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Supplementary information

Neural control of affiliative touch in prosocial interaction

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Supplementary Information for

Neural control of affiliative touch in prosocial interaction

Ye Emily Wu, James Dang, Lyle Kingsbury, Mingmin Zhang, Fangmiao Sun, Rongfeng K. Hu, and Weizhe Hong

Supplementary Table 1. Detailed statistical information.

Figure	Sample Size	Statistical test	Test statistic and P value
Fig. 1d	n = 15 male mice in each group	Two-sided Wilcoxon signed-rank test	W = 91, $P = 0.0023$
Fig. 1e	n = 15 male mice in each group	Two-sided Wilcoxon signed-rank test	W = -105, $P = 0.0001$
Fig. 1f	n = 15 male mice in each group	Two-sided Wilcoxon signed-rank test	W = 73, $P = 0.0081$
Fig. 1g	n = 15 male mice in each group	Two-sided Wilcoxon signed-rank test	W = 103, $P = 0.0002$
Fig. 1h	n = 15 male mice in each group	Two-sided Wilcoxon signed-rank test	W = 4, $P = 0.9341$
Fig. 1i	n = 15 male mice in each group	Two-sided Wilcoxon signed-rank test	W = 20, $P = 0.4131$
Fig. 1j	n = 15 male mice in each group	Two-sided Wilcoxon signed-rank test	W = -105, $P = 0.0001$
Fig. 1k	n = 10 male mice in each group	Two-sided Wilcoxon signed-rank test	W = 55, $P = 0.0020$
Fig. 1l	n = 10 male mice in each group	Two-sided Wilcoxon signed-rank test	W = -12, $P = 0.3750$
Fig. 1m	n = 12 male mice in each group	Two-sided Wilcoxon signed-rank test	W = 70, $P = 0.0034$
Fig. 1n	n = 12 male mice in each group	Two-sided Wilcoxon signed-rank test	W = 27, $P = 0.1934$
Fig. 1p	n = 9 rounds of training and evaluation for each group	Two-sided Wilcoxon signed-rank test	W = -45, $P = 0.0039$
Fig. 1q	n = 9 rounds of training and evaluation for each group	Two-sided Wilcoxon signed-rank test	W = -45, $P = 0.0039$
Fig. 1s	n = 12 male mice in each group	Two-sided Wilcoxon signed-rank test	W = 56, $P = 0.0269$
Fig. 1t	n = 12 male mice in each group	Two-sided Wilcoxon signed-rank test	W = -19, $P = 0.3750$
Fig. 1w	n = 9 male mice in each group	Two-way repeated measures ANOVA, post hoc two-sided paired t test with Bonferroni correction	ANOVA: Condition (baseline vs. foot shock): F(1, 16) = 19.03, $P = 0.0005$. Group (alone vs. reunion): F(1, 16) = 4.149, $P = 0.0586$. Interaction: F(1, 16) = 4.69, $P = 0.0458$. Pairwise comparisons: Alone, baseline vs. alone, foot shock: t(8) = -4.3134, $P = 0.0154$ (after correction) Reunion, baseline vs. reunion, foot shock: t(8) = -1.6805, $P = 0.7882$ (after correction) Alone, baseline vs. reunion, baseline: t(8) = 1.3505, $P = 1$ (after correction) Alone, foot shock vs. reunion, foot shock: t(8) = 4.8817, $P = 0.0073$ (after correction)
Fig. 1x	n = 9 male mice in each group	Two-way repeated measures ANOVA, post hoc two-sided paired t test with Bonferroni correction	ANOVA: Condition (baseline vs. foot shock): F(1, 16) = 90.87, $P < 0.0001$. Group (alone vs. reunion): F(1, 16) = 1.937×10^{-5}, $P = 0.9965$. Interaction: F(1, 16) = 0.8012, $P = 0.384$. Pairwise comparisons: Alone, baseline vs. alone, foot shock: t(8) = -9.541, $P < 0.0001$ (after correction)

