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SUPPLEMENTARY MATERIALS

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COMPARATIVE BEHAVIOR

Oxytocin-dependent consolation behavior in rodents

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Consolation behavior toward distressed others is common in humans and great apes, yet our ability to explore the biological mechanisms underlying this behavior is limited by its apparent absence in laboratory animals. Here, we provide empirical evidence that a rodent species, the highly social and monogamous prairie vole (*Microtus ochrogaster*), greatly increases partner-directed grooming toward familiar conspecifics (but not strangers) that have experienced an unobserved stressor, providing social buffering. Prairie voles also match the fear response, anxiety-related behaviors, and corticosterone increase of the stressed cagemate, suggesting an empathy mechanism. Exposure to the stressed cagemate increases activity in the anterior cingulate cortex, and oxytocin receptor antagonist infused into this region abolishes the partner-directed response, showing conserved neural mechanisms between prairie vole and human.

Consolation, which entails comforting contact directed at a distressed party, is a common empathetic response in humans that emerges in the second year of life (*1*). Until now, consolation behavior has only been documented in a few nonhuman species and only in the context of naturally occurring aggressive conflicts, as first described in great apes (*2*, *3*) and subsequently in canids (*4*, *5*), corvids (*6*, *7*), and elephants (*8*). These observations have, so far, been taken to mean that consolation behavior may require advanced cognitive capacities (*9*). Nonetheless, rodents also manifest some of the empathy-related capacities (*10*–*16*) thought to underlie consolation in humans and chimpanzees (*1*, *17*). If consolation behavior were to be observed outside of species with advanced cognition, this would suggest that it rests on much older, more widespread, and less cognitive capacities and may be variably expressed because of species-specific evolutionary context. Moreover, observing consolation behavior in a laboratory rodent under reproducible conditions would allow for empirical research on causal biological mechanisms relevant to human mental health.

Rodents in the genus Microtus display diverse mating strategies and social structures. The prairie vole (*Microtus ochrogaster*) is a socially monogamous, biparental rodent species in which both males and females may participate in philopatric cooperative breeding in the parental nest (*18*). These social traits frequently coevolve with other cooperative or altruistic behaviors that increase direct or indirect fitness, including social buffering among colony members (*19*). In contrast, closely related meadow voles (*M. pennsylvanicus*) are promiscuous breeders with no formal social structure that show comparatively abbreviated, uniparental care of pups (*20*). We hypothesized that the prairie vole, but not the meadow vole, would show consolation behavior under reproducible laboratory conditions. Additionally, we hypothesized that as suggested for humans and great apes, consolation behavior in the prairie vole would be based on an empathy mechanism. Last, we hypothesized that consolation behavior would be mediated by conserved neurobiological and neurochemical mechanisms consistent with those implicated in empathy in humans.

Consolation behavior has been defined as an increase in affiliative contact in response to and directed toward a distressed individual, such as a victim of aggression, by an uninvolved bystander, which produces a calming effect (*2*). This definition emphasizes victims of aggression due to observational constraints in naturalistic studies. In humans, the definition includes individuals experiencing stress from other sources (*1*), a strategy used in elephants (*8*) and suggested for primates (*9*). On the basis of this research, we first developed a set of laboratory conditions under which unstressed male and female prairie voles ("observers") would respond spontaneously and selectively to stressed conspecifics ("demonstrators") with a prosocial, other-directed behavior (the "consolation test") (Fig. 1A). In this protocol, an observer and a demonstrator housed together are separated from each other, and the demonstrator either sits alone in a home cage compartment or is exposed to a stressor consisting of five tones paired with light foot-shocks (0.8 mA, 0.5 s) distributed over the course of 24 min (Pavlovian fear conditioning). The demonstrator is then reunited with the naïve observer, and the natural response is recorded and measured. Under these experimental conditions, licking and grooming directed by observers toward demonstrators (or "allogrooming") was significantly longer in duration (time-treatment interaction, $F_{1,11} = 6.7$, $P < 0.025$) and shorter in latency ($t_{11} = 3.9$, $P < 0.003$) after a separation during which the demonstrator was stressed (Fig. 1B and fig. S1). Prairie vole observers did not increase allogrooming toward demonstrators after a control separation, demonstrating the selectivity of the response. Both male and female observers

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showed this behavioral response, differing only in baseline allogrooming (sex-time interaction, $F_{1,73}$ = 6.4, P < 0.015) (fig. S2). Meta-analysis across 13 experiments shows that observers initiate allogrooming within the first minute and continue for at least the first 10 min of reunion time (Fig. 1C, figs. S3 and S4, and table S1). Additionally, stressed demonstrators that rested alone in the home cage after the stressor subsequently showed increased anxiety-like behavior relative to unstressed controls, whereas those that interacted with the observer for the same period of time showed completely normalized anxiety behavior (interaction effect, $F_{2,63}$ = 3.2, P < 0.05) (Fig. 1D). This suggests that the observer provided social buffering to the demonstrator, which is consistent with other studies showing stress reduction in rodents (*21*, *22*) and primates (*3*, *23*). In contrast, meadow vole observers showed no differences in allogrooming based on the stress state of the demonstrator (fig. S5). The combination of a selective increase in directed affiliation with a social buffering effect supports the designation of the prairie vole's natural response as a consolation behavior.

The observation that prairie voles detect the stress state of conspecifics and form a directed prosocial response raises the question of whether the behavior is empathy-based. The empathy hypothesis was tested by assaying for some of its purported characteristics in human and other mammalian species, including emotional contagion, state matching, familiarity bias, and self-other differentiation (*24*–*26*). In accordance, prairie vole observers showed behavioral responses consistent with emotional contagion by mimicking the anxiety- and fear-related behaviors of stressed demonstrators (Fig. 2). Observers interacting with a stressed demonstrator after separation matched the increase in self-grooming shown by the demonstrator (main effect of time, $F_{1,23}$ = 12.7, P < 0.002) (Fig. 2A). Additionally, when observing a fear-conditioned demonstrator freezing during presentations of the conditioned stimulus (tones), the unconditioned observers showed an increase in freezing (main effect of time, $F_{1,22}$ = 22.2, P < 0.0002) (Fig. 2B) concurrently with the demonstrator's freezing (Fig. 2C). Observers separated from stressed demonstrators across a clear, perforated barrier had significantly elevated plasma corticosterone afterward (main effect of barrier, $F_{2,27}$ = 4.8, P < 0.017) (Fig. 3A), which strongly correlated with that of the demonstrator (stressor, R^2 = 0.82, P < 0.001; separation, R^2 < 0.01, P > 0.98; difference between correlations, Fisher's transformation, Z = 2.8, P < 0.006) (Fig. 3B), representing a clear example of physiological state–matching similar to that attributed to empathy in humans (*27*). Observers in full contact with demonstrators without a barrier showed no increase, suggesting that active performance of consolation behavior may ameliorate the observer's physiological stress response. Consolation behavior was significantly biased toward familiar individuals: Although baseline allogrooming did not differ between groups containing mates, siblings, cagemates, and strangers, observers directed consolation behavior only toward familiar stressed demonstrators and not toward stressed strangers (time-relation interaction, $F_{2,73}$ = 13.6, P < 0.0001; main effect of relation, $F_{2,73}$ = 26.6, P < 0.0001; cagemates, $t(8)$ = −6.1, P < 0.0003) (Fig. 3C and figs. S6 and S7). Last, although observers and stressed demonstrators both showed signs of anxiety and stress during reunion, observers increased allogrooming toward demonstrators, whereas demonstrators themselves did not alter their allogrooming (time-subject interaction, $F_{1,70}$ = 35.6, P < 0.0001) (Fig. 3D). This differential response dependent on the source of the individual's stress (vicarious or personal) is an example of self-other differentiation, which shows that the allogrooming response is not a general stress-coping behavior.

The combination of behavioral and physiological state matching in the observer shows that the observer is not neutral to the stress state of the demonstrator, as might be predicted if the allogrooming response were purely information-gathering behavior. Empathy-related responses and behaviors are biased toward familiar individuals in many species, including humans (*10*, *11*, *17*, *28*); the allogrooming response in prairie voles is also selective for familiar conspecifics (including unrelated long-term cagemates), representing a true social behavior rather than reproductive or kinship-related. Additionally, the lack of response toward strangers shows that observers are not simply reacting to aversive

