) changed little. The change in behavior for mismatched pairs was associated with changes to ultrasonic vocalization (USV) production profiles [38, in review]. We speculate that

vocal communication plays a role in the decision to adopt one coordination type over the other, as paired California mice display a rich variety of USVs that have been linked to complex social behavior [39,40]. However, the neuroendocrine mechanisms underlying this coordination-induced change to social approach have yet to be studied.

OT can context-dependently regulate approach and avoidance of a novel conspecific in female California mice [41,42]. Here we used female IN-OT administration and pre-pairing/post-pairing simulated intrusions, via playbacks of aggressive vocalizations, to show that OT is critical to mediating pair-bonding-induced changes to social approach. Two hypotheses emerge for how OT may modulate pair coordination in response to challenge: the convergence hypothesis predicts that OT will increase the similarity between pairs mismatched in their degree of social approach, increasing the amount of time pair mates spend jointly addressing an unfamiliar intruder [43,44]. Conversely, the divergence hypothesis predicts that OT can increase the dissimilarity between matched pairs, increasing the amount of time pair mates spend apart, thereby dividing labor [37].

Methods

Animals

48 adult males and 48 adult females (age 3-6 months) were housed in standard cages (48 x 27 x 16 cm) lined with aspen bedding and a nestlet with Purina 5015™ mouse chow and water available *ad libitum*, 2-4 same-sex individuals per cage. All tests occurred between 1–3 hrs after the onset of the dark cycle in dim red light in housing maintained at 20–23º C on a 14:10 h light:dark cycle (lights on at 16:00 central standard time). Males and females were tested for response to bark playbacks and were selectively paired and housed in new cages. In a separate experiment, 20 females were left unpaired and housed with their original cage mates.

Ethical statement

Animals were maintained according to the National Institute of Health *Guide for the Care and Use of Laboratory Animals*. Procedures were approved by the University of Wisconsin-Madison College of Letters and Sciences Institutional Animal Care and Use Committee (Protocol L005447). No animals were injured by any of the behavioral manipulations or assays.

Apparatus

Testing occurred in aspen bedding-lined Plexiglas cages (90 x 30 x 30 cm) equally divided into three chambers (each 30 x 30 x 30 cm) with centrally located openings (11.5 x 11.5 cm) between chambers to allow for free movement. Speakers (Vifa Dynamic Ultrasound, 1-120 kHz range, Avisoft Bioacoustics, Berlin, Germany) were placed at each end of the apparatus against a closed mesh gate.

Playback tracks

We investigated California mouse approach behavior towards playbacks of loud, aversive bark calls, adapted from [38, in review]. In a separate cohort used exclusively to produce the playback stimuli, individual male and female mice were placed in a single-chambered plexiglass apparatus (50 x 30 x 30) under normal food and water conditions for 24 hrs, a length of time demonstrated to be sufficient for the formation of residency behavior, and in which the arena becomes the individual's territory [36]. After 24 hrs, residents were introduced to a same-sex intruder for an 8-min aggressive encounter period similar to previous studies [32,33,36,45,46]. Each intruder was only used for a single encounter, and otherwise had no previous aggression testing experience. During the encounter we recorded defensive-aggressive barks using an ultrasonic microphone (Emkay/Knowles FG series, detection range: 10-120 kHz) with a 250 kHz sampling rate and 16-bit resolution, placed 30cm above the bottom of the apparatus. Spectrograms were produced using a 512 fast Fourier transform in Avisoft SASlab pro (Avisoft Bioacoustics, Berlin, Germany) in order to identify barks. Barks appear as short, high-amplitude calls with an upside down U shape that begins and ends in the audible range for humans [16,47] (Fig. 1A). We created playback tracks using these spectrograms by selecting only bark calls. Calls could not be distinguished between the resident and the intruder during the encounter, therefore both resident and intruder barks were used to construct playback tracks. Playback tracks were 2 mins in duration and contained 120 ± 5 bark calls. Output gain/volume was maintained across playback tracks. The ambient noise track control was a 2 min recording of the quiet testing room with all lights off and no mice present. We used 8 unique tracks from 8 different sets of individuals and assigned tracks to individuals randomly with each track used between 20-23 times [38, in review], ensuring that no individual heard the same track more than once over the course of the two tests (to avoid habituation and maintain consistency).

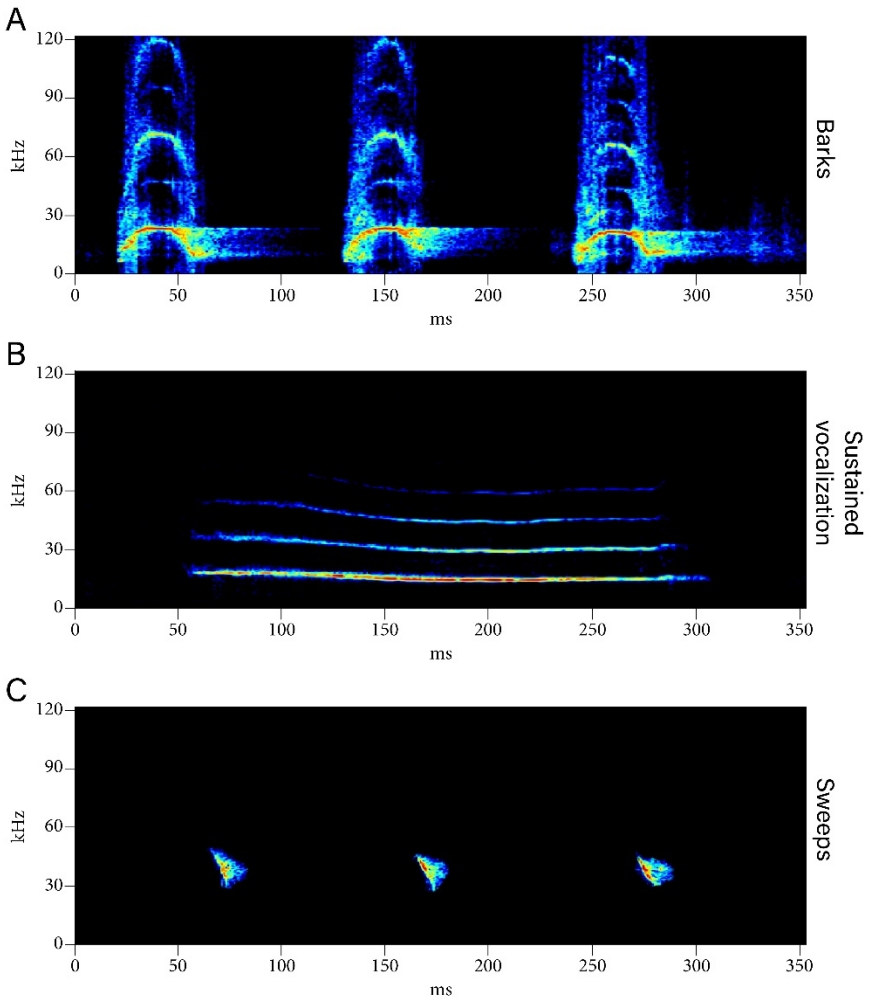


Figure 1. California mouse vocalizations. Examples of **A**. Barks, **B**. A sustained vocalization (SV), and **C**. Sweeps.

Pre-pairing playback approach test

Mice were first tested for response to bark playbacks as nonbonded, sexually naïve individuals (Fig. 2A, left). Mice were placed in the testing apparatus for 5-10 mins to habituate to all three chambers. One male and two females did not enter all three chambers by 10-min and so were removed from the experiment (resulting in 47 males and 46 females). Two-min playback tracks were played from speakers at opposite ends of the apparatus behind wire mesh, with one speaker playing a bark track and the other an ambient noise track concurrently. The bark track and ambient noise track speaker locations were randomized across trials. Video and audio recordings were made of their behavior. We recorded time spent in the chamber closest to the bark speaker ("approach chamber") as an approach score, and aggregate time spent in the chamber closest to the ambient noise speaker ("avoid chamber") as an avoidance score.

Behavioral type and pairing

3-7 days following the pre-pairing test, 46 females were selectively paired with 46 males in a stratified randomized design based on approach scores. Mice were paired such that they were "matched" in their approach scores (i.e., their approach scores were ≤ 10 s different from each other, N = 17) or they were "mismatched" (i.e., their approach scores were ≥ 30 s different from each other, N = 29). There were no pairs formed that had an approach score difference between 10 and 30 s. In a follow-up experiment, 20 female mice were left unpaired following the first playback approach test.