			Reunion, baseline vs. reunion, foot shock: t(8) = -5.8473, $P = 0.0023$ (after correction) Alone, baseline vs. reunion, baseline: t(8) = 0.68583, $P = 1$ (after correction) Alone, foot shock vs. reunion, foot shock: t(8) = -0.3418, $P = 1$ (after correction)
Fig. 2g	n = 83 unstressed conspecific-activated neurons and 87 stressed conspecific-activated neurons (from 7 independent imaging sessions in 6 male mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 2528, $P = 0.0007$
Fig. 2h	n = 7 independent imaging sessions (in 6 male mice)	Two-sided Wilcoxon signed-rank test	W = 28, $P = 0.0156$
Fig. 2p	n = 7 independent imaging sessions (in 6 male mice)	Two-sided Wilcoxon signed-rank test	"Allogrooming" vs. "Sniffing" decoder: W = 28, $P = 0.0156$ "Allogrooming" vs. "Self-grooming" decoder: W = 28, $P = 0.0156$ "Sniffing" vs. "Self-grooming" decoder: W = 28, $P = 0.0156$
Fig. 3d	n = 44 trials for GtACR2 real illumination (from 7 male mice) n = 45 trials for GtACR2 sham illumination (from 7 male mice) n = 59 trials for control real illumination (from 6 male mice)	Kruskal-Wallis test followed by post hoc Dunn's multiple comparisons test	Kruskal-Wallis statistic = 62.64, $P < 0.0001$ Control real illumination vs. GtACR2 real illumination: adjusted $P < 0.0001$ GtACR2 sham illumination vs. GtACR2 real illumination: adjusted $P < 0.0001$
Fig. 3h	n = 22 bouts for GCaMP (from 5 male mice) n = 40 bouts for control (from 7 male mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 132, $P < 0.0001$
Fig. 3j	n = 4 hemispheres independently injected with virus from 2 mice (3-6 sections per hemisphere)	NA	NA
Fig. 3m	n = 52 trials from 4 mice for Tac1-Cre (35 trials in 2 males and 17 trials 2 females n = 61 trials from 4 mice for Sst-Cre (25 trials in 2 males and 36 trials in 2 females) n = 46 trials from 4 mice for Cck-Cre (24 trials in 2 males and 22 trials in 2 females)	Kruskal-Wallis test followed by post hoc Dunn's multiple comparisons test	Kruskal-Wallis statistic = 80.37, $P < 0.0001$ Tac1-Cre vs. Sst-Cre: adjusted $P < 0.0001$ Tac1-Cre vs. Cck-Cre: adjusted $P < 0.0001$
Fig. 3n	n = 116 trials for GtACR2 real illumination (from 7 male mice) n = 97 trials for GtACR2 sham illumination (from 7 male mice) n = 84 trials for control real illumination (from 5 male mice)	Kruskal-Wallis test followed by post hoc Dunn's multiple comparisons test	Kruskal-Wallis statistic = 115.1, $P < 0.0001$ Control real illumination vs. GtACR2 real illumination: adjusted $P < 0.0001$ GtACR2 sham illumination vs. GtACR2 real illumination: adjusted $P < 0.0001$
Fig. 4c	n = 4 hemispheres independently injected with the virus from 2 mice (3-4 sections per hemisphere)	NA	NA
Fig. 4g	n = 173 trials from 8 mice for ChR2 with stressed ("foot shock") partners (115 trials in 4 females and 58 trials in 4 males). n = 79 trials from 7 mice for ChR2 with unstressed ("sep only") partners (44 trials in 4 females and 35 trials in 3 males). n = 77 trials from 6 mice for control with stressed partners (55 trials in 4 females and 22 trials in 2 males).	Kruskal-Wallis test followed by post hoc Dunn's multiple comparisons test	Kruskal-Wallis statistic = 167.3, $P < 0.0001$ "Control" vs. "Sep only": adjusted $P = 0.0003$ "Control" vs. "Foot shock": adjusted $P < 0.0001$ "Sep only" vs. "Foot shock": adjusted $P < 0.0001$
Fig. 4k	n = 19 bouts for GCaMP (from 6 male mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 84, $P < 0.0001$

