nature neuroscience

ARTICLES

https://doi.org/10.1038/s41593-017-0044-6

Social transmission and buffering of synaptic changes after stress

Toni-Lee Sterley, Dinara Baimoukhametova, Tamás Füzesi, Agnieszka A. Zurek, Nuria Daviu, Neilen P. Rasiah, David Rosenegger and Jaideep S. Bains*

Stress can trigger enduring changes in neural circuits and synapses. The behavioral and hormonal consequences of stress can also be transmitted to others, but whether this transmitted stress has similar effects on synapses is not known. We found that authentic stress and transmitted stress in mice primed paraventricular nucleus of the hypothalamus (PVN) corticotropin-releasing hormone (CRH) neurons, enabling the induction of metaplasticity at glutamate synapses. In female mice that were subjected to authentic stress, this metaplasticity was diminished following interactions with a naive partner. Transmission from the stressed subject to the naive partner required the activation of PVN CRH neurons in both subject and partner to drive and detect the release of a putative alarm pheromone from the stressed mouse. Finally, metaplasticity could be transmitted sequentially from the stressed subject to multiple partners. Our findings demonstrate that transmitted stress has the same lasting effects on glutamate synapses as authentic stress and reveal an unexpected role for PVN CRH neurons in transmitting distress signals among individuals.

In mammals, an immediate threat activates multiple, interconnected neural networks to launch an innate behavioral program that maximizes the probability of survival. These networks also drive CRH neurons in the PVN to release hormones that allow the animal to cope in the face of challenge and restore homeostasis[1]. Even a brief exposure to a stressor leaves a lasting imprint on the brain. These imprints can be overt, manifesting as changes in intrinsic activity[2], activation of specific cell ensembles or changes in the strength of synapses[3]. Stress also primes neural networks, resulting in metaplasticity that is evident at synapses on PVN CRH neurons during a subsequent challenge[4–6].

In social species such as humans[7], primates[8] and rodents[9–11], distressed individuals benefit from consolation behaviors provided by others[12]. These interactions, however, can also transmit stress to the naive individual[11,13]. Indeed, behavioral and endocrine changes in partners of stressed individuals[9–11,13] offer proof that some parameters associated with stress map from one individual to others in the group. This physiological mimicry of stress by the naive individual prompted us to hypothesize that social interactions may also transmit persistent synaptic changes, or metaplasticity, from one individual to another. To test this hypothesis, we examined glutamate synapses onto PVN CRH neurons. These inputs, which originate in key stress-sensitive brain regions, including the brainstem[14,15], the medial amygdala and local cell populations in the hypothalamus[16], are primed by acute stress[6]. This priming enables these synapses to undergo short-term potentiation (STP) in response to high-frequency afferent stimulation[6]. STP following a single stress persists for days, providing a robust readout of the lasting effect of acute stress on this system.

Using mice, we found that both authentic and transmitted stress primed glutamate synapses on PVN CRH neurons. The behaviors that transmit stress from one individual to another also buffered the synaptic consequences of stress in females, but not males. Finally, transmission between individuals required the activation of PVN CRH neurons and the release of alarm signals.

Results

Acute stress primes glutamate synapses. Subjecting a mouse to footshock stress (FS) followed by 30 min in the homecage (Fig. 1a) increased plasma levels of corticosterone (CORT) in comparison with naive controls (Fig. 1b). Whole-cell recordings were obtained from CRH neurons in hypothalamic slices[17]. To examine metaplasticity, we recorded evoked excitatory postsynaptic currents (EPSCs) before and after high-frequency electrical stimulation (HFS) of afferents in the PVN[6]. HFS potentiated glutamate synapses in FS mice (Fig. 1c). This potentiation was distinct from classically described long-term potentiation at other central synapses, with a time constant of 2.18 min (Fig. 1c). Thus, we referred to it as STP. By contrast, there was no change in the strength of glutamate synapses in naive animals (Fig. 1c). STP was greater in FS mice than in naive mice (Fig. 1c). The increase in synaptic strength was accompanied by a decrease in the paired-pulse ratio of evoked synaptic currents (Supplementary Fig. 1a) and an increase in the frequency and amplitude of spontaneous EPSCs (Supplementary Fig. 1b–d), consistent with an increase in release probability and quantal content[6].

To determine whether the induction of STP in one cell had any effect on STP in other cells in the same slice, we examined data from multiple cells in the same slice. We observed no correlation in STP between cells recorded from the same slice (Supplementary Fig. 2a). Furthermore, STP in cell 1 was not different from STP in cell 2 (Supplementary Fig. 2b). We also compared the STP data from FS and naive mice on a per-mouse, rather than a per-cell, basis. There was a significant difference in STP in naive versus stressed mice (Fig. 1d). We further quantified STP by examining EPSC amplitude distributions after HFS in FS and naive mice. This revealed a shift in the distribution resulting from FS, indicating that the effects are likely not a result of a small subset of synapses that were potentiated (Fig. 1e).

Next, we asked whether there was an effect of sex on STP. We separated data from male and female mice and observed STP in both stressed males and stressed females, but no difference in STP

Hotchkiss Brain Institute and the Department of Physiology and Pharmacology, Cumming School of Medicine, University of Calgary, Calgary, Canada.
*e-mail: jsbains@ucalgary.ca

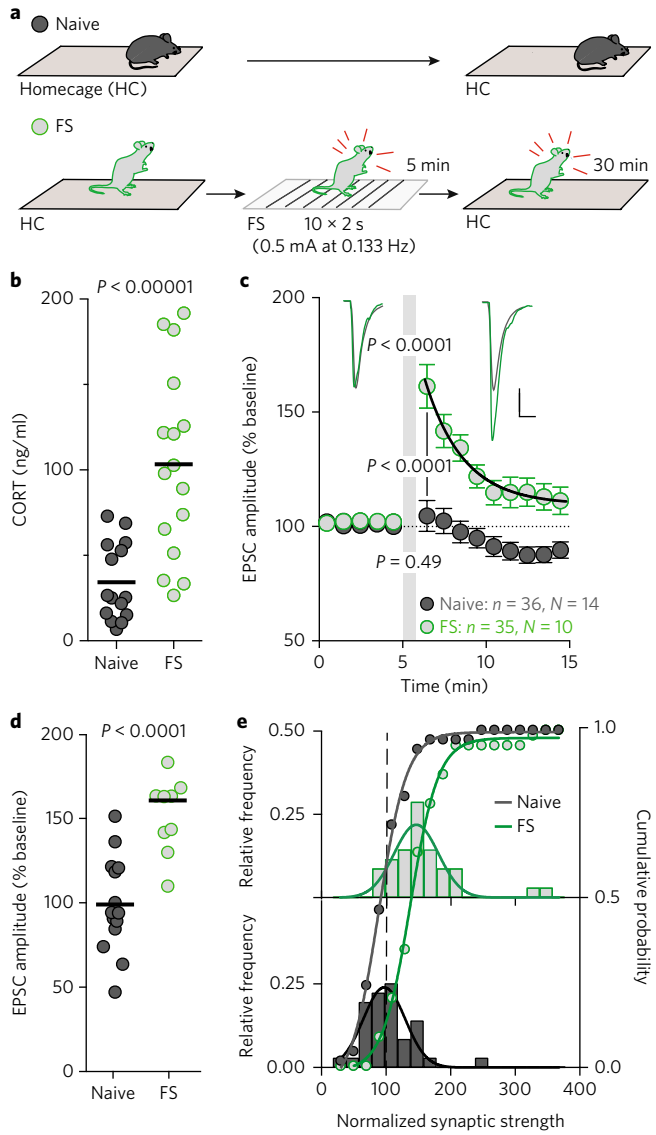

Fig. 1 | STP at glutamate synapses onto PVN CRH neurons following footshock. a, Naive mice (gray) were left undisturbed in the homecage. FS mice (green outline) were subjected to FS protocol (0.5 mA for 2 s every 30 s for 5 min) and then returned to the homecage for 30 min. **b**, Plasma corticosterone (CORT) measured 30 min after FS was elevated (mean: 103.4 ± 13.7 ng/ml, N = 16 mice) compared with naive mice (mean: 34.3.0 ± 5.8 ng/ml, N = 15 mice, $P < 0.0001$, unpaired t test, two-tailed, $t(29) = 4.54$, 95% confidence interval (CI) = 37.9 to 15.2). Horizontal bars show means. **c**, EPSCs in PVN CRH neurons potentiated following HFS (gray bar) relative to baseline in FS mice (green outline, mean: 159.3 ± 9.6%, n = 35 cells, N = 10 mice, $P < 0.0001$ versus baseline, one-sample t test, two-tailed, $t(34) = 6.19$, 95% CI = 39.8 to 78.7, tau = 2.18 min), but not in naive mice (gray, mean: 104.6 ± 6.8%, n = 36 cells, N = 14 mice, $P = 0.49$ versus baseline, one-sample t test, two-tailed, $t(35) = 0.69$, 95% CI = −9.1 to 18.3, $P < 0.0001$ versus FS, unpaired t test, one-tailed, $t(69) = 4.69$, 95% CI = 31.3 to 77.9). Inset, synaptic currents before and after HFS in FS and naive mice. **d**, STP (average ESPC amplitude first minute post-HFS relative to baseline, individual mice shown) was greater in FS mice (mean: 160.7 ± 11%, N = 10 mice) than in naive mice (mean: 99 ± 7.6%, N = 14 mice, $P < 0.0001$, unpaired t test, one-tailed, $t(22) = 4.78$, 95% CI = 34.9 to 88.4). Horizontal bars represent the means. **e**, The EPSC amplitudes after HFS are presented as binned distributions with an overlaid cumulative probability plot of amplitudes. Scale bars represent 5 ms and 20 pA (**c**). Inset currents before HFS are scaled to allow for easier comparison after HFS (**c**). Gray bar (**c**) denote HFS. Error bars represent ±s.e.m.

when comparing stressed males to stressed females (Supplementary Fig. 3). Finally, to investigate the onset and persistence of the stress-induced metaplasticity, we repeated the above experiment, but modified the time between FS and slice preparation. We observed STP in slices prepared immediately after the 5-min FS protocol as well as in slices prepared 24 h after FS (Supplementary Fig. 4). These observations demonstrate that acute stress triggers robust and persistent changes at glutamate synapses on PVN CRH neurons, revealed through HFS stimulation.

Priming and sex-specific buffering of synaptic changes following interactions with others. Using STP as a readout of persistent synaptic consequences of stress, we designed experiments to understand the effect of social interactions on metaplasticity. In these experiments, one mouse from a same-sex dyad of littermates was removed from the homecage and either subjected to FS (5-min protocol) or placed in a novel environment (NE) for 5 min, but was not subjected to FS. The FS or NE subject was then returned to its partner in the homecage for 30 min (Fig. 2a). We collected trunk blood and prepared brain slices from subjects and partners. Levels of circulating CORT in FS subjects and partners were not different than in NE subjects and partners (Fig. 2b). In subsequent whole-cell recordings from PVN CRH neurons, we observed STP in male FS subjects (Fig. 2c), but not in male NE subjects (Fig. 2c). STP was greater in male FS subjects than in male NE subjects (Fig. 2d). When we analyzed the data per animal rather than per cell (Supplementary Fig. 5), we found that STP was evident in FS subjects, but not in NE subjects. These findings suggest that NE is not sufficient to induce STP in males. In females, STP was evident in FS subjects (Fig. 2e) and in NE subjects (Fig. 2e), with no statistical difference between the two conditions (Fig. 2f). Analyzing the data per animal gave similar findings (Supplementary Fig. 5), with STP being present in both female FS and NE subjects. These findings suggest that exposure to NE and/or separation from partner is sufficient to induce STP in female subjects. Notably, STP in female FS subjects returned to their partners was significantly lower than STP in single-housed FS females ($P = 0.01$, unpaired t test, two-tailed, $t(28) = 2.69$). This finding was unique to female subjects; there was no decrease in STP in FS male subjects with partners compared to single-housed FS males ($P = 0.23$, unpaired t test, two-tailed, $t(35) = 1.22$). The sex-specific decrease resulting from the presence of a partner may be indicative of sex-specific social buffering in females[18].