Post-pairing playback approach test

All mice underwent a second playback test to determine if pairing alters responses to bark calls. Pairs were retested 10-11 days after pairing (13-17 days after the pre-pairing test). At 7 days

147 post-pairing, pairs exhibit hallmarks of pair-bonding [48], including increased affiliation and
148 decreased aggression, indicating that 10-11 days is sufficient for pair-bond formation. The
149 playback procedure was the same as in the pre-pairing test except that paired mice were tested
150 together as a pair (Fig. 2A, right). Both mice were placed into the central chamber and required to
151 enter all three chambers prior to testing. Behavior scoring was the same as in the first test and,
152 additionally, pairs were scored for time spent together in the same chamber. Females generally
153 give birth within 35 days of pairing when housed in cages of similar size to those used in this
154 experiment [37], so were expected to be pregnant at the time of the post-pairing test (gestation 31-
155 32 days, [49]). The procedure for unpaired mice was the same as the pre-pairing test. Two unpaired
156 females were removed as they did not enter all three chambers by 10 mins (resulting in 18 females).

157 *IN-OT dose and application*

158 We administered a 0.8 IU/kg dose of IN-OT (Bachem, Torrance, CA, Prod #: 4016373).
159 This dose induces changes in female behavior in rodents [50] and approximates a weight-adjusted
160 dose used in human studies [51]. Intranasal administration is an established, non-invasive route of
161 delivery for OT [52], and intranasally-administered OT enters the brain in house mice (*Mus*
162 *musculus*) at behaviorally-relevant concentrations [53]. In California mice, IN-OT produces
163 behavioral effects similar to centrally-administered OT, suggesting that it reaches the brain in this
164 species [41,54]. Following pairing, mice were randomly assigned to either saline (mismatched
165 pairs: N = 15; matched pairs: N = 8) or IN-OT treatment (mismatched pairs: N = 14; matched
166 pairs: N = 9) groups. Unpaired females in the follow-up experiment were also randomly assigned
167 to either saline (N = 10) or IN-OT treatment (N = 8) prior to the retest. Immediately prior to the 5-
168 10 min habituation preceding the retest, females were given either IN-OT or control saline, while
169 all males were administered saline, as this time course has previously been shown to result in

170 behavioral effects in California mice [40]. To administer, a mouse was scruffed and 25 µl of
171 solution was administered to the nostrils using a blunt needle attached to cannula tubing at the end
172 of a Hamilton syringe. Individual droplets were beaded at the end of the syringe, applied to the
173 surface of the nose, and allowed to absorb into the nasal mucosa. Administration lasted less than
174 30 s per animal. Only females were given IN-OT because of unpublished data suggesting that IN-
175 OT shifts the convergence/division of labor ratio for pairs defending their territories when given
176 to females, but not males [55].

177 *USV analysis*

178 We recorded USVs with two ultrasonic microphones (Emkay/Knowles FG, detection range
179 10-120 kHz) placed 85 cm apart at opposite corners of the apparatus and 30 cm from the apparatus
180 floor, with one microphone placed in the approach chamber and the other placed in the avoid
181 chamber. Microphone channels were calibrated to equal gain (-60 dB noise floor). WAV files were
182 produced using RECORDER software (Avisoft Bioacoustics, Berlin, Germany). Recordings were
183 made using a 250 kHz sampling rate with 16-bit resolution and spectrograms were produced with
184 a 512 fast Fourier transform made using Avisoft SASLab Pro (Avisoft bioacoustics). USVs were
185 differentiated by visual and auditory inspection of WAV files with sampling rates reduced to 4%
186 of normal speed (11025 kHz). Beyond barks, California mice produce a variety of ultrasonic
187 vocalizations (USVs) [39] important for communication and behavioral coordination [56].
188 Because barks are only typically produced when aggressive physical contact is made [16] and were
189 not produced by mice in this study, two call types, sustained vocalizations (SVs) and sweeps, were
190 analyzed, because they may play a role in pair bond formation and maintenance [48,57,58]. SVs
191 are low-bandwidth calls with low modulation, a peak frequency of 20 kHz and a duration of 100
192 to 500 ms for each individual syllable (Fig. 1B). Shorter SVs are used during aggressive encounters

[16]. Sweeps are of relatively short durations of 30 to 100 ms with upwards and downwards modulation ranging from 25 to 100 kHz and potentially including multiple inflection points [16] (Fig. 1C). Because audio was recorded for both pair members simultaneously, it was impossible to differentiate calls by each individual so vocalizations were analyzed at the pair level. Both the total number of USV calls produced and the proportion of each USV individual call type produced relative to all call type production were analyzed within this dataset. Four audio files were unusable due to recording setup error (mismatched saline pairs: 11; matched saline pairs: 8; mismatched OT pairs: 14; matched OT pairs: 9). The length of SVs was also analyzed, and eight pairs that did not produce SVs were removed from this analysis only (mismatched saline pairs: 11; matched saline pairs: 5; mismatched OT pairs: 12; matched OT pairs: 6).

Statistics

All statistics were analyzed using R (version 3.6.2). We analyzed the female-male approach difference before pairing by taking the absolute value of the difference between female (F) and male (M) approach, $|F_1-M_1|$ and regressing it on pair type (mismatched or matched) in a general linear model (GLM). We analyzed the degree to which pairs converged by taking the initial difference between pair members, $|F_1-M_1|$, and subtracting it from the difference at the second test, $|F_2-M_2|$, and regressed it on pair type, treatment type (saline or IN-OT), and their interaction in a GLM. We analyzed individual changes by subtracting initial approach from approach at the second test (F_2-F_1 and M_2-M_1) and regressed it on pair type, treatment type, and their interaction in a GLM. We likewise analyzed the amount of time pair members spent within the same chamber using pair type, treatment type, and their interaction as factors in a GLM. For changes in vocalizations, we summed vocalizations produced by pairs before pairing and subtracted them from the vocalizations produced in the second test, again using pair type, treatment type, and their interaction as factors

in a GLM. For the proportion of sustained vocalizations (SVs), we looked only at vocalizations produced at the post-pairing test, because there were so few vocalizations produced prior to pairing. We analyzed the proportion of SVs using a GLM with pair type, treatment, and their interaction as factors. We used linear regressions to test if behavior predicted call type proportion. For the separate experiment using unpaired females, we regressed second approach on initial approach, and also analyzed the effects of treatment on approach during the second test, using a GLM. P-values were Holm-Bonferroni corrected for multiple comparisons where appropriate.

Results

Mismatched pairs had a greater difference in approach scores before pairing than did matched pairs

Prior to pairing, a wide range in approach to bark playbacks was found in females (33.43 ± 4.07 s s.e.m.) and males (37.96 ± 4.19 s s.e.m.), with a general linear model (GLM) of approach regressed on sex revealing no mean difference between sexes ($F(1,90) = 0.597$, $p = 0.442$; Fig. 2B). Males and females were paired based on their approach, whereby "mismatched" pairs had ≥ 30 s difference in approach score while matched pairs had ≤ 10 s in their approach score ("mismatched": N=16 pairs female lower than male, N=13 pairs male lower than female; "matched": N=8 pairs female lower than male, N=9 pairs male lower than female; Fig. 2C). As expected, a GLM revealed a statistically significant gap between the approach difference for females and males of mismatched (50.59 ± 3.11 s s.e.m.) and matched (5.71 ± 0.83 s s.e.m.) pairs ($F(1,44) = 118.100$, $p < 0.001$; Fig. 2D).