	n = 28 bouts for control (from 4 male mice)		
Fig. 4n	n = 127 trials from 6 mice for ChR2 (68 trials in 4 females and 59 trials in 2 males). n = 81 trials from 6 mice for control (52 trials in 4 females and 29 trials in 2 males)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 414, $P < 0.0001$
Fig. 4o	n = 127 trials from 6 mice for ChR2 (68 trials in 4 females and 59 trials in 2 males). n = 81 trials from 6 mice for control (52 trials in 4 females and 29 trials in 2 males)	Two-sided Wilcoxon rank-sum test	Mann Whitney U = 425, $P < 0.0001$
Fig. 4r	n = 93 trials from 6 mice for ChR2 (40 trials in 3 females and 53 trials in 3 males). n = 67 trials from 6 mice for control (45 trials in 4 females and 22 trials in 2 males)	Two-sided Wilcoxon rank-sum test	Mann Whitney U = 183, $P < 0.0001$
Fig. 4s	n = 93 trials from 6 mice for ChR2 (40 trials in 3 females and 53 trials in 3 males). n = 67 trials from 6 mice for control (45 trials in 4 females and 22 trials in 2 males)	Two-sided Wilcoxon rank-sum test	Mann Whitney U = 162.5, $P < 0.0001$
Extended Data Fig. 1c	n = 10 female mice	Two-sided Wilcoxon signed-rank test	W = 36, $P = 0.0078$
Extended Data Fig. 1d	n = 10 female mice	Two-sided Wilcoxon signed-rank test	W = -36, $P = 0.0078$
Extended Data Fig. 1e	n = 10 female mice	Two-sided Wilcoxon signed-rank test	W = 36, $P = 0.0078$
Extended Data Fig. 1f	n = 10 female mice	Two-sided Wilcoxon signed-rank test	W = 32, $P = 0.0234$
Extended Data Fig. 1g	n = 10 female mice	Two-sided Wilcoxon signed-rank test	W = 25, $P = 0.2324$
Extended Data Fig. 1h	n = 10 female mice	Two-sided Wilcoxon signed-rank test	W = -4, $P = 0.5000$
Extended Data Fig. 1i	n = 10 female mice	Two-sided Wilcoxon signed-rank test	W = -55, $P = 0.0020$
Extended Data Fig. 2b	n = 9 rounds of training and evaluation for each group	Two-sided Wilcoxon signed-rank test	W = 45, $P = 0.0039$
Extended Data Fig. 2c	n = 9 rounds of training and evaluation for each group	Two-sided Wilcoxon signed-rank test	W = 41, $P = 0.0117$
Extended Data Fig. 2d	n = 9 rounds of training and evaluation for each group	Two-sided Wilcoxon signed-rank test	W = 45, $P = 0.0039$
Extended Data Fig. 2e	n = 9 rounds of training and evaluation for each group	Two-sided Wilcoxon signed-rank test	W = 45, $P = 0.0039$
Extended Data Fig. 2f	n = 9 rounds of training and evaluation for each group	One-way ANOVA, post-hoc Bonferroni's multiple comparisons test	F (4, 40) = 104.8, $P < 0.0001$ "CNN features" vs. "Tracking": adjusted $P < 0.0001$ "CNN features" vs. "Head orientation": adjusted $P < 0.0001$ "CNN features" vs. "All features: adjusted $P > 0.9999$