Next, we asked whether transmitted stress affects the synapses of partners. STP was observed in male partners of FS subjects (Fig. 2g), but not in male partners of NE subjects (Fig. 2g), indicating that transmission of synaptic changes was limited to FS subjects and partners in males. STP was greater in male FS partners than in male NE partners (Fig. 2h). Per-animal analyses showed similar results (Supplementary Fig. 5), with STP being present in FS partners, but not NE partners. In females, STP was observed in FS partners (Fig. 2i) and NE partners (Fig. 2i), and we found no significant difference between the two groups (Fig. 2j). Per-animal analyses (Supplementary Fig. 5) revealed that STP was present in both female FS partners and NE partners. These findings suggest that STP in female partners is either related to FS or NE exposure of the subject, and/or the partners are sensitive to the 5-min isolation when the subject is removed from the home cage. To distinguish between these, we collected synaptic data from females that remained alone in the homecage for 30 min following removal of their cagemate. This failed to induce STP (Supplementary Fig. 6), suggesting that re-introduction of the female FS or NE subject into the homecage is required for STP in female partners. In males, plasma CORT levels of partners were positively correlated with their respective subjects (Supplementary Fig. 7a), supporting the notion that stress is communicated between male subjects and their respective partners and that male partners map the physiological

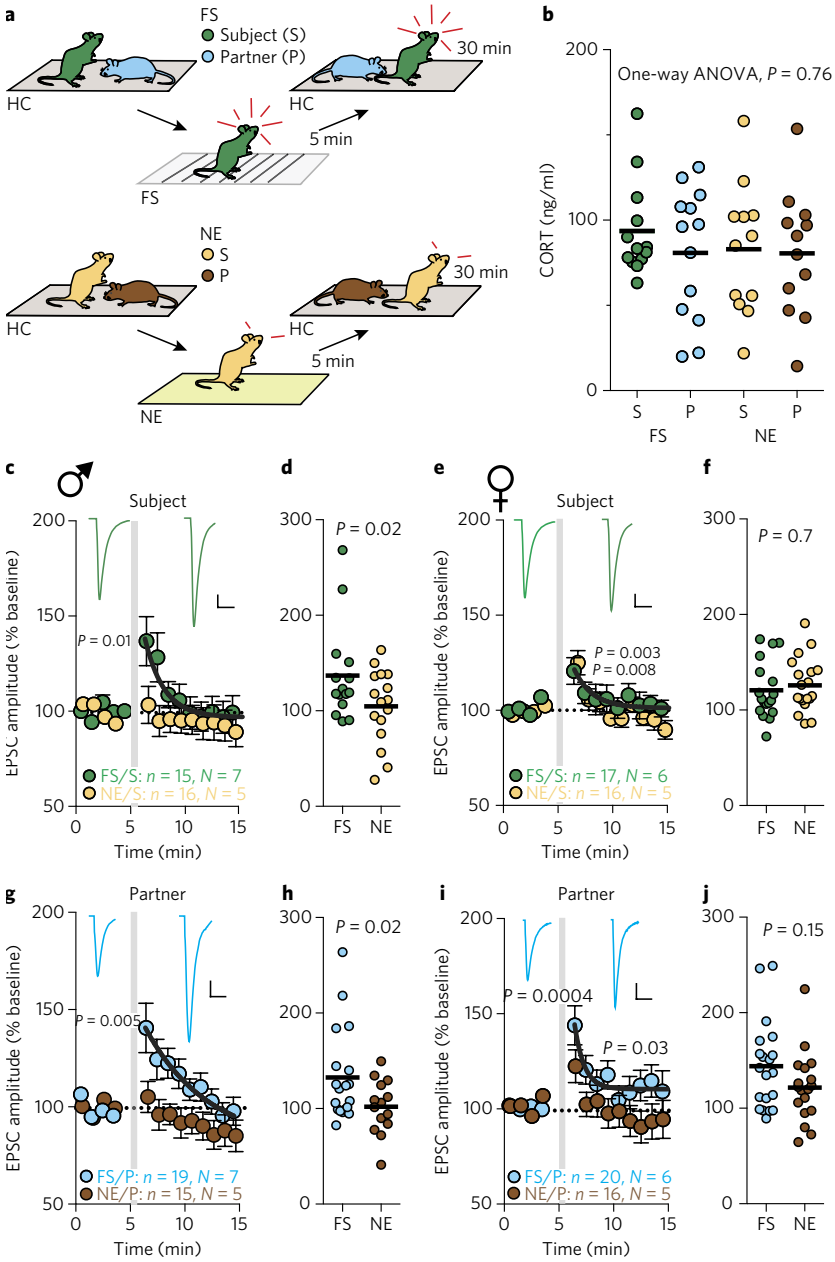

Fig. 2 | Sex-dependent modulation and transfer of STP to a partner. a, Same sex littermates were housed in pairs. In the experimental pair (above), the subject (green, $Subject_{FS}$) was removed, exposed to FS (5-min protocol) and returned to the partner (blue, $Partner_{FS}$) in the homecage for 30 min. In another pair (below), the subject (yellow) was taken to a NE ($Subject_{NE}$) for 5 min but not exposed to FS, and then returned to the partner (brown, $Partner_{NE}$) in the homecage for 30 min. **b**, CORT was elevated to a similar level in all groups ($Subject_{FS}$, mean: 93.5±7.7 ng/ml, N=13 mice; $Partner_{FS}$, mean: 80.7±10.7 ng/ml, N=13 mice; $Subject_{Novel}$, mean: 82.8±11 ng/ml, N=12 mice; $Partner_{Novel}$, mean: 80.47±10.6 ng/ml, N=12 mice, one-way ANOVA, $F(3,46)=0.39$, $P=0.76$ with adjustments for multiple comparisons). Horizontal bars represent the means. **c**, STP was observed in male $Subject_{FS}$ (green, mean: 136.8±12.8%, n=15 cells, N=7 mice, $P=0.01$ versus baseline, one-sample t test, two-tailed, $t(14)=2.85$, 95% CI=9.1 to 64.4, tau=2.01 min), but not in male $Subject_{NE}$ (yellow, mean: 104.7±9.7%, n=16, cells N=5 mice, $P=0.63$ versus baseline, one-sample t test, two-tailed, $t(15)=0.48$, 95% CI=-16 to 25.5). Inset, synaptic currents before and after HFS in $Subject_{FS}$. **d**, STP (individual cells shown) was larger in male $Subject_{FS}$ than in male $Subjects_{NE}$ ($P=0.02$, unpaired t test, one-tailed, $t(29)=2$, 95% CI=-64.8 to 0.7). Horizontal bars represent the means. **e**, STP in female $Subjects_{FS}$ (green, mean: 122.1±7.32%, n=17 cells, N=6 mice, $P=0.008$ versus baseline, one-sample t test, two-tailed, $t(16)=3.01$, 95% CI=6.5 to 37.6, tau=1.52 min) and in female $Subject_{NE}$ (yellow, mean: 126.3±7.6%, n=16 cells, N=5 mice, $P=0.003$ versus baseline, one-sample t test, two-tailed, $t(15)=3.45$, 95% CI=10 to 42.5). Inset, synaptic currents before and after HFS in $Subject_{FS}$. **f**, STP was not different in female $Subject_{FS}$ and $Subject_{NE}$ ($P=0.7$, unpaired t test, two-tailed, $t(31)=0.39$, 95% CI=-17.4 to 10.6). Horizontal bars represent the means. **g**, STP in male $Partner_{FS}$ (blue, mean: 140.7±12.7%, n=19 cells, N=7 mice, $P=0.005$ versus baseline, one-sample t test, two-tailed, $t(18)=3.2$, 95% CI=13.9 to 67.4, tau=5.68 min), but not in male $Partner_{NE}$ (brown, mean: 107.1±7.6%, n=15 cells, N=5 mice, $P=0.37$ versus baseline, one-sample t test, two-tailed, $t(14)=0.93$, 95% CI=-9.3 to 23.4). Inset, synaptic currents before and after HFS in $Partner_{FS}$. **h**, Horizontal bars represent the means. STP was larger in male $Partner_{FS}$ than in male $Partner_{NE}$ subjects ($P=0.02$, unpaired t test, one-tailed, $t(32)=2.1$, 95% CI=-65.9 to -1.2). **i**, STP in female $Partner_{FS}$ (blue, mean; 144±10.3%, n=20 cells, N=6 mice, $P=0.0004$ versus baseline, one-sample t test, two-tailed, $t(19)=4.26$, 95% CI=22.4 to 65.6, tau=0.84 min) and in female $Partner_{NE}$ (brown, mean: 122.7±9.6%, n=16 cells, N=5 mice, $P=0.03$ versus baseline, one-sample t test, two-tailed, $t(15)=2.34$, 95% CI=2 to 43.4). Inset, synaptic currents before and after HFS in $Partner_{FS}$. **j**, STP in female $Partner_{FS}$ was similar to that in female $Partner_{NE}$ ($P=0.15$, unpaired t test, two-tailed, $t(34)=1.47$, 95% CI=-50.6 to 8.1). Horizontal bars represent the means. Scale bars represent 5 ms and 20 pA (**c**,**e**,**g**,**i**). Inset currents before HFS are scaled to allow for easier comparison after HFS (**c**,**e**,**g**,**i**). Gray bar (**c**,**e**,**g**,**i**) denote HFS. Error bars represent ±s.e.m.

state of their subjects[11]. In females, however, there was no correlation in CORT levels between partners and subjects (Supplementary Fig. 7b). One interpretation of this observation is that interactions between females increase CORT levels in partners while simultaneously decreasing CORT levels in subjects. Together, our observations demonstrate that transmitted stress primes glutamate synapses in males and females with differential sensitivity and that synaptic effects of authentic stress in females are buffered or mitigated by the presence of a partner.

Stress transmission requires investigative behavior and a pheromone signal. To ask how transmission occurs between stressed subjects and naive partners, we examined the behavior between pairs after FS or exposure to NE. In rodents, stress-naive individuals initiate unreciprocated, investigative behaviors when exposed to distressed individuals[11,19,20]. These behaviors may allow the unstressed partners to detect alarm signals from stressed individuals. Studies have shown that stressed individuals alert naive partners by releasing volatile[21] chemical signals from the anogenital region and face[22] that are only detected when partners are in close contact. The pheromone from the anogenital region activates stress-responsive centers in the brain[23] and is structurally similar to predator odor[24]. Exposure to predator odor activates a neural circuit that links the olfactory bulb to the PVN via a minor cortical area, the amygdalopiriform transition area[25]. Spontaneous behavior of mouse dyads was monitored for 30 min after the FS or NE subject was returned to the homecage (Fig. 3a). The analysis for single-housed mice following FS has been described previously[26] and we extended this analysis to specific interactive behaviors, namely: anogenital sniffing (snout toward the anogenital area of conspecific), head/torso sniffing (snout toward the head or torso of conspecific) and allogrooming (grooming directed to conspecific). In both male and female FS dyads, partners engaged in anogenital sniffing that was rarely reciprocated by subjects (Fig. 3b,d). FS partners also engaged in more head/torso sniffing than the respective subjects (Fig. 3c,d), but spent more time engaged in anogenital sniffing than in head/torso sniffing (Fig. 3d). In NE dyads, partners engaged in more anogenital sniffing than subjects (Supplementary Fig. 8a,b). By contrast, NE partners did not engage in more head/torso sniffing ($P=0.16$; Supplementary Fig. 8a,b). Furthermore, NE partners showed no discrimination between anogenital sniffing and head/torso sniffing (Supplementary Fig. 8a,b). FS partners performed more allogrooming than their respective subjects (Supplementary Fig. 8c,d), but we found no difference between them and NE partners (Supplementary Fig. 8c,d). In NE dyads, allogrooming did not differ between subject and partner (Supplementary Fig. 8c,d).

