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Francesco Papaleo

Francesco.Papaleo@iit.it

Genetics of Cognition Laboratory, Neuroscience area, Istituto Italiano di Tecnologia, via Morego, 30,
16163 Genova <https://orcid.org/0000-0002-6326-0657>

Federica Antonelli

IIT

Anna Monai

Fabrizio Bernardi

IIT

Atesh Koul

Italian Institute of Technology <https://orcid.org/0000-0002-0917-9583>

Nicola Marie Engel

University Medical Center Hamburg-Eppendorf

Ilaria Carta

IIT <https://orcid.org/0000-0003-1270-573X>

Francesca Managò

Istituto Italiano di Tecnologia <https://orcid.org/0000-0003-3663-6193>

Arianna Benedetti

IIT

Anna Maria Borruto

IIT

Ludovico Spattini

IIT <https://orcid.org/0009-0007-0304-5705>

Federica Maltese

IIT

Chunzhi Yi

University Medical Center Hamburg-Eppendorf

Gian Marco Leggio

University of Catania <https://orcid.org/0000-0002-7280-4871>

Guillaume Dumas

Centre de recherche CHU Sainte-Justine and University of Montreal, and Mila, Quebec Artificial
Intelligence Institute <https://orcid.org/0000-0002-2253-1844>

Stefano Panzeri

University Medical Center Hamburg-Eppendorf <https://orcid.org/0000-0003-1700-8909>

Giacomo Novembre

Italian Institute of Technology <https://orcid.org/0000-0002-4723-4344>

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Federica Antonelli^{1,2}, Anna Monai^{1,2}, Fabrizio Bernardi¹, Atesh Koul³, Nicola Marie Engel⁴, Ilaria Carta^{1,2}, Francesca Managò¹, Arianna Benedetti^{1,2}, Anna Maria Borruto^{1,2}, Ludovico Spattini^{1,2}, Federica Maltese^{1,5}, Chunzhi Yi⁴, Gian Marco Leggio⁶, Guillaume Dumas^{7,8}, Stefano Panzeri⁴, Giacomo Novembre³, Francesco Papaleo^{1,2*}

¹*Genetics of Cognition laboratory, Neuroscience area, Istituto Italiano di Tecnologia, Genova, Italy.*

²*IRCCS Ospedale Policlinico San Martino, Largo Rosanna Benzi, 10, 16132 Genova, Italy.*

³*Neuroscience of Perception and Action Laboratory, Istituto Italiano di Tecnologia, Viale Regina Elena 291, 00161 Roma, Italy.*

⁴*Institute for Neural Information Processing, University Medical Center Hamburg-Eppendorf (UKE), Falkenried 94, 20251 Hamburg, Germany.*

⁵*Institute of Neuroscience, National Research Council, Vedano al Lambro, Italy.*

⁶*Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy.*

⁷*CHU Sainte-Justine Azrieli Research Centre, Department of Psychiatry and Addictology, University of Montréal, Montréal, Québec, Canada.*

⁸*Mila – Quebec Artificial Intelligence Institute, Montréal, Québec, Canada.*

*Corresponding author: francesco.papaleo@iit.it

Summary

Responding to others' emotional states is essential for social behavior, yet the neural mechanisms by which brains dynamically coordinate across individuals in different emotional states is unknown. Here, we show that emotions reorganize brain-to-brain coupling through distinct, cell-type-specific mechanisms that directly guide social choice. Simultaneous calcium imaging in three interacting mice revealed that anterior cingulate cortex (ACC) pyramidal neurons are inter-brain correlated during neutral interactions, but this coupling is disrupted when one partner is stressed. In contrast, stress recruits inter-brain coupling of ACC somatostatin (SOM) neurons. Behaviorally controlled, cell-type-specific optogenetic manipulations demonstrated that preventing SOM inter-brain coupling abolished emotion discrimination, whereas artificially inducing or inverting SOM or pyramidal coupling bidirectionally reshaped social preference in an emotion-dependent manner. Notably, a new closed-loop paradigm in which real-time activity from one mouse drove cell-type-specific stimulation in another promoted social preference only when targeting SOM neurons in stress conditions. Extending these findings to humans, we show that stress similarly disrupts frontal gamma-band inter-brain coupling during live social interaction, indicating conserved emotion-sensitive principles across species. Together, these findings establish a mechanistic framework in which emotion-dependent, cell-type-specific brain-to-brain dynamics causally shape socio-emotional behavior.

Emotions are key regulators of social behaviors across species, shaping communication, decision-making, conflict resolution, group cohesion, and collective dynamics¹⁻⁵. Detecting negative emotions in others can elicit a range of responses, from heightened vigilance and threat avoidance to consolatory help⁶⁻⁸. Given its fundamental impact on social life and survival, the ability to detect and respond to others' emotions is evolutionarily conserved^{1, 2, 5, 9-13}. Notably, socio-emotional exchanges depend on a continuous flow of information between interacting partners¹⁴⁻¹⁶, forming a dynamic, reciprocal feedback loop where actions, reactions, and emotional states are mutually modulated^{17, 18}. Yet, most investigations of the neural basis of socio-emotional behaviors in humans and non-human animals adopt the perspective of a single "observer" brain¹⁹⁻²¹. This approach overlooks the extent to which interacting brains operate as components of an integrated, dynamic system.

Pioneering multi-brain studies in humans, followed by mice and bats, have shown that brains of interacting individuals might function jointly, through correlated neural activity²²⁻²⁷. Indeed, correlations between interacting individuals, observed at the behavioral^{28, 29} and neural levels^{25, 27, 30, 31}, have been hypothesized to reflect an organizing principle through which brains coordinate, perceive, and adapt to other social agents in real time³². However, whether inter-brain dynamics have a consequential or causative role in modulating social interactions remains actively debated³³⁻³⁵. Most notably, the mechanistic bases of inter-brain dynamics in socio-emotional contexts remain scarcely explored and largely unknown³⁶.

The anterior cingulate cortex (ACC, Brodmann area 24) is a major hub orchestrating the detection and reaction to others' emotions in rodents and humans³⁶⁻⁴¹. Moreover, the ACC is often indicated in humans as a critical hub where others' actions and intentions might be codified through an internal mimicking of social partner's neural activity^{36, 42}. Therefore, we simultaneously recorded

neural activity in the ACC of three interacting mice engaged in an emotional state discrimination task^{20, 21, 43, 44}. In this task, an ‘observer’ mouse is placed in front of two conspecifics ('demonstrators'), one exhibiting an emotionally altered state and the other being in a neutral state. With this two-choice paradigm, we can isolate cell-type-specific neural signals underlying emotional discriminations that may not be detectable in one-on-one social interactions^{20, 21}, while simultaneously comparing real-time inter-brain dynamics in response to a neutral or an emotionally altered conspecific. The task approximates key features of human emotion recognition paradigms^{20, 21, 43, 44}. Thus, to strengthen the link to analogous processes in humans, we formally investigated inter-brain dynamics during real-time human interactions adding an emotional component. To determine whether inter-brain dynamics causally influence socio-emotional choices, we applied several optogenetic manipulations across all three interacting mice to either prevent neural correlations or induce artificial correlations. Finally, we developed a system in which real-time recordings of ACC neural subpopulations in one mouse are used to causally impose matched activity patterns in another mouse via optogenetic stimulation. Our findings uncover cell-type-specific mechanisms through which inter-brain dynamics both respond to and shape socio-emotional reactions.

Mice detect others' stress, but stressed peers break reciprocity

To simultaneously record from three interacting brains with cell-type specific resolution while performing an emotional state discrimination^{20, 21, 43, 44}, we used three miniscopes implanted in the ACC of one observer and two demonstrator mice (Fig. 1a).

To assess how inter-brain dynamics and transfer of socio-emotional information evolves throughout time, we extended the previously-used 6-min test^{20, 21} to 15 minutes. As reported before^{20, 21, 43, 44}, observer mice displayed increased sniffing towards restraint stress compared to neutral demonstrators during the first two minutes (Fig. 1b). A clear discriminatory behavior of observers' sniffing was also evident in the middle of the task (i.e. 5-10 min), but not in the last two minutes (Fig. 1b). Similar results for these three time periods were evident when measuring the time the observers spent in each demonstrator zone (Supplementary Fig. 1). To distinguish reciprocated from non-reciprocated interactions, we classified observer sniffing events based on whether the demonstrators simultaneously oriented their nose toward the observer (reciprocal sniffing) or away from it (non-reciprocated). Reciprocal sniffing was relatively more frequent and of longer duration in observer–neutral pairings than in observer–stress pairings, compared to non-reciprocated sniffing (Fig. 1c,d). However, neither the total duration nor the number of reciprocal sniffing events differed between pairing types (Supplementary Fig. 1b,c). No major signs of transfer of stress or buffering were evident, as the stress demonstrator self-groomed more and climbed less throughout the task compared to the observer and neutral mice which showed the same low levels of self-grooming and climbing (Supplementary Fig. 1d,g). Moreover, the observer exhibited more locomotor activity and rearing behaviors throughout the task compared to the demonstrators, regardless of whether it was in the stress or neutral mouse zones (Supplementary

Fig. 1e,h). Overall, observers engage more with stress demonstrators, stress diminishes demonstrators' reciprocal sniffing, and social buffering effects are not evident.

Stress disrupts inter-brain correlation of ACC pyramidal neurons

Pyramidal (PYR) excitatory neurons are the most abundant cells of the cortex^{45, 46}. To assess inter-brain correlations in socio-emotional contexts, we thus first imaged ACC PYR neurons activity simultaneously across the three interacting mice (Fig. 1e,f, Supplementary Fig. 2).

Based on our behavioral findings, and to test how inter-brain dynamics vary over time, we divided the analysis into three epochs: the first two minutes (capturing initial discrimination and enabling a direct comparison with previous single-brain studies)^{20,21,43,44}, the middle 5-10 minutes (when discrimination persists, Fig. 1b), and the last two minutes (when observer's preference is extinguished, but mice have been extensively interacting, Fig. 1b-d and Supplementary Fig. 1). We analyzed a total of 2898 PYR neurons from 6 triads of mice. We first computed the Pearson correlation between the mean PYR activity of each observer and that of the neutral or stress demonstrator upon entry into their respective zones (Fig. 1f,g). In the last 2 minutes of the task, ACC PYR neurons displayed increased correlated activity when interacting with the neutral demonstrator compared to when interacting with a stress demonstrator (Fig. 1f,g). Moreover, 5 out of 6 observers had a negative correlation with the stress demonstrator (Fig. 1f,g). These differences were not evident when mice were alone during the habituation, when the observer was in the opposite demonstrator zone, or when considering random intervals during the task (Fig. 1f,g). To assess the temporal relationship of these inter-brain dynamics, we then measured the cross-correlation of the mean ACC PYR activity between brains (Fig. 1f,h). This revealed in the last 2 minutes of the task a correlation around zero lag extending over a range of ± 1 sec which was

positive when aligned with the neutral demonstrator, and negative with the stress demonstrator (Fig. 1f,h). No differences were evident during habituation or when exploring the opposite demonstrator (Fig. 1f,h). These results indicate that inter-brain correlations of ACC PYR neurons are evident only during proximity between two neutral mice while they are disrupted if one of the two is in a stress state.

We next examined whether inter-brain correlations were shaped by finer interaction dynamics, distinguishing between reciprocal and non-reciprocated sniffing. Because of the limited number and duration of such events, we could not analyze separate interaction epochs and we used data from the entire length of the task. Again, ACC PYR neurons showed greater correlated activity between the observer and the neutral than the observer-stress demonstrator, but only during reciprocal sniffing (Supplementary Fig. 2b-d). These differences do not arise merely from coordinated movements, as inter-brain ACC PYR correlations were not detected during grooming or rearing periods examined independently of social interaction (Supplementary Fig. 2e,f). We then tested if correlation could be detected with single-cell resolution (Fig. 1i). We computed for each neuron in the observer brain its overall time-lagged inter-brain coupling with all other individual neurons in the demonstrators' brain⁴⁷. Consistent with the above analyses of correlations of aggregate activity, observer-neutral PYR neurons had stronger zero-time lag inter-brain coupling than observer-stress activity, but only during close interaction (Fig. 1j). The observer-neutral PYR inter-brain coupling was also higher during reciprocal sniffing events compared to non-reciprocated observer-only sniffing (Fig. 1j). A low but significant coupling in ACC PYR activity was also present between the two demonstrators during reciprocal sniffing with the observer, but not during non-reciprocated sniffing (Fig. 1j). This suggests that some ACC PYR inter-brain coupling may be driven by similar sniffing movement behavior. No inter-brain coupling

was observed when the observer was in the empty zone of the apparatus, when the signal was phase randomized, or when computing correlation between the observer and stress or neutral demonstrators in other cages (Fig. 1j). An index of coupling directionality (positive when demonstrator activity drives observer activity, negative for the reverse, and zero for co-activation) revealed no preferred direction in PYR inter-brain dynamics (Supplementary Fig. 3b), indicating inter-brain coactivation. Finally, the distribution of inter-brain coupling strength of ACC PYR neurons had a positive excess Kurtosis (Supplementary Fig. 3c), indicating that it has a higher proportion of large inter-brain coupling than expected with a Gaussian distribution.

These findings indicate that ACC PYR neurons are inter-brain correlated between neutral subjects, especially during reciprocal sniffing, and that the presence of stress in one subject disrupts these correlations.

Distinct patterns of intra- and inter-brain ACC PYR correlations

To assess whether inter-brain coupling reflects intra-brain dynamics or is differentially modulated across behavioral contexts, we quantified intra-brain correlations during interaction epochs, separating reciprocal from nonreciprocal sniffing. Observer's intra-brain PYR correlations were comparable during reciprocal interactions with either the stress or the neutral demonstrator (Supplementary Fig. 3f). In contrast, higher intra-brain correlation was evident during unidirectional exploration of the neutral compared to the stress demonstrator (Supplementary Fig. 3g), consistent with previous observations²¹. Within the demonstrators, intra-brain dynamics had a similar pattern between reciprocal and non-reciprocated sniffing events (Supplementary Fig. 3f,g). These data indicate a distinct pattern of intra- compared to inter-brain coupling.