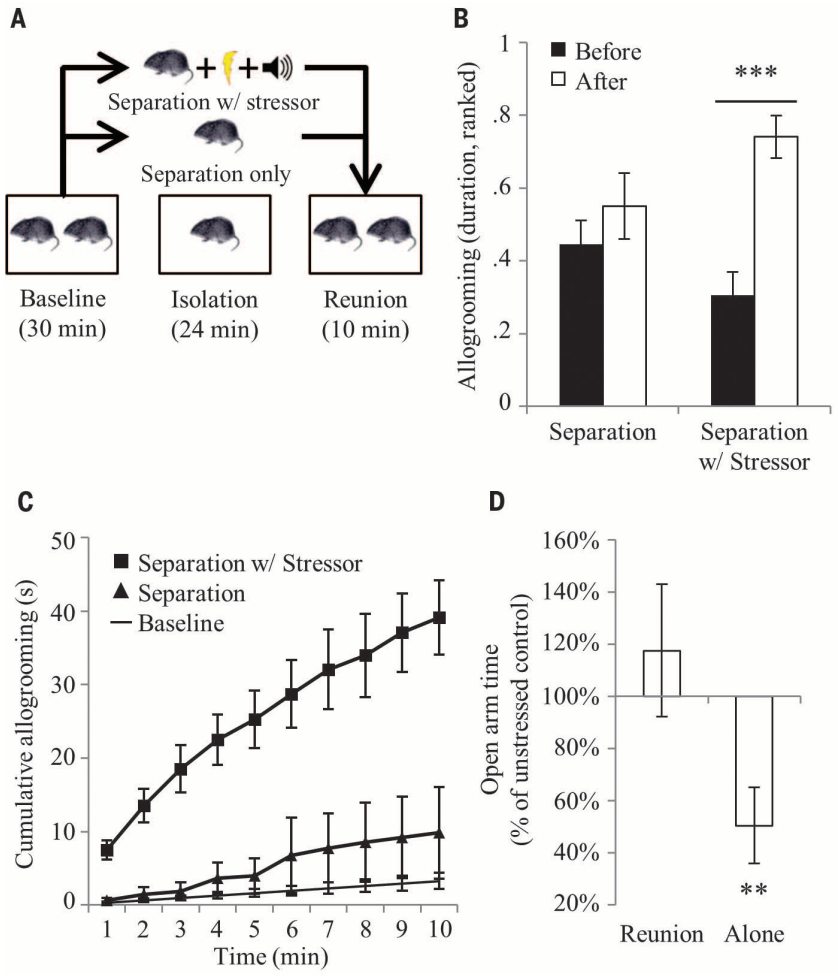

Fig. 1. The consolation test. (**A**) The consolation test protocol. (**B**) Observer-demonstrator pairs (n = 12 pairs) underwent both control separations without a stressor, and separations in which the demonstrator was stressed. Duration of allogrooming was nonparametric in these experiments and was transformed to ranks, and the ranks normalized to a 0–1 scale. Bars represent the mean ± SEM of the ranked duration of allogrooming directed by the observer toward the demonstrator. (**C**) A meta-analysis of results from 13 experiments shows the precise expected duration of observer-demonstrator allogrooming over the course of 10 min. Points represent cumulative seconds with 95% confidence intervals. (**D**) After resting alone in the home cage for 5 min, stressed demonstrators (n = 10 voles) showed a significant decrease in open-arm time on the elevated plus maze test relative to unstressed controls (n = 11 voles). Stressed (n = 11 voles) and unstressed (n = 11 voles) demonstrators reunited with the observer for 5 min showed no differences in open-arm time. Bars represent the mean ± SEM of the percent change in open-arm time between stressed and unstressed demonstrators. **P < 0.005, ***P < 0.0005.

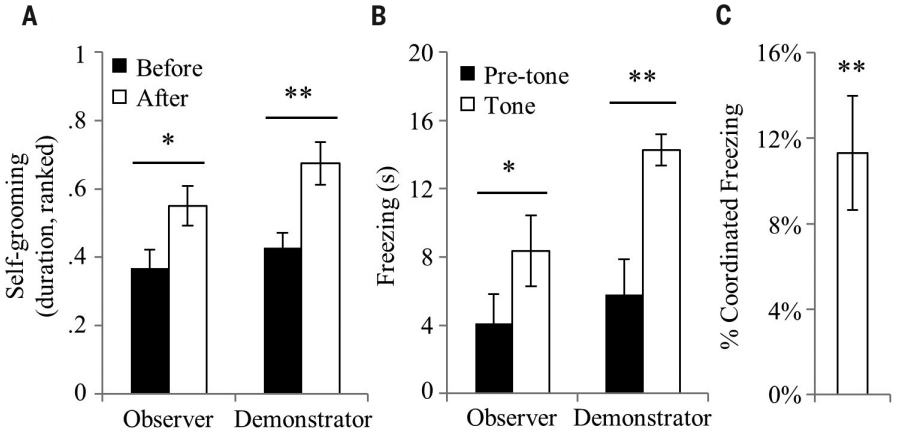

Fig. 2. Emotional contagion. Prairie vole observers exposed to a stressed demonstrator show anxiety- and fear-related responses that match the demonstrator's responses. (**A**) Anxiety-related behavior was measured in observers and demonstrators (n = 24 pairs) interacting after reunion. Bars represent the mean ± SEM of the ranked duration of self-grooming performed by the observer and demonstrator. (**B**) Freezing was measured while fear-conditioned demonstrators and unconditioned observers (n = 12 pairs) were exposed together to a 30-s conditioned stimulus (CS). Bars represent the mean ± SEM of freezing before and after the CS. (**C**) Coordinated freezing during the CS between observer and demonstrator pairs (n = 12 pairs), calculated as the within-pair difference between the observed percent of simultaneous freezing and the simultaneous freezing expected by chance. *P < 0.05, **P < 0.005.

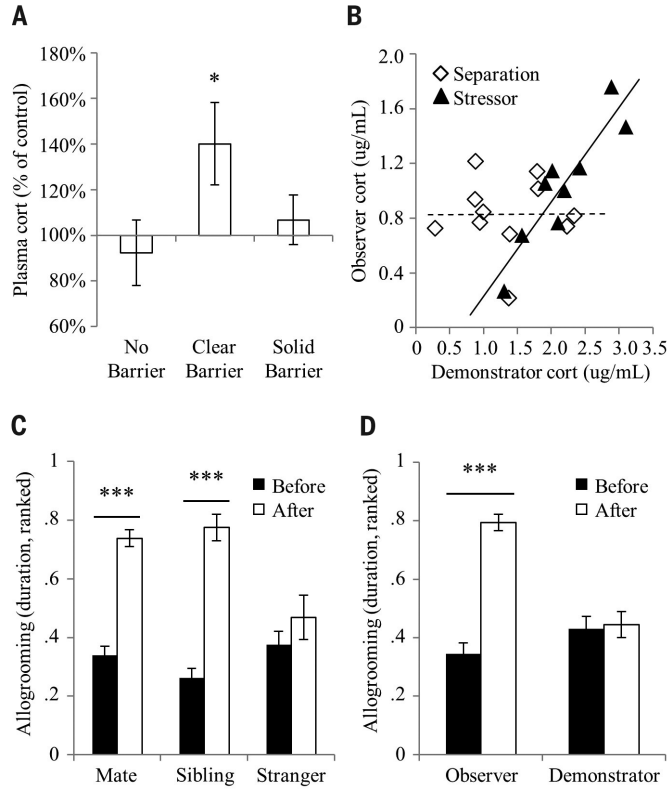

Fig. 3. State matching, familiarity bias, and self-other differentiation. (**A**) Observer-demonstrator pairs underwent either control separations or separations with stressor and subsequently were either reunited in the home cage with no barrier (separated, n = 11 pairs; stressed, n = 12 pairs), reunited across a clear perforated barrier (separated, n = 11 pairs; stressed, n = 11 pairs), or in independent sections of the home cage separated by a solid opaque barrier (separated, n = 7 pairs; stressed, n = 9 pairs). Bars represent the mean ± SEM percent change in plasma corticosterone concentration in observers between the control separations and separations with stressor in each cage configuration. (**B**) Correlations between the plasma corticosterone concentrations of observers and demonstrators that interacted across a clear perforated barrier. The dashed and solid lines represent regression lines for the separation (n = 11 pairs) and stressor (n = 9 pairs) conditions, respectively. (**C**) Prairie vole mated pairs (n = 37 pairs), same-sex sibling pairs (n = 22 pairs), and same-sex stranger pairs (n = 20 pairs) underwent separations in which one cagemate was stressed. Bars represent the mean ± SEM of the ranked duration of allogrooming directed by the observer toward the demonstrator. (**D**) Observer-demonstrator pairs (n = 37 pairs) underwent separations during which the demonstrator was stressed. Bars represent the mean ± SEM of the ranked duration of allogrooming by either the observer or the demonstrator. *P < 0.05, **P < 0.005, ***P < 0.0005.

cues. Whereas some empathy-related studies used training or conditioning (*15*, *16*, *29*, *30*), the consolation test in the present experiments was administered only once to each set of subjects and therefore captured unconditioned responses. The focus on unconditioned responses means that the consolation test does not assume or necessarily require any particular cognitive capacities, including conscious knowledge or perspective taking—which, in a multilayered view of empathy, may be included but are not required (*24*–*26*). Several empathy-related paradigms require priming the observer with direct exposure to the stressor (*12*–*15*); in contrast, observers in the present paradigm neither experienced nor witnessed the stressor, and therefore self-referential anticipation of a threat can be ruled out as an explanation. Last, a novel experience alone was not sufficient to elicit a consolation response in absence of a stressor (time-treatment interaction, $F_{1,16}$ = 7.1, P = 0.017) (fig. S8). This confluence of evidence and exclusion of alternative explanations supports the interpretation that an empathy mechanism underlies the increase in affiliative behavior in prairie voles in response to a stressed conspecific.

In humans, the oxytocin receptor (OTR) has been linked to empathy, emotion recognition, and socioemotional engagement (*31*–*33*). Observers that received an injection of an oxytocin antagonist (OTA) into the cerebral ventricle before the consolation test did not change their baseline allogrooming but showed no consolation response (time-treatment interaction, $F_{1,27}$ = 5.0, P < 0.04) (Fig. 4A), demonstrating that OTR activation in the brain is necessary for consolation behavior. The prairie vole anterior cingulate cortex (ACC), adjacent prelimbic cortex (PLC), and nucleus accumbens shell (NACS) all express high densities of OTR (Fig. 4B); in humans, the ACC and homologous medial prefrontal cortex have been linked to empathy (*34*), and the NACS is typically linked to social and nonsocial reward (*35*). Using immunohistochemistry targeting the immediate early gene protein FOS, we determined that the ACC, but not PLC or NACS, is differentially active in observers interacting with stressed demonstrators as compared with unstressed demonstrators (treatment-region interaction, $F_{2,34}$ = 6.7, P < 0.004; post-hoc t test, P < 0.02 uncorrected) (Fig. 4, C and D, and fig. S9). This result was validated in observers exposed to stressed demonstrators across a clear perforated barrier (t test, P < 0.04) (fig. S10), suggesting that the difference in activity was due to exposure to the stressed demonstrator rather than caused by the observer's behavior. Following these results, we hypothesized that oxytocin may act region-specifically on OTR in the ACC to enable consolation behavior. An injection of OTA directly into ACC abolished the consolation response in observers (time-treatment interaction, $F_{1,13}$ = 7.4, P < 0.02) (Fig. 4E and fig. S11A), whereas injections into adjacent PLC had no effect (Fig. 4F and fig. S11B); this shows that OTR signaling within the ACC modulates consolation, possibly by disrupting physiological, emotional,

