Oxytocin promotes convergence in personality between members of a monogamous pair

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Abstract

Social context is critical in shaping behavioral responses to stimuli and can alter an individual's behavioral type, which would otherwise be fixed in social isolation. For monogamous biparental vertebrates, social context is critical as interactions are frequent and consistent, involving high interindividual dependence and cooperation that can lead to large fitness impacts. We demonstrate that in the strictly monogamous and highly territorial California mouse, individuals alter approach response to an aggressive conspecific playback stimulus, barks, to become more similar to their partner during early bonding prior to pup birth; an effect distinct from assortative mating. Additionally, sustained vocalizations, an affiliative ultrasonic vocalization when used between pair members, correlate with increased behavioral convergence following pair formation suggesting a role for vocal communication in emergent pair behavior. We identified the neuropeptide oxytocin as sufficient to promote behavioral convergence in approach behavior of paired individuals who differed in their initial behavioral type. Social context, specifically pair-bonding, appears vital for behavioral responses to aggressive signals. While non-bonded animals maintained stable responses, pair-bonding led to an emergent property: convergence in behavioral responses. This convergence can be driven by oxytocin revealing a significant expansion in oxytocin's effects on behavioral coordination.

Keywords: Pair bonding; oxytocin; coordination; animal personality; *Peromyscus californicus*; California mouse

Introduction

Animals are thought to have fixed behavioral syndromes, traits that drive behavioral responses across situations(1), but the role of social context and, in particular, pair-bonding on behavioral syndromes remains unclear. Two competing hypotheses for pair-bonding effects on individual behavior exist: The social conformity hypothesis, where individuals alter behavior to become more similar to their peers and increasing group cohesion(2) and the facilitation hypothesis, where an individual's behavior becomes more polarized in the presence of a social partner(3). It is unknown if members of a pair-bond maintain their initial individual behavior, constituting a fixed behavioral syndrome, or alter their behavior based on their mate's behavioral type, constituting an emergent property of the pair.

Hormones, like social context, impact behavior(4). Notably, oxytocin (OT), an evolutionarily conserved neuropeptide, regulates social behaviors including aggression(5), affiliation(6), and communication(7), suggesting a crucial role in shaping individual personality within group-level dynamics. Social context accounts for many of OT's variable effects on behavior(8), however, how an individual's initial behavioral type determines OT's contributions to the production of pair-level behaviors in bonded animals remains unknown. Thus, pair-bonding individuals provide a unique system for integrating the study of OT and emergent properties of behavioral plasticity, as OT is a regulator of pair-bond formation and maintenance(9), and is involved in behavioral coordination(10).

To better understand how pair-bonding alters behavior, we studied the California mouse (*Peromyscus californicus*), a strictly monogamous rodent with lifelong pair-bonds(11). Individuals of both sexes show differences in boldness(12) and both sexes engage intruders to defend territory(13). Behavioral coordination between paired individuals is a fundamental

component of their compatibility(14,15). While some species(16,17) show rigid sex-specific coordination of behavior such that males invariably defend territories while females care for offspring, California mice show plasticity in their behavioral roles(18), making coordination between pair-bonded members critical. Importantly, paired California mice can show both synchronous or divided defense strategies in response to territorial intrusion, suggesting variability in pair coordination(18). Thus, it is likely that reproductive success is determined by partners' behavioral compatibility. Whether this compatibility is due to behavioral plasticity or assortative mating is unknown; as such, by studying how individual behavioral responses change before and after pair-bonding, we can gain important insights into how social context alters the behavior and coordination of animals(19).

Specifically, we investigated California mouse approach behavior towards playbacks of loud, aversive bark calls as an index of boldness, defined as a willingness to explore novel situations in spite of a threat(20). California mouse barks begin and end in the audible range (~12 kHz) with an intensity peak at ~20 kHz, consist of multiple harmonics that can extend above 50 kHz(21), and are frequently used during defensive aggression (13). Beyond barks, California mice produce a variety of ultrasonic vocalizations (USVs)(21) important for communication and behavioral coordination(13,22). We tracked two call types that may play a role in pair-bond formation and maintenance(22–25): sweeps, short contact calls in the 50-100 kHz range used during courtship(26) and in mother-to-pup communication(27)(unpublished), and sustained vocalizations (SVs), long, low bandwidth calls with harmonics that can be used to signal affiliation or aggression or act as contact calls between pair members(13,21,22).

To directly test the social conformity and the social facilitation hypotheses, we first tested if individuals maintained their approach strategy after pair-bonding. Moreover, we predicted that

pairs would produce more SVs if these USVs coordinate behavior between partners(21)., We then tested whether OT drives behavior changes by adminsitering intranasal OT (IN-OT) prior to testing, predicting greater convergence between pair members. We also predicted that OT treatment would increase affiliative USV production because of the well-characterized relationship between OT and affiliation (28).

Methods

Animals

2-3 same-sex conspecifics 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*. All tests occurred between 1–3 hrs after the onset of the dark cycle in dim red light in housing maintained at 20–23□ C on a 14:10 h light : dark cycle (lights on at 16:00 central standard time).