Based on the marked difference in anogenital sniffing between FS partners and NE partners, we hypothesized that this behavior may be involved in transmitting information from subject to partner. To test this, we used a cage with a perforated plexiglass barrier to physically separate a pair of males following FS (Fig. 3e). Males were used in this experiment because females exhibited STP following exposure to NE. FS subjects separated from partners by plexiglass showed an increase in CORT levels (Fig. 3f) and robust STP (Fig. 3g); however, partners failed to mount a CORT response (Fig. 3f) and failed to show STP (Fig. 3g,h). When STP data were analyzed per animal rather than per cell, FS subjects showed potentiation, whereas partners did not (Supplementary Fig. 9a). This suggests that investigative behaviors involving direct contact are required for transmission of stress and synaptic priming. Given that our previous analyses of the 30-min behavior revealed that FS partners are biased toward anogenital sniffing, we designed experiments to determine whether the target of the sniffing behavior (anogenital versus head/torso) had any effect on transmitted STP. We performed three separate experiments. First, we swabbed the anogenital region of an unstressed mouse and brought the swab into contact with the snout of the partner before depositing it in the homecage for 30 min (Fig. 3i). Second, we swabbed the anogenital area of a FS subject immediately following FS (Fig. 3i) and, following the same protocol described above, deposited the swab in the partner's homecage. Finally, we swabbed the head/torso region of a FS subject immediately following FS (Fig. 3i). PVN CRH neurons from partners exposed to the anogenital swab of a FS subject exhibited STP (Fig. 3j,k), whereas partners receiving a swab of an unstressed subject did not (Fig. 3j,k). Exposure to a swab from the head/torso region of a FS subject also induced STP (Fig. 3j); however, significantly less STP was induced than in partners exposed to the anogenital swab of a FS subject (Fig. 3k). Analyzing STP data per animal revealed STP in partners receiving the stressed anogenital swab, but not in partners that received the unstressed anogenital swab or in those receiving the stressed head/torso swab (Supplementary Fig. 9b). These observations demonstrate that stressed subjects release pheromone(s), predominantly from glands in the anogenital area, which are sensed by the partner during investigative behavior. Exposure to secretions alone is sufficient to cause synaptic changes that mirror those observed in FS partners following investigation of the subject.

Investigative behavior and synaptic priming requires CRHR1. Next, we conducted experiments to determine the mechanisms responsible for STP and for the transmission of the signal from subject to partner. STP was only evident in partner mice if STP occurred in the subject. We do not know whether the partner can express STP even if the subject does not. Previous work from our laboratory has shown that the activation of CRH receptor 1 (CRHR1) is required for priming glutamate synapses for STP after acute stress in rats[6]. To confirm this observation in mice and to assess whether CRHR1 activity in the subject is required for transmission of the synaptic imprint to the partner, we injected the subject with a CRHR1 antagonist, CP154256 (CP), 30 min before FS (Fig. 4a). In a control male dyad, both subject and partner received vehicle injections 30 min before FS. CRHR1 antagonism in the subject blocked STP as compared with vehicle-injected subjects (Fig. 4c,d). CP injection in the subject had no effect on the anogenital sniffing behavior of the partners (Fig. 4b); STP in the partners, however, was blunted compared with partners of vehicle-injected subjects (Fig. 4e,f). We then injected CP into the partner 30 min before FS of the subject (Fig. 4a). This reduced anogenital sniffing behavior by the partner, compared with vehicle-injected controls (Fig. 4b). STP was also significantly reduced in partners receiving the CP injection (Fig. 4e,f). Together, these results indicate that CRHR1 activity in the subject is required for development of STP in the subject; that CRHR1 activity in the subject facilitates STP in the partner; that CRHR1 activity in the subject has no effect on anogenital sniffing behavior by the partner; and that CRHR1 activity in the partner is required for both anogenital sniffing behavior and STP.

Activation of PVN CRH neurons is necessary for synaptic priming in subjects and partners. The activation of PVN CRH neurons is obligatory for mounting the endocrine response to stress[1]. To determine whether activation of CRH neurons in the PVN is necessary for STP in the subject, we expressed Archaerhodopsin 3.0 (Arch) in the CRH neurons of male mice (CRH^{Arch}; Fig. 5a). We confirmed that CRH neurons in brain slices from CRH^{Arch} mice in vitro decreased firing in response to photo-inhibition and that prolonged delivery of continuous yellow light had no lasting effects on the basal membrane properties of these cells (Supplementary Fig. 10). Next, we photo-inhibited PVN CRH neurons in vivo[26] during and after FS of a CRH^{Arch} subject (Fig. 5b). Following this manipulation, we failed to observe STP in slices from CRH^{Arch} subjects (Fig. 5b,c). STP was present in eYFP-expressing subjects following yellow light delivery (Fig. 5b), and this potentiation was

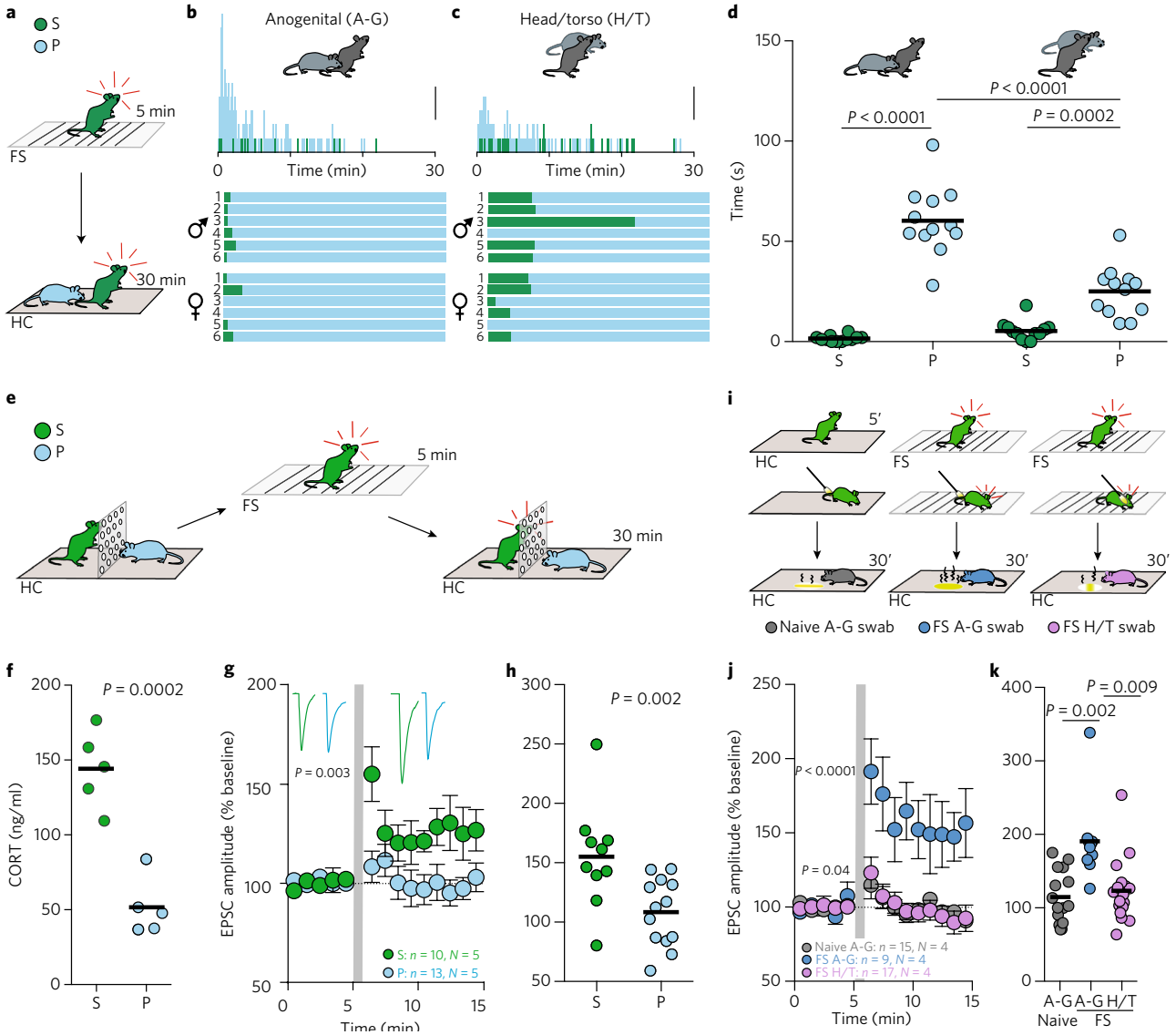

Fig. 3 | Directionally biased investigative behavior is required for STP in partner. a, Scheme of FS experiment as described in Fig. 2. **b**, Histogram (above) shows time spent engaged in anogenital sniffing of all $Subjects_{FS}$ (green) and $Partners_{FS}$ (blue) during the 30-min interaction, overlaid ($N=12$ pairs of mice). Matching color plots (below) show the ratio of time spent anogenital sniffing by each Subject-Partner pair to demonstrate directionality of behavior. The average ratio of $Subject_{FS}$:$Partner_{FS}$ was 0.97:0.03 for 12 pairs of mice. **c**, Data are presented as in **b** for head/torso sniffing behavior of $Subjects_{FS}$ (green) and $Partners_{FS}$ (blue). Matching color plots (below) show the ratio of time spent head/torso sniffing by each Subject-Partner pair. The average ratio of $Subject_{FS}$:$Partner_{FS}$ was 0.87:0.13 for 12 pairs of mice. **d**, $Partner_{FS}$ engaged in more ano-genetial sniffing (mean: 60.3±4.9 s, $N=12$ mice) than their respective $Subject_{FS}$ (mean: 1.5±0.4 s, $N=12$ mice, $P<0.0001$, 95% CI=−69.9 to −47.8, one-way ANOVA, $F(3,44)=73.37$, $P<0.0001$, followed by Sidak's multiple comparisons test), as well as more head/torso sniffing (mean: 25±3.6 s, $N=12$ mice) than their respective $Subject_{FS}$ (mean: 5.3±1.4 s, $N=12$ mice, $P=0.0002$, 95% CI=−30.7 to −8.6, Sidak's multiple comparisons test). $Partners_{FS}$ spent more time anogenital sniffing than head/torso sniffing ($P<0.0001$, 95% CI=24.3 to 46.4, Sidak's multiple comparisons test). Horizontal bars represent the means. **e**, Interactive sniffing and allogrooming behaviors between subject (green) and partner (blue) were prevented during the 30 min following FS using a perforated plexiglass barrier. **f**, CORT levels were elevated in Subject (mean: 144.2±11.5 ng/ml, $N=5$ mice), but not in respective Partner (mean: 51.5±8.6 ng/ml, $N=5$ mice, $P=0.0002$ versus Subject, unpaired t test, two-tailed, $t(8)=6.46$, 95% CI=−125.8 to −59.6). Horizontal bars represent the means. **g**, STP was present in the subject (mean: 155.2±13.8%, $n=10$ cells, $N=5$ mice, $P=0.003$ versus baseline, one-sample t test, two-tailed, $t(9)=3.99$, 95% CI=23.9 to 86.5), but not the partner (mean: 109.7±6.8%, $n=13$ cells, $N=5$ mice, $P=0.18$ versus baseline, one-sample t test, two-tailed, $t(14)=1.42$, 95% CI=−4.9 to 24.4). Inset, synaptic currents before and after HFS in subject (green) and partner (blue). Currents before HFS are scaled to allow for easier comparison after HFS. **h**, STP (individual cells shown) was larger in FS subjects than in partners ($P=0.002$, unpaired t test, two-tailed, $t(23)=3.25$, 95% CI=−74.4 to −16.5). Horizontal bars represent the means. **i**, Three separate experiments were performed (left to right): swab of the anogenital area of a naive subject (unstressed A/G swab); swab of the anogenital area of a FS subject (stressed A/G swab); and swab of the head/torso area of a FS subject (stressed H/T swab). In each experiment, the swab was placed into a partner's cage. **j**, STP was observed in partners exposed to the stressed A/G swab (blue, mean: 170.9±7.2%, $n=9$ cells, $N=4$ mice, $P<0.0001$ versus baseline, one-sample t test, two-tailed, $t(8)=9.82$, 95% CI=54.2 to 87.5) and partners that received the stressed H/T swab (purple, mean: 123.1±10.4%, $n=17$ cells, $N=4$ mice, $P=0.04$ versus baseline, one-sample t test, two-tailed, $t(16)=2.2$, 95% CI=0.8 to 45.3), but not partners that received the unstressed A/G swab (gray, mean: 114.9±9.2%, $n=15$ cells, $N=4$ mice, $P=0.13$ versus baseline, one-sample t test, two-tailed, $t(14)=1.61$, 95% CI=−4.9 to 34.7). **k**, STP (individual cells shown) was larger in stressed A/G swab partners relative to both unstressed A/G swab partners ($P=0.002$, 95% CI=18.1 to 93.8, one-way ANOVA, $F(2,38)=7.1$, $P=0.003$, followed by Tukey's multiple comparisons test) and stressed H/T swab partners ($P=0.009$, 95% CI=10.7 to 84.8, Tukey's multiple comparisons test). Horizontal bars represent the means. Scale bars represent 20% (**b**,**c**), gray bar (**g**,**j**) denote HFS. Error bars represent ±s.e.m.