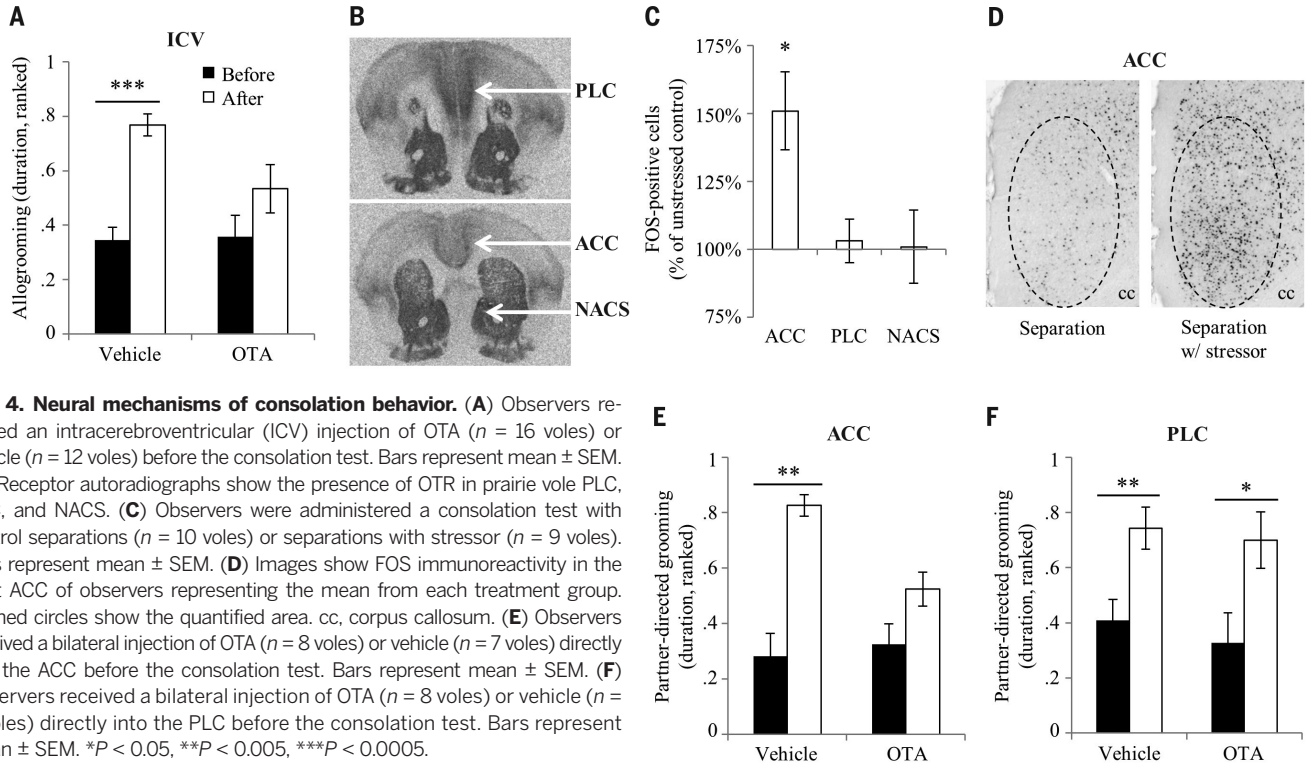

Fig. 4. Neural mechanisms of consolation behavior. (**A**) Observers received an intracerebroventricular (ICV) injection of OTA (n = 16 voles) or vehicle (n = 12 voles) before the consolation test. Bars represent mean ± SEM. (**B**) Receptor autoradiographs show the presence of OTR in prairie vole PLC, ACC, and NACS. (**C**) Observers were administered a consolation test with control separations (n = 10 voles) or separations with stressor (n = 9 voles). Bars represent mean ± SEM. (**D**) Images show FOS immunoreactivity in the right ACC of observers representing the mean from each treatment group. Dashed circles show the quantified area. cc, corpus callosum. (**E**) Observers received a bilateral injection of OTA (n = 8 voles) or vehicle (n = 7 voles) directly into the ACC before the consolation test. Bars represent mean ± SEM. (**F**) Observers received a bilateral injection of OTA (n = 8 voles) or vehicle (n = 9 voles) directly into the PLC before the consolation test. Bars represent mean ± SEM. *P < 0.05, **P < 0.005, ***P < 0.0005.

and/or behavioral responses. This evidence demonstrates that the ACC is one node where activity increases during interaction with a stressed conspecific, and where OTR activation is necessary for the expression of consolation behavior. These neural substrates suggest conserved biological mechanisms for consolation behavior between prairie vole and human.

The presence of consolation behavior in prairie voles demonstrates that this behavior does not require advanced cognitive capacities, and the conserved neurobiology of consolation between prairie voles and humans suggests a deep homology of the underlying neural substrates. The ancestral biological mechanisms supporting maternal care in mammals have likely served as the basis from which many complex social behaviors evolved, including empathy (*24*, *36*) and pair bonding (*37*), both of which involve the reorienting of parental behaviors toward adult conspecifics. Nonetheless, the confirmed absence of consolation in the closely related meadow vole and in most macaques (*9*, *38*) shows that consolation behavior emerges only under particular social and evolutionary conditions. Understanding the neurobiology of oxytocin-dependent consolation behavior in prairie voles may help us to understand the diverse deficits in detecting and responding to the emotions of others that are present in many psychiatric conditions, including autism, schizophrenia, and psychopathy.

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SUPPLEMENTARY MATERIALS

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Editor's Summary

Let me comfort you

Consolation behavior promotes stress reduction of one by another. We know that consolation occurs in humans and apes. Burkett *et al.* observed that within a pair of monogamous prairie voles, an unstressed partner increased its grooming of a stressed partner. Furthermore, the unstressed partner matched the stressed partner in its stress hormone response. Thus, consolation may be more common than assumed in animals, and prairie voles may prove a useful model for understanding the physical and neural mechanisms underlying consolation behavior.

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Supplementary Material for

Oxytocin-dependent consolation behavior in rodents

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Supplementary Materials:

Materials and Methods:

Subjects. All animal subjects and stimulus animals were sexually naïve, gonadally intact adult male and female prairie voles (*Microtus ochrogaster*, originating from individuals wild-caught in Illinois) or meadow voles (*M. pennsylvanicus*) that were raised in our breeding colony at Yerkes National Primate Research Center. Voles were weaned at 21 days of age and socially housed in same-sex duos or trios on a 14:10 light:dark cycle. Voles were provided with water and Purina rabbit chow *ad libitum* at all times. Voles were used for experiments after reaching adulthood (between 2 months and 6 months of age). All breeding, housing, and experimental procedures were approved by the Institutional Animal Care and Use Committee at Emory University. Before testing began, adult subjects to be tested as male-female mated pairs were co-housed for 2-5 days to allow for the formation of a bond. Adult subjects to be tested as same-sex siblings were co-housed since birth and weaned together. Adult subjects to be tested as cagemates were co-housed since weaning.

Drugs. The oxytocin antagonist (OTA) used in these studies was peptidergic ornithine vasotocin analog desGly–NH_2,d$(CH_2)^5$[Tyr(Me)^2, Thr^4]OVT (Bachem, Torrance, CA) (*39*). OTA was dissolved in artificial cerebrospinal fluid (aCSF) at a concentration of 2.5 ng/uL. Aliquots of aCSF from the same batch were used for vehicle injections.

Consolation test protocol. Prior to the consolation test, pair-housed subjects (one "observer" and one "demonstrator") were housed together in standard Super Mouse 750 cages (Lab Products Inc., Seaford, DE) that were cut in half and modified such that the two halves were held together with magnetic braces (Fig. S12). Subjects were subsequently habituated to the testing procedure (Fig. 1A) as follows. Home cages were moved into the testing room and all obstructive enrichment was removed from the cage. Subjects were then left undisturbed and digitally recorded for 90 minutes, the last 30 minutes of which was used for baseline behavioral analysis. Following this baseline period, subjects were separated by introducing opaque magnetic barriers into the cage seam and then splitting the home cage into two independent hemi-cages. After 24 minutes, the two hemi-cages were merged and the barriers removed. Post-separation behavior was digitally recorded for 10 minutes. This procedure was repeated for 2-4 days, and data from all but the first day were used to determine pre- and post-separation behavior.

On the day following the habituation period, subjects underwent an identical procedure, except as follows. For subjects in experimental stressor groups, the hemi-cage containing the demonstrator was moved into a separate room and the demonstrator was transferred into a fear conditioning chamber. Demonstrators habituated to the chamber for 5 minutes and then were subjected to five tones (30 s, 6 kHz, 75-80 dB) preceding light foot shocks (0.5 s, 0.8 mA) which occurred at 3-4 minute variable inter-trial intervals, for a total of 24 minutes (*40*). Demonstrators were then returned to their hemi-cage and re-united with observers as above. Post-shock behavior was digitally recorded for 10 minutes.

Behavioral Coding. Digital videos from all experiments were viewed by raters blind to the experimental groups and treatments. Videos were coded for allogrooming or other behaviors depending on the experiment, such as self-grooming and freezing. Allogrooming was defined as head contact with the body or head of another individual, accompanied by a rhythmic head movement. Grooming directed toward the genitals, anogenital region, or tail, or occurring during mating bouts, was considered genital/sexual grooming and excluded. Raters coded all

experiments using either The Observer XT (Noldus, Wageningen, The Netherlands) or Stopwatch+ (Emory University, Atlanta, GA). Each experiment was coded by a single rater using a single software package, and all raters had a minimum of 80% inter-rater reliability.