Experiment 1: 41 male and 41 female sexually naïve California mice (age 3-6 months) were tested for responses to unfamiliar conspecific bark playbacks. 62 mice (31 males, 31 females) were selectively paired with an opposite sex partner 3-7 days following the initial (i.e., pre-pairing) playback test and housed together in a standard cage. The 20 remaining individuals (10 males and 10 females) continued to be housed with their original cage mates.

Experiment 2: 48 males and 68 females were tested for response to bark playbacks. Of the 120 mice, 100 (48 males and 48 females) were selectively paired and housed as described above. The 20 remaining females were housed with their original cage mates to be used as controls.

Ethical statement

All animals were maintained according to the National Institute of Health *Guide for the care and use of laboratory animals*. All 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 Plexiglas cages (90 x 30 x 30 cm) lined with aspen bedding equally divided into three chambers (each 30 x 30 x 30 cm) with centrally located openings (11.5 x 11.5 cm Fig. 1A) between chambers to allow for free movement. A speaker (Vifa Dynamic Ultrasound, 1-120 kHz range, Avisoft Bioacoustics, Berlin, Germany) was placed at each end of the three-chambered apparatus 45 cm from the center. Speakers were positioned outside of the apparatus against a closed mesh gate (Fig. 1A).

Playback tracks

In a separate cohort of mice, barks were recorded from males and females housed under the same conditions as experimental mice to produce eight unique tracks per experiment, that were assigned randomly to individuals. To avoid habituation and maintain consistency, no individual heard the same track more than once (supplementary methods) (13).

Pre-pairing playback test

Mice were first tested for response to bark playbacks as nonbonded, sexually naïve individuals. Mice were placed in the testing apparatus for 5-10 mins to habituate and enter all three chambers (Fig. 1A). One male and two females were removed from Experiment 2 for not entering all three chambers by 10-min. Testing started with the mouse in the center chamber. 2-min playback preference tests were used with 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. Video and audio recordings were made of their behavior. We recorded time spent

in the chamber closest to the bark speaker ("bark chamber") as an approach score, and time spent in the chamber closest to the ambient noise speaker ("ambient noise chamber") as an avoidance score.

Behavioral type and pairing

Experiment 1: Following testing, mice were categorized as approachers or avoiders from a distribution of all individual responses to bark playbacks based on time spent in the bark chamber. Categories were defined using a median split (median = 30 s), with mice above the median deemed approachers and mice below the median deemed avoiders.

Mice were randomly assigned to be paired (n = 62) or remain unpaired controls (n = 20). Mice were selectively paired 3-7 days following the pre-pairing test into one of four groups: 1) male approacher with female avoider (n = 11), 2) female approacher with male avoider (n = 7), 3) male approacher with female approacher (n =5) and 4) male avoider with female avoider (n = 8). An initial 3-way interaction of sex x pair type x test indicated that groups could be split into two homogenous subsets with groups 1 and 2 making up one subset and groups 3 and 4 making up the second (supplementary methods). Thus, groups were collapsed into: 1) pairs that were initially different in response to barks (groups 1 and 2), with > 30 s difference in approach score between mice (n = 18), and 2) pairs that were initially similar (groups 3 and 4), with < 10 s difference (n = 13). The total difference in time spent in the bark chamber within a pair was also used for statistical analyses to give a continuous gradient of the similarity or difference within a pair.

Experiment 2: Following the pre-pairing test, mice were randomly assigned to be paired (n = 92) or to remain unpaired (n = 18). Mice were paired into two groups based on distribution and analyses on the Experiment 1 cohort: initially different (n = 28) or initially similar (n = 18).

Post-pairing playback tests in both Experiment 1 and Experiment 2

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 pre-pairing test). At 7 days post-pairing, pairs display hallmarks of pair-bonding(24) indicating that 10-11 days is sufficient for pair-bond formation. The playback procedure was the same as in the pre-pairing test except that paired mice were tested together as a pair. Both mice were placed into the central chamber and required to enter all three chambers prior to testing. One pair and one unpaired female in Experiment 2 were removed as they did not enter all three chambers by 10 mins. Time spent in each chamber was scored for each mouse. Pairs were scored for time spent together (both mice in the same chamber) and separate (different chambers). The procedure for unpaired mice was the same as the pre-pairing test.

Oxytocin dose and application

We administered a 0.8 IU/kg dose of IN-OT (Bachem, Torrance, CA, Prod #: 4016373). This dose induces changes in female behavior in animals(29) and approximates a weight-adjusted dose used in human studies(30). Intranasal administration is an established, non-invasive route of delivery for OT(31,32). Following pairing and before the post-pairing test in Experiment 2, mice were randomly assigned to either saline (different pairs: n = 15; similar pairs: n = 8; unpaired females: n = 10) or OT treatment (different pairs: n = 13; similar pairs: n = 10; unpaired females: n = 10) groups. Immediately prior to the 5-10 min habituation preceding the retest, females were given either IN OT or control saline (supplementary methods) while all males were administered saline. Only females were given OT because of unpublished data suggesting that IN-OT shifts the convergence/division of labor ratio for pairs defending their territories when given to females, but not males(33).

