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 members of a pair, are associated with increased behavioral convergence following pair formation suggesting a vocal communication role in emergent pair behavior. We identified the neuropeptide oxytocin as sufficient to promote behavioral convergence in paired individuals who differed in their initial behavioral type, as characterized by approach behavior. Social context, specifically pair-bonding, appears vital for behavioral responses to aggressive signals. While non-bonded animals maintained stable responses, pair-bonding led to a pair emergent property, a 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(1), traits that drive behavioral responses across situations(2), but the role of social context and, in particular, pair-bonding on behavioral syndromes remains unclear with two competing hypotheses emerging for pair-bonding effects on individual behavior. The social conformity hypothesis states that individuals alter behavior to match their social group, becoming more similar to their peers and increasing group cohesion(3). Conversely, in the facilitation hypothesis, an individual's behavioral type will become more polarized in the presence of a social partner(4). 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 the social environment, impact behavior(5). Notably, oxytocin (OT), an evolutionarily conserved neuropeptide, regulates social behaviors including aggression(6), affiliation(7), and communication(8), suggesting a crucial role in shaping individual personality within group-level dynamics. Social context accounts for many of the variable effects of OT on behavior(9–11), however, how an individual's initial behavioral type determines the contribution of OT 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(12), and is involved in behavioral coordination(13,14).

To better understand how pair-bonding alters behavior, we studied the California mouse (*Peromyscus californicus*), a strictly monogamous rodent with lifelong pair-bonds(15). Individuals of both sexes show differences in boldness(16), such that both sexes defend their

territory and engage intruders(17–19). The behavioral coordination between paired individuals is a fundamental component of their compatibility(20,21). While some species(22,23) show rigid sex-specific coordination of behavior such that males invariably defend territories while females care for offspring, monogamous rodents such as California mice show plasticity in their behavioral roles(24), making coordination between pair-bonded members critical. Thus, it is likely that reproductive success is determined by behavioral compatibility between partners. Whether this compatibility is due to behavioral plasticity or assortative mating is unknown; as such, by studying how individual behavioral responses change after a major life-history event such as pair-bonding, we can gain important insights into how social context alters the behavior and coordination of animals(25).

Specifically, we investigated California mouse approach behavior towards playbacks of aversive bark calls as an index of boldness, defined as a willingness to explore novel situations in spite of a threat(26). California mouse barks are loud, aversive calls that begin and end in the audible range (~12 kHz) with an intensity peak at ~20 kHz, and consist of multiple harmonics that can extend above 50 kHz(27). Barks are frequently used during defensive aggression (19). Importantly, paired California mice can show both synchronous or divided defense strategies in response to territorial intrusion, suggesting variability in pair coordination(24). We examined pair vocal communication as a potential mechanism of pair coordination. Beyond barks, California mice produce a variety of ultrasonic vocalizations (USVs)(27), important for communication and behavioral coordination in a variety of contexts(19,28). We tracked two call types that may play a role in pair-bond formation and maintenance(28–31): sweeps and sustained vocalizations (SVs). Sweeps are short contact calls in the 50-100 kHz range used during courtship(32) and in mother-to-pup communication(33)(unpublished), and SVs are long, low

bandwidth calls with harmonics that can be used to signal affiliation or aggression or act as contact calls between pair members(19,27,28).

In order to select between the social conformity and the social facilitation hypotheses in Experiment 1, we tested if individuals maintained their approach strategy depending on whether or not they were paired. Moreover, we predicted that pairs would produce more affiliative USV calls used in pair communication, if these USVs coordinate behavior between partners(27). In Experiment 2, we tested whether OT drives behavior changes by repeating Experiment 1 with a dose of intranasal OT (IN-OT), predicting a 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 (34).

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. Of the 82 total mice, 62 (31 males, 31 females) were selectively paired with an opposite sex partner 3-7 days following the initial (i.e., pre-pairing) playback test. Paired mice were housed together in a standard cage identical to that described above. 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 between chambers. 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 male and female mice housed under the same conditions as experimental mice to produce the bark playback recording stimuli (see supplementary methods) (19). We used eight unique tracks per experiment, and assigned tracks to individuals randomly with each track used between 15-17 times in Experiment 1, and 20-23 times in Experiment 2, ensuring that no individual heard the same track more than once over the course of the two tests (to avoid habituation and maintain consistency). Playback amplitude was

matched to the original bark amplitude produced by placing an Emkay/Knowles FG series microphone 30 cm away from the playback speaker and adjusting the output gain/volume to match the original recording(28).

Pre-pairing playback test

All animals were first tested for their response to conspecific bark playbacks as nonbonded, sexually naïve individuals. Nonbonded individual animals were placed in the central chamber 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. Once habituated, testing started with the mouse in the center chamber. 2-min playback preference tests were used with playback speakers placed at opposite ends of the apparatus behind a wire mesh with one playback speaker playing a bark track and, concurrently, the other an ambient noise track. Video and audio recordings were made to track their behavior. We recorded time spent in the chamber closest to the bark playback speaker ("bark chamber") as an approach score, and time spent in the chamber closest to the ambient noise playback speaker ("ambient noise chamber") as an avoidance score. USV production by focal animals was recorded using two Emkay/Knowles FG series microphones placed at opposite ends of the apparatus 30 cm from the cage floor.