Statistics. Statistical analyses are described within each experiment. Whenever possible, ANOVAs were used as omnibus tests, with within-subjects factors analyzed as repeated measures. Since the duration of allogrooming and self-grooming were non-parametric in our experiments, these data were transformed within each experiment into ranks, where data points from all groups are numbered ("ranked") from lowest to highest and the ranks substituted for the raw values. Ranked data were then normalized to a 0-1 scale by dividing by the number of data points in the experiment. Post-hoc tests on duration also used the ranked data. All p-values described as significant were below the Bonferroni-adjusted α after multiple-comparisons correction, unless otherwise indicated.

Effect of Separation With Versus Without a Stressor. We tested whether male prairie and meadow vole observers would show increased allogrooming toward their stressed female mates (the "consoling response"). In separate experiments, male prairie (N=12) and meadow (N=12) vole observers were paired with an age-matched female demonstrator for 2 days. In prairie voles, this is sufficient time for a pair bond to form (*41*). Mated pairs were then administered a consolation test with 3 days of habituation and one day of stress as described above.

Rank-transformed data on duration of allogrooming from each species were analyzed using a 2x2 ANOVA, with time (before, after) and treatment (separation, shock) as within-subjects factors. Post-hoc paired t-tests compared time points within each treatment condition. An additional paired t-test compared the latency to groom the partner between treatment conditions in the after time point. Results appear in Figs. 1B, S1, and S5.

Effect of Sex and Relationship. We tested whether consolation behavior would be shown preferentially toward familiar individuals, and whether the response was equal among males and females. Male and female prairie vole observers were divided into three groups: mates (male, N=24; female, N=13), siblings (male, N=11; female, N=11), and strangers (male, N=10; female, N=11). "Mates" were paired with an age-matched opposite-sex demonstrator for 3 days prior to testing. "Siblings" were pair-housed with a same-sex sibling demonstrator with which they had been continuously housed since birth. "Strangers" were pair-housed with a same-sex sibling or cagemate with which they had been continuously housed since weaning. All subjects were administered a consolation test with 3 days of habituation as described above, with the following exception. Each day immediately prior to testing, observers in the "stranger" group were separated from their cagemates and their hemi-cage was merged with a hemi-cage containing an unfamiliar same-sex demonstrator. Unfamiliar pairs were subsequently treated identically to other treatment groups, and therefore had 1 hour of habituation prior to collection of baseline data. Each observer in the "stranger" group was exposed to a different unfamiliar demonstrator on each testing day. Following testing each day, all unfamiliar demonstrators were separated back into their original hemi-cages and returned to their cagemates. Prior to mating, male and female prairie voles are generally not aggressive toward unfamiliar same-sex conspecifics (*42*); nonetheless, voles in the "stranger" group were monitored for aggressive attacks and eliminated if excessive fighting or injury occurred.

Rank-transformed data on duration of allogrooming from observers of both sexes were analyzed using a 2x2x3 ANOVA, with time (before, after) as a within-subjects factor and sex (male, female) and relationship (mate, sibling, stranger) as between-subjects factors. For the time-relationship interaction, data from both sexes were combined and post-hoc paired t-tests

compared time points within each relationship group. For the time-sex interaction, data from all relationships were combined and post-hoc paired t-tests compared sexes within each time point. An additional 2x3 ANOVA was used to analyze latency to partner-groom in the after time point, with sex (male, female) and relationship (mate, sibling, stranger) as between-subjects factors. Data from both sexes were combined for post-hoc tests, which compared different levels of relationship. Results appear in Figs. 3C, S2, and S6.

To test an independent hypothesis regarding whether prairie voles show a different response to vicarious distress than they do to personal distress (self-other differentiation), a separate 2x2 ANOVA on rank-transformed data (with time (before, after) as a within-subjects factor and subject (observer, demonstrator) as a between-subjects factor) compared allogrooming between subjects (male and female) that had either been exposed to a stressed mate (observer group) or received primary exposure to the stressor (demonstrator group). Post-hoc paired t-tests compared time points within each subject group. Results appear in Fig. 3D.

To test an independent hypothesis regarding emotional contagion, a separate 2x2 Repeated Measures ANOVA was used to analyze the rank-transformed duration of self-grooming before and after separation between male observers and their female mate demonstrators. Post-hoc paired t-tests compared time points within each subject group. Results appear in Fig. 2A.

Consolation in Unrelated Cagemates. We tested whether consolation behavior would be observed between unrelated long-term cagemates. Male cagemates (N=9 pairs) were co-housed since weaning and then administered a consolation test with 3 days of habituation as described above, with one cagemate randomly selected as the observer. Rank-transformed data on duration of observer allogrooming before and after separations with stressor were compared using a paired t-test. Results appear in Fig. S7.

Emotional Contagion of Fear. Male prairie vole observers (N=12) were paired with age-matched female demonstrators for 2 days prior to training. Subsequently, females were exposed to 3 sessions of fear conditioning as described above over the course of 9 days. On the day following the training, the male observer and female demonstrator were placed together into a novel cage and given 90 seconds to habituate. Pairs were then given five exposures to the conditioned stimulus (30 s tone, 6 kHz, 60 s inter-trial interval). Freezing duration in observers and demonstrators during each of the first 3 tones was scored and averaged. Freezing duration during the 30 seconds prior to each tone was scored and averaged to represent pre-tone behavior. Freezing duration data were analyzed using a 2x2 Repeated Measures ANOVA, with time (pre-tone, tone) as the within-subjects factor and subject (observer, demonstrator) as the between-subjects factor. Post-hoc paired t-tests compared time points within each subject group. Coordinated freezing was calculated in a separate analysis as the observed amount of concurrent freezing by both the observer and the demonstrator during the tone presentations (as a percent of total tone presentation time), minus the concurrent freezing expected by chance. The concurrent freezing expected by chance was calculated within each subject pair as the percent of time the observer spent freezing during the tones multiplied by the percent of time the demonstrator spent freezing during the tones. Coordinated freezing scores were then tested against an expected value of zero using a single-sample t-test. Results appear in Figs. 2B and 2C.

State Matching and Social Buffering. Male prairie vole observers (N=70) were paired with age-matched female demonstrators for 3-5 days prior to testing. Mated pairs were then administered a consolation test with either 2 or 3 days of habituation as described above, with the following exceptions. After separation on all testing days, observers were subjected to one of three different testing conditions: either the hemi-cages were merged as before and no barrier

was present; or the hemi-cages were merged but observers and demonstrators were separated across a transparent, perforated barrier; or observers and demonstrators remained isolated in separate hemi-cages. The post-separation reunion period in this experiment was restricted to 5 minutes. On the last day of testing, half of the demonstrators were stressed with tone-shock pairings during separation, while the other half remained in the hemi-cage as a control. The combination of these two factors, barrier and treatment, defined the six independent groups (no barrier/separation, N=12; no barrier/shock, N=12; clear barrier/separation, N=12; clear barrier/shock, N=12; solid barrier/separation, N=12; solid barrier/shock, N=10).

Following the 24-minute separation and 5-minute reunion on the last day of testing, all male and female subjects were administered an elevated plus maze (EPM) test for 5 minutes as previously described (*43*). The final data set excluded some subjects that jumped from the EPM prior to the end of testing (no barrier/separation, observers: N=11, demonstrators: N=11; no barrier/shock, observers: N=12, demonstrators: N=11; clear barrier/separation, observers: N=11, demonstrators: N=12; clear barrier/shock, observers: N=11, demonstrators: N=11; solid barrier/separation, observers: N=8, demonstrators: N=11; solid barrier/shock, observers: N=10, demonstrators: N=10). Immediately after the EPM, trunk or heart blood was collected from male and female subjects and processed for corticosterone radioimmunoassay as previously described (*43*). Some subjects jumped from the EPM following the end of the test, resulting in the exclusion of their plasma corticosterone data. Additionally, some plasma corticosterone measurements were excluded for excessive variation between technical replicates (CV > 20%). These exclusions resulted in smaller N's for the plasma cort analysis in observers (no barrier/separation, N=11; no barrier/shock, N=12; clear barrier/separation, N=11; clear barrier/shock, N=11; solid barrier/separation, N=7; solid barrier/shock, N=9). Additionally, correlations included only observer-demonstrator pairs where plasma cort data could be obtained for both observer and demonstrator (clear barrier/separation, N=11; clear barrier/shock, N=9). An average of 12 minutes passed from the beginning of the reunion period to the start of euthanasia preceding blood collection.

Plasma corticosterone data from stressor groups were divided by the average plasma corticosterone concentration of the corresponding separation control group, and then analyzed using a one-way ANOVA with barrier (none, clear, solid) as the between-subjects factor. Post-hoc single-sample t-tests compared each barrier group with an expected value of 100%. Male and female plasma corticosterone concentrations in the clear barrier groups (separation and stressor) were compared using Pearson's correlation, and the two correlations were compared using Fisher's transformation. Male and female open arm times on the EPM were analyzed with separate 2x3 ANOVAs, with treatment (separation, shock) and barrier (none, clear, solid) as between-subjects factors. Post-hoc t-tests compared treatments within each barrier group. Results appear in Figs. 1D, 3A, and 3B.

Novel Experience. Male and female prairie vole observers (N=18) were paired with opposite-sex demonstrators for 4 days prior to testing. Mated pairs were then administered a consolation test as described above, with the following exceptions. Pairs were housed in standard colony caging rather than modified Super Mouse 750 cages. Pairs were not habituated to the testing procedures prior to the day of consolation testing, and therefore all data was collected on the first day of testing. The baseline period consisted of 60 minutes of digital recording, from which 10 minutes were used for baseline behavioral analysis. On the day of testing, the demonstrators were separated from the observers and moved to a fear conditioning chamber by cup transfer. Demonstrators habituated to the chamber for 5 minutes and then were subjected to five

presentations of one of two odors (30 s, either 50% eugenol or acetophenone), counterbalanced within each group. Presentations occurred at 3-4 minute variable inter-trial intervals, for a total of 24 minutes. In one treatment group (N=12), each odor presentation preceded a light foot shock (0.5 s, 0.8 mA), while the other treatment group (N=6) experienced odors only. Demonstrators were then returned to the home cage containing the observer by cup transfer.
Rank-transformed data on duration of allogrooming were analyzed using a 2x2 ANOVA, with time (before, after) as a within-subjects factor and treatment (odor, odor with shock) as a between-subjects factor. Post-hoc paired t-tests compared time points within each treatment condition. Results appear in Fig. S8.