USV analysis

We recorded USVs with two Emkay/Knowles FG microphones (detection range 10-120 kHz) placed 85 cm apart at opposite corners of the apparatus (see supplementary methods). Because barks were not produced by mice in this study (typically produced when aggressive physical contact is made(13)), therefore two call types, sweeps and SVs, were analyzed(13). USVs (as defined by (21)) were differentiated by visual and auditory inspection of WAV files with sampling rates reduced to 4% of normal speed (11025 kHz) (see supplementary methods).

Statistics

All statistics were analyzed using SPSS v 22 (IBM Corp, Armonk, NY, USA). For Experiment 1, we analyzed changes between the pre-pairing and post-pairing tests using a mixed ANOVA with group, sex, and pre-pairing similarity of pairs as factors. For Experiment 2, we analyzed changes between the pre- and post-pairing tests using an ANOVA with group and treatment as factors. In both experiments, we analyzed USV call production by pairs and individuals using group, paired status and similarity of pairs as factors. Tukey post hoc tests were used to determine differences between groups. We used linear regressions to test if behavior predicted USV call production and call type proportion. Pairs were used as a covariate in all appropriate analyses. P-values were Holm-Bonferroni corrected for multiple comparisons.

Results

Experiment 1

Pre-pairing response by individuals to bark playbacks

During the pre-pairing test, mice showed a wide range of responses to bark playbacks. The range of time in the bark chamber was 0 – 115 out of 120 s (Fig. S1A). Time spent in the bark

chamber did not differ by sex (males: 29.71 ± 4.28 s; females: 39.76 ± 3.87 s, ANOVA, $F(1,80) = 3.032$, $p = 0.085$). Similarly, the range of time spent in the ambient noise chamber ranged from 0-115 s and the average time in the ambient noise chamber did not differ by sex (males: 35.55 ± 3.994 s; females: 37.07 ± 4.196 s, ANOVA, $F(1,72) = 0.066$, $p = 0.798$). Overall, mice did not show a preference for either the bark or the ambient noise chamber (ambient noise chamber: 36.42 ± 2.926 s, bark chamber: 36.19 ± 3.21, $t(71) = 0.041$, $p = 0.967$).

Post-pairing response to playbacks

Approach and avoidance:

Following pairing, a significant three-way interaction was found between sex, group, and pairing status on approach score such that initially different males and females altered approach behavior to be more similar to their partners. 'Approachers' decreased while 'avoiders' increased approach behavior, indicating that both behaviors can be altered (ANOVA, $F(4,72) = 4.327$, $p = 0.003$, Fig 1B). Using the overarching types of 'initially different' and 'initially similar' we found a significant two-way interaction between group and pairing status on approach score, such that mice that were initially different in their approach scores became more similar, and mice that were initially similar remained similar ($F(4,72) = 2.63$, $p = 0.041$, Fig 1C). As in the pre-pairing test, no preference was found for either the bark (26.71 ± 2.89 s) or ambient (39 ± 3.089s) noise chamber for individual mice during the second test ($t(61) = 0.443$, $p = 0.66$). There was also no difference in preference score based on pair group ($F(3,54) = 0.281$, $p = 0.839$).

Ultrasonic vocalizations:

During the post-pairing test, USV call production increased in both initially different (87.0 ± 16.37 USVs) and initially similar (66.08 ± 26.30 USVs) pairs compared to unpaired mice (0.8 ± 0.65 USVs) during bark playbacks (ANOVA, $F(2,48) = 9.78$, $p < 0.001$, Fig 2A). Specifically,

sweep production was increased in both initially different (80 ± 15.73) and initially similar (66.08 ± 25.81) compared to unpaired mice (0.80 ± 0.65) (ANOVA, $F(2,48) = 9.038$, $p < 0.001$, Fig. 2B) and total SV production was greatest in initially different pairs (7 ± 2.38) compared to both initially similar pairs (2.23 ± 1.06) and unpaired mice (0 ± 0) (ANOVA, $F(2,48) = 5.99$, $p = 0.005$ Fig. 2C). As relative proportions of USV types have been associated with pair affiliation(34), including proportion of SVs to total USVs(26), we calculated a ratio of pair SVs to total pair USVs. Initially different pairs (9.25 ± 0.28%) produced the greatest proportion of SVs as a function of total USVs compared to both initially similar pairs (2.57 ± 1.27%) and unpaired mice (0 ± 0%; Fig. 2D).

Total USVs and proportion of SVs correlated with several behavioral measures. Time spent together by pairs in the ambient noise chamber positively predicted total USVs (sweeps + SVs; linear regression, $F(1,29) = 4.253$, $R^2 = 0.1279$, $p = 0.048$, Fig. 2E). Importantly, increased similarity in approach score by pairs predicted total SVs as a dyad ($F(1,29) = 4.198$, $R^2 = 0.1265$, $p = 0.049$ Fig. 2F) and a greater proportion of SVs compared to all USVs ($F(1,29) = 5.872$, $R^2 = 0.1966$, $p = 0.023$, Fig. 2G)

Experiment 2

Individual response to bark playbacks during the pre-pairing test

As in Experiment 1, pre-paired mice displayed a wide range of responses to bark playbacks (supplementary results). The approach score difference was significantly higher in pairs with initially different (51.28 ± 3.26 s) than in pairs with initially similar approach responses (8.33 ± 1.57 s) ($X^2_1 = 503.00$, $p < 0.001$, Fig S2).