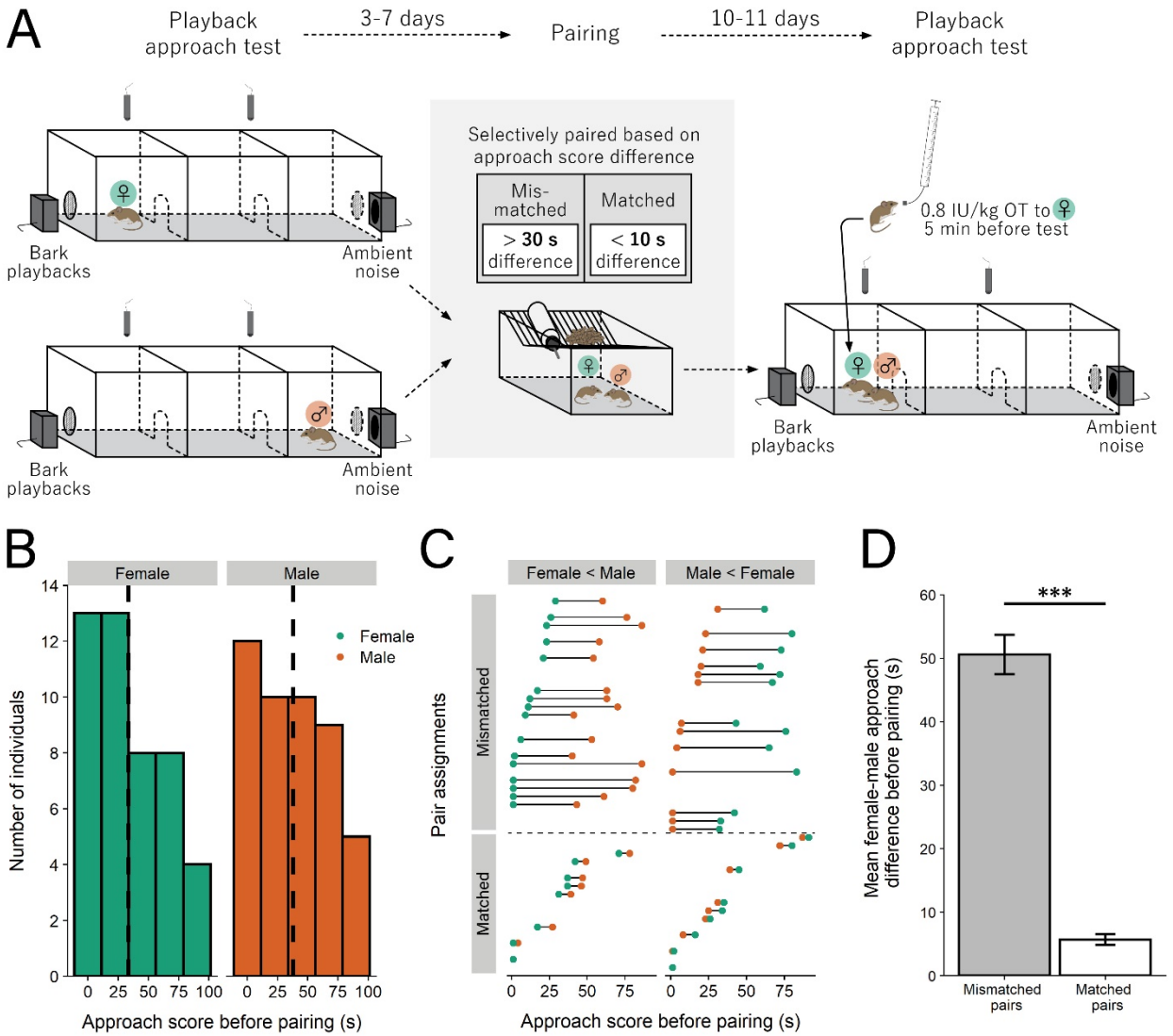


Figure 2. Selective pairing to form mismatched and matched pairs based on initial social approach score. **A**. Experimental design, adapted from [38, in review]. Approach is the amount of time spent in the chamber closet to the bark playback speaker. **B**. Distribution of approach scores for females and males before pairing; dotted line is mean approach. **C**. Stratified randomized pair assignments; mismatched pairs had ≥ 30 s difference in approach score between mates while matched pairs had ≤ 10 s difference. **D**. The female-male approch difference for mismatched pairs was significantly greater than that for matched pairs (*** = $p < 0.001$). Error bars are ± s.e.m.

IN-OT drove mismatched pairs to become more similar than saline mismatched pairs

To determine how IN-OT affected pair convergence, we first assessed the change in approach score difference for matched and mismatched pair mates from the before-pairing test to the after-pairing test. A GLM revealed a significant interaction between pair type (matched or mismatched) and treatment (saline or IN-OT) ($F(1,42) = 4.189$, $p = 0.047$; Fig. 3A; Table 1A). Controlling for treatment effects, mismatched pairs decreased their approach score difference more than matched pairs from before-pairing to after-pairing, indicating that mismatched pairs become more similar, while matched pairs remain similar ($F(1,42) = 58.346$, $p < 0.001$). Relative to saline, IN-OT treatment resulted in a further decrease in approach score difference for mismatched but not matched pairs, as shown by a simple effect of treatment within mismatched pairs, suggesting that IN-OT drove mismatched pairs to become more similar ($F(1,42) = 11.539$, $p = 0.002$).

From pre-pairing to post-pairing, mismatched and matched IN-OT and saline groups all contained pairs that had pair-averaged approach scores that decreased and pair-averaged approach scores that increased, suggesting that convergence may be driven differently by individuals within pairs (Fig. 3B). To determine whether males and females contribute differently to the overall convergence of pair approach scores, we regressed change in approach from the before-pairing test to the after-pairing test for each individual on the interaction of pair type, treatment, and sex. This GLM revealed no 3-way interaction ($F(1,84) = 0.693$, $p = 0.408$; Fig. 3C), however, IN-OT individuals in mismatched pairs had a greater change in approach from the before-pairing test to the after-pairing test than did saline individuals in mismatched pairs when controlling for sex, suggesting that the convergence in IN-OT mismatched pairs emerged from changes in approach by both sexes ($F(1,84) = 5.257$, $p = 0.0244$; Table 1B).

IN-OT increased the amount of time pairs spent in joint approach

Complementing the analyses on the degree to which pair mates changed their behavior, we also measured time spent together as an indicator of coordination. Analysis of the amount of time paired mates spent together in either the approach chamber (the chamber closest to the bark playback speaker) or the avoid chamber (the chamber closest to the ambient noise speaker), via a 3-way interaction of pair type, treatment, and location, revealed that IN-OT spent significantly more time together in the approach chamber than saline pairs ($F(1,84) = 4.311$, $p = 0.041$) but not in the avoid chamber ($F(1,84) = 0.092$, $p = 0.762$), and no main effect of location ($F(1,84) = 0.768$, $p = 0.383$), pair type ($F(1,84) = 1.645$, $p = 0.203$), or the full interaction ($F(1,84) = 0.967$, $p = 0.328$; Fig. 3D; Table 1C). Additionally, there was a nonsignificant effect of pair type in the avoid chamber for saline groups ($F(1,84) = 3.016$, $p = 0.086$). A separate analysis that combined mismatched and matched pairs into a single category also revealed a significant main effect of IN-OT ($F(1,88) = 4.066$, $p = 0.047$), with a significant simple effect whereby IN-OT increased the amount of time pairs spent together in the approach chamber ($F(1,88) = 4.543$, $p = 0.036$) but not the avoid chamber ($F(1,88) = 0.519$, $p = 0.473$; Fig. 3E). There was also no effect of location ($F(1,88) = 0.389$, $p = 0.535$) or the interaction of treatment and location ($F(1,88) = 0.996$, $p = 0.321$).