			"CNN features" vs. "Shuffle": adjusted $P < 0.0001$
Extended Data Fig. 2h	n = 7 independent imaging sessions in 6 male mice	Friedman test followed by post hoc Dunn's multiple comparisons test	Friedman statistic = 14.66, $P = 0.0021$ "allogr rnn-allogr rnn" vs. "allogr rnn-allogr h.a.": adjusted $P > 0.9999$ "allogr rnn-allogr rnn" vs. "allogr rnn-inv rnn": adjusted $P = 0.0389$ "allogr rnn-allogr rnn" vs. "allogr rnn-selfgr rnn": adjusted $P = 0.0113$
Extended Data Fig. 3e	n = 338 pairwise distances for allogrooming-encoding cells. n = 1560 pairwise distances for sniffing-encoding cells. n = 189800 pairwise distances for the "shuffle" group (generated from 100 rounds of random permutation of response type). From 7 independent imaging sessions in 6 male mice.	Kolmogorov-Smirnov test	"Allogrooming-responsive" vs. "Shuffle": $P = 0.1117$ "Sniffing-responsive" vs. "Shuffle": $P = 0.5997$
Extended Data Fig. 3h	n = 55 self-grooming-activated cells, 39 allogrooming-activated cells, 87 sniffing-activated cells, 10 self-grooming- and allogrooming-activated cells, 4 self-grooming- and sniffing activated cells, and 336 cells in total. From 7 independent imaging sessions in 6 male mice.	Hypergeometric test	Overlap between self-grooming-activated cells and allogrooming-activated cells: $P = 0.0803$ Overlap between self-grooming-activated cells and sniffing-activated cells: $P > 0.9999$
Extended Data Fig. 4c	n = 7 auROC measures (7 independent imaging sessions in 6 mice) n = 70 control auROC measures for the "shuffle" group (10 rounds of randomization for each session)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 43, $P < 0.0001$
Extended Data Fig. 4d	n = 7 independent imaging sessions in 6 male mice	Two-sided Wilcoxon signed-rank test	"Allogrooming" vs. "Sniffing" PC projection distances: W = -28, $P = 0.0156$ "Allogrooming" vs. "Self-grooming" PC projection distances: W = -28, $P = 0.0156$ "Sniffing" vs. "Self-grooming" PC projection distances: W = -28, $P = 0.0156$
Extended Data Fig. 4e	n = 7 independent imaging sessions in 6 male mice	Two-sided Wilcoxon signed-rank test	W = 28, $P = 0.0156$
Extended Data Fig. 4k	n = 89 allogrooming bouts (51 ≤ 5s, 38 > 5s, from 7 independent imaging sessions in 6 male mice) n = 1000 rounds of randomization for permutation test	Permutation test	$P = 0.002$
Extended Data Fig. 4l	n = 51 short (≤ 5s) and 38 long (> 5s) allogrooming bouts (from 7 independent imaging sessions in 6 male mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 965.5, $P = 0.9281$
Extended Data Fig. 4m	n = 51 short (≤ 5s) and 38 long (> 5s) allogrooming bouts (from 7 independent imaging sessions in 6 male mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 892.5, $P = 0.4994$
Extended Data Fig. 4n	n = 51 short (≤ 5s) and 38 long (> 5s) allogrooming bouts (from 7 independent imaging sessions in 6 male mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 963, $P > 0.9999$

Extended Data Fig. 5g	n = 35 trials for Tac1-Cre (from 2 mice) n = 25 trials for Sst-Cre (from 2 mice) n = 24 trials for Cck-Cre (from 2 mice)	Kruskal-Wallis test followed by post hoc Dunn's multiple comparisons test	Kruskal-Wallis statistic = 34.97, $P <$ 0.0001 Tac1-Cre vs. Sst-Cre: adjusted $P <$ 0.0001 Tac1-Cre vs. Cck-Cre: adjusted $P <$ 0.0001
Extended Data Fig. 5h	n = 35 trials for Tac1-Cre (from 2 mice) n = 25 trials for Sst-Cre (from 2 mice) n = 24 trials for Cck-Cre (from 2 mice)	Kruskal-Wallis test followed by post hoc Dunn's multiple comparisons test	Kruskal-Wallis statistic = 18.68, $P <$ 0.0001 Tac1-Cre vs. Sst-Cre: adjusted $P =$ 0.0003 Tac1-Cre vs. Cck-Cre: adjusted $P =$ 0.0012
Extended Data Fig. 5o	n = 17 trials for Tac1-Cre (from 2 mice) n = 36 trials for Sst-Cre (from 2 mice) n = 22 trials for Cck-Cre (from 2 mice)	Kruskal-Wallis test followed by post hoc Dunn's multiple comparisons test	Kruskal-Wallis statistic = 46.39, $P <$ 0.0001 Tac1-Cre vs. Sst-Cre: adjusted $P <$ 0.0001 Tac1-Cre vs. Cck-Cre: adjusted $P <$ 0.0001
Extended Data Fig. 5p	n = 17 trials for Tac1-Cre (from 2 mice) n = 36 trials for Sst-Cre (from 2 mice) n = 22 trials for Cck-Cre (from 2 mice)	Kruskal-Wallis test followed by post hoc Dunn's multiple comparisons test	Kruskal-Wallis statistic = 26.31, $P <$ 0.0001 Tac1-Cre vs. Sst-Cre: adjusted $P <$ 0.0001 Tac1-Cre vs. Cck-Cre: adjusted $P <$ 0.0001
Extended Data Fig. 5r	n = 40 bouts in 7 male mice for Sst^+ neurons n = 19 bouts in 6 male mice for $Tac1^+/Vgat^+$ neurons	Two-sided Wilcoxon rank-sum test	Mann Whitney U = 163, $P = 0.0003$
Extended Data Fig. 6b	n = 58 trials for ChR2 (from 4 mice) n = 22 trials for control (from 2 mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 109.5, $P < 0.0001$
Extended Data Fig. 6c	n = 58 trials for ChR2 (from 4 mice) n = 22 trials for control (from 2 mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 152.5, $P < 0.0001$
Extended Data Fig. 6e	n = 115 trials for ChR2 (from 4 mice) n = 55 trials for control (from 4 mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 247.5, $P < 0.0001$
Extended Data Fig. 6f	n = 115 trials for ChR2 (from 4 mice) n = 55 trials for control (from 4 mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 247.5, $P < 0.0001$
Extended Data Fig. 6g	n = 173 trials for ChR2 (115 trials in 4 females and 58 trials in 4 males). n = 77 trials for control (55 trials in 4 females and 22 trials in 2 males).	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 6547, $P = 0.8296$
Extended Data Fig. 8d	n = 59 trials for ChR2 (from 2 mice). n = 29 trials for control (from 2 mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 91, $P < 0.0001$
Extended Data Fig. 8e	n = 59 trials for ChR2 (from 2 mice). n = 29 trials for control (from 2 mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 95, $P < 0.0001$
Extended Data Fig. 8f	n = 68 trials for ChR2 (from 4 mice). n = 52 trials for control (from 4 mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 104, $P < 0.0001$
Extended Data Fig. 8g	n = 68 trials for ChR2 (from 4 mice). n = 52 trials for control (from 4 mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 104, $P < 0.0001$
Extended Data Fig. 8i	n = 53 trials for ChR2 (from 3 mice) n = 22 trials for control (from 2 mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 56, $P < 0.0001$