Behavioral type and pairing

Experiment 1: Following testing, animals were categorized as being either approachers or avoiders by creating a distribution of all individual responses to bark playbacks based on time spent in the bark chamber. Categories were defined using a median split on time spent in the bark chamber (median = 30 s), creating two behavioral types: mice above the median were more likely to approach (approachers) while mice below the median were less likely to approach

(avoiders). Approachers were defined as mice spending more than 30 s in the bark chamber while avoiders were defined as mice that spent less than 30 s in the bark chamber.

Mice from each category were randomly assigned to either be paired (n = 62) or remain unpaired (n = 20) as a control. Paired mice were selectively paired 3-7 days following the pre-pairing test into one of four groups based on their behavioral classification: 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 analysis of sex x pair type x test showed that groups could be split into two homogenous subsets with groups 1 and 2 making up one subset of pairs and groups 3 and 4 making up the second homogenous subset (see supplementary methods). Thus, these groups were collapsed into two overarching types: 1) pairs that were initially different in their response to bark playbacks (groups 1 and 2), with > 30 s difference in approach score between mice (n = 18), and 2) pairs that were initially similar in their response to bark playbacks (groups 3 and 4), with < 10 s difference in approach score between individuals (n = 13). Along with behavioral classification, the total difference in time spent in the bark chamber within a pair was used for statistical analyses to give a continuous gradient of the similarity or difference within a pair.

Experiment 2: We paired mice into groups based on distribution and analyses on the Experiment 1 cohort. 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: initially different (n = 28) or initially similar (n = 18). As with Experiment 1, initially similar pairs had < 10 s difference between pre-pairing approach scores, and initially different pairs had > 30s difference. There was a similar number of pairs in which the female had the higher approach score and the

male had the higher approach score (different: 15 pairs female higher, 13 pairs male higher; similar: 9 pairs female higher, 9 pairs male higher).

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). At 7 days post-pairing, pairs displayed hallmarks of pair-bonding including side by side contact, reduced aggression, increased affiliation and increased affiliative USV calls(30) 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 while nonpaired controls were tested alone. For paired mice, both 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 either one or both mice did not enter all three chambers by 10 mins. Time spent in each chamber was scored for each mouse. Pairs were scored for both time together (both mice in the same chamber) or separate (different chambers). The procedure for nonpaired mice was the same as the pre-pairing test. USV production was scored for both pairs and nonpaired mice.

Oxytocin dose and application

The 0.8 IU/kg dose of IN-OT (Bachem, Torrance, CA, Prod #: 4016373) used in this study has produced changes in female behavior in animal studies(35) and approximates a weight-adjusted, clinically-relevant dose often used in human studies(36). Intranasal administration has become an established, non-invasive route of delivery for OT (37–39), and social behavior in both animals and humans is impacted by IN-OT (40,41). Following pairing and before the post-pairing test, Experiment 2 pairs and unpaired 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, single females were given either IN OT or control saline. Members of a pair were given saline or OT at the same time. 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(42). To administer, a mouse was scruffed and 25 μl of solution was administered to the nostrils using a blunt needle attached to cannula tubing at the end of a Hamilton syringe. Individual droplets were beaded at the end of the syringe, applied to the surface of the nose, and allowed to absorb into the nasal mucosa. Administration lasted less than 30 s per animal. Habituation was conducted as described earlier, with the test initiation in the central chamber.

USV analysis

We recorded USVs with two Emkay/Knowles FG series 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 (these only appear to occur when physical contact is made(19)), therefore two call types, sweeps and SVs, were analyzed(19). USVs (as defined by (27)) 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 test and post-pairing test using a mixed ANOVA with group, sex, and pre-pairing similarity of pairs as factors. For Experiment 2,

we analyzed changes between the pre-pairing 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 the number of 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). There were no sex differences in time spent in the bark chamber, although there was a nonsignificant trend for females to spend more time in the bark chamber (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 between the sexes (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, significant three-way and two-way interactions were found. 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 partner. ‘Approachers’ decreased approach while ‘avoiders’ increased approach behavior, indicating that both behaviors can be altered (ANOVA, $F(4,72) = 4.327$, $p = 0.003$, Fig 1B). As described, we used the overarching types to form the ‘initially different’ group and the ‘initially similar’ group, which were used for all subsequent analyses. A significant two-way interaction between group and pairing status on approach score was found, such that mice that were initially more different in their approach scores became more similar, and mice that were initially more similar in approach scores 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 animals 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) in response to bark playbacks (ANOVA, $F(2,48) = 9.78$, $p < 0.001$, Fig 2A). Bark playbacks also revealed changes in specific call types. Sweep production was increased in both initially different (80 ± 15.73 sweeps) and initially similar (66.08 ± 25.81 sweeps) pairs compared to nonpaired mice (0.80 ± 0.65 sweeps) (ANOVA, $F(2,48) = 9.038$, $p < 0.001$, Fig. 2B), and pairs that were initially different (7 ± 2.38 SVs) produced the greatest total number of SV calls when compared to both pairs that were initially similar (2.23 ± 1.06 SVs) and nonpaired

individuals (0 ± 0 SVs) (ANOVA, $F(2,48) = 5.99$, $p = 0.005$ Fig. 2C). As relative proportions of USV types have been associated with pair affiliation(43), including proportion of SVs to total USVs(32), we calculated a ratio of pair SVs to total USVs for pairs. Pairs that were initially different (9.25 ± 0.28%) produced the greatest proportion of SV calls as a function of total calls when compared to both initially similar pairs (2.57 ± 1.27%) and nonpaired mice (0 ± 0%) during this post-playback period (Fig. 2D).