Stress disrupts inter-brain correlations in humans

To examine whether inter-brain correlation disruption by stress extends beyond mice, we collected EEG data from humans in which one participant was in a stress and another in a neutral state, or both in a neutral state (Fig. 1k). Stress and neutral states were induced using validated audio-visual stimuli (Supplementary Fig. 4a-c)⁴⁸. Immediately following emotional state alteration, participants engaged in a multimodal social interaction in which they looked at each other while holding their partner's right hand (Fig. 1k), without any verbal or gestural communication⁴⁹. This procedure was designed to parallel the multimodal mice interactions, which involves both visual and physical contact.

We first identified EEG electrodes carrying signals that correlated between participants, irrespective of their emotional state. We computed Pearson's correlation coefficients between electrode- and sub-band-specific EEG power time courses (i.e., envelopes) recorded from the two individuals in each dyad, in line with previous work in human and non-human species^{27, 49, 50}. We revealed that gamma-band envelopes (both in the whole 31-95Hz gamma band range, and in finer sub-bands), specifically recorded from frontal scalp electrodes, exhibited higher inter-brain correlations during social interaction compared to a condition in which participants could not see or touch each other (Fig. 1l). Next, we assessed the effect of emotional manipulation on both participants' self-reported questionnaires as well as on their inter-brain correlation, focusing on the previously identified spectral and spatial region of interest. Behavioral (self-report) data indicated that participants who watched the neutral stimuli reported feeling less negative emotions from pre- to post-experiment, while those who viewed the negative stimuli did not (Supplementary Fig. 4e). Similarly, neural data revealed that dyads including a stress participant exhibited reduced inter-brain correlation compared to dyads composed of two participants in neutral states (Fig. 1m and

Supplementary Fig. 4d). Notably, greater decreases in inter-brain correlation were associated with larger changes in participants' stress emotional state (Fig. 1n). Like in mice, these results suggest that negative emotions disrupt inter-brain correlation typically observed between individuals in a neutral state. Moreover, inter-brain correlation appears to be driven by the envelopes of EEG gamma oscillations.

Stress rewires inter-brain correlations to ACC SOM neurons

EEG primarily captures dendrosomatic dipoles of PYR activity⁵¹. However, its gamma band, preferentially modulated by inter-brain emotional dynamics in our findings, is regulated by SOM neurons⁵², which play a central role in single-brain emotion discrimination^{21, 43, 44}. Because EEG represents an aggregate population signal, it cannot resolve the contributions of neuronal subtypes. Thus, we imaged ACC SOM neurons by *in vivo* microendoscopic recordings of calcium activity in all three mice performing our emotional state discrimination task (Fig. 2a-d).

We analyzed a total of 968 SOM neurons from 9 triads of mice. Lens placement and GCaMP6f expression were confirmed histologically (Fig. 2a and Supplementary Fig. 2a). In contrast to PYR cells, ACC SOM average activity displayed high correlation between the observer and the stress mouse, only during the first 2 minutes of the task, when entering in the respective demonstrator zone (Fig. 2d,e). Cross-correlation between the average activity of the SOM neurons across brains showed a peak around zero lag with the stress demonstrator in the first two minutes (Fig. 2d-f). Moreover, 7 out of 9 observers showed strongly reduced correlation when interacting with a neutral demonstrator compared to the stress (Fig. 2d,f). No inter-brain correlations were evident during habituation, when mice were individually prepared, when considering the observer and the non-explored opposite demonstrator, or when random intervals were chosen (Fig. 2d,e,f).

These results indicate that emotional alteration in one interacting subject enhances ACC SOM inter-brain correlation.

As for PYR, we next examined whether SOM inter-brain correlations were shaped by finer-grained interaction dynamics, distinguishing between reciprocal and non-reciprocated sniffing. Again, ACC SOM neurons showed greater correlated activity between the observer and the stress than observer-neutral demonstrator, but only during reciprocal sniffing (Supplementary Fig. 2g-i). These differences were independent of coordinated movements, as inter-brain ACC SOM correlations were absent during paired grooming or rearing (Supplementary Fig. 2j,k). We then tested if these effects could be visible in inter-brain coupling between pairs of neurons measured with single-cell resolution (Fig. 2c,g,h). These analyses confirmed a correlation between the observer and the stress demonstrator during reciprocal, but not in non-reciprocated sniffing (Fig. 2g,h). This coupling was time-lag asymmetric relative to observer-neutral correlations (Fig. 2g), and showed a negative directionality index only during reciprocal sniffing with the stressed demonstrator (Fig. 2g,h). Thus, observer activity preceded that of the stressed demonstrator. The distribution of inter-brain coupling strength of ACC SOM neurons had a positive excess Kurtosis (Supplementary Fig. 5a), and thus a higher proportion of large inter-brain coupling than expected with a Gaussian distribution. No inter-brain coupling was evident when traces were phase-randomized, when the observer was in the zone without demonstrators, when the observer was interacting with the opposite demonstrator, between the two demonstrators, and between the observer with demonstrators in other cages (Fig. 2g,h). Inter-brain coupling with single-cell resolution revealed that the highest values were evident for SOM when the observer and the stress demonstrator were reciprocally interacting compared to equivalent PYR correlations, or SOM correlations between observer and neutral demonstrator, or between the brains of the two

demonstrators (Fig. 2i). In non-reciprocated events, the pattern was different with SOM showing always a higher correlation compared to PYR, but this time with SOM coupling between observer-neutral being higher than observer-stress (Fig. 2j).

Collectively, these results reveal that ACC SOM inter-brain coupling tracks reciprocal socio-emotional interactions and exhibit directional dynamics in which the observer's neural activity precedes that of the demonstrator.

Distinct patterns of intra- and inter-brain ACC SOM correlations

We next tested ACC SOM intra-brain activity in the observer and two demonstrators computing single-cell-resolution coupling during interaction epochs, either reciprocal or non-reciprocal.

In contrast to the inter-brain correlations (Fig. 2), analysis of intra-brain ACC SOM coupling in the observer revealed a peak at zero lag, with no differences between periods of interaction with stress versus neutral demonstrators (Supplementary Fig. 5d,e). Similarly, ACC SOM intra-brain coupling was higher in the stress demonstrator when interacting with the observer compared to periods without interaction, but this was not evident during non-reciprocal sniffing or in the neutral demonstrator (Supplementary Fig. 5d,e).

This data further indicates a distinct pattern of intra- compared to inter-brain correlations.

SOM encoding exceeds PYR in inter-brain emotional coupling

We finally quantified how the activity of populations of neurons intra- and inter-brain might encode and exchange emotional state information on a moment-by-moment basis.

We first computed the Shannon information⁵³ that a single time-frame of activity of an individual PYR or SOM neuron in the observer's brain carries about the emotional state of the demonstrator during reciprocal sniffing. SOM neurons were more informative than PYR (Fig. 2k). Information encoded by individual observer SOM neurons about the demonstrator's emotional state increased with the strength of inter-brain coupling to demonstrator SOM neurons, whereas PYR neurons showed no such dependence (Fig. 2l). This suggests that the emotional-state information carried by SOM neurons has a larger effect on inter-brain coupling during reciprocal behavior than the information carried by PYR neurons. Thus, SOM activity may be more directly involved in mediating emotion-dependent inter-brain correlations.

To test whether inter-brain coupling strength supported emotion discrimination at individual time points and during reciprocal interactions, we analyzed neuronal triplets within a single brain or spanning the three brains (i.e. the observer and both demonstrators). The majority of the total emotional information was carried by inter-brain coupling, which contributed more than the firing rate of the individual cells did (Fig. 2m). The information carried by SOM was then larger compared to PYR neurons (Fig. 2m).

Overall, these results further highlight the importance of SOM inter-brain dynamics in determining emotional state information encoding.

Preventing SOM inter-brain correlation abolishes emotion discrimination

Whether inter-brain dynamics arise as a consequence of social interactions or can actively modulate social choices remains an open question^{34, 35}. We therefore designed a set of cell-type-specific, emotionally contingent closed-loop optogenetic manipulations, including multi-brain photoinhibition or photostimulation paradigms that selectively disrupted, enhanced, or inverted

inter-brain correlations, as well as activity-driven photostimulation of one brain by another brain, all combined with matched control single-brain manipulations to establish which effects were genuinely due to concurrent multi-brains manipulations.

We first disrupted the naturally occurring ACC SOM inter-brain correlation between the observer and the stress demonstrator (Fig. 2) by optogenetically silencing ACC SOM neurons in the stress demonstrator whenever the observer approached (Fig. 3a,b). The neutral demonstrator received identical light delivery, but without any effect on neuronal activity (Fig. 3a,b). This manipulation abolished the observer's preference towards the stress demonstrator which was otherwise present in control experiments (Fig 3c,d, Supplementary Fig. 6a). The optoinhibition did not alter the total duration of social exploration (Supplementary Fig. 6b). This effect was equally present in male and female mice (Supplementary Fig. 6c,d). To control whether the altered activity in the targeted brain by itself might induce any change in social valence and/or visible discriminatory effect from the observer, we next inhibited ACC SOM neurons in only one of two equally neutral demonstrators. In this condition, the observer showed no preference or avoidance towards the demonstrator undergoing the opto-manipulation (Supplementary Fig. 6e). However, if two equally stress demonstrators were presented, a preference for the non-inhibited mouse was observed (Supplementary Fig. 6f). This effect is consistent with a disruption of correlation in the non-preferred stress demonstrator, while the other should retain the expected correlation with the observer (as from Fig. 2). Notably, optogenetic SOM inhibition did not alter corticosterone concentrations or demonstrator behavior (Supplementary Fig. 6g,h), ruling out that the observer choice was affected by gross changes in the opto-inhibited demonstrator. Thus, these results indicate that blocking ACC SOM inter-brain correlation between the observer and the stress demonstrator abolished observer's emotion discrimination.

We next used this design to test if the observed ACC PYR inter-brain correlation between the observer and the neutral demonstrator (Fig. 1) is implicated in emotion discrimination. Optogenetically silencing ACC PYR in the neutral demonstrator upon each visit from the observer did not alter emotion discrimination (Fig. 3g,h). Similarly, ACC PYR optoinhibition had no effect on observer's total duration of social exploration, nor induced any preference if two equally neutral or stress demonstrators were presented (Supplementary Fig. 7e,f). These results suggest that inter-brain correlation of ACC PYR neurons between the observer and the neutral demonstrator might be less important for emotion discrimination.

Inducing inter-brain correlation in ACC SOM neurons drives emotional preference

To further probe the possible causal implication of positive inter-brain correlations in shaping socio-emotional behaviors, we next artificially enhanced correlation in emotional or non-emotional contexts by using opto-stimulation to co-activate different brains.

ACC SOM of observer mice were equally stimulated when exploring two emotionally equal demonstrators (both neutral or stress), but the ACC SOM of only one demonstrator were equally optostimulated upon receiving the observer's visit (Fig. 4a, Supplementary Fig. 8a). This manipulation did not induce discrimination towards two neutral demonstrators (Fig. 4b,d), similarly to no-stimulation conditions (Supplementary Fig. 8b). In contrast, the same manipulation induced a preference for the correlated stress demonstrator over the non-correlated one during the first two minutes (Fig. 4c,d). This early preference resembles the mice's spontaneous preference for stress over neutral demonstrators (Fig 1,2 and^{21, 43, 44}). However, a preference for the non-correlated stress demonstrator emerged during the middle of the task (Fig 4c,d). This latter effect may reflect a non-physiological artifact resulting from prolonged correlation during a period when

such inter-brain correlation is not observed (Fig. 2). Indeed, the SOM opto-stimulation did not alter the stress demonstrator behavior in the first two minutes, but did so in the middle of the task (Supplementary Fig. 8i). The opto-stimulation did not alter motor behaviors in the observer mice (Supplementary Fig. 8c-e). The induced discriminatory behavior in the observer mouse was equally evident in males and females (Supplementary Fig. 8f,g). To exclude the possibility that discriminatory behavior arose solely from manipulating the demonstrator's brain, we identically stimulated only one of the two stress demonstrators, but this time without stimulating the observer. This manipulation did not induce any discrimination (Supplementary Fig. 8h). These results strengthen the conclusion that ACC SOM inter-brain correlation plays a key role in driving observer's social preference only in emotionally stress contexts.

Inducing inter-brain correlation in ACC PYR reduces preference in neutral conditions

We then applied the same optogenetic approach to test whether inter-brain correlations between ACC PYR neurons have causal relevance.

Simultaneous stimulation of ACC PYR neurons in both the observer and one of two neutral demonstrators during social interactions reduced preference (Fig. 4e,g, Supplementary Fig. 9a), in both males and females (Supplementary Fig. 9c,d). This effect is consistent with our finding of increased ACC PYR inter-brain correlation between the observer and the less preferred neutral demonstrator (Fig. 1). This same optostimulation procedure, but with two equally stress demonstrators, had no effect (Fig. 4f,g). The PYR opto-stimulation manipulation did not alter the motor behaviors of the observer and demonstrator mice (Supplementary Fig. 8c-e, Supplementary Fig. 9b,f). To test whether discriminatory behavior could be driven solely by optogenetic activation of the demonstrator, we stimulated only one of the two neutral demonstrators while leaving the

observer unstimulated. This manipulation did not elicit discriminatory behavior (Supplementary Fig. 9e). These results indicate that heightened ACC PYR inter-brain correlation plays a role in diminishing social preference for a neutral demonstrator.

Inter-brain ACC SOM anticorrelation drives aversion in neutral conditions

Our imaging experiments suggest that lack of positive correlation arises from moments in which one subject had some neuronal activity while the other did not (Supplementary Fig. 10). We therefore explored how shifting the activity of one brain in one direction and the other in the opposite direction affects socio-emotional outcomes.