Post-pairing response to playbacks and oxytocin

Approach and Avoidance:

Following pairing, significant effects were found for pair type and treatment condition on approach score convergence for pairs, where convergence is the amount of change in approach time difference from pre- to post-pairing (same, saline: 17.62 ± 5.30 s, n = 8; same, OT: 13.00 ± 4.74 s, n = 10; different, saline: 30.13 ± 3.87 s, n = 15; different, OT: 45.23 ± 4.16 s, n = 13; ANOVA, $F(3,45) = 4.53$, $p = 0.007$). OT increased the degree of convergence in initially different pairs and they converged more than initially similar pairs ($F(3,45) = 64.69$, $p < 0.001$, Fig. 3D). There were nonsignificant trends of change in approach for both sexes when analyzed individually suggesting that both mice in a pair changed their behavior, but effects only became significant when pairs were considered as a dyad (supplementary results).

In unpaired control females, initial approach score predicted second approach score, indicating high test-retest reliability for unpaired mice ($F(1,16) = 17.229$, $R^2 = 0.5185$, $p < 0.001$, Fig. 3B). There was no decrease in approach time from the initial (31.61 ± 5.55 s) to the second trial (32.83 ± 5.63 s), suggesting no habituation to the stimulus ($t(17) = 0.212$, $p = 0.834$). In addition, OT did not alter approach during the second test for unpaired females ($F(1,16) = 0.415$, $p = 0.529$, Fig. 3C).

There was a significant main effect of OT in an ANOVA of treatment by pair type on time pairs spent together, where OT pairs (53.98 ± 3.00 s) spent more time together than control pairs (41.34 ± 3.12 s) (ANOVA, $F(1,45) = 7.239$, $p = 0.010$, Fig. 3E). Behavioral convergence likely occurred by increasing the time paired mice spent together, and accordingly, there was a simple effect whereby initially different OT pairs (56.14 ± 3.95 s) spent more time together throughout the test than did initially different control pairs (38.80 ± 3.68 s) (ANOVA, $F(1,45) = 10.362$, $p = 0.002$). In contrast, there was no difference in time spent together for initially similar OT pairs

(49.80 ± 5.92 s) and initially similar control pairs (43.88 ± 5.04 s) (ANOVA, $F(1,45) = 0.768$, $p = 0.386$).

Ultrasonic vocalizations:

As in Experiment 1, Mice were significantly more likely to produce USVs once paired (supplementary results). Neither pair type nor OT impacted the number of sweeps produced by pairs (average for all pairs: 139.00 ± 17.10 sweeps; ANOVA, $F(1,42) = 1.667$, $p = 0.204$, Fig. S4B). No effects of pair type, treatment, or their interaction were observed for total SV number alone (average for all pairs: 6.48 ± 0.84 SVs; ANOVA, $F(1,42) = 0.371$, $p = 0.546$, Fig. S4C) and time together in the ambient chamber did not predict the total number of pair USVs ($F(1,43) = 2.170$, $R^2 = 0.009$, $p = 0.079$, Fig. S5A).

There was a main effect of pair type on SV proportion: initially different pairs (different, saline: 7.4 ± 2.2%; different, OT: 9.9 ± 2.3%) produced a higher percentage of SVs as a function of total calls than did initially similar pairs (similar, saline: 3.1 ± 3.0%; similar, OT: 3.3 ± 2.7%; ANOVA, $F(1,42) = 4.482$, $p = 0.040$, Fig. 4F). There was also a nonsignificant simple effect trend whereby OT initially different pairs produced a higher percentage of SVs than did OT initially similar pairs ($F(1,42) = 3.377$, $p = 0.073$). However, as in Experiment 1, increased similarity in approach score by pairs following pair-bonding (convergence) was again predictive of pairs producing a greater proportion of SVs ($F(1,42) = 5.147$, $R^2 = 0.118$, $p = 0.028$, Fig. S5B)

Discussion

Behavioral syndromes are often fixed in socially isolated animals(1), however, behavioral responses can be altered by changing an individual's social environment(35), such as when a pair-bond develops. Here we describe for the first time in the monogamous California mouse

evidence of consistent, repeatable approach behaviors in the absence of another social individual that are then altered in an emergent, context-dependent fashion by pair-bonding leading to a 'pair personality'(36), or 'pair syndrome', whereby pairs with initially different behavioral types become more similar following pairing, while initially similar pairs maintain their similarity. We then demonstrate for the first time that IN-OT rapidly drives this pair convergence, suggesting that OT influences formation of a new 'pair personality,' results consistent with the social conformity hypothesis. To fully develop the concept of 'pair personality,' future studies will examine the longevity of this new behavior and if it occurs both when together and apart. Finally, we show that behavioral convergence is correlated with increased SVs, as a proportion of total USVs, indicating a potential role of USVs in behavioral coordination. With this study, we provide a novel monogamous mammalian model of behavioral plasticity due to pair-bonding that provides insights into how and why individuals become more similar in their behavior due to social change.