FOS Immunochemistry. Male prairie vole observers (N=20) were paired with age-matched female demonstrators for 2 days prior to testing. Mated pairs were then administered a consolation test with 3 days of habituation as described above. On the last day of testing, mated pairs were divided into two groups: one in which demonstrators (N=10) were separated only, and another in which demonstrators (N=10) were separated and exposed to the stressor as described above. Observers and demonstrators were then reunited for exactly 5 minutes and then separated again. Observers then rested alone in the home cage for another 70 minutes, at which time they were euthanized and perfused as described below for FOS immunohistochemistry.
In a second experiment, an independent set of male prairie vole observers (N=20) were paired and tested exactly as before in two groups (separated, N=9; separated with stressor, N=11) except that, during the 5-minute reunion period, observers and demonstrators were separated by a clear, perforated barrier. Observers then rested alone as before for 70 minutes before being euthanized and perfused as described below for FOS immunohistochemistry.
Perfusion. Prairie vole subjects were administered an overdose of isoflurane and were immediately perfused transcardially with 40 mL of phosphate-buffered saline (PBS; Teknova, Hollister, CA) followed by 40 mL of PBS containing 4% paraformaldehyde (Polysciences, Warrington, PA) at 4 mL/minute using a perfusion pump (Easy-Load II Masterflex; Cole-Palmer, Vernon Hills, IL). Brains were then removed, post-fixed overnight in PBS containing 4% paraformaldehyde, and finally transferred to PBS containing 30% sucrose until sectioning.
Sectioning. Perfused brains were cut into 40 μm sections using a sliding microtome (Microm HM 450, Microm International, Walldorf, Germany) with a freezing stage (Physitemp BFS-30TC, Physitemp Instruments, Clifton, NJ) and were stored in 1x PBS with 0.5% sodium azide until immunohistochemical staining.
Immunochemistry. Sections were washed 3 times in PBS, incubated for 10 minutes in PBS containing 1% sodium hydroxide, and washed 3 times in PBS containing 0.5% Triton-X (PBST; Sigma-Aldrich, St. Louis, MO) before incubating in PBST containing 5% normal goat serum (Fitzgerald, Acton, MA) for 1 hour at room temperature. Sections then incubated for 48 hours in PBS containing primary rabbit polyclonal anti-FOS antibody (PC38; Calbiochem) at a dilution of 1:20,000 on an orbital shaker at 4°C. Following primary incubation, sections were washed 5 times in PBS and once in PBST containing 5% normal goat serum before incubating in secondary biotinylated goat anti-rabbit IgG antibody (1:500; Vector Labs BA-1000) for 2 hours. After secondary incubation, sections were washed 5 times with PBS and treated with an avidin-biotin peroxidase system (Vectastain ABC Kit, Vector Labs) and finally with a Nickel-DAB peroxidase substrate kit (Vector Labs). Sections were washed 3 times in PBS and stored in PBS containing 0.5% sodium azide until being mounted on slides (Superfrost Plus, Fisher Scientific, Pittsburgh, PA). Mounted sections were dehydrated in a series of ethanol solutions followed by Histo-Clear (5 minutes in 70% EtOH; 10 minutes in 95% EtOH; 10 minutes in 95% fresh EtOH;

10 minutes in 100% EtOH; 10 minutes in fresh 100% EtOH; 10 minutes in Histo-Clear; and 10 minutes in fresh Histo-Clear) and coverslipped using Krystalon (EMD Chemicals, Gibbstown, NJ). Finally, sections were imaged at 4x magnification (Eclipse E800, Nikon, Tokyo, Japan) and FOS-positive cells were counted bilaterally at three anatomical positions within the target brain regions using MCID 7.0 (GE Healthcare Life Sciences, Marlborough, MA).

In the first experiment, the average of all FOS-positive cell counts within the ACC, PLC and NACS were analyzed using a 2x3 ANOVA, with treatment (stressed, unstressed) as a between-subjects factor and brain region (ACC, PLC, NACS) as a within-subjects factor. Post-hoc t-tests looked at differences between groups within each brain region. Following a positive result in the ACC, the second experiment counted only FOS-positive cells in the ACC and analyzed them between groups using a t-test. As an exploratory analysis in each experiment, FOS-positive cell counts were averaged separately for the three rostral-caudal anatomical positions measured within ACC, and compared between groups using t-tests. Results appear in Figs. 4C, 4D, S8, and S9.

Receptor Autoradiography. Brains from subjects in some experiments were collected and processed for receptor autoradiography as previously described (*44*) targeting the oxytocin receptor. Autoradiographs are presented in Fig. 4B.

ICV Injection of OTA. Male prairie vole observers (N=28) were surgically implanted under isoflurane anesthesia with a unilateral guide cannula, dummy cannula and cap (PlasticsOne, Roanoke, VA) as previously described (*45*) targeting either the left or right lateral ventricle (AP +0.6 mm; ML ± 1 mm; DV -1.2 mm from Bregma). After 3-4 days of recovery, observers were paired with an age-matched female demonstrator for 3 days. Observers were then habituated to the consolation test protocol for three days as described above. On the morning of the last day of testing, observers were anesthetized and an ICV injection of either OTA (5 ng in 2 µL aCSF; N=16) or vehicle (2 µL aCSF; N=12) was administered via injection cannula (PlasticsOne, Roanoke, VA). Observers were allowed between 15 minutes and 2 hours to recover from anesthesia before being moved to the testing room. All subjects were then administered a consolation test as described above.

Following consolation testing, observers were euthanized and 2 µL of 3% India ink was injected into the lateral ventricle using the same procedure as above. The brain was then immediately harvested, cut in half with a razor blade, and photographed to verify the presence of ink in the ventricles. Subjects with no ink present in the ventricles after this procedure were eliminated from the analysis. One subject received no ink injection and cannula placement was confirmed through histological location of the guide cannula on slide-mounted brain sections.

Data on rank-transformed duration of allogrooming were analyzed using a 2x2 ANOVA, with time (before, after) as a within-subjects factor and treatment (vehicle, OTA) as a between-subjects factor. Post-hoc paired t-tests compared time points within each treatment group. Results appear in Fig. 4A.

Site-specific Injection of OTA. In separate experiments, male prairie vole observers were surgically implanted under isoflurane anesthesia with bilateral guide cannulae, dummy cannulae and cap (PlasticsOne, Roanoke, VA) as previously described (*45*) targeting either the pre-limbic cortex (PLC; N=28; AP +2.4 mm, ML ± 0.8 mm, DV -2.2 mm from Bregma) or anterior cingulate cortex (ACC; N=28; AP +1.4 mm, ML ± 0.8 mm, DV -1.3 mm from Bregma). After 3-5 days of recovery, observers were paired with an age-matched female demonstrator for 1-3 days. Observers were then habituated to the consolation test protocol for three days as described above. On the last day of testing, observers were anesthetized and given a bilateral injection of

either vehicle (0.2 µL aCSF/side) or vehicle containing OTA (0.5 ng in 0.2 µL aCSF/side) into the targeted brain region (aCSF in PLC, N=12; OTA in PLC, N=16; aCSF in ACC, N=12; OTA in ACC, N=16) using a bilateral internal cannula (PlasticsOne, Roanoke, VA). Infusions were delivered slowly using a microsyringe pump controller (Micro4, World Precision Instruments, Sarasota, FL) over the course of 5 minutes, an injection rate expected to limit diffusion to less than 0.5 mm (*46*). Observers were allowed between 15 minutes and 2 hours to recover from anesthesia before being moved to the testing room. Observers were then administered a consolation test as described above.

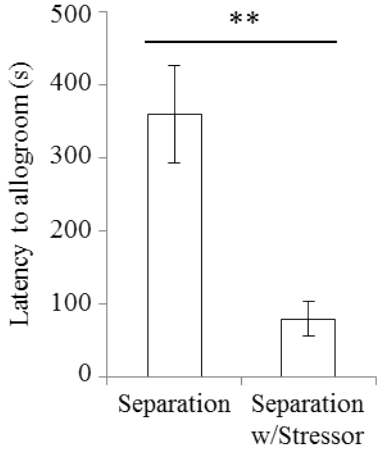
Following consolation testing, cannula placement was confirmed using 1% methylene blue dye as previously described (*44*). The location of each hit was then plotted on images from the mouse atlas (*47*). Observers with no dye in the target brain region were eliminated from analysis. Some observers did not receive dye injections and cannula placement was verified through histological location of the guide cannula on slide-mounted brain sections.

Data on rank-transformed duration of allogrooming were analyzed in each experiment using a 2x2 ANOVA, with time (before, after) as a within-subjects factor and treatment (vehicle, OTA) as a between-subjects factor. Post-hoc paired t-tests compared time points within each treatment group. Results appear in Figs. 4E, 4F and S10.

Meta-Analysis. In order to represent the observed values for the behavioral measures used in this study with the greatest possible degree of precision, a meta-analysis was performed using Comprehensive Meta-Analysis 2.0 (Biostat, Englewood, NJ). To avoid publication bias, we included all scored experiments in our laboratory where a consolation test using comparable methods was administered. Individual groups from these experiments were included in the analysis if observers were male or female prairie voles and demonstrators were familiar conspecifics. Data from experimental groups containing meadow vole observers, prairie vole observers paired with strangers, or where observers received an experimental manipulation, were excluded from the analysis. In experiments where observers were administered more than one consolation test, data from only the first test were included. These criteria resulted in the inclusion of twenty groups of subjects from thirteen experiments (Table S1). The primary measures included in the analysis were duration of allogrooming and latency to allogroom. Duration of allogrooming was subdivided into baseline (pre-separation) duration, cumulative duration per minute post-separation, and cumulative duration per minute post-separation with stressor. Latency to allogroom was subdivided into post-separation and post-separation with stressor. Groups were combined using a random-effects model to account for heterogeneity across experiments. Raw values were used to calculate point estimates and 95% confidence intervals of all outcome measures. The effect sizes and p-values of the within-experiment differences between time points were determined using rank-transformed data only from experiments where measurements were taken at each of the time points being compared. Results appear in Table 1 and Figs. 1C, S3, and S4.