Extended Data Fig. 8j	n = 53 trials for ChR2 (from 3 mice) n = 22 trials for control (from 2 mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 48, $P < 0.0001$
Extended Data Fig. 8k	n = 40 trials for ChR2 (from 3 mice) n = 45 trials for control (from 4 mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 22.5, $P < 0.0001$
Extended Data Fig. 8l	n = 40 trials for ChR2 (from 3 mice) n = 45 trials for control (from 4 mice)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 22.5, $P < 0.0001$
Extended Data Fig. 8o	n = 36 trials from 4 mice for "no lidocaine" group (24 trials from 3 females and 12 trials from 1 male) n = 58 trials from 4 mice for "lidocaine" group (39 trials from 3 females and 19 trials from 1 male)	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 981.5, $P = 0.6297$
Extended Data Fig. 8q	n = 93 trials (40 trials in 3 females and 53 trials in 3 males) for MPOA stimulation n = 118 trials (65 trials in 2 females and 53 trials in 2 males) for PMv stimulation	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 757.5, $P < 0.0001$
Extended Data Fig. 9b	n = 141 trials for ChR2 (88 trials in 7 females and 53 trials in 5 males). n = 119 trials for control (74 trials in 6 females and 45 trials in 5 males).	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 1537, $P < 0.0001$
Extended Data Fig. 9c	n = 142 trials for ChR2 (89 trials in 7 females and 53 trials in 5 males). n = 119 trials for control (74 trials in 6 females and 45 trials in 5 males).	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 6168, $P = 0.0002$
Extended Data Fig. 9d	n = 78 trials (from 5 male mice) for all condition n = 53 trials (from 5 male mice) for optimal condition	Two-sided Wilcoxon rank-sum test	Mann-Whitney U = 1500, $P = 0.0068$

Supplementary Notes:

Supplementary Note 1, related to Fig. 1b–g, 4e, g, l–s, Extended Data Fig. 1a–f, 6a–f, 8c–l.

We found that both males and females exhibited elevated allogrooming toward distressed partners, with a higher level of allogrooming in males (Fig. 1b–g, Extended Data Fig. 1a–f, two-sided Wilcoxon rank-sum test for total duration of allogrooming toward stressed partners, $P = 0.0191$, $n = 15$ male and 10 female subjects). The nature and mechanism of this sex difference remain to be determined. Interestingly, when we optogenetically activated $MeA^{Tac1\cap Vgat}$ neurons or their MPOA projection, both males and females displayed allogrooming behavior to similar degrees (Fig. 4e, g, l–s, Extended Data Fig. 6a–f, Extended Data Fig. 8c–l), suggesting that the MeA-MPOA circuit could drive this behavior in both sexes. The neural mechanisms of sex difference in affiliative allogrooming and the potential role of the MeA-MPOA circuit in this process remain an interesting question for future study.