Behavioral plasticity following pair-bonding

California mouse pairs display variation in their approach during a territorial defense paradigm(18). Thus, we focused on their approach response to aggressive bark playbacks of intruding conspecifics during the time when bonding was initiated but prior to pup birth. Across taxa, aggression and boldness are correlated and often considered within the same behavioral syndrome(37). We found in Experiment 1 (replicated in Experiment 2), that initially different pairs changed their approach to bark playbacks to become more similar after pair-bonding, while initially similar pairs remained similar, regardless of the individuals' initial type. Because we measured behavioral responses prior to and following pair-bond formation, we provide evidence that similarity can occur independently of assortative mating (19); we also found high test-retest

reliability in unpaired animals similar to measures of boldness in other animals(38), suggesting that behavioral changes were the result of pairing. Our findings are consistent with evidence that newly paired convict cichlids make post-pairing adjustments to become more similar along a proactive-reactive axis encompassing boldness, that is associated with increased fitness(14).

An emerging question regarding monogamous, biparental species is how they are capable of both maintaining bonds and coordinating labor after offspring are born. Pair-bonded California mice face many challenges including foraging, pup care, mate attendance, and territorial defense, and coordination may promote task efficiency and/or pair-bonding. Increased similarity may afford greater cooperation within the pair-bond to complete tasks related to territorial defense. This would align with data in voles(39) and California mice(18) showing that bonded males and females can participate in the same tasks. Alternatively, coordination may strengthen the pair-bond as indicated by increased contact time including huddling and grooming(40). Long term maintenance of the pair-bond may explain pair coordination since pair duration can be associated with reproductive success(41), and while California mouse pairs display hallmarks of pair-bonding including side by side contact, reduced aggression, increased affiliation and increased affiliative USV calls by 7 days following pairing(24), we expect that pair-bond maintenance is an ongoing process. We speculate that the optimal coordination strategy depends on the intensity and type of challenge. In this study, pairs were both nonparental and nonresidents; as such, in a novel environment, pairs may prefer to investigate their environment together in the absence of a nest to act as a central, safe, home location. Pairs may be less willing to act together with pups present, as this may increase pup exposure and, as seen in prairie voles, biparental pairs tend to reduce time that pups are left alone(39). Future research will determine if increased similarity is

295 due to increased pair coordination by exposing pairs to a challenge in which a different
296 coordinated strategy becomes advantageous.

297 *Behavioral convergence and oxytocin*

298 In Experiment 2, we observed that OT increased behavioral convergence of initially different
299 mice but had no effect on either the convergence of pairs that were initially similar or on total
300 time paired or unpaired females spent approaching the simulated intruder, suggesting that OT
301 effects rely on social context. Moreover, IN-OT increased total time that paired mice spent
302 together. Our findings indicate that the convergence effect observed for initially different pairs is
303 increased by OT. While OT may have influenced anxiety-like behavior(42), the lack of OT effect
304 on unpaired mice suggests that the behavioral changes only occurred at the level of the pair. OT
305 plays a role in pair-bonding, cooperation, and social recognition(43,44) indicating that OT
306 increases social cue salience(45).

307 Additionally, our findings are in line with previous research in humans indicating that OT is
308 important for synchrony(46). Synchrony is an emergent behavior that occurs between two or
309 more individuals such as eye contact, singing, or movement, and is linked to greater affiliation
310 and cooperation(47). Thus, increased similarity resulting from OT in our study suggests that OT
311 may function to increase pair affiliation. Additionally, OT may increase the ability of pair-
312 bonded individuals in monogamous, biparental species to perceive a partner's intent and thus
313 provide complimentary behaviors to efficiently address environmental challenges such as
314 foraging, defending against predators, and taking care of young.

315 *Ultrasonic vocalizations*

316 Are pairs communicating with each other and could this influence development of emergent
317 “pair personalities”? In both experiments, pairs produced more USVs than individuals,

suggesting that USVs are expressed either because of 1) an internal state change when near their partner that does not alter their partner's behavior, or 2) an attempt to influence changes in the partner's behavior to achieve an efficient unit for raising young and defending a territory. Additionally, since the proportion of SVs to total USVs correlated with increasing similarity in approach behaviors between pairs, USVs may mediate behavioral coordination within an aggressive context. Across species, communication is important for coordinating social behaviors, such as responses to threats(48), territorial defense(49), and information sharing(50). As such, communication may play an important role in coordinating behavioral similarities within pairs.

Moreover, OT did not significantly alter USVs, in Experiment 2 suggesting that OT is not involved in the relationship between USVs and behavioral convergence, aligning with unpublished research indicating that OT affects USVs when given to males but not females during paired territorial defense(33). However, different results might have occurred if we had recorded individual USVs as opposed to pair USVs. We did, however, find anecdotal evidence that both sexes produced calls, namely that overlapping SVs and overlapping sweep and SVs were detected across behavioral trials in pairs.