We first activated ACC SOM neurons in observer mice every time they entered the zone of two equally neutral or stress demonstrators (Fig. 4h). Simultaneously, only one of the two demonstrators received ACC SOM inhibition upon the observer's entry into their zone, whereas the other demonstrator received light without inhibition (Fig. 4h). Reduced observer sniffing was directed towards the neutral anticorrelated compared to the neutral control demonstrator (Fig. 4i,k, Supplementary Fig. 11a). This was due to an avoidance towards the neutral anticorrelated as we saw reduced timing of sniffing, with same number of entries in the zone (Supplementary Fig. 11a,b). No confounding effects of observers' locomotion were evident (Supplementary Fig. 8c-e). No behavioral differences were observed between neutral demonstrators with or without ACC SOM inhibition (Supplementary Fig. 11g). In contrast, no effects were evident with two equal stress demonstrators (Fig. 4j,k). Reversing the stimulation/inhibition protocol, so that ACC SOM neurons were inhibited in the observer when entering zones containing two neutral or two stressed demonstrators, and activating them in only one of the two demonstrators, did not induce any discriminatory behavior (Supplementary Fig. 11c-f).

These results indicate that ACC SOM inter-brain anticorrelation is meaningful in reducing preference, but only in an emotionally-neutral situation, with the observer brain driving it.

Inducing inter-brain anticorrelation in ACC PYR neurons induces social preference

We next applied the same optogenetic approach to test whether ACC PYR anticorrelation patterns (Supplementary Fig. 10) contribute to socio-emotional responses.

Activating ACC PYR neurons in observer mice each time they entered the zone of two equally neutral or stress demonstrators, while simultaneously inhibiting the ACC PYR neurons in only one of the two demonstrators upon the observer's visit (Fig. 4h), induced a preference for the anticorrelated demonstrator in both neutral and stress conditions (Fig. 4l-n, Supplementary Fig. 12a,b). No alterations in locomotor behaviors were detected (Supplementary Fig. 12c,d). Moreover, ACC PYR inhibition did not affect demonstrator behavior (Supplementary Fig. 12i,j). These results are in line with the imaging data (Fig. 1), indicating that ACC PYR inter-brain activity is anticorrelated with the most preferred mouse, even though the timing of the induced social preference differed depending on the emotional context. In contrast, inhibiting ACC PYR in observer mice upon entering the zone of two equally neutral or stress demonstrators, while simultaneously activating ACC PYR in only one demonstrator had no effects (Supplementary Fig. 12e-h).

These data indicate that ACC PYR inter-brain anticorrelation is meaningful to induce social preference independently of emotional contexts, and again driven by the observer.

Real-time close-loop induction of inter-brain correlation in ACC SOM drives emotional preference

Our previous inter-brain optogenetic manipulations relied on sustained, behaviorally triggered stimulation, which may not capture the natural dynamical structure of physiological neural activity. Thus, we set up a paradigm in which the recorded neuronal activity of one mouse (either the observer or one demonstrator) optogenetically drove brain activity in another mouse, in stress or neutral conditions (Fig. 5a, Supplementary Fig. 13a). Here, the induced correlated brain activity occurred independently of mice's position, thereby disentangling inter-brain dynamics from movement- or position-related confounders.

When the observer mouse's ACC SOM activity drove optogenetic stimulation of ACC SOM in one of two equally neutral demonstrators (Fig. 5b,d), no effects were evident. In contrast, when the observer faced two equally stress demonstrators, a preference for the correlated demonstrator emerged during the first two minutes (Fig. 5c,d, Supplementary Fig. 13b). This mirrored the results obtained with the imaging experiments (Fig. 2e,f,g). The optostimulation did not affect reciprocal sniffing, nor other motor behaviors of the three interacting mice (Supplementary Fig. 13c-g). When the roles were inverted (i.e. activity recorded from the demonstrator drove stimulation in the observer), no effects were evident (Fig. 5e-g, Supplementary Fig. 13h). These latter findings are consistent with the indications from single-neuron pairwise analyses (Fig. 2g). Using the same approach to drive PYR inter-brain correlation produced no effects in either the observer-to-demonstrator and demonstrator-to-observer configuration, and in both neutral-neutral and stress-stress conditions (Fig. 5h-m, Supplementary Fig. 13i,j).

These results provide first evidence that physiological ACC SOM inter-brain correlations initiated by the observer mouse drive emotion discrimination towards a stress conspecific.

Discussion

We discovered that emotions modulate inter-brain dynamics in a neuron-type-specific manner. Inter-brain ACC PYR correlations arise when two subjects are in an emotionally neutral state and are disrupted if one interacting subject is in a stress state. Conversely, ACC SOM inter-brain correlations appear when one partner is in a stress state. Importantly, we established a causal link between inter-brain dynamics and socio-emotional choices, identifying SOM neurons as key drivers of socio-emotional inter-brain connectivity.

Altered emotional state in one interacting subject changed inter-brain dynamics. This extends previous inter-brain findings in humans, monkeys, bats and mice that were conducted in neutral emotional states^{25, 27, 30, 31, 54, 55}, by adding an emotional component. Our initial comparative study with humans suggests that the same organizing principles of emotion-dependent inter-brain dynamics operate across species, pointing to conserved neural mechanisms. Specifically, inter-brain cortical correlations between two subjects in neutral states, evident in EEG signal in humans and PYR activity in mice, are weakened if one partner is in a stress state. These findings mirror prior human reports of enhanced inter-brain correlation during positive emotional interactions⁵⁶⁻⁵⁸, or when two subjects share a similar emotional state through emotional contagion^{36, 56}. Collectively, our findings position emotional state as a key regulator of inter-brain coupling, pointing to conserved, emotion-sensitive mechanisms that dynamically shape neural coordination across individuals.

We demonstrated that distinct neural substrates support inter-brain coupling depending on the socio-emotional context. Stress initially tunes interacting brains dynamics by correlating ACC SOM activity across individuals. These results expand on prior single-brain findings on cortical SOM neurons' roles in emotion discrimination^{21, 43}, and acute stress responses⁵⁹ to contexts

entailing multiple interacting individuals. Remarkably, analyzing multiple brains simultaneously revealed that triplets of neurons across interacting brains encode emotion-dependent signals at levels that surpass what reported within individual brains. By contrast, correlations among pairs or triplets of neurons within a single brain usually capture only a small fraction of the total information about sensory or cognitive variables⁶⁰. This highlights the unique properties of emotional states coding in inter-brain communication. We also demonstrated the existence of multiple types of inter-brain dynamics during socio-emotional choices, having different functional significances. This aligns with previously theorized importance of alternating correlated and anticorrelated brain and behavioral patterns in supporting social goals^{61, 62}. Notably, our comparative analysis of SOM and PYR neurons further emphasizes differences between intra- and inter-brain circuit dynamics. Whereas elevated cortical SOM activity in single-brain studies is linked to enhanced local inhibition of PYR neurons^{21, 43, 59}, inter-brain correlations in ACC PYR activity displayed emotional and temporal dynamics distinct from ACC SOM, being strongest during neutral interactions and developing progressively over time. Intriguingly, GABAergic neurons in the prelimbic PFC have been linked to shared-space inter-brain dynamics during neutral one-to-one interactions⁶³. Because this study did not distinguish among GABAergic subtypes, and given our observation of minimal SOM involvement in emotionally neutral interactions, together with prior single-brain evidence implicating PFC PV neurons engagement in neutral social interactions^{21, 64}, our results raises the possibility that PV neurons may drive inter-brain correlations in non-emotional social contexts. Thus, our findings reveal that inter-brain correlated activity arises from distinct, molecularly defined neural substrates, shaped by the emotional states of the interacting individuals.

Our data indicate that inter-brain dynamics can actively modulate social decisions in emotional contexts. This finding represents an initial step toward resolving, at fine-grained level, whether inter-brain correlations are simply byproducts of social interaction or play a causal role as modulators^{33-35, 65}. We first demonstrated that preventing inter-brain correlation by silencing SOM neurons, but not PYR, in the stress demonstrator impaired emotion discrimination. In line with our imaging observations, optogenetic manipulations that enhanced inter-brain correlations revealed that the coupling dynamics of SOM and PYR populations are selectively involved and shape socio-emotional responses based on the emotional relationship between interacting individuals. Similarly, ACC PYR anticorrelation drove social approach toward stressed demonstrators, while ACC SOM anticorrelation induced avoidance of neutral demonstrators. By directly probing inter-brain perturbations across diverse socio-emotional contexts, with real-time control over a non-correlated mouse, our study goes beyond earlier approaches that inferred inter-brain coupling from indirect manipulations during neutral social interactions⁶⁶. By introducing an ethologically inspired closed-loop framework, where neural activity in one mouse dynamically shaped corresponding activity in another, we more directly manipulated inter-brain coupling. This approach revealed a directional influence of observer ACC SOM activity on the stressed demonstrator, driving approach behavior toward the emotionally altered partner. This setting avoids artifacts of artificial stimulation and strengthens our conclusions that different neural substrates drive social choices in different socio-emotional contexts.

In summary, we provide evidence of emotional inter-brain dynamics during social interactions. Notably, cell-specific correlated and anticorrelated inter-brain dynamics are both causative in determining social choices towards others' emotions. This constitutes a new vision in evolutionary conserved socio-emotional responses, with fundamental impact on animals' lives.