Total USV calling and proportion of SV calling correlated with several behavioral measures. Time spent together by pairs in the ambient noise chamber positively predicted total USV calling (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 following pair-bonding was predictive of both pairs producing more total SVs as a dyad ($F(1,29) = 4.198$, $R^2 = 0.1265$, $p = 0.049$ Fig. 2F) and a greater proportion of SV calls compared to all calls ($F(1,29) = 5.872$, $R^2 = 0.1966$, $p = 0.023$, Fig. 2G)

Experiment 2

Individual response to bark playbacks during the pre-pairing test

As in Experiment 1, individual mice displayed a wide range of responses to bark playbacks during the pre-pairing test, with time spent in the bark chamber ranging from 0 – 101 s for all mice combined with no difference between males (38.46 ± 4.15 s) and females (32.59 ± 3.32 s) (ANOVA, $F(1,111) = 1.239$, $p = 0.268$). Time spent in the ambient noise chamber ranged from 0 – 112 s, again, with no difference in the average time spent in the ambient noise chamber between males (38.35 ± 3.97 s) and females (44.39 ± 3.80 s) (ANOVA, $F(1,111) = 1.131$, $p = 0.290$). Similar to Experiment 1, there was no significant preference for either the bark (35.05 ± 2.60 s) or ambient noise (41.86 ± 2.80 s) ($t(110) = 0.041$, $p = 0.967$) chambers. As expected, the

approach score difference was significantly higher in pairs with ‘different’ approach response (51.28 ± 3.26 s) than in pairs with ‘similar’ approach response (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 the pre-pairing to the post-pairing test (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 ‘different’ pairs and, as expected, ‘different’ pairs converged more than ‘similar’ pairs ($F(3,45) = 64.69$, $p < 0.001$, Fig. 3D). There were also nonsignificant main and simple trends of change in approach for both sexes when analyzed separately instead of as pairs, suggesting that both mice in a pair changed their behavior, but effects only became significant when pairs were considered as a dyad. Females from OT ‘different’ pairs tended to alter their approach score more from pre-pairing to post-pairing (same, saline: 21.75 ± 4.73 s; same, OT: 19.1 ± 4.23 s, different, saline: 17.73 ± 3.46 s, different, OT: 27.46 ± 3.71 s; ANOVA, $F(1,45) = 3.06$, $p = 0.087$, Fig. S3), as did males from OT pairs (same, saline: 19.88 ± 4.05 s; same, OT: 23.10 ± 3.62 s, different, saline: 19.27 ± 2.96 s, different, OT: 27.92 ± 3.18 s; ANOVA, $F(1,45) = 2.92$, $p = 0.095$, Fig. S3).

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). Moreover, there was no decrease in approach time from the initial trial (31.61 ± 5.55 s)

to the second trial (32.83 ± 5.63 s), suggesting no habituation occurred to the stimulus ($t(17) = 0.212$, $p = 0.834$). In addition, OT did not alter approach during the second test for these unpaired females in the absence of social stimuli, suggesting that OT only alters behavioral approach in a social context ($F(1,16) = 0.415$, $p = 0.529$, Fig. 3C).

In an ANOVA of treatment by pair type on time pairs spent together overall, there was a significant main effect of OT, 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 'different' OT pairs (56.14 ± 3.95 s) spent more time together throughout the test than did '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 'similar' OT pairs (49.80 ± 5.92 s) and 'similar' control pairs (43.88 ± 5.04 s) (ANOVA, $F(1,45) = 0.768$, $p = 0.386$).

Ultrasonic vocalizations:

As in Experiment 1, there was a main effect of pair type on SV proportion, whereby '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 '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). Additionally, there was a nonsignificant simple effect trend whereby OT 'different' pairs produced a higher percentage of SVs than did OT 'similar' pairs ($F(1,42) = 3.377$, $p = 0.073$).

Mice were significantly more likely to produce USVs once paired, as in Experiment 1 (pre-pairing: 1.60 ± 3.09 USVs, post-pairing: 145.45 ± 113.52 USVs; $t(43) = 67.583$, $p < 0.001$). As expected, unpaired females (1.17 ± 1.82 USVs) produced many fewer USVs than pairs (145.45 ±

113.52 USVs) during the post-pairing test (ANOVA, $F(1,58) = 28.792$, $p < 0.001$, Fig. S4A). Neither pair type nor OT impacted the total 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). 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 SV calls ($F(1,42) = 5.147$, $R^2 = 0.118$, $p = 0.028$, Fig. S5B).

Discussion

Behavioral syndromes are often fixed in socially isolated animals(2), however, behavioral responses can be altered by changes to an individual's social environment(44,45), such as when a pair-bond develops. Our results are consistent with the social conformity hypothesis(46), whereby paired individuals altered their behavior to match their partner. 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'(47), 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 can rapidly drive this pair convergence, suggesting that OT may be critical for formation of a new 'pair personality'. To fully develop the concept of 'pair personality,' future studies will examine if this new emerging behavior lasts over multiple tests and occurs both when together and apart. Finally, we show that behavioral convergence is

correlated with increased SV calls, as a proportion of total USV production, 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 can provide 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(24). Thus, we focused on the time of post-pairing, when bonding was initiated but prior to pup birth, and their approach response to playbacks of intruding conspecifics emitting aggressive barks. Across taxa, aggression and boldness are correlated and often considered within the same behavioral syndrome(48,49). When we created pairs consisting of mice that were similar or different in their approach to a speaker playing barks, we found in Experiment 1 (replicated in Experiment 2), that initially different pairs changed their approach 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 (25); we found high test-retest reliability in unpaired animals similar to that found for measures of boldness in other animals(50), 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 interestingly associated with increased fitness(20).