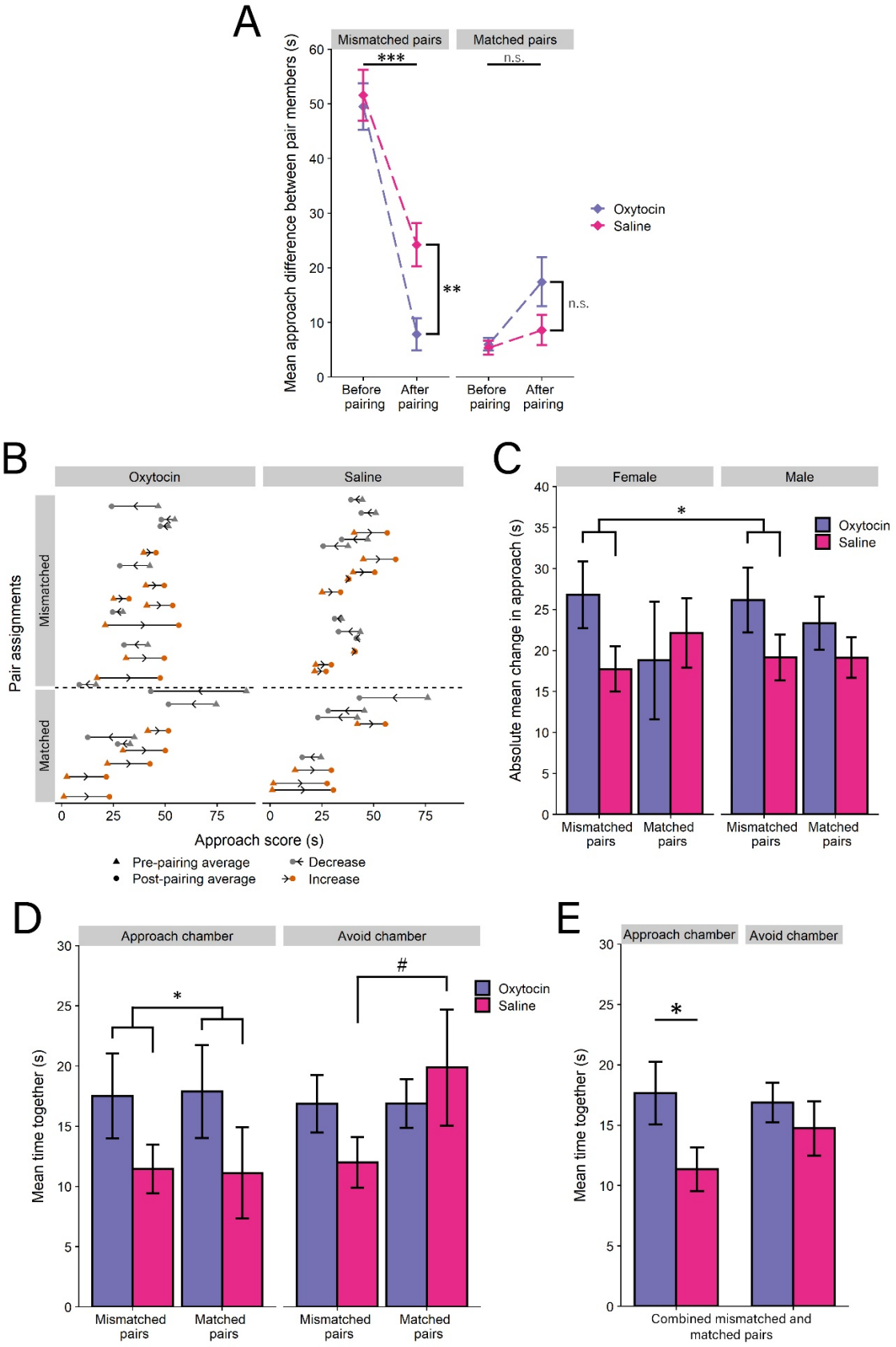

A
Mismatched pairs
Matched pairs
Mean approach difference between pair members (s)
***
n.s.
**
n.s.
Oxytocin
Saline
Before pairing
After pairing
B
Oxytocin
Saline
Pair assignments
Mismatched
Matched
Approach score (s)
Pre-pairing average
Post-pairing average
Decrease
Increase
C
Female
Male
Absolute mean change in approach (s)
*
Oxytocin
Saline
Mismatched pairs
Matched pairs
D
Approach chamber
Avoid chamber
Mean time together (s)
*
#
Oxytocin
Saline
Mismatched pairs
Matched pairs
E
Approach chamber
Avoid chamber
Mean time together (s)
*
Combined mismatched and matched pairs

Figure 3. IN-OT drove mismatched pair mates to converge more in their social approach and pairs to increase their joint approach. **A**. Mismatched pair mates became more matched, an effect enhanced by OT, while matched pairs remained matched and were unaffected by OT. **B**. Within-pair average approach increased and decreased by pair for mismatched and matched OT and saline groups. **C**. Females and males of OT mismatched pairs changed their approach more than females and males of saline mismatched pairs. **D**. IN-OT increased the amount of time OT pair mates spent together in the approach chamber. **D**. Time together in the approach chamber and avoid chamber, aggregated for all pairs. (n.s. = not significant; # = $p < 0.10$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$). Error bars are ± s.e.m.

Mismatched pairs produced a higher proportion of sustained vocalizations after pairing, which was not affected by IN-OT

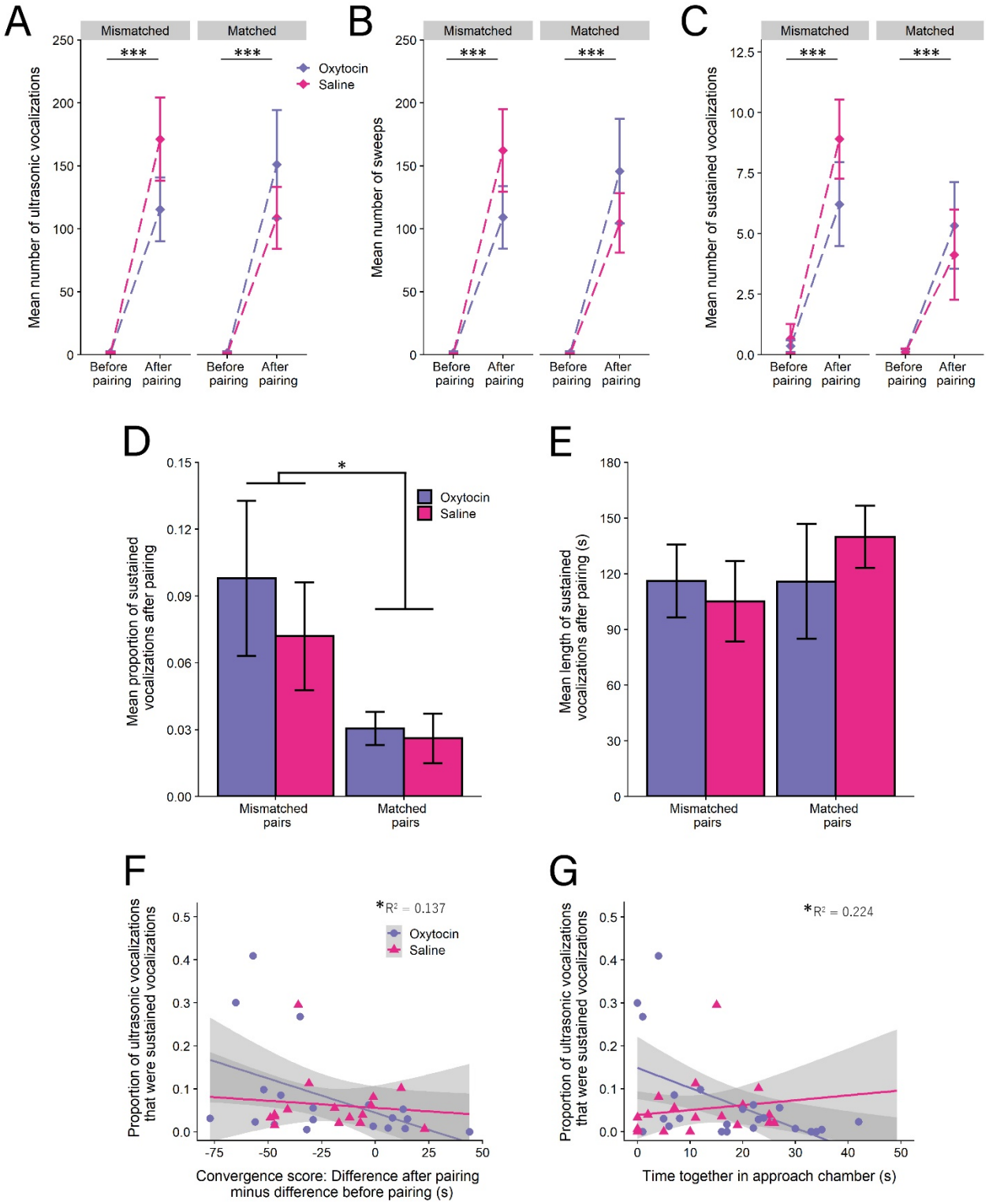
To determine how pair type and IN-OT affected vocal production, we assessed the number and type of USVs produced in the pre-pairing and post-pairing tests. A GLM revealed a significant increase in the total number of vocalizations produced by pairs relative to the sum of vocalizations for pair members before pairing ($F(1,38) = 70.267$, $p < 0.001$), but no significant interaction between pair type and treatment ($F(1,38) = 2.182$, $p = 0.148$; Fig. 4A). Similarly, a GLM for number of sweeps ($F(1,38) = 66.182$, $p < 0.001$) indicated significant increases in sweep production after pairing, but no significant interaction ($F(1,38) = 2.184$, $p = 0.148$; Fig. 4B). A GLM for number of sustained vocalizations (SVs) revealed a significant increase in SV production after pairing ($F(1,38) = 35.168$, $p < 0.001$) as well as a nonsignificant increase in SV production by mismatched pairs ($F(1,38) = 3.801$, $p = 0.059$), but no main effect of treatment ($F(1,38) = 0.799$, $p = 0.377$) or significant interaction ($F(1,38) = 0.106$, $p = 0.747$; Fig. 4C; Table 1D). Taken together, this suggests that pairing increases the total number of sweeps and SVs that are produced, and that pair type and IN-OT do not affect the total number of either sweeps or SVs.