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Figures and Figure Legends

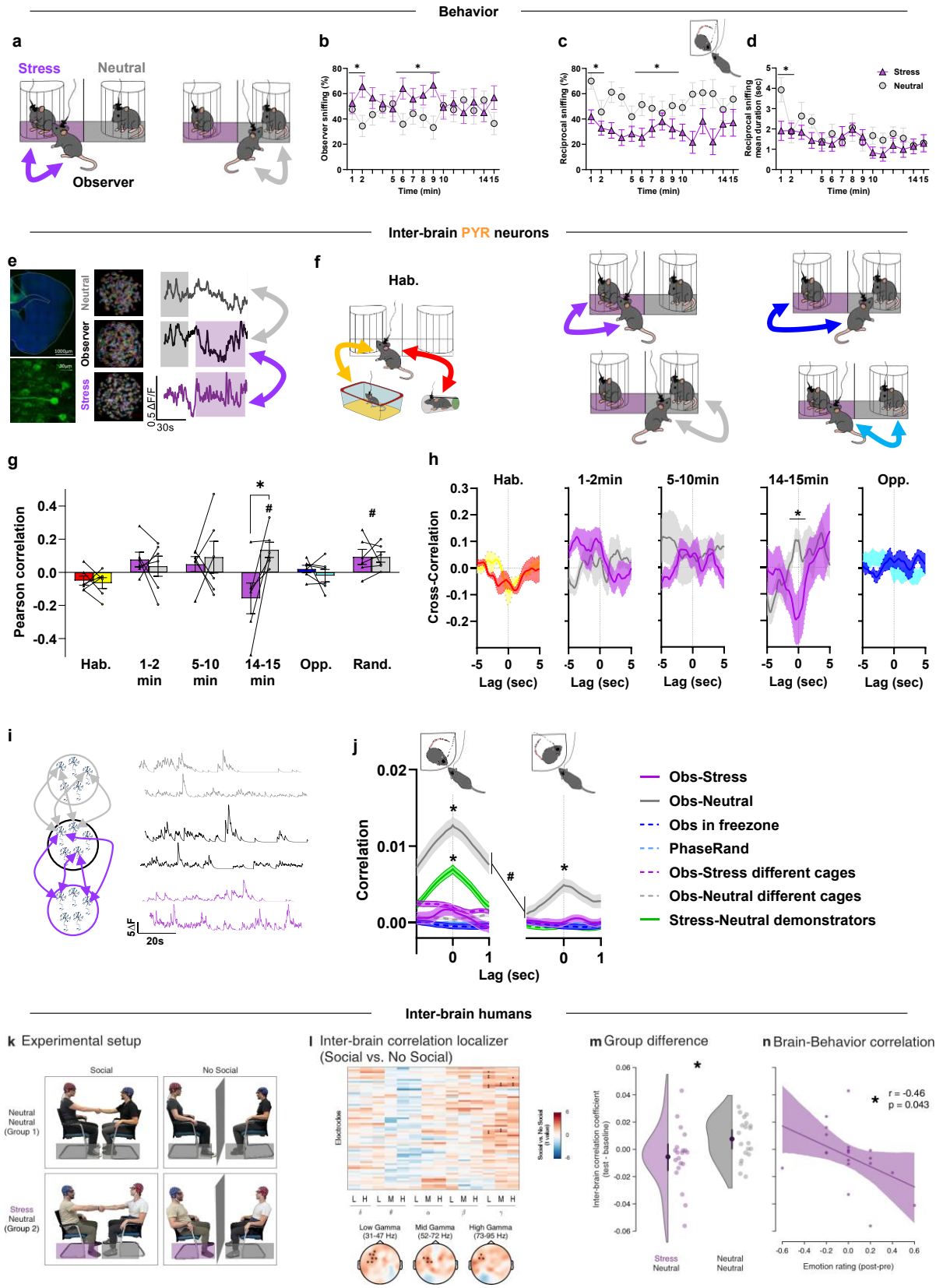


Figure 1

Figure 1. Negative emotional state in one subject disrupts inter-brain correlations of ACC PYR neurons. (a) Mice experimental design in which one observer and two demonstrators are concurrently recorded through microendoscopic calcium imaging during the emotional state discrimination task (EDT). (b) Observers' social investigation expressed as percentage of sniffing toward the stress (purple) or the neutral (grey) demonstrator (nStress=15, nNeutral=15, two-way repeated measures ANOVA; interaction effect, $F_{14,392}=2.53$, $P=0.0018$, with Bonferroni post hoc test). (c) Reciprocal sniffing between the observer and the stress or neutral demonstrators expressed as percentage of observer's sniffing (two-way repeated measures ANOVA; demonstrator effect, $F_{1,4}=18.19$, $P=0.013$, with Bonferroni post hoc test). (d) Mean duration (in seconds) of reciprocal sniffing events between the observer and the two demonstrators (two-way repeated measures ANOVA; demonstrator effect, $F_{1,28}=8.58$, $P=0.0067$, with Bonferroni post hoc test). (e) Left: representative GCaMP expression and lens placement in the ACC (top), single PYR neuron (bottom). Center: example ROIs for single cell calcium imaging recordings from the 3 mice. Right: example average traces from the 3 interacting mice. (f) Inter-brain correlations were computed during the different phases of the task. Hab: habituation phase in which the observer was alone in the testing cage while the stress demonstrator was under restraint in a falcon tube and the neutral demonstrator was in its home cage. Test – zone visit: considering when the observer visits one of the two demonstrators. Test – opposite zone: periods during which the observer was in the zone of one demonstrator but considering the brain activity of the opposite non-visited demonstrator. (g) Pearson correlation between PYR average activity in the observer and in each demonstrator during the different phases of the test. Rand.: inter-brain correlations between the observer and each demonstrator irrespectively of observer position in the box. (nObserver=6, nStress=6, nNeutral=6, two-way repeated measures ANOVA; interaction effect, $F_{5,30}=3.07$, $P=0.023$, with Bonferroni post hoc test; T-Test of means against 0 value, $t_5=3-4$, $P<0.05$). (h) Cross-correlation between PYR average activity in the observer and each demonstrator during the different phases of the task (two-way repeated measures ANOVA; interaction effect, $F_{19,190}=1.78$, $P=0.027$, with Bonferroni post hoc test). (i) Schematic of the pairwise correlation analyses between the observer, neutral and stress demonstrators' brains with representative traces from PYR single neurons from each of the 3 interacting mice. (j) Time-lagged coupling between individual PYR neurons of the observer and the two demonstrators during reciprocal (left) or non-reciprocated (right) sniffing intervals and control conditions (n=2898 neurons; two-way repeated measures ANOVAs; interaction effect for reciprocal sniffing, $F_{240,313840}=12.84$, $P<0.0001$, with Bonferroni post hoc test; interaction effect for non-reciprocal sniffing, $F_{160,282960}=4.02$, $P<0.0001$, with Bonferroni post hoc test; interaction effect for reciprocal versus non-reciprocal sniffing, $F_{40,67760}=1.54$, $P<0.02$, with Bonferroni post hoc test). Coupling was computed with correlations partialized with respect to fluctuations of mean activity of both cells across interaction windows to discount the effect of rate covariations across windows and reveal only within-interaction-window interactions between the neurons. (k) We recorded EEG from 40 human dyads (80 participants) during a social interaction task in which participants faced each other and held their partner's right hand, and a no social-contact control condition in which an occluder prevented visual and haptic interaction. Each dyad completed both conditions following the viewing of neutral audio-visual stimuli (baseline phase), as well as after exposure to either stress-inducing or additional neutral audio-visual stimuli (test phase), depending on group assignment (Stress–

Neutral or Neutral–Neutral). **(l)** Inter-brain correlation was computed between homologous electrodes across frequency sub-bands, based on power envelope time courses. A data-driven localizer analysis identified a cluster of central and frontal electrodes within the low, mid, and high gamma bands that showed significantly higher inter-brain correlation during social compared to no social conditions. (cluster-corrected $P=0.025$; $N=1000$ permutations). **(m)** We compared inter-brain correlation between dyads composed of one stressed and one neutral participant versus dyads composed of two neutral participants, focusing specifically on the electrode–frequency cluster identified in **(l)**. Correlation coefficients were baseline-corrected by subtracting values obtained in the baseline social interaction block. This analysis revealed that dyads including a stressed individual exhibited significantly reduced inter-brain correlation [$t(38)=2.21$, $P=0.03$, Cohen’s $d=0.7$]. **(n)** Reductions in inter-brain correlation were associated with the intensity of stress-induced negative emotion, as quantified by self-report questionnaires administered before and after the experiment (Spearman’s rank correlation $\rho=-0.46$, $P=0.043$). This suggests that the greater the emotional impact of stress induction, the stronger the reduction in inter-brain correlation within the Stress–Neutral group.

* $P<0.05$ Stress vs Neutral; # $P<0.05$ vs 0 value; with Bonferroni post hoc test. Error bars represent mean $\pm$ SEM throughout.

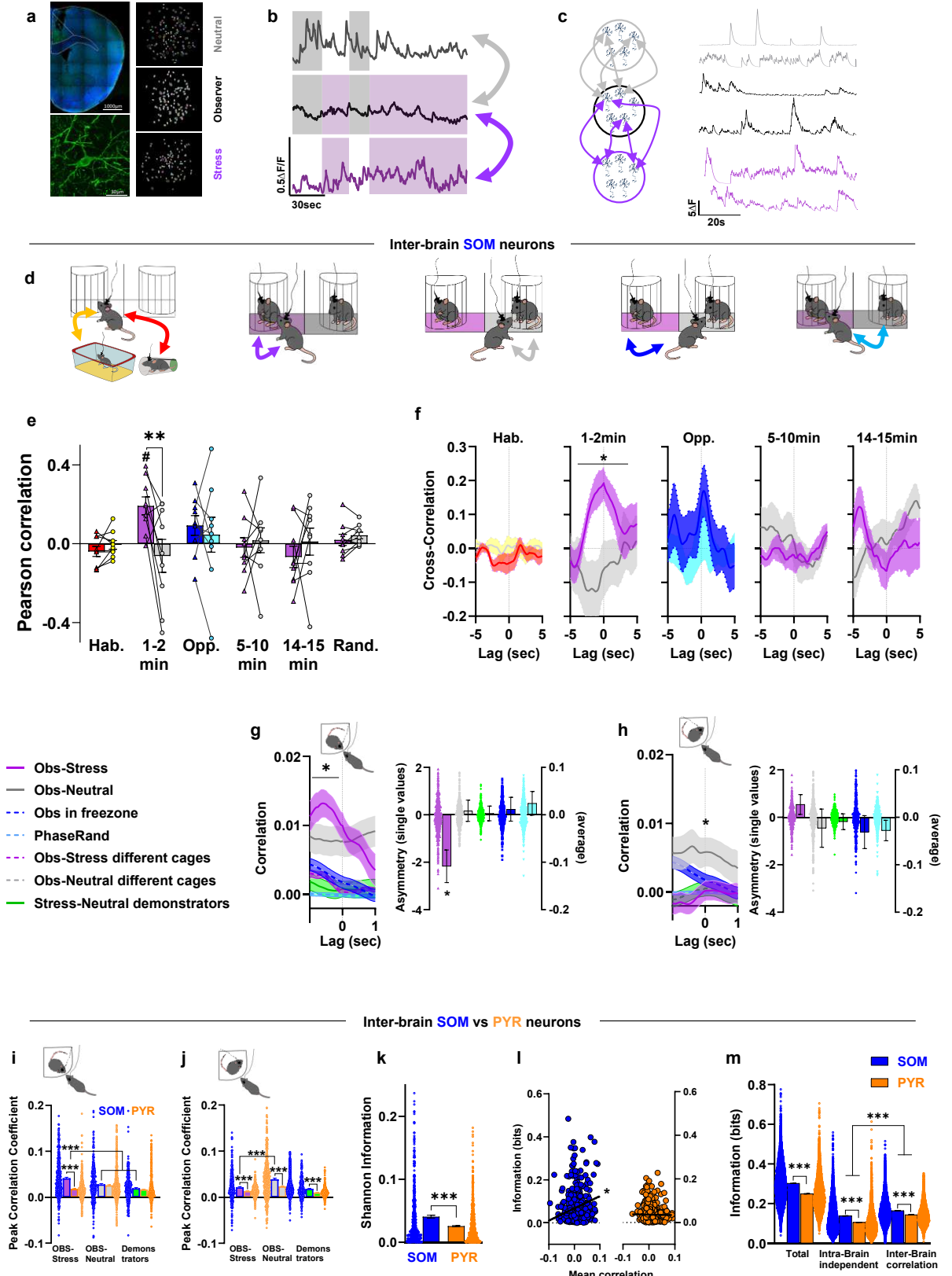
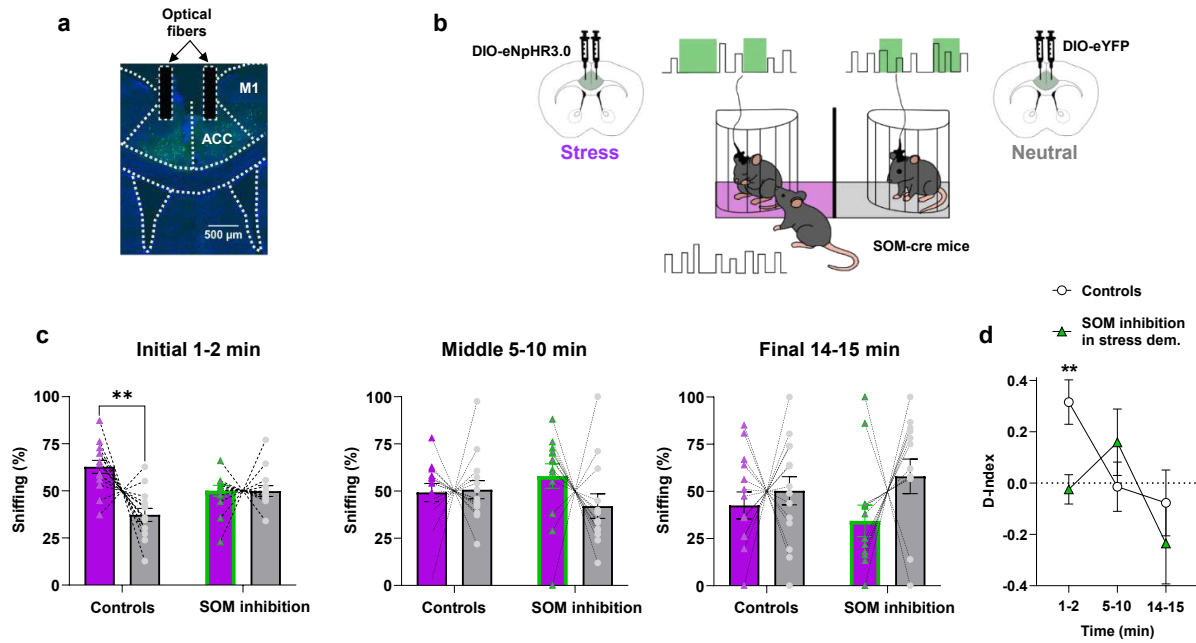


Figure 2

Figure 2. Negative emotional state in one subject reveals inter-brain correlations of ACC SOM neurons. (a) Left: representative GCaMP expression and lens placement in the ACC (top), single SOM neuron (bottom). Right: example ROIs for single SOM cell calcium imaging recordings from the 3 interacting mice. Example (b) average traces and (c) SOM single-neurons from the 3 interacting mice. (d) Inter-brain correlations were computed during the different phases of the task. Hab: habituation phase in which the observer was alone in the testing cage while the stress demonstrator was under restraint in a falcon tube and the neutral demonstrator was in its home cage. Test – zone visit: considering when the observer visits one of the two demonstrators. Test – opposite zone: periods during which the observer was in the zone of one demonstrator but considering the brain activity of the opposite non-visited demonstrator. (e) Pearson correlation between SOM average activity in the observer and in each demonstrator during the different phases of the test. Rand.: inter-brain correlations between the observer and each demonstrator irrespectively of observer position in the box. (nObserver=9, nStress=9, nNeutral=9, two-way repeated measures ANOVA; interaction effect, $F_{5,48}=3.02$, $P=0.019$, Bonferroni post hoc; T-Test of means against 0 value, $t_8=4.25$, $P=0.003$). (f) Cross-correlation between SOM average activity in the observer and each demonstrator during the different phases of the task (two-way repeated measures ANOVA; interaction effect, $F_{19,304}=2.49$, $P=0.0006$, with Bonferroni post hoc). (g,h) Time lagged coupling computed between individual SOM neurons of the observer and the two demonstrators during (g) reciprocal or (h) non-reciprocated sniffing intervals and control conditions (n=968 neurons; two-way repeated measures ANOVAs; interaction effect for reciprocal sniffing, $F_{240,159080}=6.57$, $P<0.0001$, Bonferroni post hoc test; interaction effect for non-reciprocal sniffing, $F_{160,126600}=3.32$, $P<0.0001$, Bonferroni post hoc test). (g,h) Directionality index with asymmetry measures between individual SOM neurons of the observer and the two demonstrators during (g) reciprocal or (h) non-reciprocated sniffing intervals and control conditions (one-way ANOVA; condition effect, $F_{4,2283}=4.59$, $P=0.0010$, Bonferroni post hoc). (i,j) Peak Pearson correlation coefficient for inter-brain correlations between SOM and PYR individual neurons during (i) reciprocal and (j) non-reciprocated sniffing between the observer and the demonstrators. Obs-stress: observer-stress interactions. Obs-neutral: observer-neutral interactions. Demonstrators: inter-brain correlation between the two demonstrators (two-way ANOVA; interaction effect, $F_{2,4100}=47.62$, $P<0.0001$, with Bonferroni post hoc). (k) Shannon information that observer's SOM (blue) and PYR (orange) neurons carry about the demonstrators' emotional state during reciprocal sniffing (one-way ANOVA; neuron type effect, $F_{1,1316}=48.12$, $P<0.0001$, Bonferroni post hoc). (l) Correlation between the mean inter-brain coupling (averaged across all time lags and the two conditions of interacting with stress or neutral demonstrators) of an observer's neuron and the Shannon information that this SOM (blue) and PYR (orange) observer's neuron carry about demonstrators' emotional state during reciprocal sniffing (for SOM, $r=0.21$, $P=0.0092$). (m) Information breakdown for triplets of SOM (blue) and PYR (orange) neurons of observer, stress and neutral demonstrators considering the sum of information containing both the contribution of individual cells within each brain and of correlations between the activity of cells in different brains (Total), the contribution of intra-brain individual cells (intra-brain independent), and the information that inter-brain correlations add because their strength is modulated by the emotional state (inter-brain correlation). (two-way ANOVA; interaction effect, $F_{3,59992}=422.5$, $P<0.0001$, with Bonferroni post hoc). * $P<0.05$, *** $P<0.0005$; Bonferroni post hoc test.