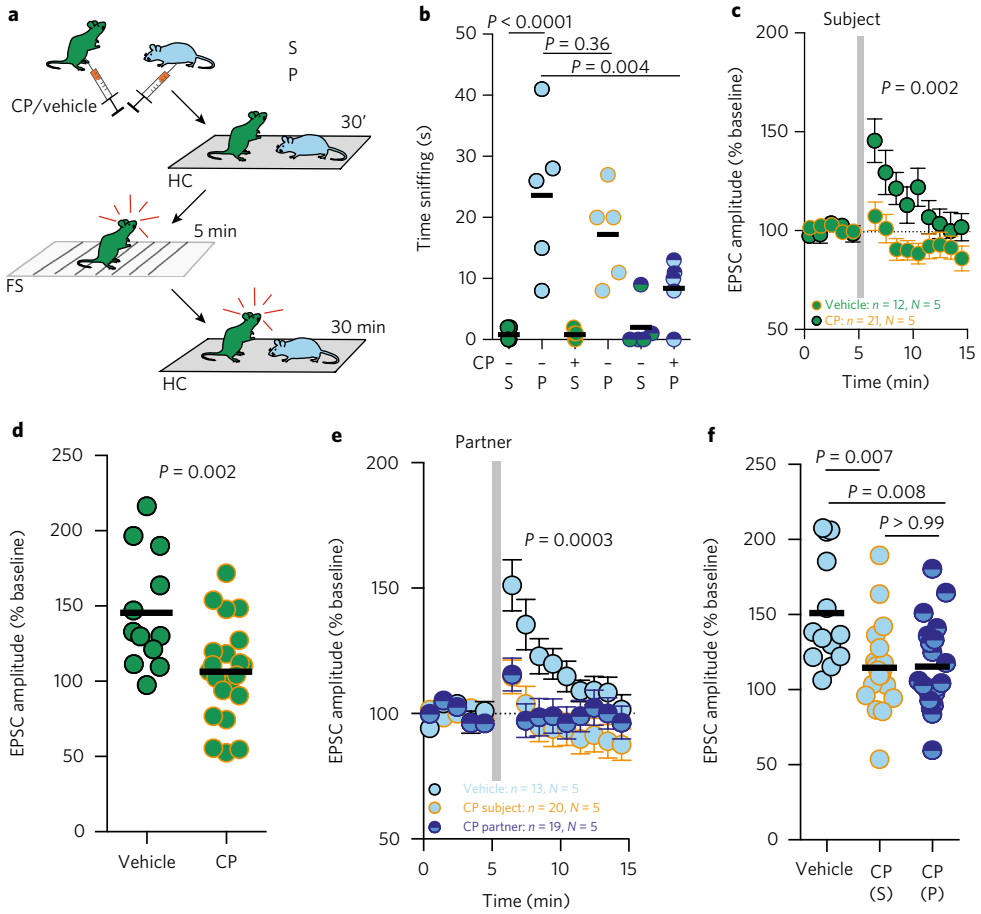

Fig. 4 | CRHR1 antagonist blocks STP and prevents transmission form subject to partner. a, Male mice were housed in littermate pairs. Three separate experiments were carried out: both the subject (green) and partner (blue) received vehicle injections; the subject received a CP154256 injection (CP, 30 mg/kg, CRHR1 antagonist) while the partner received a vehicle injection; the subject received a vehicle injection while the partner received a CP injection. 30 min post-injections, the subject was exposed to FS and then returned to the partner in the homecage for 30 min. **b**, Vehicle-injected partners of vehicle-injected subjects engaged in more anogenital sniffing (mean: 23.6 ± 5.6 s, $N = 5$ mice) than their respective vehicle-injected subjects (mean: 0.8 ± 0.49 s, $N = 5$ mice, $P < 0.0001$, 95% CI = -33.5 to -12.1, one-way ANOVA, $F(5,24) = 10.6$, $P < 0.0001$, followed by Sidak's multiple comparisons test). Vehicle-injected partners of vehicle-injected subjects also spent more time anogenital sniffing than CP-injected partners of vehicle-injected subjects (mean: 8.4 ± 2.2 s, $N = 5$ mice, $P = 0.004$, 95% CI = -25.9 to -4.4, Sidak's multiple comparisons test), but not more than vehicle-injected partners of CP-injected subjects (mean: 17.2 ± 3.4 s, $N = 5$ mice, $P = 0.36$, 95% CI = -14.1 to 4.3, Sidak's multiple comparisons test). Horizontal bars represent the means. **c**, STP was evident in vehicle-injected subjects (partner also received vehicle, mean: 145.5 ± 11%, $n = 12$ cells, $N = 5$ mice, $P = 0.002$ versus baseline, one-sample t test, two-tailed, $t(11) = 4.14$, 95% CI = 21.3 to 69.6), but was not present in the CP-injected subjects (partner received vehicle, mean: 106.2 ± 7.1%, $n = 21$ cells, $N = 5$ mice, $P = 0.4$ versus baseline, one-sample t test, two-tailed, $t(20) = 0.86$, 95% CI = -8.7 to 21.1). **d**, STP (individual cells shown) was larger in vehicle-injected subjects than in CP-injected subjects (both had vehicle-injected partners, $P = 0.002$, unpaired t test, one-tailed, $t(31) = 3.13$, 95% CI = 13.7 to 64.9). Horizontal bars represent the means. **e**, STP was evident in vehicle-injected partners of vehicle-injected subjects (mean: 151.1 ± 10.2%, $n = 13$ cells, $N = 5$ mice, $P = 0.0003$ versus baseline, one-sample t test, two-tailed, $t(12) = 4.98$, 95% CI = 28.7 to 73.4), CP-injected partners of vehicle-injected subjects (mean: 115 ± 6.6%, $n = 20$ cells, $N = 5$ mice, $P = 0.03$ versus baseline, one-sample t test, two-tailed, $t(19) = 2.3$, 95% CI = 1.6 to 29.4) and vehicle-injected partners of CP-injected subjects (mean: 114.7 ± 6.8%, $n = 19$ cells, $N = 5$ mice, $P = 0.04$ versus baseline, one-sample t test, two-tailed, $t(18) = 2.16$, 95% CI = 0.4 to 29). **f**, STP (individual cells shown) was larger in vehicle-injected partners of vehicle-injected subjects than in both CP-injected partners of vehicle-injected subjects ($P = 0.008$, 95% CI = -62.8 to -8.3, one-way ANOVA, $F(2,49) = 6.3$, $P = 0.004$, followed by Tukey's multiple comparisons test) and vehicle-injected partners of CP-injected subjects ($P = 0.007$, 95% CI = -63.8 to -8.8, Tukey's multiple comparisons test). STP of CP-injected partners was not different to that of vehicle-injected partners of CP-injected subjects ($P > 0.99$, 95% CI = -23.2 to 25.3, Tukey's multiple comparisons test). Horizontal bars represent the means. Gray bars (**c**,**e**) denote HFS. Error bars represent ±s.e.m.

significantly greater than that in CRH^{Arch} subjects (Fig. 5c). Next, we examined anogenital sniffing behavior and STP in partners of CRH^{Arch} subjects (Fig. 5d). Inhibiting CRH neuronal firing in the PVN of CRH^{Arch} subjects during FS and for 30 min with the partner did not reduce the anogenital sniffing behavior of the partner (Fig. 5e). Directional anogenital sniffing behavior was still as evident in CRH^{Arch} subjects versus partners as it was in CRH^{eYFP} subjects versus partners (Fig. 5e). Weak STP was still evident in the partners of CRH^{Arch} subjects (Fig. 5f). However, it was significantly reduced compared with that of the partners of CRH^{eYFP} subjects (Fig. 5f,g). Photo-inhibition of PVN CRH neurons in the partner during separation from and interaction with FS subjects (Fig. 5h) inhibited anogenital sniffing (Fig. 5i). Anogenital sniffing was no longer directional in the pairs with CRH^{Arch} partners, but directional sniffing was maintained in CRH^{eYFP} pairs (Fig. 5i). STP was blocked in CRH^{Arch} partners (Fig. 5j), but not in CRH^{eYFP} partners (Fig. 5j,k). These findings indicate that firing of CRH neurons is necessary for synaptic imprint of authentic stress in FS subjects;