We next assessed whether other features of SV call production were affected by pair type or IN-OT. A GLM revealed that mismatched pairs significantly increased the proportion of SVs produced after pairing (as used in [57]) ($F(1,38) = 4.141$, $p = 0.048$), but that there was no significant interaction with IN-OT ($F(1,38) = 0.298$, $p = 0.588$; Fig. 4D, Table 1E). A GLM indicated no significant effect of pair type ($F(1,31) = 0.475$, $p = 0.496$), treatment ($F(1,31) = 0.069$, $p = 0.795$), or interaction ($F(1,31) = 0.489$, $p = 0.490$) on SV length (Fig. 4E, Table 1E). These results indicate that SV proportion may be involved in pair convergence.

Sustained vocalization proportion positively correlated with pair similarity and negatively correlated with time IN-OT-treated pairs spent together in the approach chamber

To determine whether SV proportion after pairing was associated with pair coordination, we correlated SV proportion of pairs with convergence and treatment, and then joint approach and treatment. A GLM revealed a significant main effect of the degree of convergence ($|F2-M2| - |F1-M1|$) on the proportion of SVs produced after pairing ($F(1,38) = 4.476$, $p = 0.041$; Fig. 4F), showing that the degree to which pairs become more similar is positively correlated with SV production. However, there was no significant interaction between pair type and treatment ($F(1,38) = 0.595$, $p = 0.445$) or main effect of treatment ($F(1,38) = 0.105$, $p = 0.748$), suggesting that IN-OT did not have an effect on the relationship between SV proportion and convergence.

A separate GLM revealed a significant interaction between the amount of time pairs spent together in the approach chamber and treatment type on the proportion of SVs produced ($F(1,38) = 5.000$, $p = 0.031$, Fig. 4G), with a significant simple effect of IN-OT ($F(1,38) = 10.440$, $p = 0.003$) but not saline ($F(1,38) = 0.280$, $p = 0.600$), indicating that increased time spent together in the approach chamber for IN-OT pairs was associated with decreasing SV proportion.


A
Mismatched
Matched
***
***
Oxytocin
Saline
Mean number of ultrasonic vocalizations
Before pairing
After pairing
B
Mean number of sweeps
C
Mean number of sustained vocalizations
D
*
Mean proportion of sustained vocalizations after pairing
Mismatched pairs
Matched pairs
E
Mean length of sustained vocalizations after pairing (s)
F
*R² = 0.137
Proportion of ultrasonic vocalizations that were sustained vocalizations
Convergence score: Difference after pairing minus difference before pairing (s)
G
*R² = 0.224
Time together in approach chamber (s)

346 *Figure 4*. Pair type and IN-OT altered the production of ultrasonic vocalizations. **A**, **B**, **C**. Pairing
347 resulted in a profound increase in the number of ultrasonic vocalizations produced, including
348 sweeps and sustained vocalizations, across pair type and treatment. **D**. Mismatched pairs produced
349 a greater proportion of sustained vocalizations. **E**. Length of sustained vocalizations did not differ
350 by pair type or treatment condition. **F**.The degree to which pair mates converged predicted the
351 proportion of sustained vocalzations produced after pairing. A negative number on the x-axis
352 shows pair mates decreased the difference in approach score from the first test to the second test,
353 indicating convergence. **G**. IN-OT and time spent together in the approach chamber negatively
354 predicted the proportion of SVs produced. (* = $p < 0.05$; *** $p < 0.001$). Error bars are ± s.e.m.

355

356

357 *Unpaired females respond to bark playbacks in a consistent manner*

358 In order to demonstrate that approach behavior is repeatable and to investigate the effects
359 of the IN-OT treatment in unpaired individuals, a separate cohort of unpaired females was tested
360 for initial response and response 13-17 days later, the same length of time as that between the pre-
361 pairing and post-pairing test for paired mice. A GLM revealed that initial approach reliably
362 predicts later approach ($F(1,14) = 14.59$, $p = 0.002$; Fig. 5A), with no significant main effect of
363 treatment ($F(1,14) = 0.313$, $p = 0.585$) or interaction ($F(1,14) = 0.227$, $p = 0.641$). A separate GLM
364 indicated no effect of treatment on approach during the second test ($F(1,16) = 0.415$, $p = 0.529$;
365 Fig. 5B).

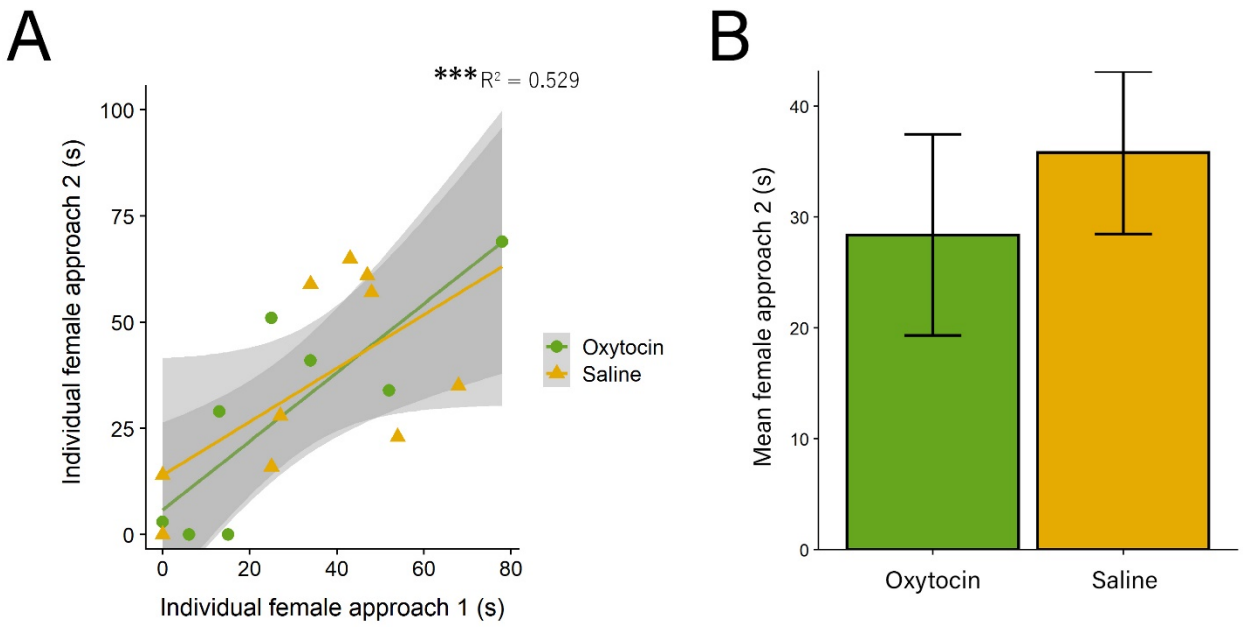


Figure 5. A separate cohort of unpaired females showed high test-retest reliability. **A**. Unpaired females showed reliable response to the playback approach test 13-17 later. **B**. IN-OT did not significantly change female approach at the second test. (*** = $p < 0.001$). Error bars are ± s.e.m.

A

	Female-male approach difference before pairing (|F1-M1|) (s) ± s.e.m.		Female-male approach difference after pairing (|F2-M2|) (s) ± s.e.m.		Δ approach difference from before pairing to after pairing (|F2-M2| - |F1-M1|) (s) ± s.e.m.	
	Saline	Oxytocin	Saline	Oxytocin	Saline	Oxytocin
Mismatched	51.60 ± 4.66	49.50 ± 4.24	24.20 ± 3.98	7.86 ± 2.92	-27.40 ± 4.88	-41.64 ± 5.83
Matched	5.38 ± 1.28	6.00 ± 1.13	8.63 ± 2.78	17.44 ± 4.47	3.25 ± 3.62	11.44 ± 4.66

B

	Female Δ approach from before pairing to after pairing (|F2-F1|) (s) ± s.e.m.		Male Δ approach from before pairing to after pairing (|M2-M1|) (s) ± s.e.m.	
	Saline	Oxytocin	Saline	Oxytocin
Mismatched	17.73 ± 2.76	26.79 ± 4.06	19.13 ± 2.80	26.14 ±3.97
Matched	22.13 ± 4.21	18.78 ± 7.20	19.13 ± 2.47	23.33 ± 3.25

C

	Time together in approach chamber (s) ± s.e.m.		Time together in avoid chambers (s) ± s.e.m.	
	Saline	Oxytocin	Saline	Oxytocin
Mismatched	11.47 ± 2.01	17.50 ± 3.53	12.00 ± 2.10	16.86 ± 2.38
Matched	11.13 ± 3.78	17.89 ± 3.86	19.88 ± 4.81	16.89 ± 2.01
Aggregate	11.35 ± 1.81	17.65 ± 2.57	14.74 ± 2.24	16.87 ± 1.62