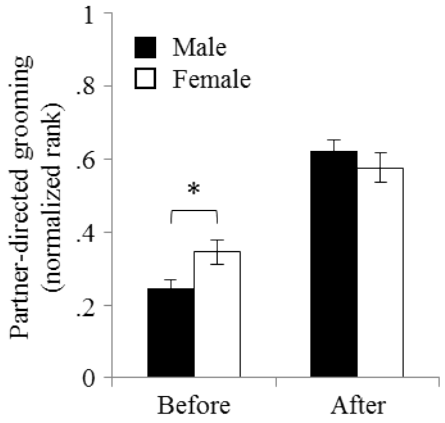
Supplementary Figures:

Figure S1



Latency. Observers (N=12) are faster to initiate allogrooming toward demonstrators after separations during which the demonstrator was stressed. Bars represent the mean ± SEM of the observer's latency to allogroom the demonstrator. ** $p<0.005$.

Figure S2

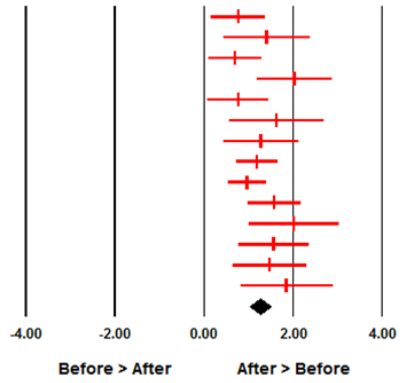


Sex difference in allogrooming. Male and female observers differed only in baseline allogrooming and not in their response to stressed demonstrators. Bars represent the mean ± SEM of the ranked duration of allogrooming performed by the observer. * $p<0.007$.

Figure S3

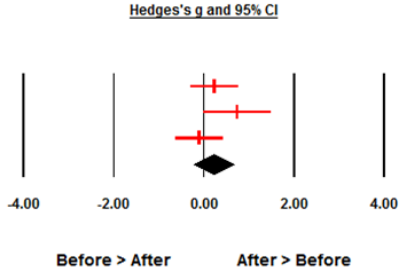
A

Study name	Subgroup	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Experiment 2	1.000	0.767	0.311	0.097	0.158	1.377	2.469	0.013563189
Experiment 3	1.000	1.404	0.499	0.249	0.426	2.382	2.815	0.004880533
Experiment 4	1.000	0.684	0.303	0.092	0.091	1.278	2.261	0.023756732
Experiment 5	1.000	2.028	0.430	0.185	1.186	2.871	4.717	0.000002389
Experiment 7	1.000	0.767	0.351	0.123	0.079	1.455	2.184	0.028935130
Experiment 8	1.000	1.620	0.544	0.296	0.555	2.686	2.980	0.002879504
Experiment 9	1.000	1.275	0.425	0.181	0.441	2.109	2.997	0.002723182
Experiment 10	1.000	1.185	0.242	0.059	0.709	1.660	4.885	0.000001032
Experiment 10	2.000	0.967	0.221	0.049	0.534	1.400	4.380	0.000011888
Experiment 11	1.000	1.571	0.301	0.090	0.982	2.161	5.226	0.000000173
Experiment 11	2.000	2.017	0.512	0.262	1.013	3.021	3.937	0.000082341
Experiment 11	3.000	1.562	0.402	0.161	0.775	2.349	3.890	0.000100413
Experiment 11	4.000	1.465	0.418	0.175	0.645	2.286	3.502	0.000461164
Experiment 13	1.000	1.849	0.530	0.281	0.811	2.887	3.490	0.000482161
		1.259	0.116	0.014	1.031	1.487	10.828	0.000000000



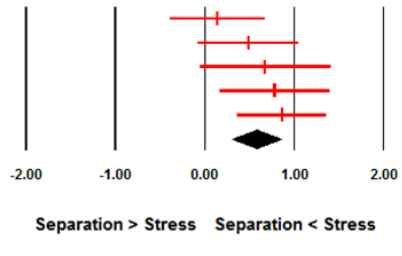
B

Study name	Subgroup	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Experiment 2	1	0.231	0.273	0.074	-0.303	0.765	0.847	0.396921589
Experiment 3	1	0.731	0.382	0.146	-0.019	1.480	1.911	0.055996476
Experiment 4	1	-0.107	0.269	0.073	-0.635	0.421	-0.398	0.690749505
		0.223	0.222	0.049	-0.212	0.659	1.007	0.314137091



C

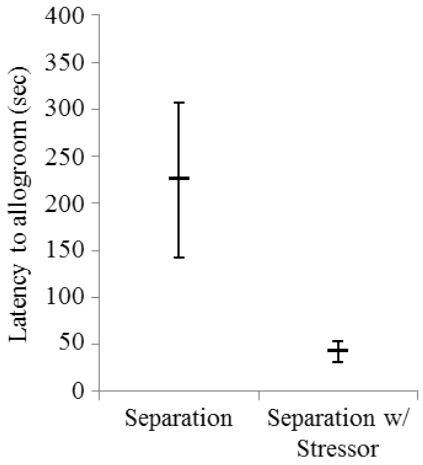
Study name	Subgroup	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Experiment 1	1, 2	0.138	0.270	0.073	-0.391	0.668	0.513	0.608274614
Experiment 2	1	0.487	0.286	0.082	-0.074	1.048	1.701	0.088858045
Experiment 3	1	0.673	0.375	0.140	-0.061	1.408	1.797	0.072357319
Experiment 4	1	0.779	0.312	0.097	0.167	1.390	2.495	0.012580913
Experiment 12	1, 2	0.862	0.254	0.065	0.363	1.360	3.389	0.000700358
		0.578	0.138	0.019	0.307	0.850	4.176	0.000029690



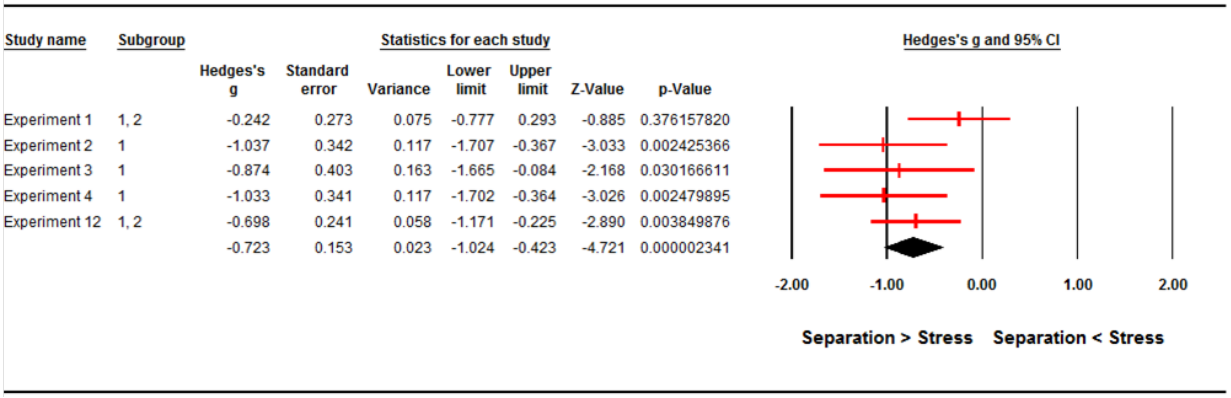
Meta-analysis of duration. Forest plots show the effect sizes of the within-experiment differences between the observer's allogrooming at (A) baseline and after a separation with stressor (Hedges' g=1.3, $p<3x10^{-26}$), (B) baseline and after a control separation (Hedges' g=0.22, p>0.31), and (C) separation with and without a stressor (Hedges' g=0.58, p<0.0001). Hedges' g for each study was calculated using ranked duration. The last row of each plot shows the overall effect size and the lower and upper limits of the 95% confidence interval. Experiment numbers refer to the experiments in Table S1.

Figure S4

A



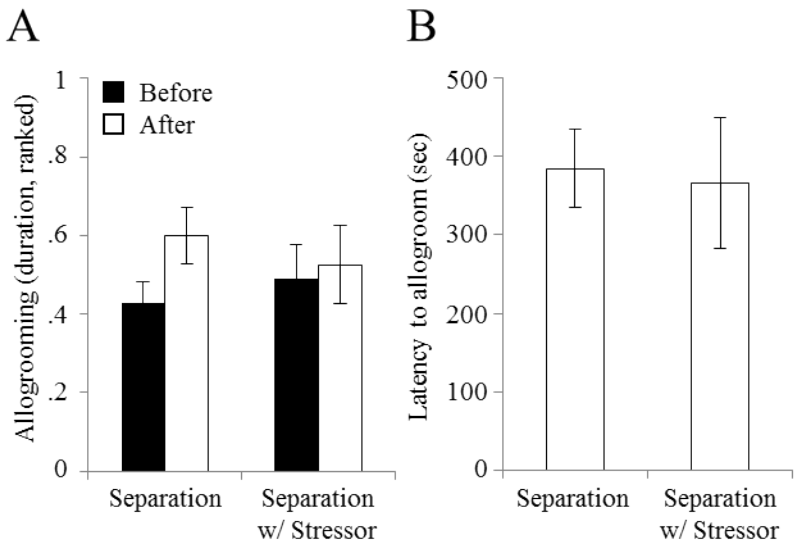
B



Study name	Subgroup	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Experiment 1	1, 2	-0.242	0.273	0.075	-0.777	0.293	-0.885	0.376157820
Experiment 2	1	-1.037	0.342	0.117	-1.707	-0.367	-3.033	0.002425366
Experiment 3	1	-0.874	0.403	0.163	-1.665	-0.084	-2.168	0.030166611
Experiment 4	1	-1.033	0.341	0.117	-1.702	-0.364	-3.026	0.002479895
Experiment 12	1, 2	-0.698	0.241	0.058	-1.171	-0.225	-2.890	0.003849876
		-0.723	0.153	0.023	-1.024	-0.423	-4.721	0.000002341

