



A Brain-to-Brain Interface for Real-Time Sharing of Sensorimotor Information

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WHISKER SYSTEM

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A brain-to-brain interface (BTBI) enabled a real-time transfer of behaviorally meaningful sensorimotor information between the brains of two rats. In this BTBI, an “encoder” rat performed sensorimotor tasks that required it to select from two choices of tactile or visual stimuli. While the encoder rat performed the task, samples of its cortical activity were transmitted to matching cortical areas of a “decoder” rat using intracortical microstimulation (ICMS). The decoder rat learned to make similar behavioral selections, guided solely by the information provided by the encoder rat’s brain. These results demonstrated that a complex system was formed by coupling the animals’ brains, suggesting that BTBIs can enable dyads or networks of animal’s brains to exchange, process, and store information and, hence, serve as the basis for studies of novel types of social interaction and for biological computing devices.

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In his seminal study on information transfer between biological organisms, Ralph Hartley wrote that “*in any given communication the sender mentally selects a particular symbol and by some bodily motion, as his vocal mechanism, causes the receiver to be directed to that particular symbol*”¹. Brain-machine interfaces (BMIs) have emerged as a new paradigm that allows brain-derived information to control artificial actuators² and communicate the subject’s motor intention to the outside world without the interference of the subject’s body. For the past decade and a half, numerous studies have shown how brain-derived motor signals can be utilized to control the movements of a variety of mechanical, electronic and even virtual external devices^{3–6}. Recently, intracortical microstimulation (ICMS) has been added to the classical BMI paradigm to allow artificial sensory feedback signals^{7,8}, generated by these brain-controlled actuators, to be delivered back to the subject’s brain simultaneously with the extraction of cortical motor commands^{9,10}.

In the present study, we took the BMI approach to a new direction altogether and tested whether it could be employed to establish a new artificial communication channel between animals; one capable of transmitting behaviorally relevant sensorimotor information in real-time between two brains that, for all purposes, would from now on act together towards the fulfillment of a particular behavioral task. Previously, we have reported that specific motor^{11,12} and sensory parameters^{13,14} can be extracted from populations of cortical neurons using linear or nonlinear decoders in real-time. Here, we tested the hypothesis that a similar decoding performed by a “recipient brain” was sufficient to guide behavioral responses in sensorimotor tasks, therefore constituting a Brain-to-Brain Interface (BTBI)¹⁵ (Figure 1). To test this hypothesis, we conducted three experiments in which different patterns of cortical sensorimotor signals, coding a particular behavioral response, were recorded in one rat (heretofore named the “encoder” rat) and then transmitted directly to the brain of another animal (i.e. the “decoder” rat), via intra-cortical microstimulation (ICMS). All BTBI experiments described below were conducted in awake, behaving rats chronically implanted with cortical microelectrode arrays capable of both neuronal ensemble recordings and intracortical microstimulation¹⁶. We demonstrated that pairs of rats could cooperate through a BTBI to achieve a common behavioral goal.

Results

In our training paradigm, animals learned basic elements of the tasks prior to participating in any BTBI experiments. First, prospective encoder rats were trained to respond to either tactile or visual stimuli until they reached 95% correct trials accuracy. Meanwhile, decoder rats were trained to become proficient while receiving ICMS as a stimulus. A train of ICMS pulses instructed the animal to select one of the levers/nose pokes, whereas a single ICMS pulse instructed a response to the other option. Decoder rats reached a 78.77% ± 2.1 correct trials