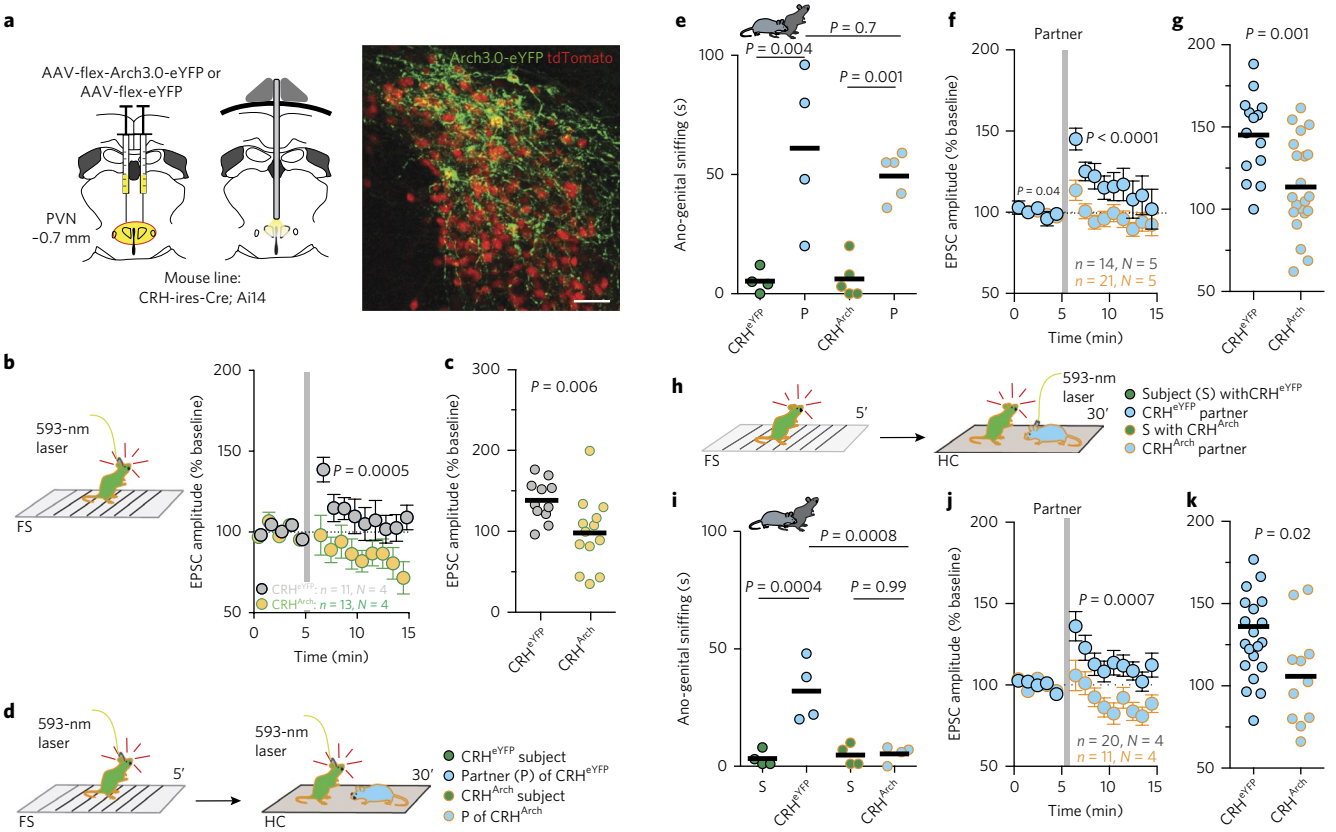

Fig. 5 | Photoinhibition of PVN CRH neurons decreases STP in subject and partner. a, Cre-dependent AAV-DIO-Arch3.0-eYFP virus (ARCH) was injected bilaterally into the PVN of Crh-IRES-Cre; Ai14 mice. Confocal image shows expression of eYFP (green, labeling ARCH) and tdTomato (red, labeling CRH cells) in the PVN of virus-infected animals. Such expression was seen in the PVN of CRH^{ARCH} subjects and partners ($N = 13$ mice). **b**, Yellow light delivered into the PVN of CRH^{ARCH} subjects (yellow with green outline) in vivo during and following FS prevented STP (mean: 98.1 ± 11.37%, $n = 14$ cells, $N = 4$ mice, $P = 0.87$ versus baseline, one-sample t test, two-tailed, $t(13) = 0.17$, 95% CI = -26.4 to 22.6). STP was still evident in CRH^{eYFP} subjects (gray) that received yellow light into PVN during and following FS (mean: 138.2 ± 7.7%, $n = 11$ cells, $N = 5$ mice, $P = 0.0005$, one-sample t test, two-tailed, $t(10) = 4.99$, 95% CI = 21.1 to 55.3). **c**, STP (individual cells shown) was reduced in CRH^{ARCH} mice compared with CRH^{eYFP} ($P = 0.006$, unpaired t test, one-tailed, $t(23) = 2.76$, 95% CI = 10 to 70.2). Horizontal bars represent the means. **d**, Yellow light was delivered into the PVN of CRH^{Arch} subjects (green with yellow outline) during FS and during the 30-min interaction with their respective partners (blue with yellow outline). **e**, During the 30-min interaction following FS, partners of CRH^{eYFP} engaged in more anogenital sniffing than their respective subjects (mean: 61 ± 16.9 s versus mean: 5.2 ± 2.7 s, $N = 4$ pairs of mice, $P = 0.001$, 95% CI = -88.9 to 22.7, one-way ANOVA, $F(3,14) = 12.29$, $P = 0.0003$, followed by Sidak's multiple comparisons test). Partners of CRH^{ARCH} subjects engaged in more anogenital sniffing than their respective subjects (mean: 49.4 ± 4.4 s versus mean: 6.2 ± 3.7 s, $N = 5$ pairs of mice, $P = 0.004$, 95% CI = -72.8 to -13.6, Sidak's multiple comparisons test). Partners of CRH^{ARCH} subjects and partners of CRH^{eYFP} subjects engaged in a similar amount of anogenital sniffing ($P = 0.7$, 95% CI = -43 to 19.8, Sidak's multiple comparisons test). Horizontal bars represent the means. **f**, STP was evident in partners of CRH^{eYFP} subjects (mean: 145.1 ± 6.7%, $n = 14$ cells, $N = 3$ mice, $P < 0.0001$ versus baseline, one-sample t test, two-tailed, $t(13) = 6.68$, 95% CI = 30.5 to 59.7). STP was also still evident in partners of CRH^{ARCH} subjects (mean: 113.6 ± 6.2%, $n = 21$ cells, $N = 4$ mice, $P = 0.04$ versus baseline, one-sample t test, two-tailed, $t(20) = 2.19$, 95% CI = 0.6 to 26.5). **g**, STP (individual cells shown) was reduced in partners of CRH^{ARCH} subjects compared with partners of CRH^{eYFP} subjects ($P = 0.001$, unpaired t test, one-tailed, $t(33) = 3.36$, 95% CI = 12.5 to 50.7). Horizontal bars represent the means. **h**, Yellow light was delivered into the PVN of CRH^{Arch} partners (blue with yellow outline) during the 30-min interaction with FS subject. **i**, CRH^{eYFP} partners engaged in more anogenital sniffing than their respective subjects (mean: 32 ± 6.6 s versus mean: 3.2 ± 1.6 s, $N = 4$ pairs of mice, $P = 0.0004$, one-way ANOVA, $F(3,12) = 13.71$, $P = 0.0003$, followed by Sidak's multiple comparisons test). Yellow light delivered into the PVN of CRH^{ARCH} partners prevented typical anogenital sniffing behavior; anogenital sniffing of CRH^{ARCH} partners was not different from their respective subjects (mean: 5.2 ± 1.7 s versus mean: 4.7 ± 2.2 s, $N = 4$ pairs of mice, $P > 0.99$, 95% CI = -15.1 to 14.1, Sidak's multiple comparisons test) and was less than that of CRH^{eYFP} partners ($P = 0.0008$, 95% CI = -41.4 to -12.1, Sidak's multiple comparisons test). Horizontal bars represent the means. **j**, STP was evident in CRH^{eYFP} partners following interaction with FS subject (mean: 136.1 ± 9%, $n = 21$ cells, $N = 4$ mice, $P = 0.0007$, one-sample t test, two-tailed, $t(20) = 4.01$, 95% CI = 17.3 to 54.9). STP was not observed in CRH^{ARCH} partners (mean: 105.9 ± 9.3%, $n = 11$ cells, $N = 3$ mice, $P = 0.54$, one-sample t test, two-tailed, $t(10) = 0.63$, 95% CI = -14.8 to 26.6). **k**, STP (individual cells shown) was reduced in CRH^{ARCH} partners compared with CRH^{eYFP} partners ($P = 0.02$, unpaired t test, one-tailed, $t(30) = 2.13$, 95% CI = 1.3 to 59.2). Horizontal bars represent the means. Gray bars (**b**,**f**,**j**) denote HFS. Error bars represent ±s.e.m.

that CRH neuron firing in the subject is not required to trigger anogenital sniffing behavior of the partner, but instead facilitates STP in the partner; and that CRH neuron firing in the partner is required for anogenital sniffing and STP in response to transmitted stress.

PVN:CRH activation is sufficient for priming synapses and transmitting stress. PVN CRH neurons are a crucial hub in a network of hypothalamic survival circuits[27]. Given that one benefit of social groups is to increase the chances for survival, we hypothesized that PVN CRH neurons may have a key role