D

	Δ total ultrasonic vocalizations ± s.e.m.		Δ sustained vocalizations ± s.e.m.		Δ sweeps ± s.e.m.		Δ barks ± s.e.m.	
	Saline	Oxytocin	Saline	Oxytocin	Saline	Oxytocin	Saline	Oxytocin
Mismatched	171.09 ± 33.05	115.36 ± 25.34	8.00 ± 1.76	5.86 ± 1.65	161.09 ± 32.62	107.57 ± 24.86	0.00 ± 0.00	0.00 ± 0.00
Matched	108.75 ± 24.67	148.89 ± 42.60	4.00 ± 1.91	3.00 ± 1.39	103.88 ± 23.80	144.22 ± 41.42	0.00 ± 0.00	0.00 ± 0.00

E

	Sustained vocalization proportion after pairing ± s.e.m.		Sustained vocalization length after pairing (ms) ± s.e.m.	
	Saline	Oxytocin	Saline	Oxytocin
Mismatched	0.072 ± 0.024	0.098 ± 0.035	105.20 ± 21.72	116.08 ± 19.72
Matched	0.026 ± 0.011	0.031 ± 0.007	139.82 ± 16.80	115.84 ± 30.94

Table 1. Descriptive table of mean group values. **A**. Approach differences between paired mates. **B**. Approach differences for individuals. **C**. Time spent together by pairs. **D**. Changes in aggregate vocalizations from before pairing to after pairing. **E**. Proportion of ultrasonic vocalizations that were sustained vocalizations after pairing.

392 **Discussion**

393 The social salience hypothesis argues that oxytocin (OT) attunes individuals to social
394 context [24], possibly explaining why OT is able to induce both social avoidance [19] and social
395 approach [41]. However, the roles of affiliative bonds in this process have remained elusive. Here
396 we used a pre-post pairing design and found that OT facilitates pair-bonding-induced changes in
397 social behavior and coordination. Intranasal OT (IN-OT), when given to female California mice,
398 produced pair-level shifts in social approach of an aggressive bark stimulus driving mismatched
399 pair mates to become more similar. Analysis of time spent in proximity showed that IN-OT drove
400 pairs to spend more time together approaching the stimulus, likely producing the shift towards pair
401 similarity and indicating coordinating effects of OT beyond bonding in these already bonded pair
402 members. Recordings of USVs revealed that IN-OT drove the negative correlation between the
403 ratio of SVs and time spent together in approach. Finally, unpaired females demonstrated a high
404 degree of repeatability in their approach, which was not affected by IN-OT. These data suggest
405 that the social context of pair bonding alters social approach, that the degree to which an individual
406 alters their social approach depends on their partner, and that OT facilitates pair-bonding-
407 dependent changes in social approach. Our results are consistent with a social convergence theory,
408 whereby OT unites pair-bonded individuals in the face of challenge, and that this coordination may
409 be related to changes in the proportions of vocalization types produced.

410 Recently, two other Peromyscus species were shown to have repeatability in behavior and
411 correlation between boldness behaviors [59], and other consistent individual differences have been
412 found in Peromyscus species [60], including California mice [39]. We extend these findings to
413 consider the change in behavior type resulting from the presence of a pair member, and
414 vocalizations and OT signaling as behavioral and hormonal mechanisms, respectively, under

415 affiliative social contexts. We observed that IN-OT given to females increased convergence in
416 approach scores for initially mismatched pairs but had no effect on either the convergence of pairs
417 that were initially matched or on total time unpaired females spent approaching the simulated
418 intruder, suggesting that OT changes in behavior may be related to a motivation to maintain partner
419 proximity in response to a single stimulus. While convergence has been shown to be asymmetrical
420 in convict cichlids, with the more reactive partners being more flexible than proactive partners
421 [43], we show that in California mice both individuals with low and high initial levels of social
422 approach change their behavior in response to pairing. Our results can be interpreted as
423 adjustments by both pair members to become more similar in behavioral type to their partners, and
424 not solely within-individual variation in approach response from the pre-pairing to the post-pairing
425 test, because unpaired individuals showed repeatable responses to the vocalization playback
426 stimuli. Taken together, our study provides experimental evidence of pair type-dependent changes
427 to social behavior that are facilitated by female OT signaling, and is consistent with prior evidence
428 of convergence by mismatched pairs [38, in review]. Moreover, it suggests that although individual
429 unpaired females demonstrate reliable and consistent approach behavior even in response to IN-
430 OT, the change in approach behavior induced by pairing mismatched individuals is malleable in
431 response to IN-OT.

432 Several hypotheses emerge for why IN-OT drives changes in behavior. First, it is possible
433 that social approach reflects underlying differences in stress and stress susceptibility, as the
434 stressed state of an individual influences both social approach and OT signaling. Social defeat
435 stress decreases OT receptor (OTR) gene expression in female California mice [19] and reduced
436 OTR binding in the NAc corresponds to reduced social approach following defeat [61]. Moreover,
437 plasma OT levels have been positively linked to attachment anxiety in romantic human

438 relationships [62], and pair bonding influences affective state across taxa [63,64]. Because we did
439 not directly measure stress or anxiety, we are unable to correlate individual differences in stress
440 response to social approach or pair bonding-induced convergence. However, while IN-OT may
441 have influenced anxiety-like behavior, the lack of IN-OT to change social approach in unpaired
442 individuals suggests no baseline interaction between stress and OT.

443 Another hypothesis for why IN-OT drove changes in behavior is that it increased the
444 motivation to bond. While it is possible that the behavioral convergence reflects a motivation to
445 stay closer together to maintain the pair bond, the second test occurred after the pairs were stably
446 bonded. IN-OT likely induced coordination of behavior beyond bonding, as evidenced by the
447 increase in time IN-OT pairs spent jointly approaching the stimulus, but not jointly avoiding.
448 Further evidence is suggested by the vocalization data: In this study we tracked two call types,
449 sustained vocalizations (SVs) and sweeps, because they may play a role in pair bond formation
450 and maintenance [58]. While mismatched pairs produced a higher proportion of sustained
451 vocalizations, indicating a potential behavioral mechanism underlying this coordination, IN-OT
452 drove a negative correlation between SV proportion and time spent together in the approach
453 chamber, suggesting that OT may be involved in the relationship between decreased SV
454 production and behavioral convergence. We found no difference in SV length between groups,
455 which has previously been shown to be a relevant characteristic for bonding pairs, with longer SVs
456 produced over the first week of pairing [56]. However, different results might have occurred if we
457 had recorded individual USVs as opposed to pair USVs. Moreover, the SVs in this study were
458 relatively short compared to those found by other studies (an average of about 115 ms compared
459 to, for example, an average of about 175 ms in [48]), but similar to those observed during

460 aggressive encounters (for example, an average of about 105 ms in [16]) which may indicate
461 negotiation or negative affect by pairs in this novel context.

462 Finally, another hypothesis for why IN-OT drove changes in behavior relates to pair
463 coordination and synchrony. Synchrony, an emergent behavior that occurs between two or more
464 individuals, such as eye contact, singing, or movement, is linked to greater affiliation and
465 cooperation [65]. California mice are socially monogamous and form strong affiliative bonds that
466 last for life [66]; OT may increase the ability of pair-bonded individuals in monogamous,
467 biparental species to perceive a partner's intent and thus provide complimentary behaviors to
468 efficiently address environmental challenges such as foraging, defending against predators, and
469 taking care of young.

470 It is interesting to note that IN-OT did not increase sweeps as it has previously been shown
471 to do so when given to mothers interacting with their pups [40]. This effect was context-dependent
472 such that OT increased sweeps in mothers only when mothers were in physical contact with their
473 pups. In the current study we observed an increase in sweeps in response to pairing as expected by
474 [48], but IN-OT did not increase production of sweeps. The reason for this is unknown, however,
475 it is possibly because the playbacks resulted in a less affiliative interaction, or that IN-OT effects
476 are restricted to mother-pup interactions.

477 Overall our results suggest that OT modulates social approach in a social context-
478 dependent fashion by driving initially mismatched pairs to become more matched by increasing
479 time spent in joint navigation, consistent with previous findings that OT signaling is critical to
480 both bonding [67] and social coordination [23]. Taken together these results suggest that affiliative
481 social context is critical for OT-regulated social approach and vigilance. An important direction
482 for future studies is determine the neural circuitry overlap between social approach and affiliative

bonding. Our results are in line with calls for researchers to consider social context when studying behavioral types [68,69].