An emerging question regarding monogamous and biparental species is how they are capable of both bonding and then 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(51) and California mice(24) showing that bonded males and females can participate in the same tasks. Alternatively, coordination may strengthen the pair-bond. One indicator of both forming and maintaining pair-bonds is increased contact time, such as huddling or grooming(52). Long term maintenance of the pair-bond may explain pair coordination since pair duration can be associated with reproductive success(53), 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(30), 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(51). Future research will determine if the increased similarity is due to increased pair coordination by exposing pairs to a challenge in which a different coordinated strategy becomes advantageous.

Behavioral convergence and oxytocin

In Experiment 2, we observed that OT increased behavioral convergence of initially different mice but had no effect on either the convergence of pairs that were initially similar or on total time paired or unpaired females spent approaching the simulated intruder, suggesting that OT

effects rely on social context. Moreover, IN-OT increased total time that paired individuals spent together. Our combined findings suggest that the convergence effect observed for initially different pairs is increased by OT. While OT may have influenced anxiety-like behavior(41), the lack of OT effect on unpaired mice suggests that the behavioral changes only occurred at the level of the pair. OT plays a role in pair-bonding, cooperation, and social recognition(54–56), and extensive literature on OT and vertebrate social behavior suggests that OT increases social cue salience(57).

Additionally, our findings are in line with previous research in humans suggesting that OT is important for synchrony(58). Synchrony is an emergent behavior that occurs between two or more individuals such as eye contact, singing, or movement, and is linked to greater affiliation and cooperation(59). Thus, increased similarity resulting from OT in our study suggests that OT may function to increase pair affiliation. Additionally, OT may increase the ability of pair-bonded individuals in monogamous, biparental species to perceive a partner's intent in order to provide complimentary behaviors that efficiently address environmental challenges such as foraging, defending against predators, and taking care of young.

Ultrasonic vocalizations

Are pairs communicating with each other and could this influence development of emergent “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 they are near their partner that does not alter their partner’s behavior, or 2) an attempt to influence a change 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(60), territorial defense (61) and information sharing(62). As such, communication may play an important role in coordinating behavioral similarities within pairs.

In addition, in Experiment 2, OT did not significantly alter USVs. This suggests 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(42). However, different results may have been obtained if we had been able to record individual USVs as opposed to pair USVs. We did, however, find anecdotal data that both sexes produced calls, namely that overlapping SV calls and overlapping sweep and SV calls 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, relevant to other taxa such as fish(20), birds(3), and primates(63), 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 their responses to aversive stimuli leading to greater similarity in behavior, and that IN-OT increased this effect. Furthermore, this increased similarity of approach behaviors corresponded with increased communication in the form of SVs suggesting, for the first time in a monogamous rodent, the potential importance of vocal communication for coordinating behavior between mates in order to increase behavioral similarity. Our research adds to a growing body of literature underscoring

the importance of accounting for individual-level variation and its role in producing emergent variation at the level of the pair.

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Research was conducted at the UW-Madison, which occupies the ancestral Ho-Chunk land known as Teejop. Following an 1832 treaty forcing the Ho-Chunk nation to cede this territory, federal and state governments perpetrated ethnic cleansing to unsuccessfully remove the Ho-Chunk from Wisconsin. We challenge ourselves and others to reflect on 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) and the Wisconsin Alumni Research Foundation.

Competing interests

Authors declare no competing interests

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.

References

1. Bell AM, Hankison SJ, Laskowski KL. The repeatability of behaviour: a meta-analysis. Vol. 77, Animal Behaviour. 2009. p. 771–83.

2. Sih A, Bell A, Johnson JC. Behavioral syndromes: An ecological and evolutionary overview. Vol. 19, Trends in Ecology and Evolution. 2004. p. 372–8.

3. King AJ, Williams LJ, Mettke-Hofmann C. The effects of social conformity on Gouldian finch personality. Anim Behav. 2015;99:25–31.

4. Conradt L, Roper TJ. Conflicts of interest and the evolution of decision sharing. Philos Trans R Soc B Biol Sci. 2009;364(1518):807–19.

5. Taff CC, Vitousek MN. Endocrine Flexibility: Optimizing Phenotypes in a Dynamic World? Vol. 31, Trends in Ecology and Evolution. Elsevier Ltd; 2016. p. 476–88.

6. Veenema AH, Neumann ID. Central vasopressin and oxytocin release: regulation of complex social behaviours. Progress in Brain Research Elsevier; 2008 p. 261–76.

7. Insel TR. The Challenge of Translation in Social Neuroscience: A Review of Oxytocin, Vasopressin, and Affiliative Behavior. Vol. 65, Neuron. Cell Press; 2010. p. 768–79.

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

9. Bartz JA, Zaki J, Bolger N, Ochsner KN. Social effects of oxytocin in humans: Context and person matter. Vol. 15, Trends in Cognitive Sciences. Elsevier Current Trends; 2011. p. 301–9.

10. Bredewold R, Smith CJW, Dumais KM, Veenema AH. Sex-specific modulation of juvenile social play behavior by vasopressin and oxytocin depends on social context. Front Behav Neurosci. 2014;8(JUNE):216.

11. Ma X, Zhao W, Luo R, Zhou F, Geng Y, Xu L, Gao Z, Zheng X, Becker B, Kendrick KM. Sex- and context-dependent effects of oxytocin on social sharing. Neuroimage. 2018;183:62–72.