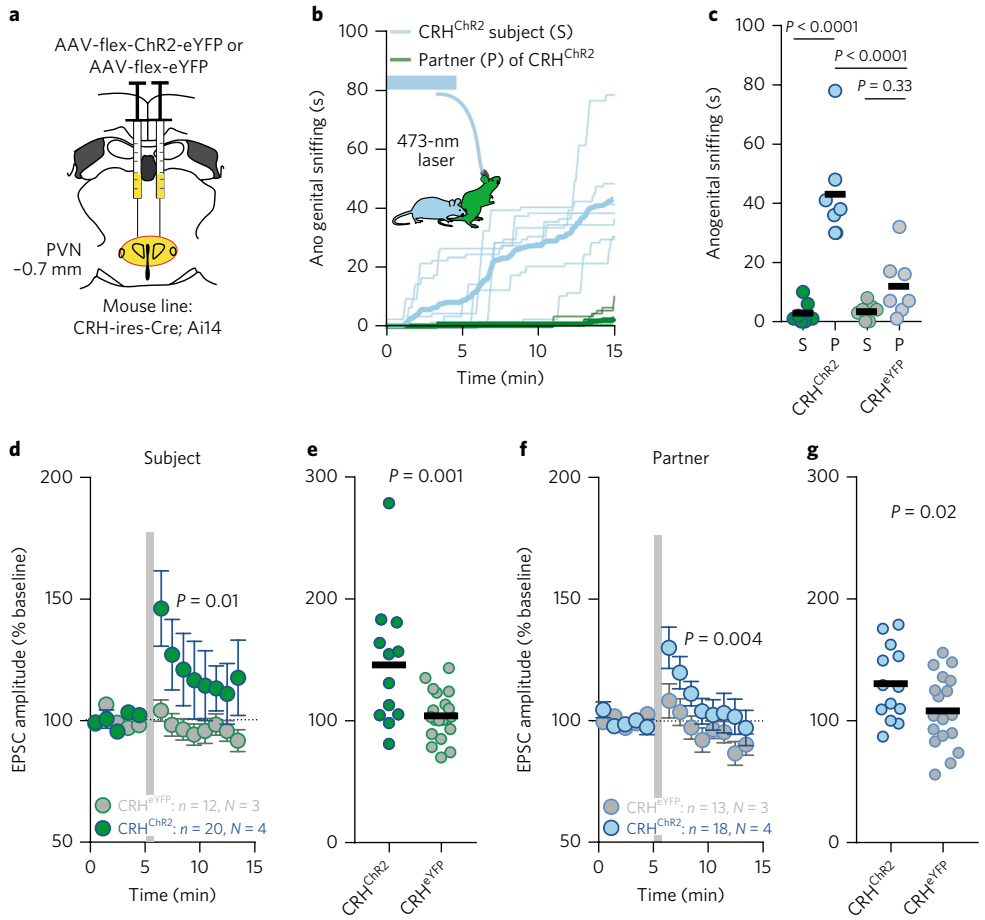

Fig. 6 | Photoactivation of PVN CRH neurons induces STP in subject and partner. a, Cre-dependent AAV-DIO-ChR20-eYFP virus (ChR2) was injected bilaterally into the PVN of Crh-IRES-Cre; Ai14 male mice. **b**, Cumulative plot of anogenital sniffing behavior over 15 min showing that delivery of blue light (10 Hz, 5 min, shown by blue bar) into the PVN of CRH^{ChR2} subjects (green) resulted in anogenital sniffing behavior of their respective partners (blue). Thin lines show individual mice and thick lines show the mean, for subjects (green) and partners (blue) respectfully. **c**, Partners of CRH^{ChR2} subjects engaged in more anogenital sniffing (mean: 43 ± 6.3 s, $N = 7$ mice) compared with their respective CRH^{ChR2} subjects (mean: 2.8 ± 1.4 s, $N = 7$ mice, $P < 0.0001$, 95% CI = −54 to 26.2, one-way ANOVA, $F(3,24) = 24.3$, $P < 0.0001$, followed by Sidak's multiple comparisons test) and compared with partners of CRH^{eYFP} subjects (mean: 12 ± 4 s, $N = 7$ mice, $P < 0.0001$, 95% CI = 17.1 to 44.9, Sidak's multiple comparisons test). There was no difference in anogenital sniffing time between between CRH^{eYFP} subjects (mean: 3.4 ± 1 s, $N = 7$ mice) and their respective partners ($P = 0.33$, 95% CI = −22.5 to 5.3, Sidak's multiple comparisons test). Horizontal bars represent the means. **d**, STP following delivery of blue light into PVN in vivo in CRH^{ChR2} subjects (mean: 146 ± 15.5%, $n = 12$ cells, $N = 3$ mice, $P = 0.01$ versus baseline, one-sample t test, two-tailed, $t(11) = 2.96$, 95% CI = 11.7 to 80.2), but not in CRH^{eYFP} subjects (mean: 104.2 ± 4.4%, $n = 20$ cells, $N = 4$ mice, $P = 0.35$ versus baseline, one-sample t test, two-tailed, $t(19) = 0.95$, 95% CI = −5 to 13.4). **e**, STP (individual cells shown) of CRH^{ChR2} subjects was larger than that of CRH^{eYFP} subjects ($P = 0.001$, unpaired t test, one-tailed, $t(30) = 3.16$, 95% CI = −68.7 to 13.2). Horizontal bars represent the means. **f**, STP was observed in partners of CRH^{ChR2} subjects (mean: 130.4 ± 8.5%, $n = 13$ cells, $N = 3$ mice, $P = 0.003$ versus baseline, one-sample t test, two-tailed, $t(12) = 3.57$, 95% CI = 11.9 to 49), but not in partners of CRH^{eYFP} subjects (mean: 108.2 ± 6.9%, $n = 18$ cells, $N = 4$ mice, $P = 0.25$ versus baseline, one-sample t test, two-tailed, $t(17) = 1.17$, 95% CI = −6.5 to 22.9). **g**, STP (individual cells shown) of partners of CRH^{ChR2} subjects was larger than that of partners of CRH^{eYFP} subjects ($P = 0.02$, unpaired t test, one-tailed, $t(29) = 2.03$, 95% CI = −44.6 to 0.11). Horizontal bars represent the means. Blue bar (**b**) denotes blue light delivery (10 Hz, 5 min). Gray bars (**d**,**f**) denote HFS. Error bars represent ±s.e.m.

in transmitting stress-relevant signals from one individual to another. To test whether activation of PVN CRH neurons, in the absence of stress, is sufficient to induce STP in a subject and its partner, we expressed channelrhodopsin 2 (ChR2) in the CRH neurons (CRH^{ChR2}) of male subjects (Fig. 6a and Supplementary Fig. 11). We confirmed that photostimulation with blue light in vitro excited CRH^{ChR2} cells (Supplementary Fig. 11). In response to photostimulation in CRH^{ChR2} subjects (Fig. 6b), partners initiated anogenital (Fig. 6b,c) and head/torso sniffing (Supplementary Fig. 12). Partners of CRH^{ChR2} subjects engaged in more anogenital sniffing than their respective subjects and partners of CRH^{eYFP} subjects (Fig. 6c). Partners of CRH^{ChR2} subjects also engaged in more head/torso sniffing behavior than their respective subjects and partners of CRH^{eYFP} subjects (Supplementary Fig. 12b). Subsequent whole-cell recordings revealed STP in CRH^{ChR2} subjects (Fig. 6d) and their respective partners (Fig. 6f). STP in CRH^{ChR2} subjects was significantly greater than that observed in CRH^{eYFP} subjects (Fig. 6e), whereas STP in partners of CRH^{ChR2} subjects was greater than that of partners of CRH^{eYFP} subjects (Fig. 6g). Together, these findings indicate that firing of PVN CRH neurons in one individual is sufficient to trigger investigative sniffing by the partner and prime glutamate synapses for STP in both the subject and partner.

Consequences of transmitted stress are persistent and can be propagated to others. Although these findings demonstrate that

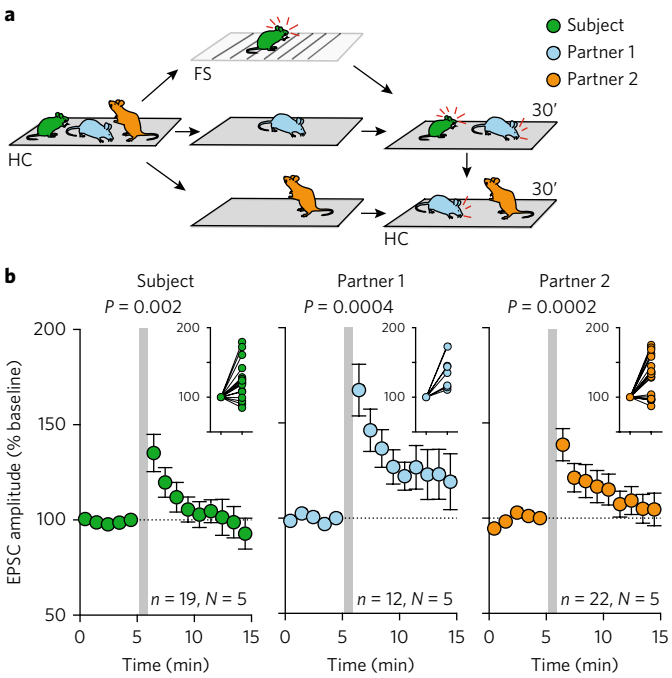

Fig. 7 | STP in multiple group members following interaction with stressed individual or proxy. a, Experiment for sequential transmission. One male subject (green) from a group of three male littermates was exposed to FS and then returned to one partner (blue). Following a 30-min interaction, the partner was transferred to a cage containing the third group member (orange). **b**, STP was evident in cells from $Subject_{FS}$ (mean: 135.1 ± 9.9%, $n = 19$ cells, $N = 5$ mice, $P = 0.002$ versus baseline, one-sample t test, two-tailed, $t(18) = 3.57$, 95% CI = 14.4 to 55.8), $Partner1_{FS}$ (mean: 167.2 ± 13.6%, $n = 12$ cells, $N = 5$ mice, $P = 0.0004$ versus baseline, one-sample t test, two-tailed, $t(11) = 4.94$, 95% CI = 37.3 to 97.2) and $Partner2_{FS}$ (mean: 138.6 ± 8.5%, $n = 22$ cells, $N = 5$ mice, $P = 0.0002$ versus baseline, one-sample t test, two-tailed, $t(21) = 4.57$, 95% CI = 21 to 56.2). Insets show data of individual cells. Gray bars (**b**) denote HFS. Error bars represent ±s.e.m.

second-order stress primes synapses in a similar fashion to authentic stress, we have no information about the permanence of these synaptic change or whether they are constrained to one partner. To assess the stability of synaptic priming following transmitted stress, we returned a FS subject to its partner in the homecage for 24 h (Supplementary Fig. 13a). PVN CRH neurons in the stressed individual exhibited STP 1 d after stress induction. Furthermore, the partner also exhibited a synaptic imprint, and the STP of the partner was no different than that of the subject (Supplementary Fig. 13).

Next, we asked whether the transmission was limited to the subject and partner or whether the partner could in turn transmit to another individual. This latter scenario could have additional value in a social group. Male mice were housed in groups of three and separated immediately before the experiment. The subject experienced FS and was then returned to a cage containing one partner (Partner1; Fig. 7a). After a 30-min interaction, Partner1 was transferred to a cage containing the third group member (Partner2) for another 30 min (Fig. 7a). Partner2 did not have contact with the FS subject. In a control experiment, FS was replaced with 5 min of NE. The FS subject had higher levels of plasma CORT relative to Partner1, Partner2 and NE subjects (Supplementary Fig. 14). CORT levels were similar in the NE subjects and their respective partners (Supplementary Fig. 14). STP was evident in the FS subject, Partner1 and Partner2 (Fig. 7b). The STP in FS Partner2 was greater than that of NE Partner2 (Supplementary Fig. 15). These observations demonstrate that synaptic priming induced by a transmitted stress can be propagated by a partner to a tertiary group member. Furthermore, the STP observed following transmitted secondary stress and tertiary stress is similar to that observed following authentic stress.

Discussion

We found that stress primed glutamate synapses on PVN CRH neurons. This synaptic load was transmitted to naive individuals from the stressed subject. In addition, in females, but not males, the partner buffered the synaptic load in stressed individuals. Activation of PVN CRH neurons in the stressed subject seemed to be necessary to release a putative alarm pheromone. In the naive partner, PVN CRH neuron activation was required for investigative behavior and synaptic priming. Finally, the synaptic load could be transferred by the partner to a third member of the group (Supplementary Fig. 16).

Priming of glutamate synapses in response to either authentic or transmitted stress unmasked associative STP. This STP, which lasted for at least a day after the stress, but might persist for several days[6], requires the availability of CRHR1; this is consistent with earlier descriptions of STP in rats following immobilization or predator odor stress[6]. The CRHR1-dependent downregulation of NMDA receptors allows for multi-vesicular glutamate release immediately following tetanization[6]. Photostimulation of PVN CRH neurons, even in the absence of stress, was sufficient to unmask STP, whereas photoinhibition during stress prevented STP. These observations demonstrate that activation of PVN CRH neurons is both necessary and sufficient for the induction of STP. Combined with the finding that CRHR1 is required for STP, we conclude that locally released CRH binds to CRHR1, creating a synaptic environment that is permissive for STP. Abnormalities in the CRH system are evident in post-traumatic stress disorder (PTSD) and other stress-related affective disorders, such as anxiety and depression[28], and recent work has implicated PVN CRH neurons as drivers of anxiety-like behaviors[26,29].

Although STP is a reliable consequence of acute stress, the endocrine response is not an accurate predictor of STP. More specifically, elevated CORT levels do not predict the occurrence of STP. CORT levels were elevated in both male and female subjects following exposure to either FS or NE; only female subjects, however, showed STP following NE exposure. This suggests that the consequence of stress on synapses is both graded and sex dependent and is consistent with our previous findings that relatively mild stressors have profound consequences for CRH neurons in females[2]. Although we have not explored the mechanisms responsible for this differential sensitivity, they may result from previously described sex differences in CRHR1 signaling[30].

Synaptic priming in both male and female mice is transmissible. Once synapses in a subject are loaded, regardless of the stress (FS or NE), transmission of the synaptic load to a partner occurs reliably following social interaction with the stressed subject. Thus, not only is stress transmitted from a stressed subject to partners, as previously reported in rodents[9,11,13] and humans[31], but the enduring synaptic consequence of stress, or the synaptic load, is also transmitted from subject to partners. These findings suggest that, in addition to consoling the stressed individual, affiliative behaviors in humans[7], primates[8] and rodents[9–11] may serve a strategic purpose by communicating information about a stressful event.

Social interaction also modifies synaptic load in female subjects. Specifically, STP was reduced in stressed female subjects returned to a partner in the homecage, suggesting that the presence of a partner buffers the lingering effects of acute stress in females. This is consistent with previous work suggesting that females, through a 'tend and befriend' strategy, may buffer the effects of stress more effectively than males[32]. Given that STP is induced even if no time elapses between FS and slice preparation, CRH neurons must encode the

biochemical signals of stress very rapidly. This also means that the 30-min interaction between females is not buffering the induction of the stress-associated biochemical changes necessary for STP, but instead is likely reducing the changes that have already occurred. The mechanisms through which this occurs are not known, although a recent report showing that oxytocin—a hormone that has been implicated in pro-social[33], attachment[34] and consolation behaviors[11]—decreases spontaneous glutamatergic drive to CRH neurons[35], providing an interesting avenue for future studies.