Acknowledgements

Research was conducted at the University of Wisconsin-Madison, which occupies the ancestral Ho-Chunk land known as Teejop. Following an 1832 treaty, federal and state governments repeatedly, but unsuccessfully, sought to forcibly remove the Ho-Chunk from Wisconsin [70]. As members of a land grant institution we directly benefit from land theft, and we challenge ourselves and others to reflect on the perpetuation of the colonialist roots of western scientific progress. A. Auger, L. Riters, C. Guoynes, and C. Malone provided manuscript feedback, and Z. Herro and T. Nguyen helped with data collection. We also thank the UW-Madison animal research technicians. Research was supported by the National Science Foundation (IOS-1946613 and DGE-1747503).

Competing interests

Authors declare no competing interests.

Contributions

PKM, NSR, and CAM designed the study, PKM and JS conducted the experiments and data collection, PKM analyzed data, PKM wrote the initial draft of the manuscript, CAM and NSR provided guidance during analyses, and all authors contributed to the writing and review of the manuscript.

Data accessibility

Data available from the Open Science Framework: https://osf.io/2a8gm/

References

1. Dillard JR, Westneat DF. Disentangling the Correlated Evolution of Monogamy and Cooperation. Trends Ecol Evol. 2016;31(7):503–13.
2. French JA, Cavanaugh J, Mustoe A, Carp S. Social Monogamy in Nonhuman Primates: Phylogeny, Phenotype, and Physiology. J Sex Res. 2018;55(4–5):410–34.
3. Klug H. Why monogamy? A review of potential ultimate drivers. Front Ecol Evol. 2018;6:30.
4. Mayer M, Zedrosser A, Rosell F. Extra-territorial movements differ between territory holders and subordinates in a large, monogamous rodent. Sci Rep. 2017;7(1):1–10.
5. Dally JM, Emery NJ, Clayton NS. The Social Suppression of Caching in Western Scrub-Jays (Aphelocoma californica). Behaviour. 2005;142(7):961–77.
6. Borries C, Savini T, Koenig A. Social monogamy and the threat of infanticide in larger mammals. Behav Ecol Sociobiol. 2011;65(4):685–93.
7. Both C;, Visser ME. Breeding territory size affects fitness: an experimental study on competition at the individual level. J Anim Ecol. 2000;69(6):1021–30.
8. Thomas DW, Blondel J, Perret P, Lambrechts MM, Speakman JR. Energetic and Fitness Costs of Mismatching Resource Supply and Demand in Seasonally Breeding Birds. Science (80-). 2001;291(5513):2598–600.
9. Agrell J, Wolff JO, Ylönen H. Counter-Strategies to Infanticide in Mammals: Costs and Consequences. Oikos. 1998;83(3):507–17.
10. Rogers W. Parental Investment and Division of Labor in the Midas Cichlid (Cichlasoma citrinellum). Ethology. 1988;79(2):126–42.
11. Brotherton, Peter NM, Pemberton JM, Komers PE, Malarky G. Genetic and behavioural evidence of monogamy in a mammal, Kirk's dik-dik (Madoqua kirkii). Proc R Soc B Biol Sci. 1997;264(1382):675–81.
12. Thomas JA, Birney EC. Parental care and mating system of the prairie vole, Microtus ochrogaster. Behav Ecol Sociobiol. 1979;5(2):171–86.
13. Ahern TH, Hammock EAD, Young LJ. Parental division of labor, coordination, and the effects of family structure on parenting in monogamous prairie voles (Microtus ochrogaster). Dev Psychobiol. 2011;53(2):118–31.
14. Frazier CRMM, Trainor BC, Cravens CJ, Whitney TK, Marler CA. Paternal behavior influences development of aggression and vasopressin expression in male California mouse offspring. Horm Behav. 2006;50(5):699–707.
15. Marler CA, Trainor BC, Gleason ED, Bester-Meredith JK, Becker EA. The Effects of Paternal Behavior on Offspring Aggression and Hormones in the Biparental California Mouse. Neurobiol Parent Brain. 2008;435–48.
16. Rieger NS, Marler CA. The function of ultrasonic vocalizations during territorial defence by pair-bonded male and female California mice. Anim Behav. 2018;135:97–108.

562 17. Olff M, Frijling JL, Kubzansky LD, Bradley B, Ellenbogen MA, Cardoso C, Bartz JA,
563 Yee JR, van Zuiden M. The role of oxytocin in social bonding, stress regulation and
564 mental health: An update on the moderating effects of context and interindividual
565 differences. Psychoneuroendocrinology. 2013;38(9):1883–94.

566 18. Ditzen B, Schaer M, Gabriel B, Bodenmann G, Ehlert U, Heinrichs M. Intranasal
567 Oxytocin Increases Positive Communication and Reduces Cortisol Levels During Couple
568 Conflict. Biol Psychiatry. 2009;65(9):728–31.

569 19. Williams A V., Duque-Wilckens N, Ramos-Maciel S, Campi KL, Bhela SK, Xu CK,
570 Jackson K, Chini B, Pesavento PA, Trainor BC. Social approach and social vigilance are
571 differentially regulated by oxytocin receptors in the nucleus accumbens.
572 Neuropsychopharmacology. 2020;45(9):1423–30.

573 20. Eckstein M, Scheele D, Weber K, Stoffel-Wagner B, Maier W, Hurlemann R. Oxytocin
574 facilitates the sensation of social stress. Hum Brain Mapp. 2014;35(9):4741–50.

575 21. Shamay-Tsoory SG, Fischer M, Dvash J, Harari H, Perach-Bloom N, Levkovitz Y.
576 Intranasal Administration of Oxytocin Increases Envy and Schadenfreude (Gloating). Biol
577 Psychiatry. 2009;66(9):864–70.

578 22. DeWall CN, Gillath O, Pressman SD, Black LL, Bartz JA, Moskovitz J, Stetler DA. When
579 the Love Hormone Leads to Violence: Oxytocin Increases Intimate Partner Violence
580 Inclinations Among High Trait Aggressive People. Soc Psychol Personal Sci.
581 2014;5(6):691–7.

582 23. De Dreu CK, Greer LL, Kleef GA Van, Shalvi S, Handgraaf MJ. Oxytocin promotes
583 human ethnocentrism. Proc Natl Acad Sci. 2011;108(4):1262–6.

584 24. Shamay-Tsoory SG, Abu-Akel A. The Social Salience Hypothesis of Oxytocin. Biol
585 Psychiatry. 2016;79(3):194–202.

586 25. Harari-Dahan O, Bernstein A. A general approach - avoidance hypothesis of Oxytocin:
587 Accounting for social and non-social effects of oxytocin. Neurosci Biobehav Rev.
588 2014;47:506–19.

589 26. Kemp AH, Guastella AJ. The role of oxytocin in human affect: A novel hypothesis. Curr
590 Dir Psychol Sci. 2011;20(4):222–31.

591 27. Cavanaugh J, Mustoe A, Womack SL, French JA. Oxytocin modulates mate-guarding
592 behavior in marmoset monkeys. Horm Behav. 2018;106:150–61.

593 28. Marsh N, Scheele D, Feinstein JS, Gerhardt H, Strang S, Maier W, Hurlemann R.
594 Oxytocin-enforced norm compliance reduces xenophobic outgroup rejection. Proc Natl
595 Acad Sci. 2017;114(35):9314–9.

596 29. Wey TW, Vrana PB, Mabry KE. Mating system as a possible driver of behavioral
597 diversity in Peromyscus. Behav Ecol Sociobiol. 2017;71(11):163.

598 30. Merritt JF. Peromyscus californicus. Mamm Species. 1978;(85):1.

599 31. Gubernick DJ, Cully Nordby J. Behavioral Ecology and Sociobiology Mechanisms of
600 sexual fidelity in the monogamous California mouse, Peromyscus californicus. Behav
601 Ecol Sociobiol. 1993;32:211–9.

602 32. Trainor BC, Marler CA. Testosterone, Paternal Behavior, and Aggression in the
603 Monogamous California Mouse (Peromyscus californicus). Horm Behav. 2001;40(1):32–
604 42.

605 33. Bester-Meredith JK, Young LJ, Marler CA. Species differences in paternal behavior and
606 aggression in Peromyscus and their associations with vasopressin immunoreactivity and
607 receptors. Horm Behav. 1999;36(1):25–38.

608 34. Davis ES, Marler CA. The progesterone challenge: Steroid hormone changes following a
609 simulated territorial intrusion in female Peromyscus californicus. Horm Behav.
610 2003;44(3):185–98.

611 35. Marler CA, Oyegbile TO, Marler CA. Winning fights elevates testosterone levels in
612 California mice and enhances future ability to win fights. Horm Behav. 2005;48(3):259–
613 67.