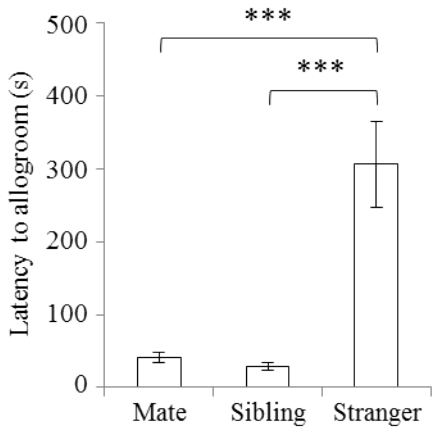
Meta-analysis of latency. (A) A meta-analysis of results from 13 experiments shows the observer's precise latency to groom the demonstrator after control separations and after separations with a stressor. Lines represent the mean ± 95% confidence interval. (B) A forest plot shows the effect size of the within-experiment difference between the observer's latency to allogroom after control separations and after separations with stressor. The last row of the plot shows the overall effect size (Hedges' g=-0.72, $p<0.0001$) and the lower and upper limits of the 95% confidence interval. Experiment numbers refer to the experiments in Table S1.

Figure S5



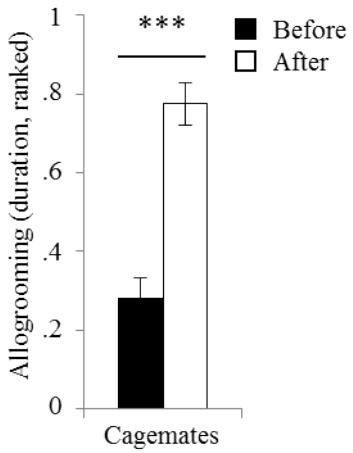
Consolation test in meadow voles. Meadow vole males (N=12) do not show an increase in allogrooming toward stressed female mates. (A) Bars represent the mean ± SEM of the ranked duration of allogrooming performed by the male observer. (B) Bars represent the mean ± SEM of the male observer's latency to allogroom the female mate.

Figure S6



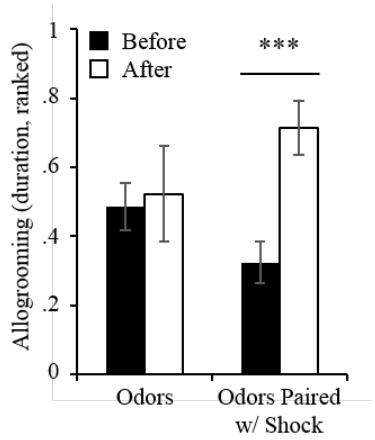
Familiarity bias in latency. Observers are faster to initiate allogrooming toward stressed mates and siblings than toward stressed strangers. Bars represent the mean ± SEM of the observer's latency to allogroom the demonstrator. *** $p<0.0001$.

Figure S7



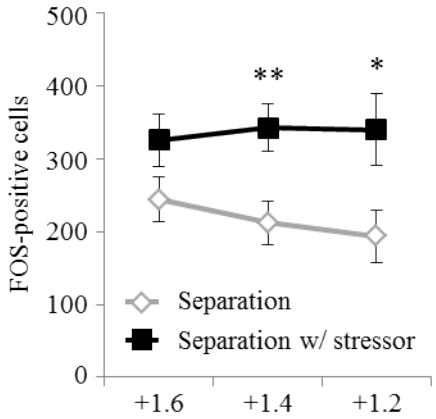
Cagemates. Unrelated same-sex cagemates, housed together since weaning, underwent separations where one cagemate was stressed. Bars represent the mean ± SEM of the ranked duration of allogrooming directed by the observer toward the demonstrator. *** $p<0.0005$.

Figure S8



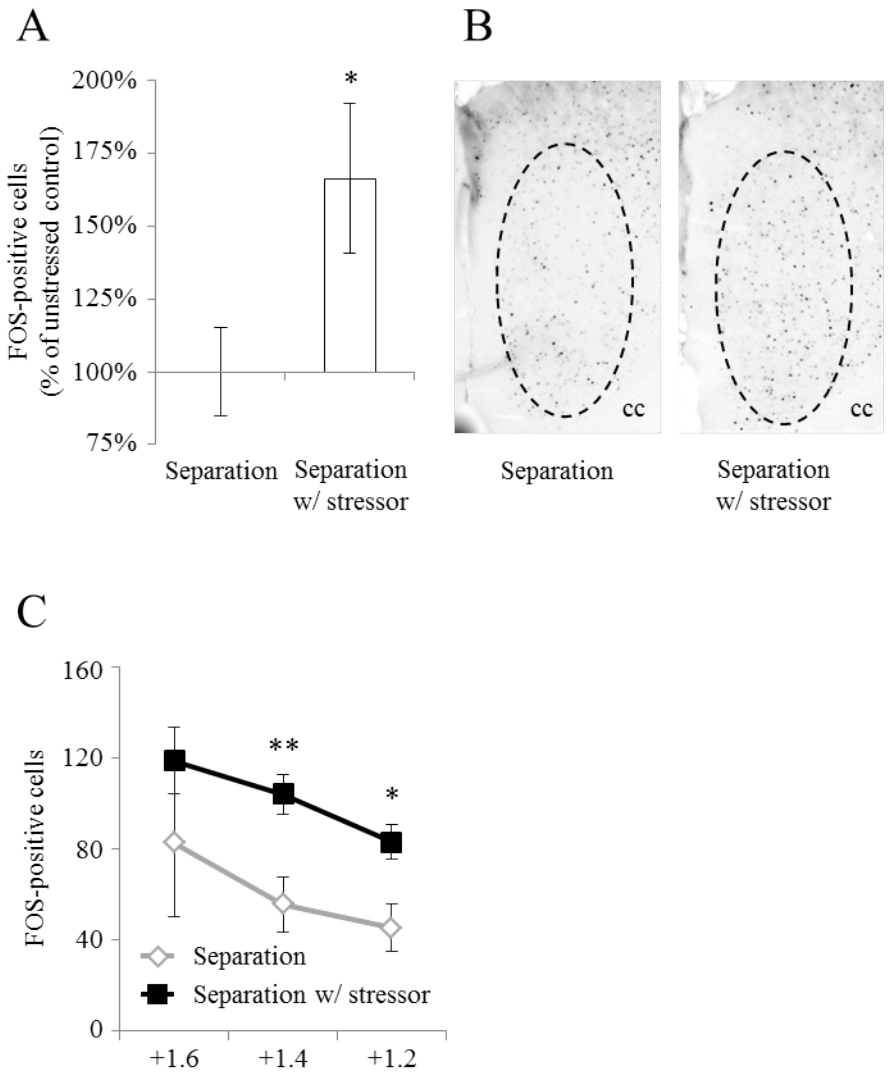
Novel Experience. Observer-demonstrator pairs underwent separations where the demonstrator was moved into a chamber and either exposed to odors alone, or to odors paired with light foot shocks. Bars represent the mean ± SEM of the ranked duration of allogrooming directed by the observer toward the demonstrator. *** $p<0.0005$.

Figure S9



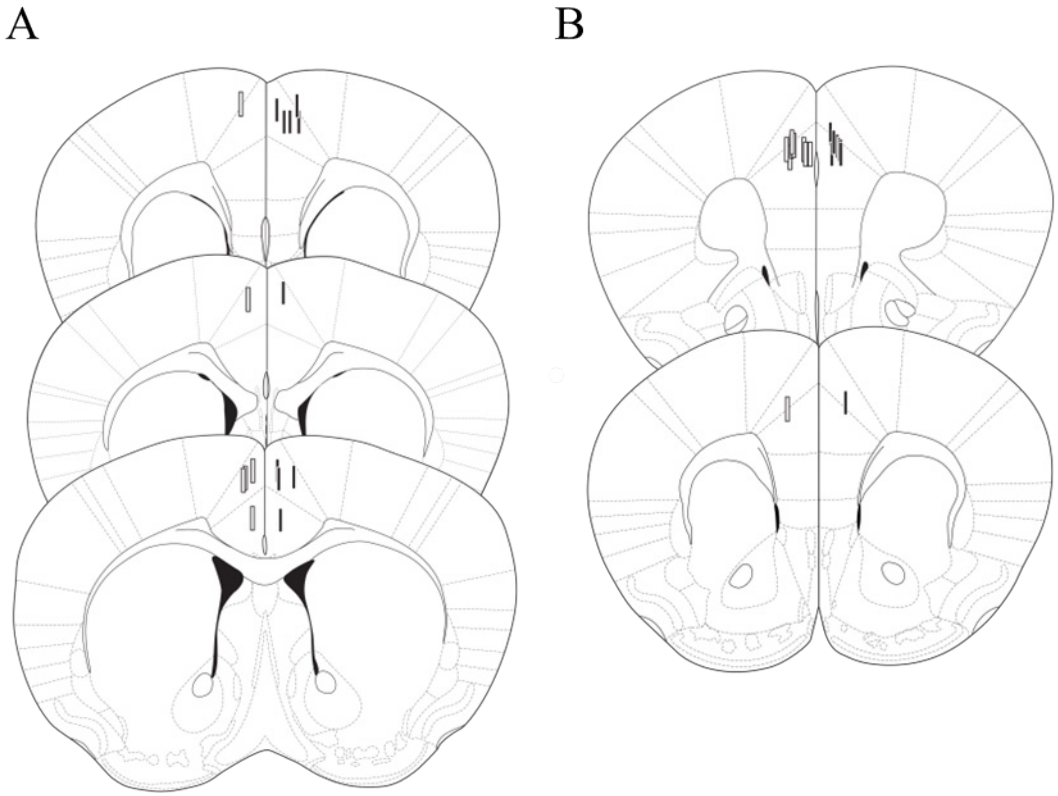
FOS in ACC by coordinates. Observers (from Figs. 4C-D) were administered a consolation test with either control separations or separations with stressor. An exploratory analysis looked at the relationship between FOS-positive cell counts in both groups at the three rostral-caudal anatomical positions within the ACC that were averaged for the primary analysis. The largest statistical difference occurred at +1.4 from Bregma, the coordinates targeted for drug injections. Data points represent the mean ± SEM of the count of FOS-positive cells. * p=0.03, ** p=0.01.