12. Lieberwirth C, Wang Z. The neurobiology of pair bond formation, bond disruption, and social buffering. Vol. 40, Current Opinion in Neurobiology. Elsevier Ltd; 2016. p. 8–13.

13. Jiang Y, Platt ML. Oxytocin and vasopressin flatten dominance hierarchy and enhance behavioral synchrony in part via anterior cingulate cortex. Sci Rep. 2018;8(1):1–14.

14. Spengler FB, Scheele D, Marsh N, Kofferath C, Flach A, Schwarz S, Stoffel-Wagner B, Maier W, Hurlemann R. Oxytocin facilitates reciprocity in social communication. Soc Cogn Affect Neurosci. 2017;12(8):1325–33.

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

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

17. Trainor BC, Marler CA. Testosterone, Paternal Behavior, and Aggression in the Monogamous California Mouse (Peromyscus californicus). 2001;

18. Davis ES, Marler CA. c-fos changes following an aggressive encounter in female California mice: A synthesis of behavior, hormone changes and neural activity. Neuroscience. 2004;127(3):611–24.

19. Rieger N, Marler C. The function of ultrasonic vocalizations during territorial defence by pair-bonded male and female California mice. Anim Behav. 2018;135:97–108.

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

21. Rangassamy M, Dalmas M, Féron C, Gouat P, Rödel HG. Similarity of personalities speeds up reproduction in pairs of a monogamous rodent. Anim Behav. 2015;103:7–15.

22. Rogers W. Parental Investment and Division of Labor in the Midas Cichlid (Cichlasoma citrinellum). Ethology. 79(2):126–42.

23. Brotherton PNM, 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.

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

25. Munson AA, Jones C, Schraft H, Sih A. You're Just My Type: Mate Choice and Behavioral Types. Vol. 35, Trends in Ecology and Evolution. Elsevier Ltd; 2020. p. 823–33.

26. Stamps JA. Growth□mortality tradeoffs and "personality traits" in animals [Internet]. Ecology Letters John Wiley & Sons, Ltd; May 1, 2007 p. 355–63.

27. Kalcounis-Rueppell MC, Pultorak JD, Marler CA. Ultrasonic Vocalizations of Mice in the Genus Peromyscus. Handbook of Behavioral Neuroscience Elsevier B.V.; Jan 1, 2018 p. 227–35.

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

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

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

31. Marler CA, Monari PK. Neuroendocrine control of vocalizations in rodents (in press). In: Neuroendocrine Regulation of Animal Vocalization.

32. Pultorak JD, Fuxjager MJ, Kalcounis-Rueppell MC, Marler CA. Male fidelity expressed through rapid testosterone suppression of ultrasonic vocalizations to novel females in the monogamous California mouse. Horm Behav. 2015;70:47–56.

33. Guoynes CD, Marler CA. Unpublished data.

34. Ross HE, Young LJ. Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. Vol. 30, Frontiers in Neuroendocrinology. Academic Press; 2009. p. 534–47.

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

36. Leppanen J, Ng KW, Tchanturia K, Treasure J. Meta-analysis of the effects of intranasal oxytocin on interpretation and expression of emotions. Vol. 78, Neuroscience and Biobehavioral Reviews. Elsevier Ltd; 2017. p. 125–44.

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

38. Quintana DS. Most oxytocin administration studies are statistically underpowered to reliably detect (or reject) a wide range of effect sizes. Compr Psychoneuroendocrinology. 2020;100014.

39. Lee MR, Shnitko TA, Blue SW, Kaucher A V., Winchell AJ, Erikson DW, Grant KA, Leggio L. Labeled oxytocin administered via the intranasal route reaches the brain in rhesus macaques. Nat Commun. 2020;11(1):1–10.

40. Rilling JK, DeMarco AC, Hackett PD, Thompson R, Ditzen B, Patel R, Pagnoni G. Effects of intranasal oxytocin and vasopressin on cooperative behavior and associated brain activity in men. Psychoneuroendocrinology. 2012;37(4):447–61.

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

42. Rieger NS, Schefelker J, Marler CA. Unpublished data.

43. Musolf K, Hoffmann F, Penn DJ. Ultrasonic courtship vocalizations in wild house mice, Mus musculus musculus. Anim Behav. 2010;79(3):757–64.

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

45. Mcdonald ND, Rands SA, Hill F, Elder C, Ioannou CC. Consensus and experience trump leadership, suppressing individual personality during social foraging. Behav Ecol. 2016;

46. King A, Cowlishaw G. Communicative & Integrative Biology Leaders, followers, and group decision-making. 2009;

47. Ruuskanen S, Groothuis TGG, Baugh AT, Schaper S V., Vries B, Oers K. Maternal egg hormones in the mating context: The effect of pair personality. Moore I, editor. Funct Ecol. 2018;32(2):439–49.

48. Hurtado G, Mabry KE. Aggression and boldness in Merriam's kangaroo rat: An urban-tolerant species? J Mammal. 2017;98(2):410–8.

49. Martins EP, Bhat A. Population-level personalities in zebrafish: Aggression-boldness across but not within populations. Behav Ecol. 2014;25(2):368–73.

50. Mazué GPF, Dechaume-Moncharmont FX, Godin JGJ. Boldness-exploration behavioral syndrome: Interfamily variability and repeatability of personality traits in the young of the convict cichlid (Amatitlania siquia). Behav Ecol. 2015;26(3):900–8.

51. 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.