We observed that the partner acquires information from a stressed subject via olfaction. Partners engaged in sniffing behavior that was directed predominantly toward the anogenital region of the stressed subject, but also directed sniffing behavior toward the head/torso region, likely detecting pheromones from perianal glands and whisker pads, respectively[22]. This directional sniffing behavior toward a stressed conspecific has been reported previously[13,20]. Notably, exposing a single mouse to alarm pheromone while restricting its behavior in a NE results in avoidance behavior toward the alarm pheromone[36]. By contrast, mice housed in groups of three and exposed to alarm pheromone in their homecage show increased activity and seek out, rather than avoid, the source of the odor[36]. This suggests that social context and environment influence behaviors of mice toward alarm pheromones.

Alarm pheromones released from the anal glands induce a stress response in recipients and are hypothesized to be critical for communicating stress[21,22,37]. Our findings support this hypothesis, as partners of FS mice discriminated between anogenital sniffing and head/torso sniffing, spending more time anogenital sniffing; partners of NE subjects did not. Furthermore, mice that were exposed to a swab from the anogenital region of a stressed subject showed reliable STP, similar to stressed individuals; this STP was greater than that of mice exposed to a swab from the head/torso region of a stressed subject. Thus, although we cannot dismiss the involvement of other modes of communication, such as ultrasonic vocalizations[38,39], our findings strongly support alarm pheromone, specifically from the anogenital region, as the predominant method of communication of stress and STP from subject to partner.

The volatile chemicals released by mice under alarm conditions share common features with predator scents (kairomones)[24]. Both are detected by the vomeronasal organ[40,41] and Grueneberg ganglion cells[42] in mice, and may recruit parallel pathways[43] that converge in the ventromedial hypothalamus[44]. Alarm pheromones activate key stress nuclei, including the bed nucleus of the stria terminalis, amygdala, dorsomedial hypothalamus and the PVN[23]. Although the pathway through which mouse alarm pheromone specifically activates PVN CRH neurons is not known, work using predator odors implicates a pathway from the olfactory bulb to the amygdalo-piriform transition area, which projects directly to PVN CRH neurons[23,25].

Our data indicate that the activity of PVN CRH neurons and recruitment of CRHR1 in the partner is required for anogenital sniffing to occur. This may be important in the initial arousal of the partner following the return of the subject to the homecage; in the absence of this arousal, the partner fails to approach or investigate the subject. When CRHR1 was inhibited in the stressed subject during and following stress, partner mice still exhibited anogenital sniffing. Similarly, photo-inhibition of PVN CRH neurons in the stressed subject during and following stress had no effect on sniffing by the partner. In both experiments, the initial arousal of the partner following the return of the subject to the homecage likely triggered this investigative behavior. In both experiments, however, STP in the partner was significantly reduced, as if the signal antecedent to the synaptic changes was not fully transmitted from subject to partner. It is plausible that, although partner mice engaged in anogenital sniffing behavior, the signal required to activate and prime PVN CRH neurons was not released by the subject. In support of this hypothesis are findings that photoactivation of PVN CRH neurons in a subject mouse in the absence of stress triggered anogenital sniffing behavior by the partner and resulted in STP in the partner. Here, activation of PVN CRH neurons in the subject initiated a currently unknown signaling cascade that culminated in the release of an alarm pheromone. PVN CRH neurons are therefore upstream of the alarm pheromone production in stressed subjects and are essential for generating the specific behaviors required for seeking out and detecting alarm pheromones in partners. These observations position PVN CRH neurons as central controllers in communication via alarm signals.

From an ethological perspective, the ability to buffer the effects of stress[11] while simultaneously extracting experiential information from the distressed individual has clear adaptive benefits. This information may promote coalition formation during times of stress[45] while editing neural circuits to prepare for subsequent challenges without subjecting all group members to danger directly. In humans, buffering or consolation behavior is nearly universal[32], yet our findings suggest that the partner, or consoling individual, may experience long-term synaptic consequences similar to those of the distressed individual. This may, for example, offer a potential explanation for why individuals who have themselves not experienced a trauma develop PTSD symptoms after learning of the trauma of others[46].

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41593-017-0044-6.

Received: 24 March 2017; Accepted: 12 November 2017;
Published online: 8 January 2018

References

1. Denver, R. J. Structural and functional evolution of vertebrate neuroendocrine stress systems. *Ann. NY Acad. Sci.* **1163**, 1–16 (2009).
2. Senst, L., Baimoukhametova, D., Sterley, T.-L. & Bains, J. S. Sexually dimorphic neuronal responses to social isolation. *eLife* **5**, 5904 (2016).
3. Bains, J. S., Wamsteeker Cusulin, J. I. & Inoue, W. Stress-related synaptic plasticity in the hypothalamus. *Nat. Rev. Neurosci.* **16**, 377–388 (2015).
4. Wamsteeker Cusulin, J. I., Füzesi, T., Inoue, W. & Bains, J. S. Glucocorticoid feedback uncovers retrograde opioid signaling at hypothalamic synapses. *Nat. Neurosci.* **16**, 596–604 (2013).
5. Inoue, W. et al. Noradrenaline is a stress-associated metaplastic signal at GABA synapses. *Nat. Neurosci.* **16**, 605–612 (2013).
6. Kuzmiski, J. B., Marty, V., Baimoukhametova, D. V. & Bains, J. S. Stress-induced priming of glutamate synapses unmasks associative short-term plasticity. *Nat. Neurosci.* **13**, 1257–1264 (2010).
7. Zahn-Waxler, C., Radke-Yarrow, M., Wagner, E. & Chapman, M. Development of concern for others. *Dev. Psychol.* **28**, 126–136 (1992).
8. Clay, Z. & de Waal, F. B. M. Bonobos respond to distress in others: consolation across the age spectrum. *PLoS One* **8**, e55206 (2013).
9. Langford, D. J. et al. Social modulation of pain as evidence for empathy in mice. *Science* **312**, 1967–1970 (2006).
10. Ben-Ami Bartal, I., Decety, J. & Mason, P. Empathy and pro-social behavior in rats. *Science* **334**, 1427–1430 (2011).
11. Burkett, J. P. et al. Oxytocin-dependent consolation behavior in rodents. *Science* **351**, 375–378 (2016).
12. Martin, L. J. et al. Reducing social stress elicits emotional contagion of pain in mouse and human strangers. *Curr. Biol.* **25**, 326–332 (2015).
13. Bruchey, A. K., Jones, C. E. & Monfils, M.-H. Fear conditioning by-proxy: social transmission of fear during memory retrieval. *Behav. Brain Res.* **214**, 80–84 (2010).
14. Swanson, L. W. et al. An immunohistochemical study of the organization of catecholaminergic cells and terminal fields in the paraventricular and supraoptic nuclei of the hypothalamus. *J. Comp. Neurol.* **196**, 271–285 (1981).
15. Khan, A. M. et al. MAP kinases couple hindbrain-derived catecholamine signals to hypothalamic adrenocortical control mechanisms during glycemia-related challenges. *J. Neurosci.* **31**, 18479–18491 (2011).
16. Boudaba, C., Schrader, L. A. & Tasker, J. G. Physiological evidence for local excitatory synaptic circuits in the rat hypothalamus. *J. Neurophysiol.* **77**, 3396–3400 (1997).

17. Wamsteeker Cusulin, J. I., Füzesi, T., Watts, A. G. & Bains, J. S. Characterization of corticotropin-releasing hormone neurons in the paraventricular nucleus of the hypothalamus of Crh-IRES-Cre mutant mice. *PLoS One* **8**, e64943 (2013).
18. Hennessy, M. B., Kaiser, S. & Sachser, N. Social buffering of the stress response: diversity, mechanisms, and functions. *Front. Neuroendocrinol.* **30**, 470–482 (2009).
19. Knapska, E. et al. Between-subject transfer of emotional information evokes specific pattern of amygdala activation. *Proc. Natl. Acad. Sci. USA* **103**, 3858–3862 (2006).
20. Knapska, E., Mikosz, M., Werka, T. & Maren, S. Social modulation of learning in rats. *Learn. Mem.* **17**, 35–42 (2009).
21. Inagaki, H. et al. The volatility of an alarm pheromone in male rats. *Physiol. Behav.* **96**, 749–752 (2009).
22. Kiyokawa, Y., Kikusui, T., Takeuchi, Y. & Mori, Y. Alarm pheromones with different functions are released from different regions of the body surface of male rats. *Chem. Senses* **29**, 35–40 (2004).
23. Kiyokawa, Y., Kikusui, T., Takeuchi, Y. & Mori, Y. Mapping the neural circuit activated by alarm pheromone perception by c-Fos immunohistochemistry. *Brain Res.* **1043**, 145–154 (2005).
24. Brechbühl, J. et al. Mouse alarm pheromone shares structural similarity with predator scents. *Proc. Natl. Acad. Sci. USA* **110**, 4762–4767 (2013).
25. Kondoh, K. et al. A specific area of olfactory cortex involved in stress hormone responses to predator odours. *Nature* **532**, 103–106 (2016).
26. Füzesi, T., Daviu, N., Wamsteeker Cusulin, J. I., Bonin, R. P. & Bains, J. S. Hypothalamic CRH neurons orchestrate complex behaviours after stress. *Nat. Commun.* **7**, 11937 (2016).
27. Sternson, S. M. Hypothalamic survival circuits: blueprints for purposive behaviors. *Neuron* **77**, 810–824 (2013).
28. Risbrough, V. B. & Stein, M. B. Role of corticotropin releasing factor in anxiety disorders: a translational research perspective. *Horm. Behav.* **50**, 550–561 (2006).
29. Zhang, R. et al. Loss of hypothalamic corticotropin-releasing hormone markedly reduces anxiety behaviors in mice. *Mol. Psychiatry* **22**, 733–744 (2017).
30. Valentino, R. J., Van Bockstaele, E. & Bangasser, D. Sex-specific cell signaling: the corticotropin-releasing factor receptor model. *Trends Pharmacol. Sci.* **34**, 437–444 (2013).
31. Buchanan, T. W., Bagley, S. L., Stansfield, R. B. & Preston, S. D. The empathic, physiological resonance of stress. *Soc. Neurosci.* **7**, 191–201 (2012).
32. Taylor, S. E. et al. Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychol. Rev.* **107**, 411–429 (2000).
33. Andari, E. et al. Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. *Proc. Natl. Acad. Sci. USA* **107**, 4389–4394 (2010).
34. Insel, T. R. & Young, L. J. The neurobiology of attachment. *Nat. Rev. Neurosci.* **2**, 129–136 (2001).
35. Jamieson, B.B., Nair, B.B. & Iremonger, K.J. Regulation of hypothalamic CRH neuron excitability by oxytocin. *J. Neuroendocrinol.* **29**, e12532 (2017).
36. Zalaquett, C. & Thiessen, D. The effects of odors from stressed mice on conspecific behavior. *Physiol. Behav.* **50**, 221–227 (1991).
37. Kiyokawa, Y., Shimozuru, M., Kikusui, T., Takeuchi, Y. & Mori, Y. Alarm pheromone increases defensive and risk assessment behaviors in male rats. *Physiol. Behav.* **87**, 383–387 (2006).
38. Grimsley, J. M. S. et al. Contextual modulation of vocal behavior in mouse: newly identified 12 kHz "mid-frequency" vocalization emitted during restraint. *Front. Behav. Neurosci.* **10**, 38 (2016).
39. Mun, H.-S., Lipina, T. V. & Roder, J. C. Ultrasonic vocalizations in mice during exploratory behavior are context-dependent. *Front. Behav. Neurosci.* **9**, 316 (2015).
40. Papes, F., Logan, D. W. & Stowers, L. The vomeronasal organ mediates interspecies defensive behaviors through detection of protein pheromone homologs. *Cell* **141**, 692–703 (2010).
41. Chamero, P., Leinders-Zufall, T. & Zufall, F. From genes to social communication: molecular sensing by the vomeronasal organ. *Trends Neurosci.* **35**, 597–606 (2012).
42. Brechbühl, J., Klaey, M. & Broillet, M.-C. Grueneberg ganglion cells mediate alarm pheromone detection in mice. *Science* **321**, 1092–1095 (2008).
43. Sosulski, D. L., Bloom, M. L., Cutforth, T., Axel, R. & Datta, S. R. Distinct representations of olfactory information in different cortical centres. *Nature* **472**, 213–216 (2011).
44. Pérez-Gómez, A. et al. Innate predator odor aversion driven by parallel olfactory subsystems that converge in the ventromedial hypothalamus. *Curr. Biol.* **25**, 1340–1346 (2015).
45. Berger, J., Heinrichs, M., von Dawans, B., Way, B. M. & Chen, F. S. Cortisol modulates men's affiliative responses to acute social stress. *Psychoneuroendocrinology* **63**, 1–9 (2016).
46. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders (DSM-5)* (2013).