Figure S10



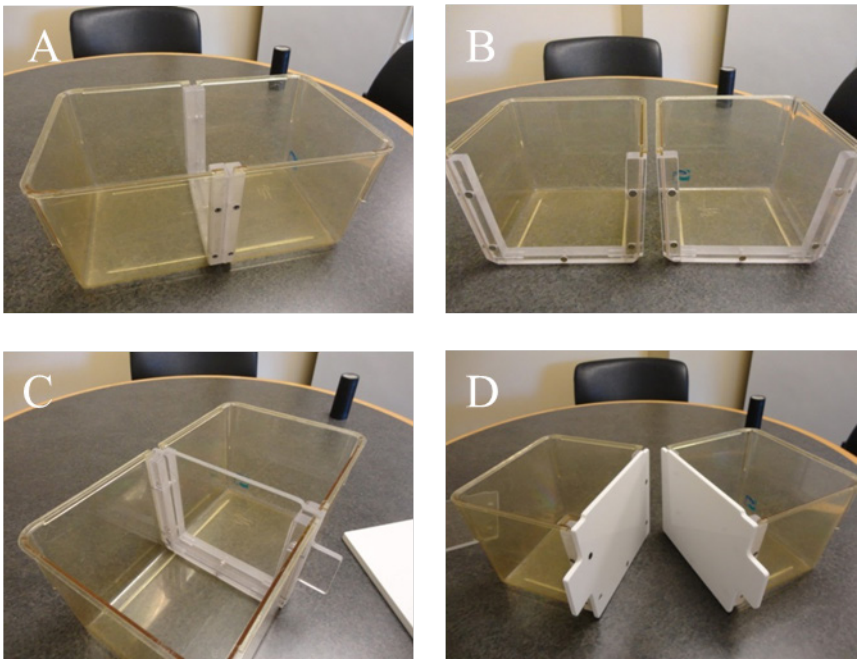
ACC activation during observation of stressed demonstrator. Observers were administered a consolation test where they were exposed to either stressed (N=10) or unstressed (N=10) demonstrators across a clear, perforated barrier for 5 minutes. (A) Observers exposed to stressed demonstrators (relative to those exposed to unstressed demonstrators) showed increased activity in the ACC. Bars represent the mean ± SEM of the count of FOS-positive cells as a percent of the unstressed control. (B) Representative brain sections showing FOS immunostaining in the ACC from both treatment groups. Dotted circles show the quantified area. cc: corpus callosum. (C) An exploratory analysis looked at the relationship between FOS-positive cell counts in both groups at the three rostral-caudal anatomical positions within the ACC that were averaged for the primary analysis. The largest statistical difference occurred at +1.4 from Bregma, the coordinates targeted for drug injections. Data points represent the mean ± SEM of the count of FOS-positive cells. * $p<0.05$, ** $p<0.005$.

Figure S11



Cannula placement. The location of intracranial injections into (A) ACC and (B) PLC is shown by bars representing the 0.5 mm path of the injection cannula, from the end of the implanted guide cannula to the target brain region. Injections occurred at the bottom of the bar. Open bars (left) show vehicle injections; solid bars (right) show drug injections.

Figure S12



The consolation testing cage. (A) The cage with the two halves connected together to form a single space. In this configuration, the cage can be used as standard housing. Cage lids and feeders not shown. (B) The two cage halves pulled apart to show the braces with embedded magnets. (C) The cage with a clear, perforated barrier inserted in between the cage halves. The clear barrier has embedded magnets matching the cage magnets. (D) The two cage halves pulled apart with solid barriers inserted to form two independent hemi-cages.

Table S1

				Latency - Separation		Latency - Separation w/stressor		Duration - Baseline		Cumulative duration (by minute) - Separation																								Cumulative duration (by minute) - Separation w/stressor																			
Experiment	Groups	Observer Sex	N	Mean	SD	Mean	SD	Mean	SD	M1 Mean	M1 SD	M2 Mean	M2 SD	M3 Mean	M3 SD	M4 Mean	M4 SD	M5 Mean	M5 SD	M6 Mean	M6 SD	M7 Mean	M7 SD	M8 Mean	M8 SD	M9 Mean	M9 SD	M10 Mean	M10 SD	M1 Mean	M1 SD	M2 Mean	M2 SD	M3 Mean	M3 SD	M4 Mean	M4 SD	M5 Mean	M5 SD	M6 Mean	M6 SD	M7 Mean	M7 SD	M8 Mean	M8 SD	M9 Mean	M9 SD	M10 Mean	M10 SD
Experiment 1	1	Male	6	108.03	241.06	18.77	21.54			4.73	4.76	6.35	6.88	7.1	8.32	11.4	11.29	11.52	11.18	12.27	10.52	12.95	10.76	13.22	10.47	13.77	9.99	15.17	11.19	9.53	13.56	16.4	28.5	22.5	39.80	25.3	42.21	25.3	42.21	33.15	59.69	35.07	60.00	36.25	59.74	37.4	59.35	41.48	58.06
Experiment 2	1	Male	12	359	232.16	79.07	83.45	3.14	4.40	0.75	0.93	1.58	2.32	3.93	8.07	4.83	10.24	5.55	12.20	7.46	16.02	10.07	17.66	11.98	20.39	12.93	21.08	13.78	21.80	4.08	7.26	7.22	11.76	10	16.52	12.72	16.52	17.72	26.72	17.72	26.72	21.37	28.64	22.32	28.87	24.1	30.79	26.6	32.02
Experiment 3	1	Male	7	240.9	177.56	124.51	211.58	2.62	3.33	0.34	0.37	1.33	1.85	2.23	2.59	7.6	14.27	9.7	18.68	10.54	18.92	10.54	18.92	10.89	18.72	10.89	18.72	11.07	19.2	6.80	7.09	16.49	16.27	21.11	16.78	23.2	17.68	23.2	17.68	24.03	18.99	25.83	22.17	28.06	26.79	28.29	26.51	32	25.58
Experiment 4	1	Male	12	360.27	252.5	60.83	69.73	6.72	10.30	0.26	0.89	0.33	0.89	0.8	1.23	2.65	6.27	2.65	6.27	2.73	6.28	4.23	6.89	4.23	6.89	4.71	6.91	4.79	6.85	4.65	4.42	8.49	9.7	11.99	16.68	16.61	25.25	21.17	39.97	25.71	47.35	29.74	55.37	35.08	62.79	35.3	62.80	35.96	62.73
Experiment 5	1	Male	16			32.17	36.8	2.41	4.32																					14.84	15.40	22.11	20.82	26.51	28.95	27.82	29.98	28.91	30.26	29.41	30.52	33.1	32.93	38	41.27	42.76	51.77	43.64	54.20
Experiment 6	1	Male	12	142.48	136.49					2.02	2.94	5.46	8.56	6.93	12.73	7.87	15.70	9.56	19.10																																
	2	Male	12			22.43	30.35																							6.11	4.44	9.58	6.93	11.86	7.69	16.47	13.58	17.45	14.93										
Experiment 7	1	Male	9			132.94	89.61	11.52	10.59																					6.27	8.69	12.02	15.94	18.58	24.78	19.41	24.36	20.37	25.07	28.64	29.75	33.58	33.41	33.76	33.64	37.09	31.32	39.53	31.18
Experiment 8	1	Male	7			55.46	27.98	3.68	5.69																					8.86	7.37	22.43	22.15	25.31	25.33	31.97	23.54	37.3	26.71	40.27	24.13	43.2	23.56	50.81	34.34	59.14	30.89	65.93	35.67
Experiment 9	1	Male	9			61.96	96.51	4.26	5.16																					6.64	6.26	9.24	9.25	11.24	13.22	12	14.48	12.49	15.76	15.18	14.97	15.62	14.81	17.6	15.96	22.26	20.38	25.06	23.97
Experiment 10	1	Male	28			94.38	154.19	7.04	9.31																					11.90	11.25	18.69	18.21	23.88	22.76	29.67	28.63	33.85	31.34	37.6	37.7	41.53	37.5	43.43	38.15	45.28	40.12	47	40.41
	2	Male	29			83.15	145.94	8.32	11.90																					6.24	7.20	13.24	14.97	17.8	20.05	23.73	25.67	26.92	28.78	28.53	30.19	31.11	30.63	33.1	32.39	36.91	33.6	38.11	34.51
Experiment 11	1	Male	24			50.33	52.18	2.22	4.57																					13.13	14.38	18.71	20.54	23.18	25.05	28.19	32.21	29.62	32.92	32.34	34.81	35.31	37.61	38.72	42.05	42.76	45.47	43.31	45.11
	2	Male	11			7.74	5.47	0.98	1.63																					9.20	11.13	21.26	17.96	34.07	31.23	39.43	33.15	49.26	40.44	57.98	54.49	62.64	62.18	67.75	67.19	67.75	67.19	69.29	67.91
	3	Female	13			43.48	15.76	6.03	9.81																					5.99	5.25	12.72	11.7	23.32	18.61	27.53	18.76	30.06	22.29	32.29	23.03	35.98	25.96	36.08	25.91	36.28	25.99	37.87	26.68
	4	Female	11			52.01	20.49	1.59	2.52																					11.20	12.80	17.45	16.81	23.19	20.42	25.58	20.29	29.32	27.09	32.39	30.64	36.92	31.80	38.67	33.69	38.98	33.92	41.18	36.08

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