52. Savage A, Ziegler TE, Snowdon CT. Sociosexual development, pair bond formation, and mechanisms of fertility suppression in female cotton-top tamarins (Saguinus oedipus oedipus). Am J Primatol. 1988;14(4):345–59.

53. Kvarnemo C. Why do some animals mate with one partner rather than many? A review of causes and consequences of monogamy. Biol Rev. 2018;93(4):1795–812.

54. Ophir AG, Gessel A, Zheng DJ, Phelps SM. Oxytocin receptor density is associated with male mating tactics and social monogamy. Horm Behav. 2012;61(3):445–53.

55. Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E. Oxytocin increases trust in humans. Nature. 2005;435(7042):673–6.

56. Blocker TD, Ophir AG. Social recognition in paired, but not single, male prairie voles. Anim Behav. 2015;108:1–8.

57. Shamay-Tsoory SG, Abu-Akel A. The Social Salience Hypothesis of Oxytocin. Vol. 79, Biological Psychiatry. Elsevier USA; 2016. p. 194–202.

58. Feldman R, Bakermans-Kranenburg MJ. Oxytocin: a parenting hormone [Internet]. Current Opinion in Psychology Elsevier B.V.; Jun 1, 2017 p. 13–8.

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

60. Townsend SW, Zöttl M, Manser MB. All clear? Meerkats attend to contextual information in close calls to coordinate vigilance. Behav Ecol Sociobiol. 2011;65(10):1927–34.

61. Wiewandt TA. Vocalization, Aggressive Behavior, and Territoriality in the Bullfrog, Rana catesbeiana. Copeia. 1969;1969(2):276.

62. Brudzynski SM. Ethotransmission: communication of emotional states through ultrasonic vocalization in rats. Curr Opin Neurobiol. 2013;23(3):310–7.

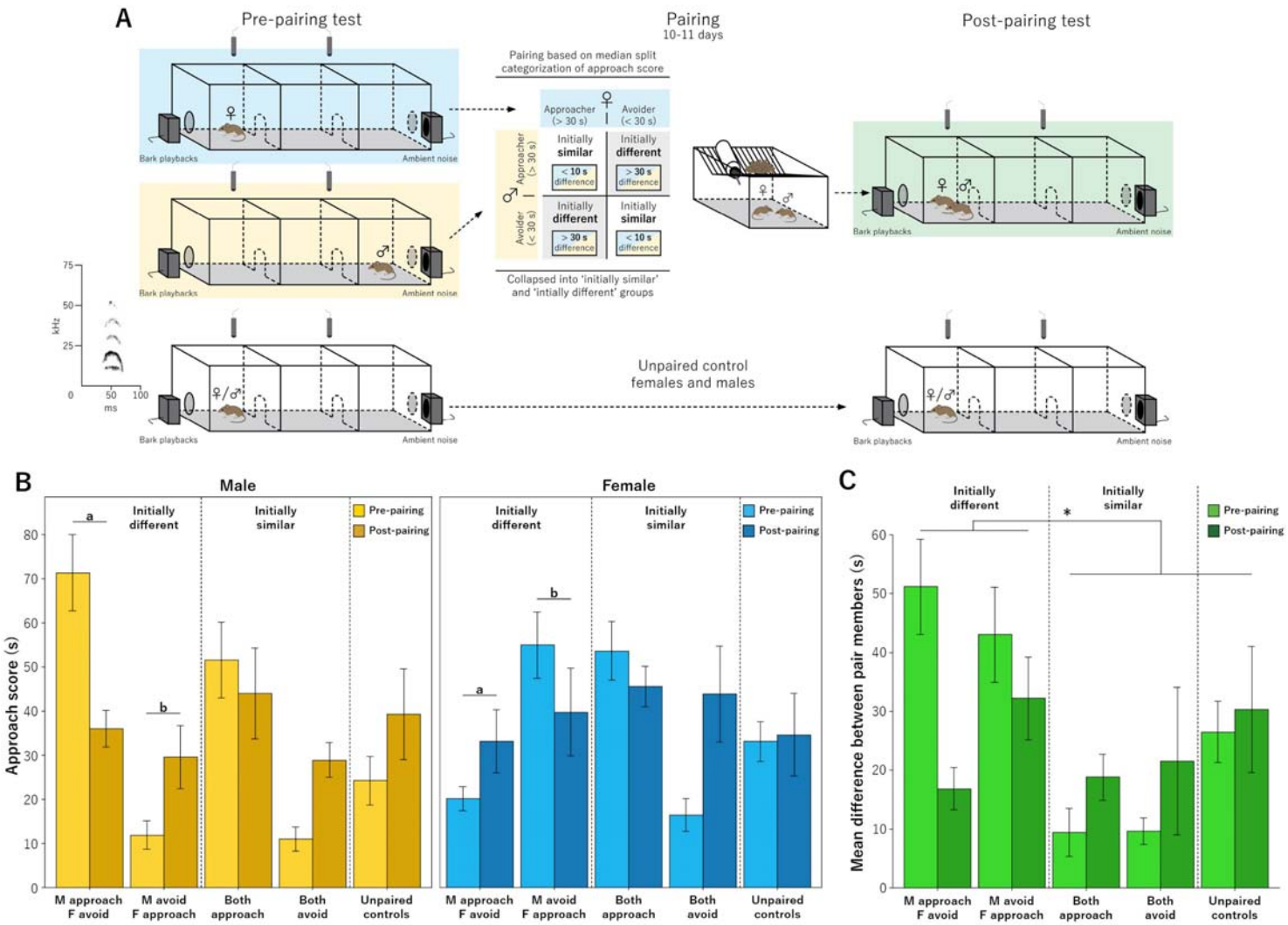
63. van de Waal E, Borgeaud C, Whiten A. Potent social learning and conformity shape a wild primate's foraging decisions. Science (80-). 2013;340(6131):483–5.