Preventing SOM inter-brain correlation



Preventing PYR inter-brain correlation

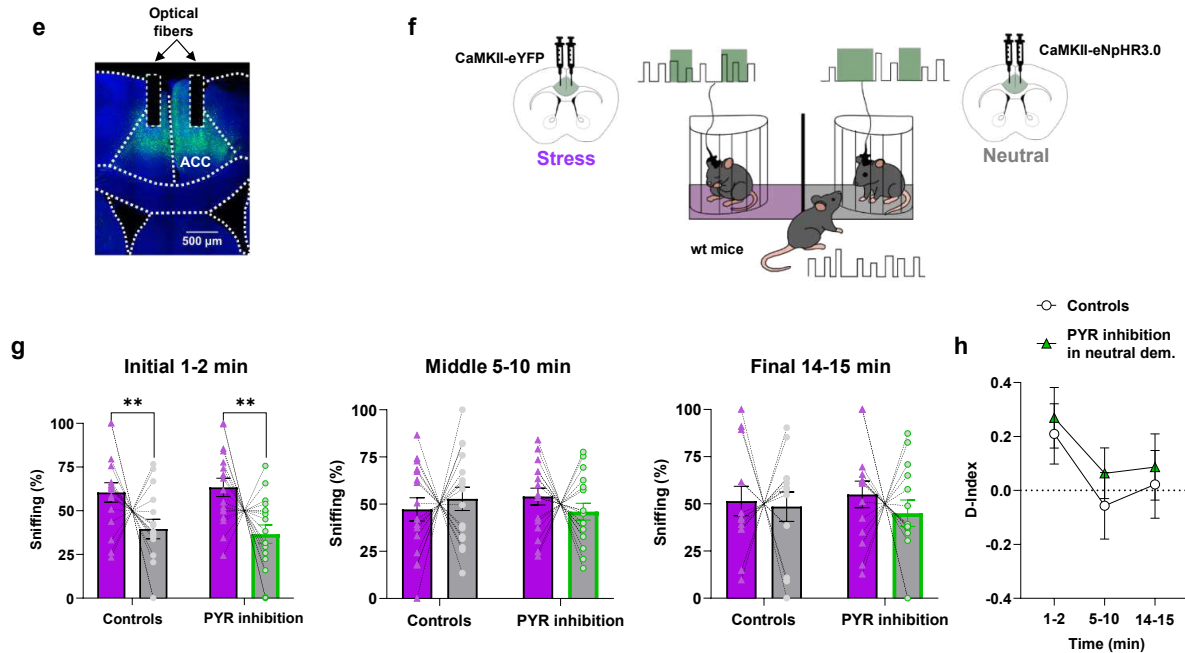


Figure 3

Figure 3. Preventing ACC SOM inter-brain correlation abolishes emotion discrimination.

(a) Representative fiber and opsin placement for SOM inhibition. **(b)** Experimental design: Stress halorodopsin-expressing mice were paired with neutral fluorophore-expressing mice in the EDT stress paradigm. Both demonstrators received green light in the ACC during observer visits to the respective zone, resulting in SOM inhibition only in the stress demonstrator. **(c)** Observers' social investigation expressed as percentage of sniffing toward the stress (purple) or the neutral (grey) demonstrator for controls and SOM inhibition in stress demonstrator optomanipulations (nControls=14, nSOMinhibition=13, two-way repeated measures ANOVA; interaction effect, $F_{1,25}=10.38$, $P=0.0035$, with Bonferroni post hoc test). **(d)** Discrimination index (sniffing time stress – sniffing time neutral / total sniffing time) for Controls and SOM inhibition in stress demonstrator group (two-way repeated measures ANOVA; interaction effect, $F_{2,50}=3.58$, $P=0.035$, with Bonferroni post hoc test). **(e)** Representative fiber and opsin placement for PYR neurons inhibition. **(f)** Experimental design: neutral halorodopsin-expressing mice were paired with stress fluorophore-expressing mice in the EDT stress paradigm. Both demonstrators received green light in the ACC during observer visits to the respective zone, resulting in PYR inhibition only in the neutral demonstrator. **(g)** Observers' social investigation expressed as percentage of sniffing toward the stress (purple) or the neutral (grey) demonstrator for controls and PYR inhibition in neutral demonstrator optomanipulations (nControls=16, nPYRinhibition=17, two-way repeated measures ANOVA; demonstrator effect, $F_{1,31}=9.61$, $P=0.0041$, with Bonferroni post hoc test). **(h)** Discrimination index for Controls and PYR inhibition in neutral demonstrator group (two-way repeated measures ANOVA; no significant effects).

** $P<0.005$; with Bonferroni post hoc test.

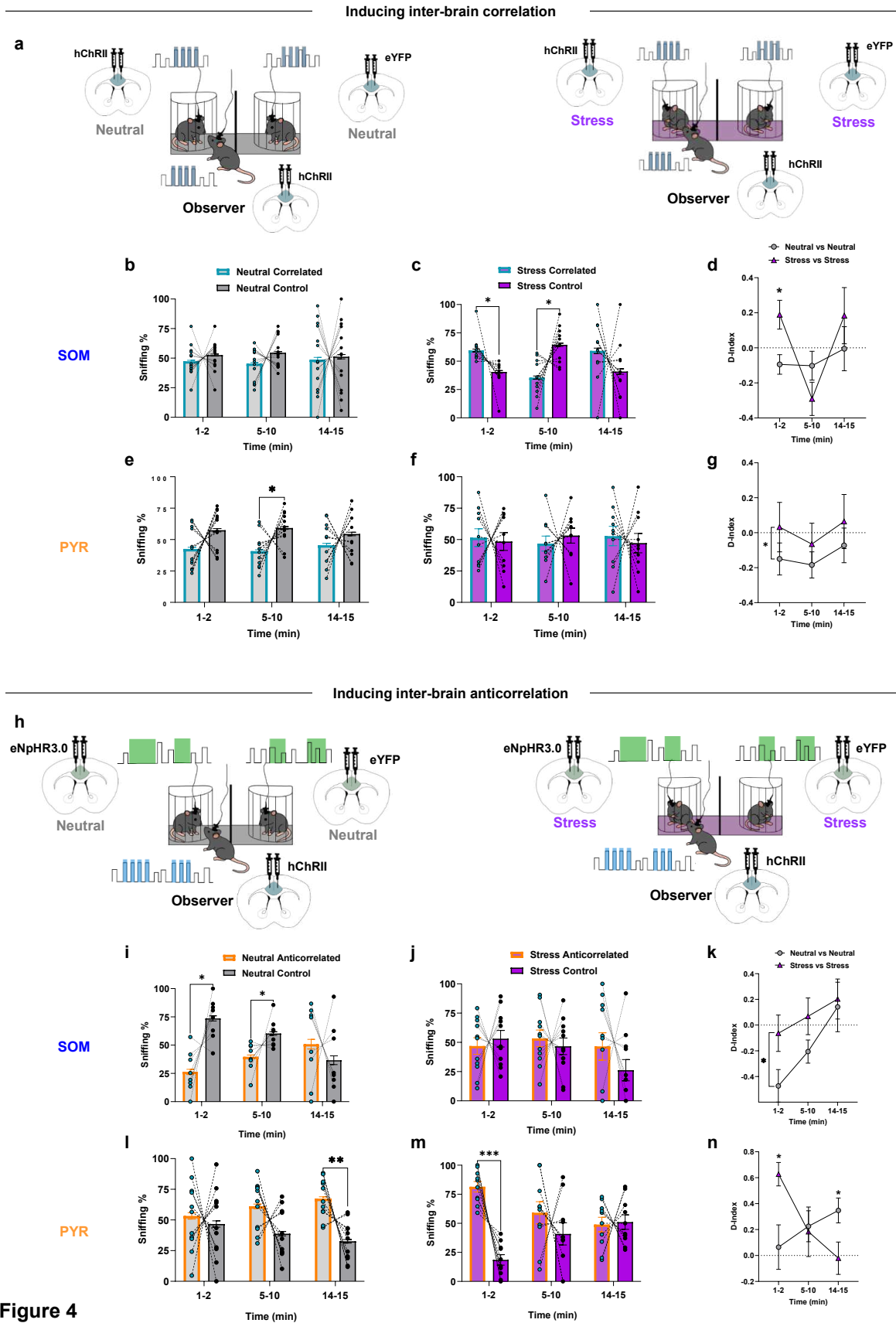
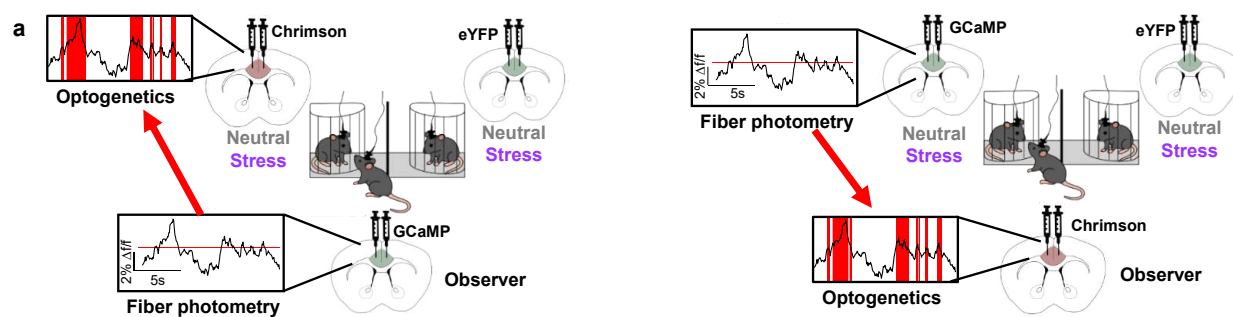


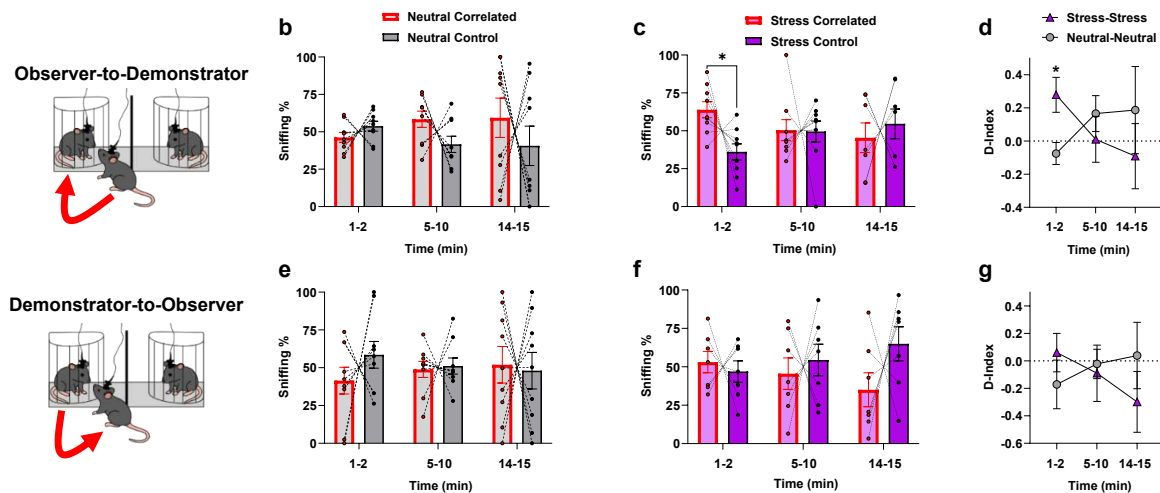
Figure 4

Figure 4. Inducing inter-brain correlations or anticorrelations modulates social preference in a cell-type and emotion-dependent way. (a) Experimental design: concurrent optogenetic stimulation of ACC SOM or PYR neurons in the observer and in one of two emotionally-equivalent demonstrator mice upon zone entry. (b) Observers' social investigation expressed as percentage of sniffing toward the neutral SOM opto-correlated (light blue ticker outline) or the neutral control (black outline) demonstrator (nCorrelated=15, nControls=15, one-way repeated measures ANOVA; no significant effects). (c) Observers' social investigation toward the stress SOM opto-correlated (light blue ticker outline) or the stress control (black outline) demonstrator (nCorrelated=11, nControls=11, one-way repeated measures ANOVA; demonstrator effect, 0-2min $F_{1,10}=5.33$, $P=0.044$, 5-10min $F_{1,10}=9.26$, $P=0.012$, with Bonferroni post hoc test). (d) Discrimination index for neutral-neutral and stress-stress SOM opto-correlation manipulations (two-way repeated measures ANOVA; interaction effect, $F_{2,44}=3.09$, $P=0.050$, with Bonferroni post hoc test). (e) Observers' social investigation expressed as percentage of sniffing toward the neutral PYR opto-correlated (light blue ticker outline) or the neutral control (black outline) demonstrator (nCorrelated=12, nControls=12, one-way repeated measures ANOVA; demonstrator effect at 5-10min, $F_{1,11}=6.03$, $P=0.032$, with Bonferroni post hoc test). (f) Observers' social investigation toward the stress PYR opto-correlated (light blue ticker outline) or the stress control (black outline) demonstrator (nCorrelated=10, nControls=10, one-way repeated measures ANOVA; no significant effects). (g) Discrimination index for neutral-neutral and stress-stress PYR opto-correlation manipulations (two-way repeated measures ANOVA; condition effect, $F_{1,20}=4.41$, $P=0.048$, with Bonferroni post hoc test). (h) Experimental design: concurrent optogenetic stimulation of ACC neurons in the observer and inhibition in one of two emotionally-equivalent mice. (i) Observers' social investigation expressed as percentage of sniffing toward the neutral SOM opto-anticorrelated (orange ticker outline) or the neutral control (black outline) demonstrator (nAnticorrelated=8, nControls=8, one-way repeated measures ANOVA; demonstrator effect, $F_{1,7}=1.36$, $P=0.0078$, with Bonferroni post hoc test). (j) Observers' social investigation toward the stress SOM opto-anticorrelated (orange ticker outline) or the stress control (black outline) demonstrator (nAnticorrelated=11, nControls=11, one-way repeated measures ANOVA; no significant effects). (k) Discrimination index for neutral-neutral and stress-stress SOM opto-anticorrelation manipulations (two-way repeated measures ANOVA; condition effect, $F_{1,17}=4.60$, $P=0.046$, with Bonferroni post hoc test). (l) Observers' social investigation expressed as percentage of sniffing toward the neutral PYR opto-anticorrelated (orange ticker outline) or the neutral control (black outline) demonstrator (nAnticorrelated=11, nControls=11, one-way repeated measures ANOVA; demonstrator effect, $F_{1,10}=1.30$, $P=0.0048$, with Bonferroni post hoc test). (m) Observers' social investigation toward the stress PYR opto-anticorrelated (orange ticker outline) or the stress control (black outline) demonstrator (nAnticorrelated=10, nControls=10, one-way repeated measures ANOVA; demonstrator effect, $F_{1,9}=48.36$, $P<0.0001$, with Bonferroni post hoc test). (n) Discrimination index for neutral-neutral and stress-stress PYR opto-anticorrelation manipulations (two-way repeated measures ANOVA; interaction effect, $F_{2,38}=7.92$, $P=0.0013$, with Bonferroni post hoc test).