Conclusion

In collective behaviors, individuals often follow simple rules resulting in group-level characteristics difficult to predict based on individual-level behaviors. We provide evidence in monogamous rodents, that formation of a social partner bond can lead to conformity in social behavior. Overall, we found that nonparental, pair-bonded California mice showed changes in aversive responses to stimuli leading to greater similarity in behavior, and that IN-OT increased this effect. Furthermore, this increased behavioral similarity corresponded with increased

341 communication in the form of SVs indicating, for the first time in a monogamous rodent, the
342 potential importance of vocal communication for coordinating behavior between mates in order
343 to increase behavioral similarity. Our research adds to a growing body of literature underscoring
344 the importance of accounting for individual-level variation and its role in producing emergent
345 variation at the level of the pair.

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356

357 **Competing interests**

358 Authors declare no competing interests

359

360 **Contributions**

PKM, NSR, and CAM designed the study, PKM, NSR, KH and JS conducted the experiments and data collection, PKM and NSR analyzed data, PKM led writing of the manuscript, CAM provided guidance during analyses, and all authors contributed to and reviewed the manuscript.

Data accessibility

Data available from the Open Science Framework: https://osf.io/kv6um/

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Figure legends

Figure 1. Experiment 1. Paired individuals changed their approach to bark playbacks to become more similar. **A**. Experimental overview: example of paired approacher female (blue box) and avoider male (yellow box) tested together (green box). **B.** A three-way interaction occurred between sex, group, and approach score pre- and post-pairing; sex differences occurred when pairs were initially different pre-pairing. Approacher male and avoider female (left, 'M approach F avoid') decreased time in the bark chamber from pre- (light bars) to post-pairing (dark bars) while the female (right) increased time in the bark chamber in the post-pairing test, creating a significant sex difference. Conversely, avoider males paired with approacher females ('M avoid F approach') increased time in the bark chamber following pairing, while the females decreased time in the bark chamber. Neither sex showed differences from pre- to post-pairing. **C.** A two-way interaction revealed that pairs with one approacher and one avoider decreased their difference in approach score from the pre- (light bars) to the post- test (dark bars).

499 Overall, initially different pairs became more similar, and initially similar pairs and unpaired individuals
500 remained similar. (a, b, * = $p < 0.05$). Significant lower-order effects are not indicated for any analyses.

501

502 ***Figure 2. Experiment 1.*** USVs correlated with visual behavioral coordination. **A**. Total SV calls
503 increased in both pairs with initially different approach scores (dark gray) and initially similar approach
504 scores (light gray) compared to unpaired individuals. **B**. Total number of sweeps increased in both
505 initially different and initially similar pairs compared to unpaired individuals. **C**. Total mean number of
506 SV calls increased in initially different pairs compared to all other groups. **D**. The proportion of SV calls
507 as a function of total calls increased in initially different pairs compared to all other groups. **E**. Pairs that
508 spent more total time together in the ambient noise chamber in response to bark playbacks produced
509 significantly more total USV calls, including sweeps and SVs. **F**. Pairs with greater similarity in post-
510 pairing approach scores produced more SVs as a dyad. Pairs that became more similar (pre-pairing
511 approach score difference - post-pairing approach score difference) as denoted by positive numbers on the
512 x-axis, produced more total SV calls as well as **G**. a significantly greater proportion of SV calls to total
513 USV calls produced (* = $p < 0.05$).

514

515 ***Figure 3. Experiment 2.*** OT increased behavioral coordination in pairs that were initially different. **A.**
516 Experimental overview. **B**. Initial approach to bark playbacks predicted approach to bark playbacks 13-17
517 days later in unpaired females (light circles: saline, dark circles: OT). **C**. OT (dark bar) did not impact
518 unpaired female approach during the second test. **D.** Change in pair difference from pre- to post-pairing
519 that indicates how much pairs became more similar. Initially different pairs converged more, and OT
520 treatment increased this effect. **E.** Overall OT pairs spent more time together than control pairs during
521 post-pairing. **F.** Initially different pairs produced a higher proportion of SVs, with a nonsignificant trend
522 for OT to increase SV proportion in initially different pairs. (# = $p < 0.10$; * = $p < 0.05$; ** = $p < 0.005$;
523 *** = $p < 0.001$).