Figure legends

Figure 1. Experiment 1. Paired individuals changed their approach to bark playbacks to become more similar. **A**. Experimental overview: example of an approacher female (blue box) and an avoider male (yellow box) that were paired and then tested together (green box). **B.** A three-way interaction between sex, group and approach score before and after pairing was found; in general, sex differences within groups occurred when pairs were initially different prior to pairing. Approacher males paired with an avoider female (left, 'M approach F avoid') decreased their time spent in the bark chamber from the pre-pairing test (light bars) to the post-pairing test (dark bars) while their female mate (right) increased their time spent 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 their time spent in the bark chamber following pairing, while their female mate decreased their time in the bark chamber. Males and females of all other groups showed no behavioral sex differences from the pre-pairing to the post-pairing test. **C.** A two-way interaction was found such that pairs with one approacher and one avoider decreased their difference in approach score from the pre-pairing test (light bars) to the post-pairing test (dark bars). Overall, initially different pairs became more similar, and initially similar pairs and nonpaired individuals remained similar. (a, b, * = $p < 0.05$). For the sake of clarity, significant lower-order effects are not indicated for any analyses.

Figure 2. Experiment 1. Ultrasonic vocalizations (USVs) correlated with behavioral coordination. **A**. Total SV calling was increased in both pairs with initially different approach scores (dark gray) and initially similar approach scores (light gray) compared to individuals. **B**. The total number of sweeps produced was increased in both initially different and initially similar pairs compared to individuals. **C**. The total mean number of SV calls produced was increased in pairs that were initially different compared to all other groups. **D**. Similarly the proportion of SV calls as a function of total calls was also increased in pairs which were initially different compared to all other groups. **E**. Pairs that spent more total time together in the ambient noise chamber in response to bark playbacks produced significantly more total USV calls, including both sweeps and SVs. **F**. Pairs that showed greater similarity in approach scores after pairing showed greater production of SV calls as a dyad. Pairs that became more similar (pre-pairing approach score difference - post-pairing approach score difference) as denoted by positive numbers on the x-axis, produced significantly more total SV calls and **G**. A significantly greater proportion of SV calls to total USV calls produced (* = $p < 0.05$).

***Figure 3*. Experiment 2.** Oxytocin (OT) increased the degree of behavioral coordination in pairs that were initially different. **A.** Experimental overview. **B**. Initial approach response to bark playbacks predicted approach response to bark playbacks 13-17 days later in unpaired females (light circles: saline, dark circles: OT). **C**. OT (dark bar) did not impact unpaired female approach during the second test. **D.** Change in pair difference from pre-pairing to post-pairing. Indicates the degree to which pairs became more similar to each other. Initially different pairs converged more, and OT treatment increased this effect. **E.** Amount of time individuals within a pair spent together during the post-pairing test. OT pairs spent more time together overall. **F.** Initially different pairs produced a higher proportion of SVs, and there was a nonsignificant trend whereby OT increased the SV proportion in initially different pairs. (# = $p < 0.10$; * = $p < 0.05$; ** = $p < 0.005$; *** = $p < 0.001$).



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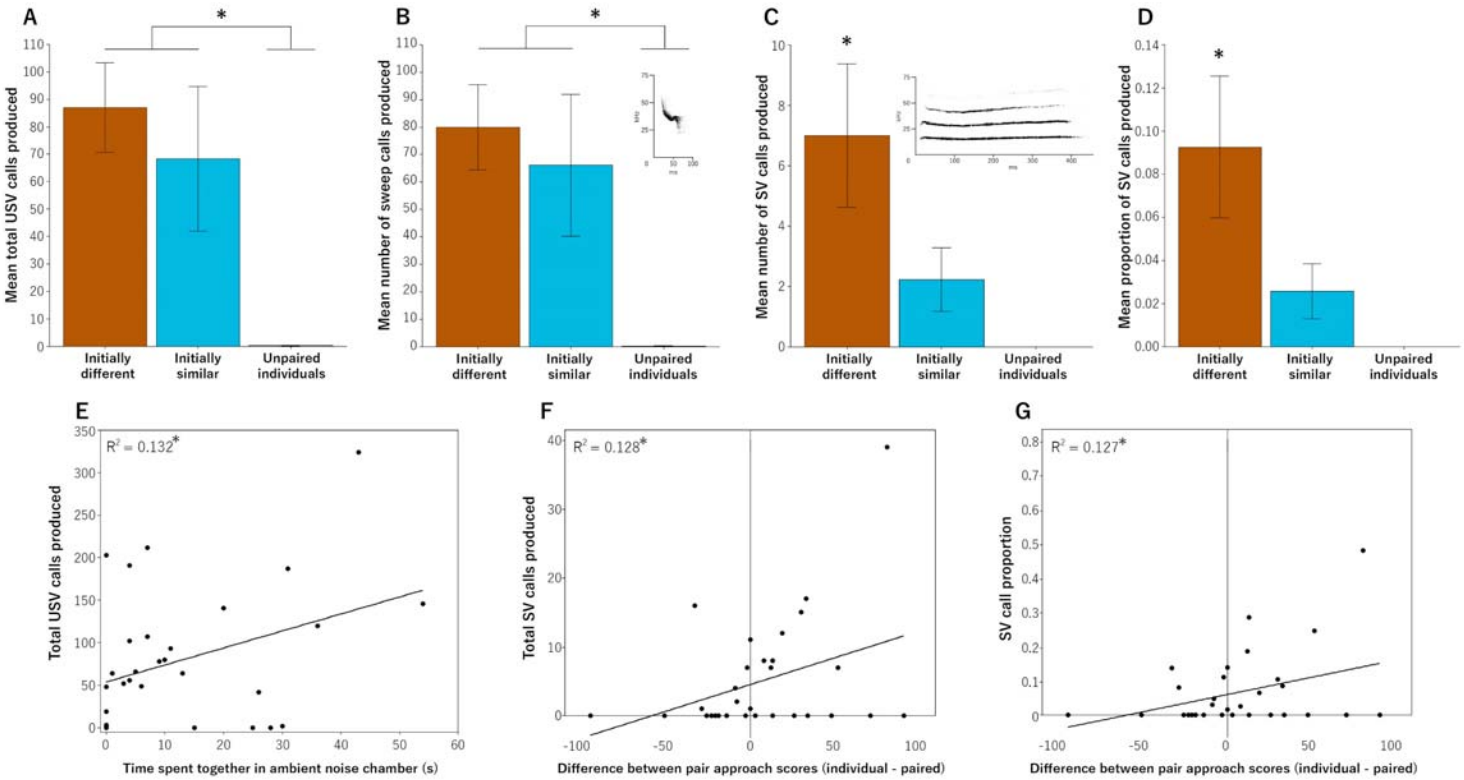