* $P<0.05$, * $P<0.005$, and *** $P<0.0005$; with Bonferroni post hoc test.



Physiologic inter-brain **SOM** coupling



Physiologic inter-brain **PYR** coupling

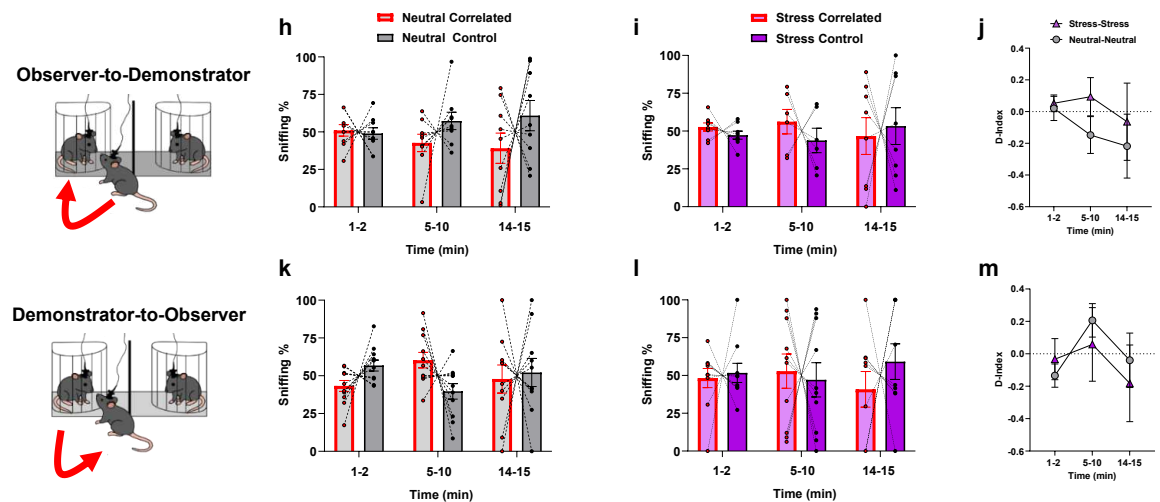


Figure 5

Figure 5. Real-time close-loop induction of physiological inter-brain correlation shows that ACC SOM drives emotional preference while ACC PYR induce avoidance in neutral states.

(a) Experimental design. Left: observers' SOM or PYR activity was recorded and used to control optogenetic stimulation to one of two emotionally equivalent stress or neutral demonstrators. Right: the SOM or PYR neuronal activity from one of two emotionally equivalent demonstrators was recorded and used to deliver optogenetic stimulations to the observer. **(b)** Observers' social investigation expressed as percentage of sniffing during observer-driven SOM inter-brain correlation toward the neutral SOM opto-correlated (red ticker outline) or the neutral control (black outline) demonstrator (nCorrelated=9, nControls=9, one-way repeated measures ANOVA; no effects). **(c)** Observers' social investigation expressed as percentage of sniffing during observer-driven SOM inter-brain correlation toward the stress SOM opto-correlated (red ticker outline) or the stress control (black outline) demonstrator (nCorrelated=9, nControls=9, one-way repeated measures ANOVA; demonstrator effect, $F_{1,8}=5.86$, $P=0.041$, Bonferroni post hoc). **(d)** Discrimination index for neutral-neutral and stress-stress SOM observer-to-demonstrator opto-correlation manipulations (one-way ANOVA; condition effect 0-2min, $F_{1,16}=7.10$, $P=0.017$, Bonferroni post hoc. T-Test means against 0, stress-stress condition 0-2min $t_8=2.42$, $P=0.042$). **(e)** Observers' social investigation expressed as percentage of sniffing during demonstrator-driven SOM inter-brain correlation toward the neutral SOM opto-correlated (red ticker outline) or the neutral control (black outline) demonstrator (nCorrelated=9, nControls=9, one-way repeated measures ANOVA; no effects). **(f)** Observers' social investigation expressed as percentage of sniffing during demonstrator-driven SOM inter-brain correlation toward the stress SOM opto-correlated (red ticker outline) or the stress control (black outline) demonstrator (nCorrelated=9, nControls=9, one-way repeated measures ANOVA; no effects). **(g)** Discrimination index for neutral-neutral and stress-stress SOM demonstrator-to-observer opto-correlation manipulations (one-way ANOVA; no effects). **(h)** Observers' social investigation expressed as percentage of sniffing during observer-driven PYR inter-brain correlation toward the neutral PYR opto-correlated (red ticker outline) or the neutral control (black outline) demonstrator (nCorrelated=9, nControls=9, one-way repeated measures ANOVA; no effects). **(i)** Observers' social investigation expressed as percentage of sniffing during observer-driven PYR inter-brain correlation toward the stress PYR opto-correlated (red ticker outline) or the stress control (black outline) demonstrator (nCorrelated=8, nControls=8, one-way repeated measures ANOVA; no effects). **(j)** Discrimination index for neutral-neutral and stress-stress PYR observer-to-demonstrator opto-correlation manipulations (one-way ANOVA; no effects). **(k)** Observers' social investigation expressed as percentage of sniffing during demonstrator-driven PYR inter-brain correlation toward the neutral PYR opto-correlated (red ticker outline) or the neutral control (black outline) demonstrator (nCorrelated=11, nControls=11, one-way repeated measures ANOVA; no effects). **(l)** Observers' social investigation expressed as percentage of sniffing during demonstrator-driven PYR inter-brain correlation toward the stress PYR opto-correlated (red ticker outline) or the stress control (black outline) demonstrator (nCorrelated=10, nControls=10, one-way repeated measures ANOVA; no effects). **(m)** Discrimination index for neutral-neutral and stress-stress PYR demonstrator-to-observer opto-correlation manipulations (one-way ANOVA; no effects).

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Methods

Mouse Subjects

All procedures were approved by the Italian Ministry of Health and the local Animal Use Committee (permit n. 201/2024-PR 176AA.161 IIT272; 392/2021-PR 176AA.112.EXT.162 IIT215; 639/2020-PR 176AA.82.EXT.133 IIT190; n. 749/2017-PR 176AA.34 IIT126), and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the European Community Council Directives. Routine veterinary care and animal maintenance are provided by dedicated and trained personnel. Adult mice between 2-6 months old with C57Bl/6J background were used. For the miniscope recording experiments, only male mice were used, as they were better able to support the weight of the devices. For all other experiments, both male and female mice were used. Heterozygous somatostatin cre-expressing mice (SOM-cre, Jackson: 013044) and Wild-Type (WT, Jackson: 000664) used for all experiments were bred in our animal facility. Mouse genotypes were determined by Polymerase Chain Reaction (PCR) of ear snip tissue. Animals were housed in cages of 2-to-4 cagemates in a climate-controlled facility (22°C) with *ad libitum* access to food and water, with standard environmental enrichment (material for nest and cardboard house), and maintained on a 12hr light-dark cycle (light on: 7am-7pm). All experiments were run during the light phase (within 10am-5pm).

Stereotaxic surgeries

C57Bl/6J and SOM-cre naïve mice were subjected to stereotaxic surgeries at 2-3 months old. All mice were anesthetized with a mix of isoflurane (4%) in Oxygen (O₂, 1%) before being mounted on a stereotaxic frame (Kopf Instruments), then kept at 1-2% isoflurane throughout the surgery. Brain coordinates for the ACC were defined based on the Mouse brain Atlas ⁶⁷ as follows (AP/ML/DV, in mm from Bregma and the skull): AP, +1.0 mm and ML, +0.3 mm; at two different DV coordinates, -1.75 and -1.55. 0.2 µl of virus mix were infused in each site through a borosilicate micropipette connected to a 10µl Hamilton syringe filled with mineral oil, with a motorized injector (WPI). The injection rate was set to 0.15µl /min and the pipette

was maintained in position for at least 5 min to allow for proper diffusion. After viral injection mice were given between 2 and 3 weeks to recover before the lens or fiber implantation. Mice underwent a second surgery for the implantation of a 0.5 mm outer diameter gradient-index (GRIN) lens (ProView lenses, Inscopix) at the same coordinates as the virus injections, 10–14 d following virus injections. Before lowering the GRIN lens, a pre-track was performed using a blunted needle (25 gauge) to reduce tissue damage and minimize brain pressure. GRIN lens insertion was monitored and positioned at the focal point presenting the highest Ca^{2+} signal. Optogenetic dual fiber implants (Doric Lenses) and optic fibers (RWD) for fiber photometry recordings were placed at the same AP and ML coordinates as the viral injection, and at DV: -1.50 and -1.55 respectively. All implants were maintained in position using a thin layer of Kwik-Sil (World Precision Instruments), two blunted skull screws, and adhesive dental cement (Sun Medical Super-Bond C&B Kit, Dental Leader). Mice were allowed to recover at least 7 days before starting the handling phase.

Apparatus

To allow neuronal recording and manipulation, the testing cage was placed inside a custom-made canopy structure where the cables and two high-resolution monochrome cameras (Imaging source, DMK 22AUC03) were accommodated during testing. The emotional state discrimination task (EDT) apparatuses were 3D printed onto a square apparatus (35.5 x 23.5 x 19) containing a two quarter circles (4cm radius) on two opposite sides as previously described^{20, 21, 43, 44}. Each quarter circle is delimited by metal rods (0.8cm wide) that allow sufficient space for social contact between the demonstrator (freely moving in the apparatus) and the stimuli (freely moving in the quarter-circle). A 3D-printed separator (11 x 19cm) is placed in the middle of the apparatus between the two quarter-circles to block the reciprocal view of the demonstrator mice. Both a top view and an upper-front view of the apparatus were acquired through ANYmaze software (Anymaze 7.4, Stoelting). For automated scoring, a virtual square (11 x 11 cm) is drawn between each quarter-circle and the separator to define a zone associated with each stimulus. Videos were collected at 15FPS using the behavioral tracking system (Anymaze 7.4, Stoelting). In optogenetic

experiments, live tracking of the mice was used to deliver 5V- TTL through an Anymaze interface (Ami1) to the acquisition hardware (DAQs) calcium data or LEDs and Lasers for optogenetics, based on the zone visited.

Behavioral procedure

All mice were group-housed and separated based on their experimental role (observer or demonstrator). All mice were handled once a day for 1 min for at least 3 days before behavioral testing procedures. Additionally, mice assigned to miniscope recordings were habituated to wear the miniscope for 15 minutes over 3 consecutive days in their home cage. Observers and demonstrators were individually habituated to the experimental apparatus with the cable attached for three days with daily sessions of 15 minutes, in their respective positions (demonstrators inside their quarter-circle, observers in the free zone). Demonstrator mice were matched by age, sex, and strain to the observers. To avoid confounders due to side preference, neutral-, stress-, manipulated-, controls- stimulus were counterbalanced in the two sides of the testing arena. Neutral demonstrators were naïve and used for a maximum of 2-to-3 times, with at least 1 week between each experiment. Observers were always exposed to novel unfamiliar demonstrators.

On the day of testing, all mice were brought into the experimental room at least 1h before the experiment began, and kept distant to avoid visual or olfactory contact between observers and demonstrators. Observer mice were gently moved into the arena connected to the patch cord for optogenetics/fiber photometry, or the electric cord for miniscope recordings. Neutral demonstrators stayed in their home-cage while stress demonstrators were subjected to a 15-minutes restraint inside a 50mL falcon tube with a hole in the tip for air exchange and a long and narrow opening on top for the cable. The demonstrators were then moved immediately to their respective side of the arena, and the observer was allowed to explore the two demonstrators for 15 minutes. Following each experiment, the apparatus was thoroughly cleaned with 50% ethanol, wiped, and allowed to air dry. At the end of the day the apparatuses were clean with soap, water, and then with 70% ethanol.

Miniscope acquisition setting

For calcium imaging experiments, AAV1.Syn.Flex.GCaMP6f.WPRE.SV40 (Addgene, 100833-AAV1) was injected in SOM-cre heterozygous mice and pENN.AAV1.CamKII.GCaMP6f.WPRE.SV40 (Addgene, 100834-AAV1) was injected in WT mice. Both viral vectors were diluted 1:6 in saline before the injection. To concurrently image the GCaMP6 calcium indicator from 3 mice, we took advantage of three miniature fluorescent microscopes (nVoke 3.0, Inscopix) with an integrated blue LED (EX-LED, 435–460 nm, average power 1 mW mm⁻²) and red LED (OG-LED, 590–650 nm, light power 0–5 mW mm⁻²). On the first day of mice habituation to the cables, the miniature microscope was positioned on the head of the mice, and the focal plane was adjusted through the Acquisition Software (version 1.2.0, Inscopix). Optimum laser power, imaging gain, and focal distance were selected for each animal and conserved across all experiments. On the experiment day, Ca²⁺ signals were collected at 20 Hz during the habituation of the observer, the restrain-stress phase for the stressed demonstrator, while neutral demonstrators were in their home-cage, and during the entire 15 min period of the EDT. To synchronize the 3 miniscopes and the behavioral video, calcium recording was controlled by the tracking software and interface (Anymaze, Amil) through a TTL concurrently delivered to the miniscopes at the start of the habituation and the test phases.