Supplementary Note 2, related to Fig. 1b–g, k–n.

We found that mice displayed increased allogrooming toward partners that had experienced different types of acute stressors (Fig. 1b–g, k–n). To further examine whether allogrooming can be induced by chronic stress in partners, we subjected partners to chronic restraint stress (Methods) and found no significant increase of allogrooming by subject animals ($n = 8$ male mice for the chronic restraint group and 8 male mice for the control group, two-sided Wilcoxon rank-sum test for total duration of allogrooming, $P = 0.9883$, 9.3 ± 7.1 s [mean ± s.e.m.] in control group, 11.4 ± 9.3 s [mean ± s.e.m.] in chronic stress group), suggesting that this type of chronic stress may not be sufficient to induce increased allogrooming.

Supplementary Note 3, related to Fig. 3i–m, Extended Data Fig. 5a–p.

In a previous single-cell RNAseq study[45], we reported a neuronal cluster in the MeA that contains neurons expressing Tac1 and Cck. It is noteworthy that in single-cell analysis, cell types are clustered by similarities in a high-dimensional space defined by transcriptional profiles of thousands of genes. When cells are classified into the same cell type or subtype, not all cells within a single type necessarily uniformly express the same genes. Indeed, in our more recent, larger-scale single-cell RNAseq dataset[18], we found that majority (77%) of $Tac1^+$ neurons do not express Cck at the level of individual cells. Using *in situ* hybridization analysis, we confirmed that the majority of $Tac1^+$ and Cck^+ neurons do not overlap in the MeA (Fig. 3i, j). As a substantial fraction of $Tac1^+$ neurons are not Cck-positive, it is conceivable that activation of $Tac1^+$ neurons evokes allogrooming and self-grooming but activation of Cck^+ neurons does not (Fig. 3k–m, Extended Data Fig. 5a–p). Moreover, our finding that activation of $Tac1^+$ neurons elicits allogrooming does not imply that all $Tac1^+$ neurons play a unitary role in positively promoting allogrooming. It remains possible that a fraction of $Tac1^+$ neurons are not involved or play a distinct role in allogrooming.

Supplementary Note 4, related to Fig. 3n, 4d, e, g, l–s, Extended Data Fig. 6a–f, 8c–o.

We show that the activity of MeA^{Tac1} neurons is required for natural allogrooming (Fig. 3n) and that, under the stimulation frequencies, intensities, and durations used in the current study (Methods), optogenetic activation of $MeA^{Tac1\cap Vgat}$ neurons or their MPOA projection is capable of eliciting allogrooming that resembles the native behavior (4d, e, g, l–s, Extended Data Fig. 6a–f, 8c–o, Supplementary Video 3, 4). We did not optogenetically activate these neurons with longer durations or excessively higher stimulation intensities or frequencies. The execution of allogrooming behavior requires a natural response from the recipient. Excessive artificial activation of $Tac1^+/Vgat^+$ neurons that does not reflect the physiological activity patterns of these neurons may potentially evoke unnatural behaviors that lead to aversive experiences in the recipients.

Supplementary Note 5, related to Fig. 4e, g.

We found that activating $MeA^{Tac1\cap Vgat}$ neurons induced allogrooming toward unstressed partners and that the level of evoked allogrooming is significantly higher than that in controls (Fig. 4e, g). This suggests that activation of these neurons partially bypasses the dependency of allogrooming on conspecific stress. On the other hand, we also found that the stimulation-induced allogrooming is further elevated by the presence of distressed partners (Fig. 4e, g). This suggests that the behavioral effects of photostimulation could also be modulated by different social contexts. One model to explain this is that the MeA does not act alone and that social context could modulate neural activity of brain areas that function downstream of or in parallel to the MeA.

Supplementary Note 6, related to Extended Data Fig. 9a–d, 10a–f.