Figure 2. Experiment 1.

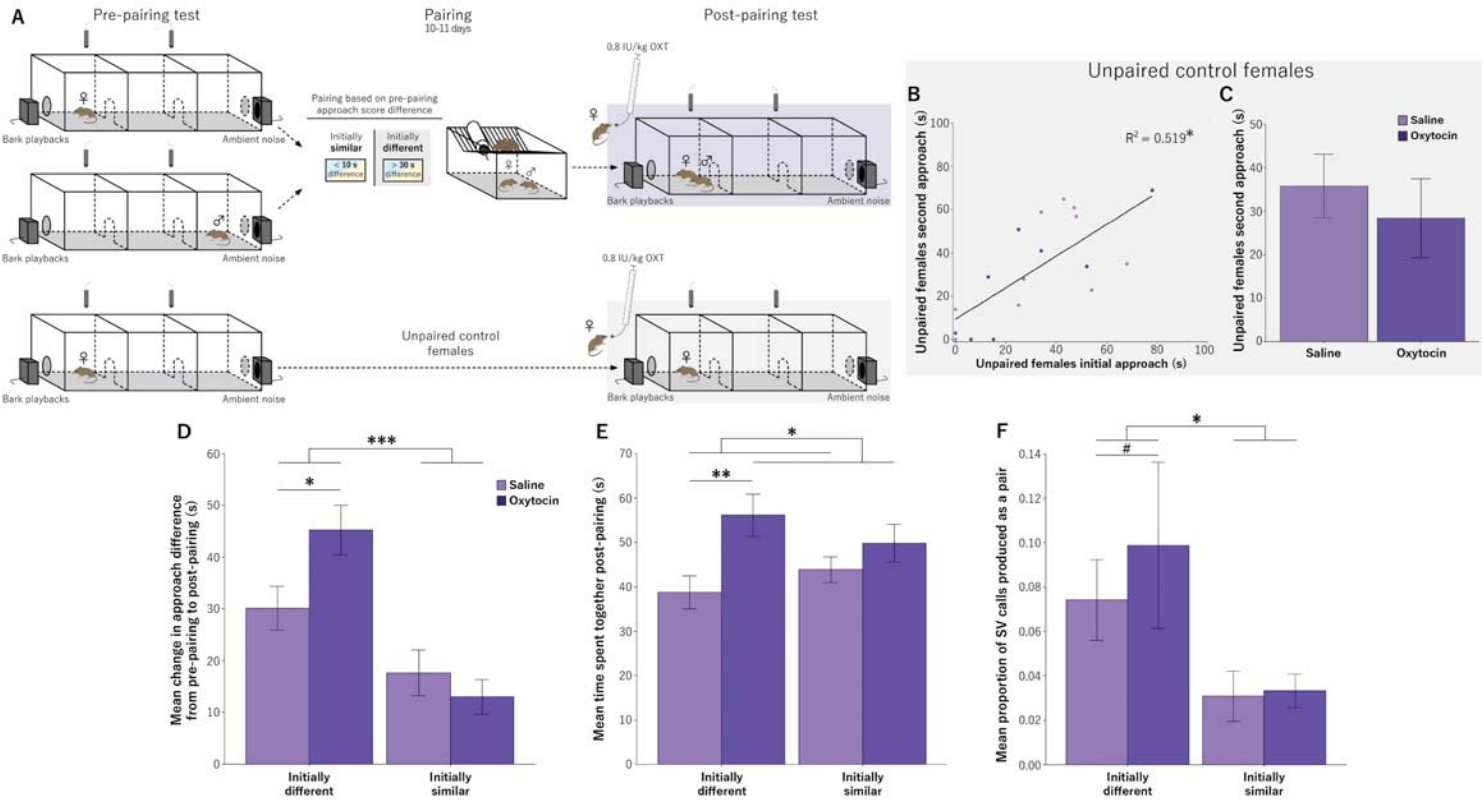

Figure 3. Experiment 2.