Calcium signal analyses

Before proceeding with behavior-based segmentation of calcium activity and inter-brain correlation analyses, the signal was processed using data analysis software (Inscopix Data Processing Software version 1.2, Inscopix). Both signals recorded during the EDT and the respective habituation periods were merged in a time series and analyzed together to track the same neurons before and after exposure to the demonstrators. Dropped frames were corrected by a smooth correction of the edges. Data were pre-processed through the following steps: spatial and temporal downsampling, using x2 scale factors of space and time; spatial filtering of the image to remove low and high frequencies with a bandpass filtering; motion

correction between different frames of the videos; pixel normalization over the mean value of the movie. The selection of neurons was done using manual regions of interest (ROIs), for which a selection tool was used to delimitate the borders of putative GCaMP-expressing neurons. After neurons selection, an OASIS algorithm was applied to deconvolve the traces. At the end of these steps, the final data available consisted of a list of every neuron detected, and the corresponding $\Delta F/F$ value for each frame. To make Ca^{2+} signals traces comparable with each other, individual neuron traces were normalized by their standard deviation.

Pearson inter-brain correlation of neuronal activity averaged within each brain. To test inter-brain correlations during emotion discrimination, we first averaged the Ca^{2+} activity of all recorded cells (except those active only during habituation) for each animal and applied a linear detrending function to exclude the effect of photobleaching on correlations. We then computed the Pearson correlation coefficient to consider possible non-linear correlations across brains.

Cross-correlation. To gain more insight into the timescale at which inter-brain correlations occur, we performed a cross-correlation analysis using the neural activity from interacting animals in the EDT. The cross-correlation curve between the two activities is expressed over different values of the reciprocal lag between the two. A peak in the cross-correlation curve expresses a state of synchronization of the two signals, one being shifted in time from the other by the corresponding lag value. Therefore, once computing the cross-correlation curve between two time-dependent activities, one can identify its maximum value and retrieve the corresponding value of the lag, estimating the delay between the signals. We calculated the correlation between $\Delta F/F$ activity traces during intervals in which mice were close (observer in demonstrator's zone) with different time shifts, ranging from -5 seconds to +5 seconds, and plotted the correlation as a function of time lag.

Across-neuron correlation computed with single-cell resolution. For each observer neuron i , we computed the time-lagged partial correlation between the activity of i and the time-shifted activity of another neuron j (either in the observer brain for the within brain condition, or in the demonstrator brain for the across brain condition). Positive (negative) time lags in the computation of the time-lagged correlation correspond to

shifting the neuron j forward (back) in time. Negative time shifts indicate that the activity of neuron i precedes that of the other neurons j (observer is preceding the demonstrator's activity), while positive shifts indicate that neuron i follows the activity of neurons j (demonstrator is preceding the observer's activity). To emphasize the short-timescale correlations, slow rate variations across behavioural interaction windows were regressed out by partializing out the mean activity of both signals within each interaction window during the correlation computation.

From the time-lagged partial correlations between pairs of neurons we averaged over j to obtain a within-brain and an across brain *time-lagged coupling function*⁶⁸ for each neuron i in the observer brain, from which we extracted the following coupling indices of each neuron i in the observer brain. The first was the *peak coupling* defined as the maximum value of the time-lagged coupling function for each neuron i in the time lag interval $[-1,1]$ s. The second was the *mean coupling* taken as averaged over time lags of the coupling function in the time lag interval $[-1,1]$ s. We then computed the *coupling directionality index* as the integral of the coupling function over positive time shifts minus the integral of the coupling function over negative time shifts. Because higher coupling values with negative (positive) time shift indicate that the observer precedes (follows) the demonstrator's activity, the coupling directionality index captures the balance of directional influences: if it is positive (negative) it indicates that the demonstrator drives (follows) the observer.

Shannon information carried by single neurons about emotional state. We quantified how much information the activity of a single neuron of the observer in a given imaging time frame (R) carries about the emotional state of the demonstrators (S) using Shannon information $I(S;R)$ between the emotional state and the neuron's activity. This quantifies (in units of bits, with one bit corresponding to a reduction of uncertainty by a factor of two) the reduction in uncertainty about the emotional state of the demonstrator from the observation of the observer's neural response in a single imaging frame as follows:

$$I(S;R) = \sum_{r,s} p(r,s) \cdot \log_2 \left(\frac{p(r,s)}{p(r) \cdot p(s)} \right)$$

where R and S representing the sets of all possible discrete values of neural responses and affective states, $p(r, s)$ is the joint probability of response r and affective state s , $p(r)$ and $p(s)$ the marginal probabilities of response and affective state

Information was computed using the MINT toolbox. For each neuron, the activity ($\Delta F/F$) was discretized into four equipopulated bins, and mutual information (MI) estimates were corrected for the limited sampling bias using the Panzeri–Treves bias correction ⁶⁹.

MI between neural activity and demonstrator affective state (stressed vs. neutral) was computed using only imaging frames in which the observer was engaged in reciprocal sniffing of either the stressed or the neutral demonstrator. To ensure balanced sampling across conditions, the number of frames per condition was matched by restricting the analysis to the minimum number of total available frames across stressed- and neutral-reciprocal sniffing.

Breakdown of emotional state information carried by triplets of neurons across three brains into contributions of single cells and across-brain correlations. To assess the extent to which inter-brain correlations contribute to encode information about the emotional state of the demonstrator that is interacting with the observer, we proceeded as follows. We first computed the Shannon information about emotional state carried by the simultaneously recorded activity of triplets of neurons consisting of one neuron in the observer, in the stressed demonstrator and neutral demonstrator brain, using the above Shannon information formula replacing the activity of one neuron r with the activity of the simultaneously recorded neurons r_1, r_2, r_3 . For each observer, only imaging frames in which the observer engaged in reciprocal sniffing of either the stressed or the neutral demonstrator were included. Neural activity ($\Delta F/F$) for each neuron in the triplet was discretized into four equipopulated bins. This mutual information was computed using the MINT toolbox with shuffle correction to correct conservatively for the limited-sampling (20 shuffles). For each observer, a random subset of 500 triplets was analysed to ensure balanced sampling.

We then used the Information breakdown ⁷⁰ to decompose this Shannon information into the sum of two components: the “independent information” I_{ind} that would be carried if each single neurons had the same properties but correlations across brains were absent (it thus quantifies the contribution of individual cells to encoding), and the correlation information I_{cor} (the information that across-brain correlations add because their strength is modulated by the emotional state). We verified that the information carried by inter-brain correlations I_{cor} was due exclusively to modulations of inter-brain correlations by emotional state by splitting it as previously described in ⁷⁰ into its stimulus-dependent and stimulus-independent components and verifying that the stimulus-independent component was null in these data.

Optogenetic manipulations

All optogenetic manipulations involved bilateral AAV injections in the ACC and subsequent implantation with dual fiber optic implants terminating dorsally to the injection area (Doric Lenses, DFC_200/245-0.37_2mm_GS0.7_FLT). During behavioral experiments, implants were connected to splitter branching patchcord (Doric Lenses, SBP(2)_200/230/900-0.37_1m_FCM-GS0.7), which were in turn connected to blue (480 nm) or green light (530 nm) LEDs (Prizmatix Laser) using a 1×1 fiberoptic rotary joint (Prizmatix) located above the cubicle containing the testing arena. Laser power was adjusted such that the light at the tip of the fiberoptic cable was approximately 3 mW. Behaviorally-controlled light delivery was achieved through TTL signals from the behavioral tracking software and interface (Anymaze, Ami1) to the LEDs. In all experiments, opsin-expressing and fluorophore demonstrators were paired and both received light, to concurrently exclude possible effects of the light on observer preference and control for non-specific effects of LED light delivery in the demonstrator. Both observers and demonstrators were tested up to 3-4 times, always keeping their original roles. Demonstrators always started being tested as neutral, 1-2 times, and then became stressed demonstrators 2 more times.

Preventing interbrain correlation

For optogenetic inhibition experiments we used AAV carrying: (1) for SOM, a Cre-dependent halorhodopsin (AAV5-EF1a-DIO-eNpHR3.0-eYFP, Addgene, 26966) or a cre-dependent eYFP (AAV5-EF1a-fDIO-EYFP, University of Pennsylvania, CS0378); (2) for PYR, a CaMKIIa-dependent halorhodopsin (AAV1-CaMKIIa-eNpHR 3.0-eYFP.WRGE.hGH, University of Pennsylvania, V4188MI-R) or a CaMKII-dependent eYFP (AAV1-CaMKII0.4-eGFP.WRGE.rGH, University of Pennsylvania, CS0934) into the ACC of SOM- or C57BL/6J mice. During behavioral experiments we delivered a continuous green light to each demonstrator only during observer visits to their zone. For SOM experiments, demonstrators were pseudo-randomly paired as the stress demonstrators expressed halorhodopsin while the neutral demonstrator eYFP. For PYR experiments, demonstrators were pseudo-randomly paired as the neutral demonstrators expressed halorhodopsin while the stress demonstrator eYFP. In control experiments both demonstrators expressed eYFP and received light during observer's visits.

Inducing inter-brain correlation

For optogenetic excitation experiments we injected AAV expressing: (1) for SOM, a Cre-dependent channelrhodopsin (AAV5-EF1a-DIO-hChR2(H134R)-EYFP-WPRE-HGHpA, Addgene, 20298) or a cre-dependent eYFP; (2) for PYR, a CaMKIIa-dependent channelrhodopsin (AAV1-CaMKIIa-hChR2(H134R)-eYFP.WRGE.hGH, University of Pennsylvania, CS0717-3CS) or a CaMKII-dependent eYFP into the ACC of SOM- or C57BL/6J mice. Photo-stimulation protocol involved 5 ms pulses of blue light delivered at 5Hz controlled by observer position in the arena. All observers expressed ChR2 and received optogenetic stimulation during the visits to both demonstrators. Synchronized demonstrators (ChR2-expressing) were paired with YFP-expressing demonstrators and both received light when the observer was exploring their specific compartment. Overall, resulting in synchronous observer-demonstrator stimulation in the synchronized demonstrator side and observer-only stimulation in the control demonstrator side.

Inducing inter-brain anti-correlation

We injected AAV carrying: (1) for SOM, a Cre-dependent ChR2 or a cre-dependent halorhodopsin or a cre-dependent eYFP; (2) for PYR, a CaMKIIa-dependent ChR2 or a CaMKIIa-dependent halorhodopsin

or a CaMKII-dependent eYFP into the ACC of SOM- or C57BL/6J mice. Two experimental designs were used to achieve anti-correlation:

- Observer photo-stimulation and concurrent demonstrator photo-inhibition: in this configuration observers expressed ChR2 and received pulsed blue light in both demonstrators' compartments. Anti-correlated demonstrators (Halorhodopsin-expressing) were paired with YFP-expressing demonstrators and both received continuous green light when the observer was exploring their specific compartment.
- Observer photo-inhibition and concurrent demonstrator photo-stimulation: halorhodopsin expressing observers received continuous green light in both demonstrators' compartments. Anti-correlated demonstrators (hChR2-expressing) were paired with YFP-expressing demonstrators and both received pulsed blue light when the observer was exploring their specific compartment.

Fiber photometry and closed-loop optogenetic setting

We injected a 1:6 mix of either AAV1.Syn.Flex.GCaMP6f.WPRE.SV40 (Addgene, 100833-AAV1) and AAV5-Syn-FLEX-rc[ChrimsonR-tdTomato] (Addgene, 62723-AAV5) or a 1:6 mix of pENN.AAV1.CamKII.GCaMP6f.WPRE.SV40 (Addgene, 100834-AAV1) and AAV5-CamKIIa-ChrimsonR-mScarlet-KV2.1 (Addgene, 124651-AAV9) in SOM-cre or wt mice respectively. Optic-fiber implants connected to metal or zirconia ferrules were cleaned and connected to a low-autofluorescence patch cord (2.5 m long, 0.5 NA, Doric Lenses). A second dummy patch cord was attached and connected to the dummy ferrule to reduce oscillations and thus prevent artifacts. Calcium signals were acquired through an integrated fiber photometry system (Aquineuro, Fiber Photometry recording System), delivering alternated light at 470nm for GCaMP excitation and at 410nm for isosbestic control.

For closed loop stimulation, activity dependent optogenetic stimulation was delivered. During the experiment, the recording software computed real-time data normalization from the signal acquired from the mouse chosen to drive the stimulation and a 2% variation of fluorescence was set as threshold to deliver

optogenetic stimulation to the mouse chosen to receive stimulation, through a TTL from the fiber photometry system and the 635nm Laser (Aquineuro, Intelligent Optogenetics System).

The estimated latency between the recorded signal crossing the 2% threshold and stimulation delivery is approximately 15ms.

Behavioral analyses

All behaviours were manually scored by three expert scorers, blinded to the treatments, through the software BORIS⁷¹, allowing frame-by-frame videos inspection. Observer sniffing was defined as observer approaching and nose-touching demonstrator's cages. Reciprocal sniffing was defined as closeness between mice noses displaying interest for each other. Grooming, rearing and climbing were scored one at a time for each video. Data collected were then extracted as start/stop time for each event and analysed in Matlab, graphs and statistics were done using Graphpad Prism software.