Our previous studies show that optogenetic activation of MeA^{Vgat} neurons can promote attack behavior towards unfamiliar intruder animals in a resident-intruder assay[17]. While we also observed sporadic allogrooming towards intruders under a low stimulation intensity, this induced allogrooming only occurred in a fraction of stimulated animals

and its ethological context and physiological relevance were unclear[17]. Consistent with previous findings, we found that optogenetic activation of MeA Vgat$^+$ neurons could promote allogrooming toward stressed familiar partners without eliciting aggressive behavior (attack or mounting) when light stimulation was delivered at lower laser power and duty cycles (Extended Fig. 9a–d, Methods). However, under the same low-intensity stimulation conditions, the level of triggered allogrooming was significantly lower when unfamiliar intruders were presented and a mixture of aggression and allogrooming was induced, consistent with previous observations[17] (allogrooming duration during 15-s photostimulation: 5.34 ± 0.69 s [mean ± s.e.m.] toward stressed partner, n = 56 trials in 3 male mice**,** 2.26 ± 0.48 s [mean ± s.e.m.] toward unfamiliar intruders, n = 71 trials in 3 male mice, two-sided Wilcoxon rank-sum test, $P < 0.0001$; attack duration during 15-s photostimulation: 0 ± 0 s [mean ± s.e.m.] toward stressed partner, n = 56 trials in 3 male mice**,** 3.32 ± 0.64 s [mean ± s.e.m.] toward unfamiliar intruders, n = 71 trials in 3 male mice, two-sided Wilcoxon rank-sum test, $P < 0.0001$). These findings suggest that MeA^{Vgat} neurons may contain mixed subsets of neurons that differentially evoke allogrooming versus attack and that the effects of photostimulation of these neurons may be modulated by social context.

Using a Cre-off/Flp-on viral vector for expression of ChR2 in Cre$^-$ and Flp$^+$ neurons in Tac1-Cre/Vgat-Flp animals (Extended Data Fig. 10a–c), we found that activating MeA Tac1$^-$/Vgat$^+$ neurons elicited a substantial level of attack behavior but minimal allogrooming toward stressed partners (Extended Data Fig. 10d–f, Methods). Under the same stimulation conditions, activation of Tac1$^+$/Vgat$^+$ neurons elicited allogrooming but not attack (Fig. 4e, g, Methods). These results suggests that Tac1$^-$/Vgat$^+$ neurons may preferentially promote aggressive behavior. However, whether each population (Tac1$^+$/Vgat$^+$ or Tac1$^-$/Vgat$^+$) contains a subset of neurons that may exert a distinct, or even opposite, behavioral function remains an interesting question for further investigation.

Supplementary Note 7.

Humans and other animals are capable of sensing and sharing the emotional states of social partners, and this empathetic sharing can motivate prosocial behaviors, such as comforting, helping, and sharing of resources, toward partners in need[1–3]. While prosocial behavior is often motivated by empathy, it represents a distinct neural process that entails active, other-benefitting behavior directed toward conspecifics in need beyond affective sharing. Previous studies in humans, non-human primates, and rodents have shed light on the neural basis of empathy, highlighting the role of several brain areas such as the anterior cingulate cortex (ACC), insular cortex, and prefrontal cortex in vicarious experience[1,10,16,19,20,53–55]. However, how affective empathy is linked to prosocial behavior is not well understood. A previous study in prairies voles found that the ACC is activated in response to stressed partners[7]. While the activation of ACC does not require prosocial behavior, blocking oxytocin signaling in this area inhibits prosocial consolation behavior in voles[7]. While mice have been shown to exhibit social transmission of emotions such as fear and stress, direct physical interactions between bystanders and distressed individuals have rarely been examined[9,19–21,53,54,56–58]. By demonstrating that mice display prosocial comforting behavior and identifying the MeA as a key node that encodes and directly drives this behavior, our study may provide a starting point for investigating how the amygdala cooperates with other brain areas such as the ACC to link emotional empathy to prosocial behavioral responses.

Supplementary Note 8, related to Fig. 2–4.

While the MeA has been shown to regulate parenting, a type of offspring-directed caring behavior[18], we uncover an additional behavioral role for the MeA in a distinct social context (i.e. controlling adult-directed affiliative behavior). The fact that activity of MeA neurons encodes native allogrooming behavior and that acute inhibition of these neurons disrupts naturally occurring allogrooming toward stressed individuals demonstrate that these neurons are engaged in and essential for affiliative allogrooming in an ethological context. This argues against the possibility that optogenetically induced allogrooming simply reflects a displacement of parenting-related behavior in an irrelevant social context.

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