614 36. Fuxjager MJ, Mast G, Becker EA, Marler CA. The "home advantage" is necessary for a
615 full winner effect and changes in post-encounter testosterone. Horm Behav.
616 2009;56(2):214–9.

617 37. Rieger NS, Stanton EH, Marler CA. Division of labour in territorial defence and pup
618 retrieval by pair-bonded California mice, Peromyscus californicus. Anim Behav.
619 2019;156:67–78.

620 38. Rieger NS, Monari PK, Hartfield K, Schefelker J, Marler CA. Pair-bonding leads to
621 convergence in approach behavior to conspecific vocalizations in California mice
622 (Peromyscus californicus). Manuscript submitted for publication. 2021.

623 39. Kalcounis-Rueppell MC, Pultorak JD, Marler CA. Ultrasonic Vocalizations of Mice in the
624 Genus Peromyscus. Handb Behav Neurosci. 2018;25:227–35.

625 40. Guoynes CD, Marler CA. An acute dose of intranasal oxytocin rapidly increases maternal
626 communication and maintains maternal care in primiparous postpartum California mice.
627 PLoS One. 2021;16(4 April 2021):e0244033.

628 41. Steinman MQ, Duque-Wilckens N, Greenberg GD, Hao R, Campi KL, Laredo SA,
629 Laman-Maharg A, Manning CE, Doig IE, Lopez EM, Walch K, Bales KL, Trainor BC.
630 Sex-specific effects of stress on oxytocin neurons correspond with responses to intranasal
631 oxytocin. Biol Psychiatry. 2016;80(5):406–14.

632 42. Duque-Wilckens N, Torres LY, Yokoyama S, Minie VA, Tran AM, Petkova SP, Hao R,
633 Ramos-Maciel S, Rios RA, Jackson K, Flores-Ramirez FJ, Garcia-Carachure I, Pesavento
634 PA, Iniguez SD, Grinevich V, et al. Extrahypothalamic oxytocin neurons drive stress-
635 induced social vigilance and avoidance. Proc Natl Acad Sci. 2020;117(42):26406–13.

636 43. Laubu C, Dechaume-Moncharmont F-XX, Motreuil S, Schweitzer C. Mismatched
637 partners that achieve postpairing behavioral similarity improve their reproductive success.
638 Sci Adv. 2016;2(3):e1501013.

639 44. Arueti M, Perach-Barzilay N, Tsoory MM, Berger B, Getter N, Shamay-Tsoory SG.
640 When Two Become One: The Role of Oxytocin in Interpersonal Coordination and
641 Cooperation. J Cogn Neurosci. 2013;25(9):1418–27.

642 45. Trainor BC, Bird IM, Marler CA. Opposing hormonal mechanisms of aggression revealed

643 through short-lived testosterone manipulations and multiple winning experiences. Horm
644 Behav. 2004;45(2):115–21.

645 46. Oyegbile TO, Marler CA. Weak winner effect in a less aggressive mammal: Correlations
646 with corticosterone but not testosterone. Physiol Behav. 2006;89(2):171–9.

647 47. Kalcounis-Rueppell MC, Metheny JD, Vonhof MJ. Production of ultrasonic vocalizations
648 by Peromyscus mice in the wild. Front Zool. 2006;3(1):1–12.

649 48. Pultorak JD, Alger SJ, Loria SO, Johnson AM, Marler CA. Changes in Behavior and
650 Ultrasonic Vocalizations During Pair Bonding and in Response to an Infidelity Challenge
651 in Monogamous California Mice. Front Ecol Evol. 2018;6:125.

652 49. Gubernick DJ. Reproduction in the California Mouse, Peromyscus californicus. J
653 Mammal. 1988;69(4):857–60.

654 50. Bales KL, Solomon M, Jacob S, Crawley JN, Silverman JL, Larke RH, Sahagun E,
655 Puhger KR, Pride MC, Mendoza SP. Long-term exposure to intranasal oxytocin in a
656 mouse autism model. Transl Psychiatry. 2014;4(11):480.

657 51. Leppanen J, Ng KW, Tchanturia K, Treasure J. Meta-analysis of the effects of intranasal
658 oxytocin on interpretation and expression of emotions. Neurosci Biobehav Rev.
659 2017;78:125–44.

660 52. Guoynes CD, Simmons TC, Downing GM, Jacob S, Solomon M, Bales KL. Chronic
661 Intranasal Oxytocin has Dose-dependent Effects on Central Oxytocin and Vasopressin
662 Systems in Prairie Voles (Microtus ochrogaster). Neuroscience. 2018;369:292–302.

663 53. Smith AS, Korgan AC, Young WS. Oxytocin delivered nasally or intraperitoneally
664 reaches the brain and plasma of normal and oxytocin knockout mice. Pharmacol Res.
665 2019;146:104324.

666 54. Duque-Wilckens N, Steinman MQ, Busnelli M, Chini B, Yokoyama S, Pham M, Laredo
667 SA, Hao R, Perkeybile AM, Minie VA, Tan PB, Bales KL, Trainor BC. Oxytocin
668 Receptors in the Anteromedial Bed Nucleus of the Stria Terminalis Promote Stress-
669 Induced Social Avoidance in Female California Mice. Biol Psychiatry. 2018;83(3):203–
670 13.

671 55. Rieger NS, Spool JA, Riters L V., Marler CA. Unpublished data.

672 56. Pultorak JD, Matusinec KR, Miller ZK, Marler CA. Ultrasonic vocalization production
673 and playback predicts intrapair and extrapair social behaviour in a monogamous mouse.
674 Anim Behav. 2017;125:13–23.

675 57. Timonin ME, Kalcounis-Rueppell MC, Marler CA. Testosterone pulses at the nest site
676 modify ultrasonic vocalization types in a monogamous and territorial mouse. Ethology.
677 2018;124(11):804–15.

678 58. Marler CA, Monari PK. Neuroendocrine control of vocalizations in rodents. In:
679 Neuroendocrine Regulation of Animal Vocalization. Elsevier; 2021. p. 201–16.

680 59. Underhill V, Pandelis GG, Papuga J, Sabol AC, Rife A, Rubi T, Hoffman SMG, Dantzer
681 B. Personality and behavioral syndromes in two Peromyscus species: presence, lack of
682 state dependence, and lack of association with home range size. Behav Ecol Sociobiol.

683 2021;75(1):1–16.

684 60. Careau V, Bininda-Emonds ORP, Thomas DW, Réale D, Humphries MM. Exploration
685 strategies map along fast-slow metabolic and life-history continua in muroid rodents.
686 Funct Ecol. 2009;23(1):150–6.

687 61. Wang L, Hou W, He Z, Yuan W, Yang J, Yang Y, Jia R, Zhu Z, Zhou Y, Tai F. Effects of
688 chronic social defeat on social behaviors in adult female mandarin voles (Microtus
689 mandarinus): Involvement of the oxytocin system in the nucleus accumbens. Prog Neuro-
690 Psychopharmacology Biol Psychiatry. 2018;82:278–88.

691 62. Marazziti D, Dell'Osso B, Baroni S, Mungai F, Catena M, Rucci P, Albanese F,
692 Giannaccini G, Betti L, Fabbrini L, Italiani P, Del Debbio A, Lucacchini A, Dell'Osso L.
693 A relationship between oxytocin and anxiety of romantic attachment. Clin Pract
694 Epidemiol Ment Heal. 2006;2(1):1–6.

695 63. Laubu C, Louâpre P, Dechaume-Moncharmont FX. Pair-bonding influences affective state
696 in a monogamous fish species. Proc R Soc B Biol Sci. 2019;286(1904).

697 64. Shaver PR, Hazan C. A biased overview of the study of love. J Soc Pers Relat.
698 1988;5(4):473–501.

699 65. von Zimmermann J, Vicary S, Sperling M, Orgs G, Richardson DC. The Choreography of
700 Group Affiliation. Top Cogn Sci. 2018;10(1):80–94.

701 66. Ribble DO, Salvioni M. Social organization and nest co-occupancy in Peromyscus
702 californicus, a monogamous rodent. Behav Ecol Sociobiol. 1990;26(1):9–15.

703 67. Bosch OJ, Young LJ. Oxytocin and social relationships: From attachment to bond
704 disruption. Curr Top Behav Neurosci. 2018;35:97–117.

705 68. Webster MM, Ward AJW. Personality and social context. Biol Rev. 2011;86(4):759–73.

706 69. Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. Integrating animal
707 temperament within ecology and evolution. Biol Rev. 2007;82(2):291–318.

708 70. Ostler J. Surviving Genocide: Native Nations and the United States from the American
709 Revolution to Bleeding Kansas. Yale University Press; 2019.

710