Immunohistochemistry

All animals used for behavioral experiments or tracing were deeply anesthetized following all experiments with an overdose of urethane (>2 g per kg body weight) and then transcardially perfused with ice-cold 0.05 M phosphate buffer saline (PBS) pH 7.4, followed by 4% paraformaldehyde (PFA) in PBS (0.1 M, pH 7.4). Brains were removed and stored in 4% PFA for 24h post-fixation. Brains were then placed in in 30% sucrose solution (in PBS) for at least 48 h of cryoprotection until sectioning. Brains were mounted on the sectioning chamber and frozen, and 40 µm slices were cut along the coronal axis using a microtome (HM45, Thermo Fisher). Sections were collected in PBS with 0.02% NaN₃ until staining. For immunohistochemistry, all sections were first incubated in a blocking solution consisting of 10% normal goat serum (NGS) in PBS containing 1% Triton X-100 for a minimum of 1 h. Following 3–5 washes in

PBS solution, primary antibodies were selected based on the target (GFP or mCherry). The primary solution consisted of the primary antibody diluted in 1% NGS, 0.03% Triton X-100 in PBS. Primary incubation occurred at 4 °C for 24–48 h. Following primary incubation, slices were washed 3–5 times in PBS and prepared for the secondary incubation that consists of a solution containing the secondary antibody diluted in 1% NGS, 0.03% Triton X-100 in PBS. Secondary incubation occurred at room temperature (RT) for 4–6 h. Primary antibodies used were: rabbit anti-GFP (1:1,000 dilution; Invitrogen, A11122), chicken anti-GFP (1:1,000 dilution; Abcam, AB290), and rabbit anti-dsRed (1:1,000 dilution; Thermo Fisher, 150128). Secondary antibodies were: Alexa Fluor 488 (goat anti-rabbit, 1:1,000 dilution; A11034, ABclonal), Alexa Fluor 488 (goat anti-chicken, 1:1,000 dilution; A11039, ABclonal) and anti-568 (goat anti-rabbit, 1:1,000 dilution; A11036, ABclonal). Fluorescence images were obtained with a confocal microscope (Nikon, CTR6500) or with an inverted fluorescence microscope (Zeiss Axiozen) using the following filter: 490 nm for Alexa Fluor 488, 560 nm for Alexa Fluor 568, and 650 nm for. Images were collected at $\times 20$ in air or $\times 10$, $\times 20$. All fluorescence images were treated using the software ImageJ and Adobe Illustrator CS6. All primary and secondary antibodies have been previously validated.

ELISA corticosterone assay

The corticosterone concentration was analyzed from plasma. Thirty minutes after exposure to the laser stimulation, each mouse was killed by decapitation. The blood was quickly collected in tubes coated with 0.5 M EDTA and centrifuged at 664 ref for 10 min; the supernatant obtained was immediately processed. The corticosterone concentration was detected by a commercially available Detect X corticosterone ELISA kit (Arbor Assays, K014-H1) following the manufacturer's protocol. Original samples were diluted 1:100 in assay buffer. Each standard and sample was loaded in duplicate and plates were read using a 450 nm wavelength into a multi-plate reader (Tecan). Measures of absorbance were converted in concentration expressed as mg/ml using a Four Parameter Logistic (4PL) Regression fit, and normalized to the blank

(negative control). The level of corticosterone was expressed as fold changes compared to the control group average.

Statistics

Results are expressed as the mean $\pm$ s.e.m. throughout the paper. Each observer's behavior toward the two different demonstrator mice was analyzed using a within-groups RM-ANOVA. Data distribution was tested using the D' Agostino and Pearson normality test. The discrimination index with optogenetics stimulation was analyzed by two-way ANOVAs with optostimulation (no light, ChR2, NphR) as between-subjects factors, and time intervals (0–2 min, 5–10 min and 13–15 min) as RM within-subjects factors. Bonferroni's post hoc test with multiple-comparison corrections was used for making comparisons within groups when the overall ANOVA showed statistically significant differences. All tests were two sided. Spearman correlation coefficients were compared using a two-tailed paired or unpaired *t*-test. For optogenetic experiments, sniffing time, distance travelled, number of entries in each zone and time in each zone was then compared across different conditions to the control (no stimulation) experiments. The accepted value for significance was $P \leq 0.05$. Statistical analyses were done using GraphPad Prism. The number of mice is reported in the legends and corresponds to similar numbers used in previous publications. Experiments reported in the paper were replicated three to four times for a different group, cage, sex or generation. No statistical measures were taken to predetermine the sample size of the experiments. For each animal, only entries to the stimulus area that lasted more than 5 s were used for analyses. Mouse exclusion criteria were defined before the beginning of the experiments and include genotyping errors, viral injection and fiber misplacement, and uncompleted experiments due to loss of implants. For all experiments, the side of the allocated stimulus mice and the order of the testing were randomized.

Human experiment

Participants

Eighty participants (mean age = 27.64 years, range = 18-49 years) were recruited and randomly assigned to two groups. To maintain experimental consistency with the mice studies, the sample was restricted to male participants, and all dyad members were unfamiliar with each other. All participants had normal or corrected to normal vision and reported no history of psychological or neurological disorders. Written informed consent was obtained from all participants, who were compensated with 25 Euros for their participation. All experimental procedures were approved by the local ethical committee and were carried out in accordance with the principles of the revised Helsinki Declaration (World Medical Association General Assembly, 2008). The sample size was determined a priori based on previous studies investigating interpersonal neural synchronization ⁷²⁻⁷⁵.

Experimental stimuli selection

The experimental stimuli comprised 3 stress-inducing and 3 neutral audio-video clips (Supplementary Fig. 4a). Audio-video clips, i.e. images paired with background music, were chosen as stimuli because such a combination is known to effectively evoke negative affect in humans ⁴⁸. To ensure the effectiveness of the stress-inducing stimuli, the images were pre-selected through an online survey ⁷⁶. For this, we first compiled an initial set of 120 negative affect images obtained from both previously validated image databases and web sources. Specifically, we included 23 images from the Nencki Affective Picture System ⁷⁷, 21 images from the Open Affective Standardized Image Set ⁷⁸, 10 images from the Geneva Affective Picture Database ⁷⁹, and 66 additional images from the web. The web-sourced images were selected to highlight negative emotions concerning contemporary themes such as climate change, natural disasters, and war-related destruction.

Participants taking part to the online survey were located in Italy at the time of the experiment and were recruited via the Prolific platform (www.prolific.com). They were compensated at a rate of €8 per hour. During the survey, participants observed the images on a computer screen and rated their valence i.e., how negative they perceived each image to be (Supplementary Fig. 4b). To ensure participants were paying attention to the task, we included six control images. For these images, participants were instructed to enter a specific valence rating (e.g., “Please enter a value of 3 for valence”). Each image was displayed for 5 seconds, and participants had a maximum of 6 seconds to respond.

All participants demonstrated high accuracy on the control task, with the exception of one individual who achieved only 33% accuracy and was consequently excluded from further analysis. The final sample comprised 21 participants (11 males; mean age = 26.57 years; mean accuracy = 99.20%). We then averaged the valence ratings across participants and selected the 30 images with the highest average valence (see Supplementary Fig. 4c). We also confirmed that the selected images were rated as significantly more negative than the non-selected images (Welch Two Sample t-test $t(117.67) = 14.46$, $p < 0.001$; Supplementary Fig. 4c).

Next, we selected the three audio clips with the highest negative valence ratings from the validated Database for Emotional Analysis in Music (DEAM)⁸⁰. The three audio clips were combined with the 30 previously selected visual images. The images were divided into three groups of ten, with each group paired with one of the audio clips. Within each audio-visual stimulus, each image was presented for 4 seconds, followed by a 2-second blank screen.

Finally, three neutral audio-video clips were created using 30 images previously rated as neutral in the validated datasets described above. Specifically, the neutral set consisted of 13 images from NAPS, 12 from OASIS, and 5 from GAPED. These images were combined with neutral audio clips—identified as such in the DEAM database—following the same procedure used for the stress-inducing stimuli.

Experimental design and procedure

The study employed a between-subjects design comprising a stress group (Stress–Neutral) and a control group (Neutral–Neutral) (Fig. 1k). Emotional content was manipulated only in the Stress–Neutral group, where one participant in each dyad (counterbalanced across participants) viewed stress-inducing audio-video stimuli, while the partner viewed neutral stimuli. In contrast, participants in the Neutral–Neutral group viewed only neutral stimuli. The experiment comprised three different procedures: emotion induction, social interaction, and no social interaction (Supplementary Fig. 4d). During the emotion induction, participants viewed experimental stimuli on a monitor (placed at a distance of approximately 1 m) with no visual contact permitted between partners. Immediately following the emotion induction, participants engaged in the social interaction condition, during which they were asked to look towards their partner and hold each other's right hand. Finally, in no social condition, participants' view of one another was obstructed by an occluder placed between them, preventing any visual or haptic social contact.

Experimental procedures were structured into repeated trials, each lasting one minute, and implemented in the following sequence: (1) no social interaction, (2) emotion induction (neutral-neutral for all groups), (3) social interaction (baseline), (4) no social interaction, (5) emotion induction (neutral-neutral or stress-neutral, depending on group assignment), (6) social interaction (test), and (7) a final no social interaction phase (see Supplementary Fig. 4d). Each no social interaction condition was composed of three consecutive trials. Similarly, the emotion induction followed by social interaction block was repeated three times. To prevent stimulus-driven synchrony and maintain independence of perceptual experience, stimulus order was randomized across dyad members, such that the two participants viewed different stimuli at any given time. In the second social interaction block (test phase), participants in the Stress-Neutral group were exposed to asymmetric emotional stimulation: one participant viewed stress-inducing stimuli while the other viewed neutral stimuli. In the Neutral-Neutral group, both participants were presented with neutral stimuli.

Behavioral and neural recordings

Behavioral data: We assessed participants' current affect using the Italian version of the short-form Positive and Negative Affect Schedule (PANAS-SF)⁸¹. PANAS-SF is a self-report questionnaire with 10-items assessing positive and negative affect. Participants were asked to rate their current affective state i.e., how intensively they felt each emotion in that particular moment. This measure has proven to be a reliable and valid self-report tool⁸². We collected these reports both at the start and at the end of the experiment.

Neural recordings: We recorded neural activity, simultaneously from both participants, using dual electroencephalography (EEG). EEG was recorded from 64 Ag/AgCl active electrodes placed on the scalp according to the extended international 10–10 system (Biosemi ActiveTwo system). EEG signal was locally amplified and digitized using a sampling rate of 1024 Hz.

Data analysis

Behavioral data: To assess the effectiveness of emotion induction, we analysed participants' subjective ratings from the questionnaire administered before and after the experiment. We averaged participants' responses to the negative items on the questionnaire and computed the difference in ratings (post-experiment minus pre-experiment) for each individual. To evaluate whether these ratings changed significantly from pre- to post-experiment within each group, we conducted a one-sample test against a reference value of zero, indicating no change. As the data did not meet the assumptions of normality (Shapiro-Wilk test, $p < 0.05$), we employed non-parametric Wilcoxon signed-rank tests for the analysis.

Neural data analysis: EEG data preprocessing was performed using a fully data-driven pipeline that was previously developed for denoising EEG data in humans and awake monkeys^{83, 84}. This pipeline combines algorithms from Fieldtrip and EEGLAB toolboxes^{85, 86}. Specifically, the recorded EEG data were band-pass filtered (cutoff frequencies: 0.3 Hz and 95 Hz, Butterworth, filter-order: 3). A notch filter (47–53 Hz) was also used to remove electrical noise. We used an automated pipeline to remove noisy electrodes [defined as flat-lined, uncorrelated channels (correlation with other channels < 0.8) or channels carrying

amplitude values that deviated more than 2.75 standard deviations from the mean of all channels]. We used Artifact Subspace Reconstruction (ASR)⁸⁷ to remove transient and large-amplitude movement artifacts⁸⁷. Artifact Subspace Reconstruction (ASR) is an adaptive algorithm that uses principal component analysis (PCA) to automatically identify clean segments of data, which are then used to establish variance thresholds. Based on these thresholds, the algorithm rejects components exhibiting abnormally high variance. Based on the comparative results from⁸⁸, we used a threshold value of 10. We then re-referenced the data using Common Average Reference. Next, we submitted the data to ICLabel⁸⁹, which uses independent component analysis to automatically remove eye blinks or eye movements artifacts. Finally, previously removed channels were reconstructed through interpolation, using the mean voltage of adjacent electrodes with denoised signals. The resulting pre-processed data were then analyzed as in⁴⁹. EEG timeseries were time frequency transformed using a sliding window Fourier analysis with a tapering function. To reduce spectral leakage and control the frequency smoothing, we used a Hanning-tapered window with a frequency dependent time window that linearly decreased from 500 ms (1 Hz) to 100 ms (95 Hz) (using the ‘ft_freqanalysis’ function with ‘mtmconvol’ method as implemented in FieldTrip)⁹⁰. We computed power estimates for all frequencies between 1 and 95 Hz in steps of 1 Hz and a time resolution of 100 ms (10 Hz). To avoid overrepresentation of certain bands embracing a disproportionate amount of frequency bins (in particular, gamma band spanning from 31–95 Hz), we defined sub-bands⁴⁹. Next, we computed inter-brain correlation by calculating Pearson’s correlation coefficients between electrode- and frequency bin-specific normalized EEG power time courses (i.e., power envelopes) recorded from the two individuals within each dyad^{27, 30, 49, 91, 92}. We normalized the power time series as below:

$$normalized\ data_c = \frac{data_c - mean(data_c)}{data_c + mean(data_c)}$$

Where $data_c$ is the (channel- and frequency bin- specific) power time course data for the entire duration of a single trial (0–60 s) and $mean(data_c)$ is the average (over time) of this data. The inter-brain correlation measure was both channel- and frequency bin-specific and was only computed among homologous

channels and bins. We averaged the correlation coefficients across trials belonging to the same condition and Fisher z-transformed (inverse hyperbolic tangent function) them to obtain averaged normalized correlation coefficients for each channel and each frequency bin (across conditions within each dyad). Next, we averaged the coefficients according to frequency sub-bands. Finally, we conducted a data-driven localizer analysis to identify regions exhibiting greater inter-brain correlation during the social condition compared to the no social condition. The localizer was defined using a cluster-based non-parametric permutation test⁹³. This procedure controls for false-alarm rate by using a cluster statistic that is evaluated under a single permutation distribution. More specifically, this algorithm compares power estimates between conditions, separately for each channel and frequency sub-band, using two-sided independent-samples t-tests, yielding one t-value for each channel and frequency sub-band. The algorithm then forms clusters of (at least two) neighboring channels whose t-values exceed the significance criterion (here $p < .05$) and computes a cluster-level statistic value (sum of t-values within the cluster). Next, cluster-level statistics from 1000 random partitions of the data are used to build a distribution upon which the significance probability (here $p < .05$) of the experimental cluster can be estimated. To assess whether inter-brain correlation during the social conditions varied as a function of emotion induction, we computed the difference in inter-brain correlation between the two social interaction blocks (social test – social baseline; Supplementary Fig. 4d). This difference was extracted specifically from the inter-brain correlation localizer cluster and compared between the Stress-Neutral and Neutral-Neutral groups using an independent-samples t-test.

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