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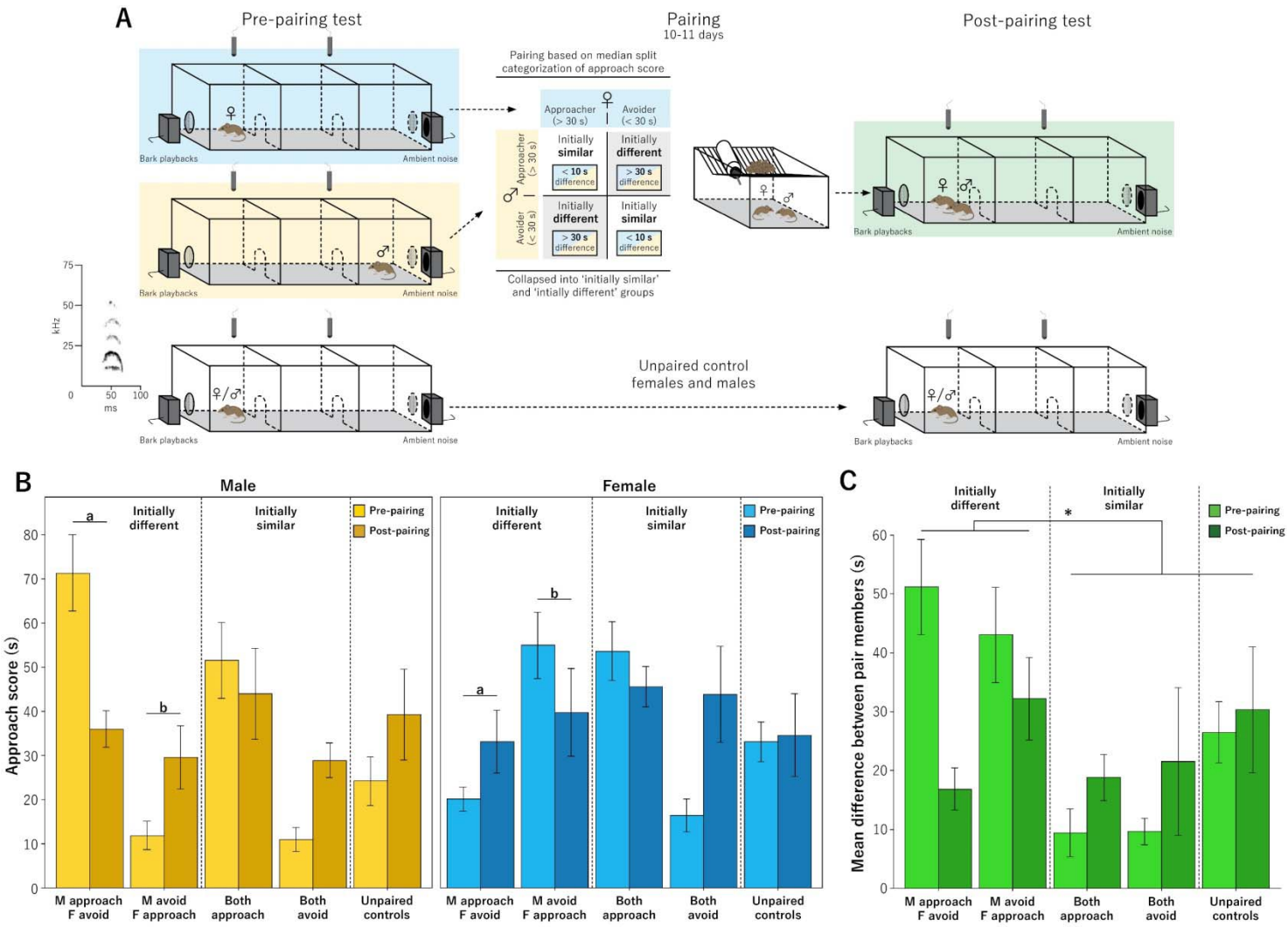

Figure 1.