Acknowledgements

We thank C. Breiteneder, M. Tsutsui and R. Barasi for technical assistance with tissue processing, injections and mouse colony maintenance. We thank K. Gorzo for assisting with behavioral analysis and R.P. Bonin (University of Toronto) for providing us with the original macro for analyzing behaviors. We thank K. Deisseroth (Stanford University) for kindly providing the viral constructs used for optical silencing of CRH neurons. We are grateful for the support of the Hotchkiss Brain Institute in creating the HBI Advance Light and Optogenetics core facility. This research was funded by operating grants to J.S.B. from the Canadian Institutes for Health Research (CIHR 86501) and Brain Canada Multi-Investigator Research Initiative and the Brain Canada Neurophotonics Platform. T.-L.S. and N.D. are supported by Fellowships from Alberta Innovates-Health Solutions (AIHS) and the UCalgary Eyes High Program. A.Z. is a CIHR Banting Fellow with additional support from AIHS.

Author contributions

T.-L.S. designed and conducted experiments, analyzed the data, and prepared the manuscript. D.B. designed and conducted experiments and assisted with data analyses. T.F. organized viral injections for optogenetic experiments and formatted figures. A.Z., N.D. and N.P. all contributed to electrophysiology data collection. D.R. assisted with organization of optogenetic experiments. J.B. designed experiments, prepared the manuscript, created figures and supervised the project. All of the authors contributed to intellectual discussion and direction of the project.

Competing interests

The authors declare no competing financial interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41593-017-0044-6.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to J.S.B.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Methods

Animals. All animal protocols were approved by the University of Calgary Animal Care and Use Committee. Male and female Crh-IRES-Cre; Ai14 mice in which CRH neurons express tdTomato fluorophore[17] were used in the present study, at 4–8 weeks of age. Mice were housed on a 12-h:12-h light:dark cycle (lights on at 6:00 a.m.) in whole litters until 1–2 d before use. Mice were then housed either alone, in same same-sex littermate pairs, or triplets, depending on the experiment. Mice were randomly assigned to experimental groups with no more than two mice being used from the same litter per group. Sample sizes were determined based on previous work of a similar nature by our lab and others. Mice were provided with food and water ad libitum. The FS protocol consisted of a 0.5-mA, 2-s FS delivered every 30 s for a period of 5 min in a FS chamber. FS was applied between 8:30 and 9:30 a.m. during the light phase. Thereafter the stressed mouse was returned to the home cage. In NE experiments, one mouse out of the pair was removed from the cage and placed in a separate cage for 5 min. In experiments involving injection of CP154256 (CP), CP was injected at a dose of 30 mg/kg. CP was dissolved in PEG with 0.4% lidocaine, final volume 50 µl. Vehicle-injected mice received PEG with 0.4% lidocaine. Pairs of mice were video-recorded during the 30 min following FS or NE. These videos were later analyzed and the following behaviors were scored: anogenital sniffing (directing the snout toward the anogenital area of conspecific); head/torso sniffing (directing the snout toward the head or torso of conspecific); and allogrooming (grooming directed to conspecific rather than self). Mice that engaged in fighting behavior during the 30-min interaction were excluded from the study since this behavior is stressful. Each video was analyzed by two different individuals; one individual was blind to the experimental groups. Results were tabulated post hoc.

Optogenetics. Crh-IRES-Cre; Ai14 mice, aged 6–8 weeks old, were kept under isoflurane anesthesia in stereotaxic apparatus. Glass capillaries were lowered into the brain (anteroposterior (AP), −0.7 mm; lateral (L), −0.3 mm from the bregma; dorsoventral (DV), −4.5 mm from the dura). Recombinant adeno-associated virus (AAV) carrying Arch3.0-eYFP (rAAV2-EF1a-double floxed-eArch3.0-eYFP; 5×10^{11} GC per ml; UNC Vector Core), ChR2-eYFP (Adgene plasmid 20298, pAAV-EF1a-double floxed-gChR2(H134R)-eYFP-WPRE-HGHpA; 5×10^{11} HC per mil; Penn Vector Core), or eYFP only (Addgene plasmid 20296, pAAV-EF1a-double floxed-eYFP-WPRE-HGHpA; 5×10^{11} GC per ml; Penn Vector Core) was pressure injected with Nanoject II apparatus (Drummond Scientific Company) in a total volume of 210 nl. After a 2-week wait period, mono fiber optic cannulas (Doric Lenses) were stereotactically implanted under similar conditions (AP, −0.7 mm; L, 0.0 mm from the bregma; DV, −4.0 mm from the dura). Following 1–2 d recovery time, mice were handled for at least 5 min daily for three consecutive days. Thereafter they were habituated daily to having a fiber optic cable (200-µm core diameter, Doric Lenses) attached to the mono fiber optic cannula for another 3 d (no light). For experiments, the light source (for Arch3.0: 532 nm, LRS-0532-100-OP, Laserglow Technologies; for ChR2: 473 nm, LRS-0473-GFM, Laserglow Technologies) was connected to the implanted ferrule with a fiber optic cable (200µm core diameter, Doric Lenses). The lasers were controlled with a manually programmable Master 8 unit (A.M.P.I.). For Arch3.0, yellow light (15-mW laser intensity) was used continuously for a total time of 35 min. For ChR2, blue light (15-mW laser intensity) was delivered for 5 min (10 Hz, 10-ms pulse width).

Slice preparation. 30 min following FS or 5-min separation, the mice were anesthetized with isofluorane and decapitated. Brains were rapidly removed and immersed in ice-cold slicing solution containing, in mM: 87 NaCl, 2.5 KCl, 0.5 $CaCl_2$, 7 $MgCl_2$, 25 $NaHCO_3$, 25 D-glucose, 1.25 NaH_2PO_4, 75 sucrose, saturated with 95% O_2/5% CO_2. Coronal sections (250 µM) containing the PVN were obtained using a vibratome (Leica). Slices were allowed to recover for 1 h in 30 °C artificial cerebrospinal fluid (aCSF) containing, in mM: 126 NaCl, 2.5 KCl, 26 $NaHCO_3$, 2.5 $CaCl_2$, 1.5 $MgCl_2$, 1.25 NaH_2PO_4, 10 glucose, saturated with 95% O_2/5% CO_2.

Electrophysiology. Data for electrophysiology experiments were acquired by multiple individuals. Only one individual was aware of the allocation of the animal during the experiment, while the others were bind. All recordings took place in aCSF containing picrotoxin (100 µM) at 30–2 °C, perfused at a rate of 1 ml/min. Neurons were visualized using an upright microscope fitted with differential interference contrast and epilfluorescence optics and camera. Borosilicate pipettes (2.5–4.5 mΩ) were filled with internal solution containing, in mM: 108 potassium-gluconate, 2 $MgCl_2$, 8 sodium-gluconate, 8 KCl, 1 potassium-EGTA, 4 potassium-ATP, 0.3 sodium-GTP, 10 HEPES. In current-clamp recordings, the initial membrane potential was −70 mV. In paired-pulse recordings, cells were voltage-clamped at −70 mV. A monopolar aCSF-filled electrode placed in the vicinity of the cell (~20 µM) was used to evoke EPSCs 50 ms apart at 0.2-Hz intervals. The high-frequency stimulation consisted of four 100-Hz stimulations for 1 s every 10 s. Access resistance (<20 MΩ) was continuously monitored and recordings were accepted for analysis if changes were < 15%.

For experiments involving virally injected mice, slices were transferred to paraformaldehyde at the end of the recording day and stored at 4 °C overnight. The following day slices were transferred to 30% sucrose solution and stored at 4 °C. Slices were mounted on slides and an experimenter blind to the electrophysiological data viewed the slices under a confocal microscope to determine accuracy of the viral injections. Data collected in viral injection experiments was only included in the final dataset if the electrophysiologist noted an effect of light during experiments, in vitro, and experimenter saw evidence of viral expression under the confocal microscope post hoc.

Corticosterone immunoassay. Trunk blood was collected in Eppendorf tubes containing 25 µl EDTA (0.5 M, pH 8) at the time of decapitation and was centrifuged (3,000 rpm, 4 °C, 10 min). Aliquots of plasma were stored at −80 °C until assay using DetectX Corticosterone Immunoassay Kit (Arbor Assay). Plasma samples were run in triplicate on the same day and an average value per animal obtained per day. Different aliquots of plasma from the same animal were run on different days. Values were averaged across days per animal.

Data analysis and statistics. Signals were amplified using a Multiclamp 700B amplifier (Molecular Devices), low-pass filtered at 1 kHz and digitized at 10 kHz using the Digidata 1440 (Molecular Devices). Data were recorded (pClamp 10.2, Molecular Devices) for offline analysis. Evoked EPSC amplitude was calculated from the baseline current before stimulation to the peak synaptic current. The magnitude of STP was calculated as the average amplitude of 12 eEPSCs immediately following HFS (0–1 min post-HFS). sEPSCs were detected using a variable threshold (MiniAnalysis, Synaptosoft). Data following HFS were normalized and expressed as percentage of the baseline values (0–5 min before HFS). One-sample parametric *t* test (two-tailed) was used when comparing post-HFS measured to 100% baseline. When comparing the means of two independent groups, unpaired *t* tests were used. When comparing the means of two dependent groups, paired *t* tests were used. When comparing the means of multiple groups, one-way ANOVA was used followed by Tukey's or Sidak's post hoc, multiple comparisons test. For correlations, Pearson's correlation was performed.

Life Sciences Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary.

Data and code availability. All relevant data and analysis tools are available upon reasonable request from the authors.

nature research

Corresponding author(s): Jaideep Bains

☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

Sample sizes were based on previous experiments conducted by us and many other labs in the field.

2. Data exclusions

Describe any data exclusions.

Data were excluded from any pairs of mice that showed fighting behavior when reunited after a separation. This increases HPA activation that we cannot control and confounds the experiments.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Our observation that stress leads to synaptic priming and STP has been reproduced multiple times by different experimenters (at least 7 different people in the lab) in both mice and rats (previous work from our lab). Furthermore, in all electrophysiological experiments, multiple experimenters contributed to the data set and thus findings were not due solely to one experimenter working on one rig.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Mice were randomly selected. If multiple mice were used from one litter the mice were spread across experimental groups to avoid a litter effect. Not more than 2 mice were used from one litter in each experimental group.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Due to the nature of the experiments, one experimenter overseeing the experiment could not be blind to the experimental groups. However, where possible, the experimenters collecting electrophysiology data were blind to the experimental group.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	GraphPad Prism 7, Minianalysis, Clampfit, Excel

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	No restrictions.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	No antibodies were used.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	N/A
b. Describe the method of cell line authentication used.	N/A
c. Report whether the cell lines were tested for mycoplasma contamination.	N/A
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	N/A

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	Male and female CRH-IRES-Cre; A14 mice in which CRH neurons express td-Tomato fluorophore were used (CRH-IRES-Cre X td-Tomato). Mice were 4-8 weeks old.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	N/A