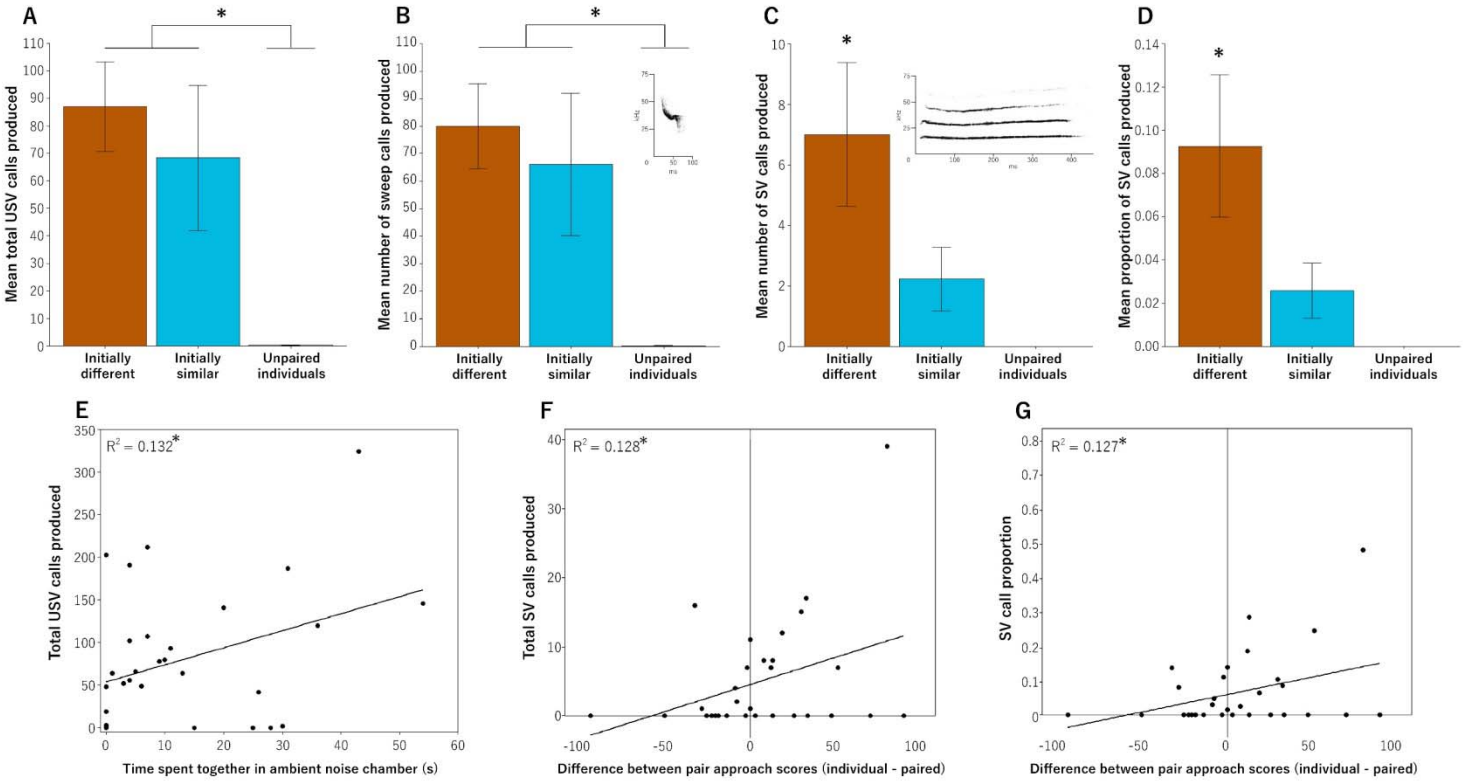

Figure 2.

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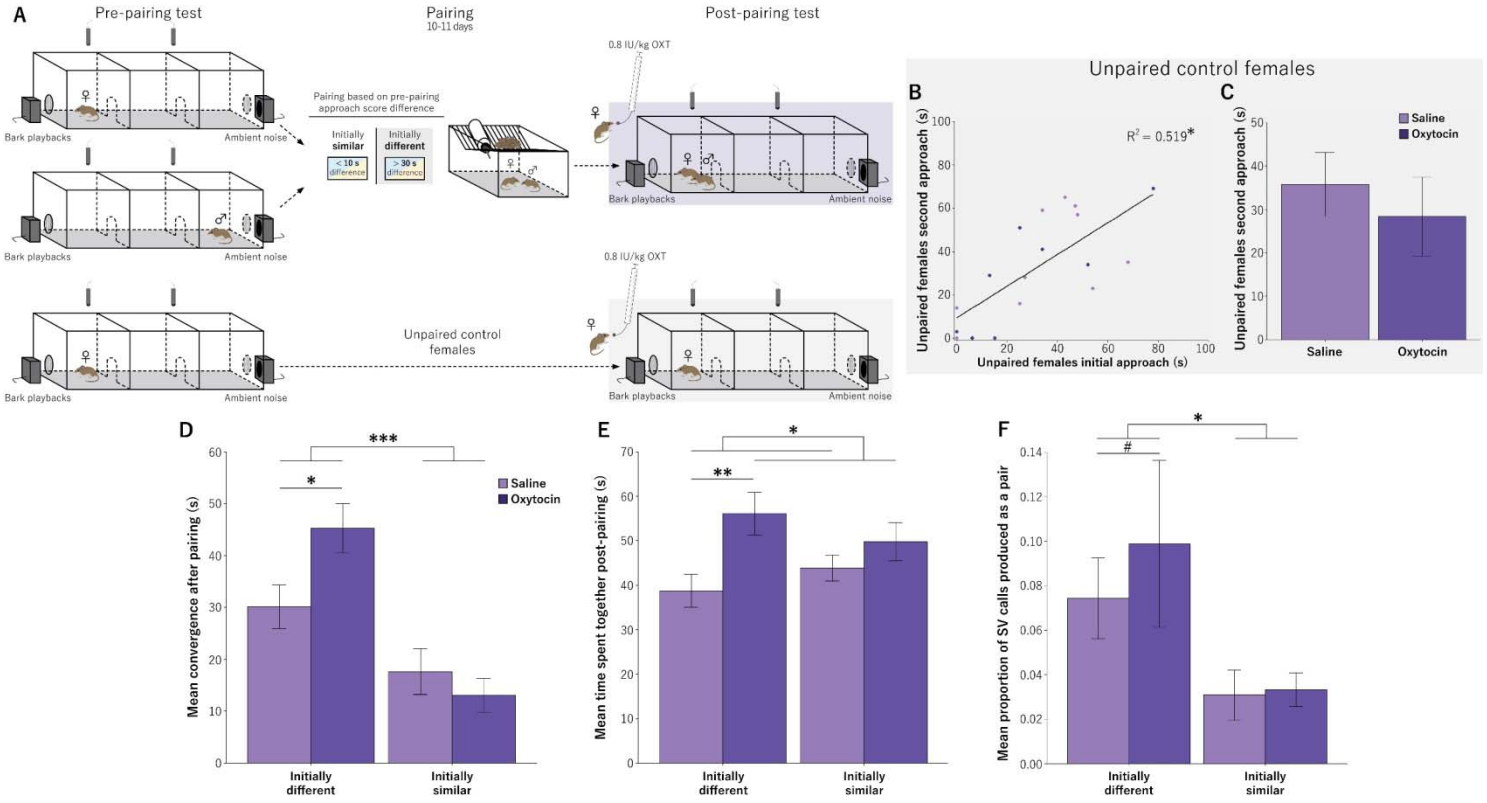

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551 ***Figure 3.***