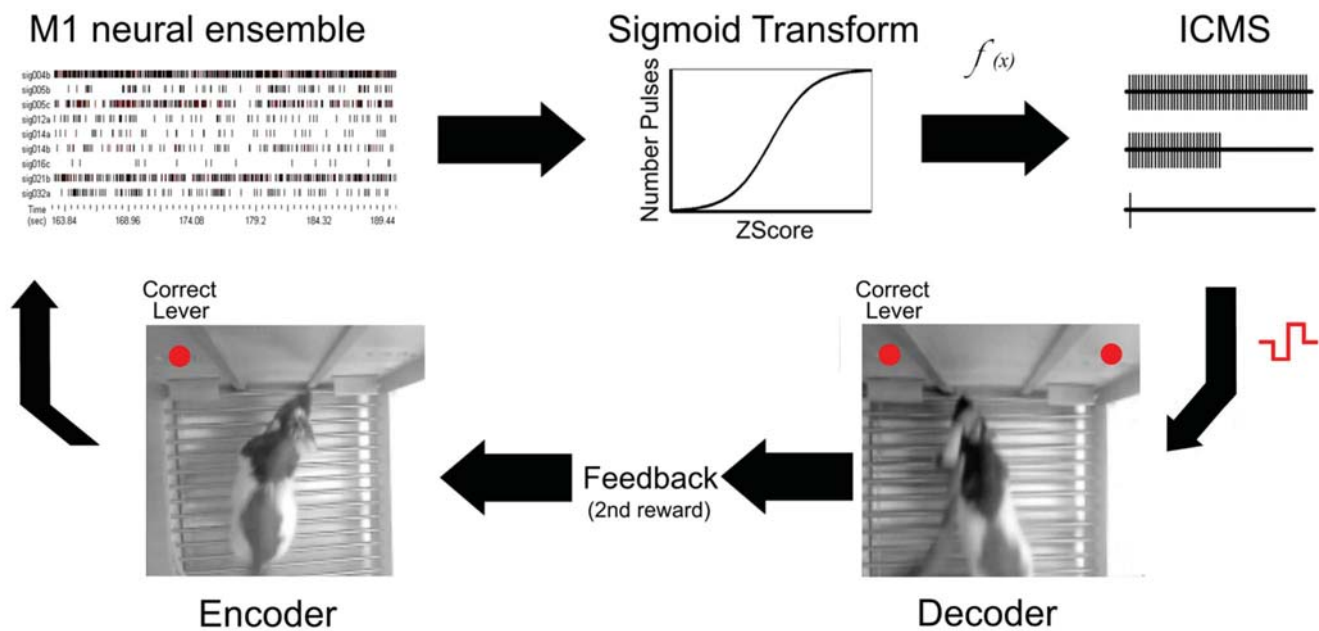


Figure 1 | Experimental apparatus scheme of a BTBI for transferring cortical motor signals. Arrows represent the flow of information from the encoder to the decoder rat. In the motor task, the encoder rat has to identify a visual stimulus, signaled by an LED (red circle), and then press one of two levers to receive a small water reward. Meanwhile, M1 neural activity is recorded from the encoder rat and transmitted to the decoder animal, by comparing the pattern of the encoder's M1 to a template trial (previously built with the firing rate average of a trial sample). The difference between the number of spikes in a given trial and the template trial is used to calculate a Zscore. The Zscore is then converted, through a sigmoid function centered on the mean of the template trial, into an ICMS pattern. Thus, the microstimulation patterns varied in real time, according to the number of spikes recorded from the encoder rat's M1, on a trial by trial basis. Once microstimulation is delivered to the M1 cortex of the decoder rat, this animal has to select the same lever pressed by the encoder. Notice that the correct lever to press is cued only by the pattern of the decoder's M1 microstimulation. If the decoder rat pressed the correct lever, both rats were rewarded. Thus, when the information transfer between the brains of the two rats was successful, the encoder rat received an additional reward that served as positive reinforcement.

performance level. After this preliminary training was completed, the animals were run in pairs, each one in a separate operant box.

The next phase of training began with the encoder rat performing ~10 trials of the motor or tactile task, which were used to construct a cortical ensemble template, i.e. the mean cortical neuronal activity for one of the responses. The increased firing rate associated with the right lever press was selected as the parameter extracted from the neuronal ensemble in the motor task. The increased firing rate associated with the "Narrow" trials was selected as the parameter extracted from the neuronal ensemble in the tactile task. A BTBI session followed in which ICMS trains applied to the cortex of the decoder rat reflected the difference between the template and single-trial neuronal ensemble rates produced by a sample of the encoder rat's M1 or S1 activity. ICMS duration (i.e. number of pulses delivered) was proportional to the difference between the sampled neuronal ensemble firing rate recorded during a given trial and the template normalized by the standard deviation. The time window for the analysis of neuronal activity and ICMS parameters was adjusted in each recording session to maximize the directional signal. The decoder rats reacted to ICMS patterns and not any other cues, as was evident from control experiments in which the performance of those rats dropped to chance level after the ICMS cable was disconnected from the stimulator. Furthermore, the encoder rat received feedback information describing the single trial performance of the decoder rat; each time the decoder rat responded correctly to the ICMS cue, the encoder rat received an additional reward (i.e. water).

In experiment 1 (Figure 1), encoder rats ($N = 3$) pressed one of two levers after an LED on top of the lever was turned on. While the rats did so, M1 neuronal activity was recorded, compared to the template and transformed into ICMS trains applied to M1 of the decoder rats ($N = 4$) who performed the same lever press task. As would be expected, the encoder rats performed better ($95.87\% \pm 1.83$

correct trials) (Figure 2 A) than the decoder rats ($64.32 \pm 1.1\%$; range: 60 – 72% correct trials; Binomial test: $P < 0.05$ in all sessions) (Figure 2 A and B). Yet, the performance of the decoder animals was above chance and highly significant. Indeed, in some experiments the decoder rat's performance using the BTBI was very close to the maximum performance obtained when ICMS was used alone in these animals (72% BTBI vs 78% ICMS alone, see above).

The primary factor that influenced the decoder rat's performance was the quality of spatial information extracted from the encoder rat's M1. The performance was high if the chosen neuronal ensemble accurately encoded left versus right presses (Figure 2 C and Figure 3 A–D). The higher the deviation from the template and hence the larger the duration of the ICMS (i.e. number of pulses delivered), the better was the decoder rat's performance (Figure 2 C and 3 B–D). For this first experiment, a total of 538 units and 110 multiunits were recorded from encoder rats. Sessions were comprised of 48.04 ± 1.5 trials. The response latency of the encoder rats was 20.06 ± 1.0 seconds, while decoder animals responded at 13.59 ± 0.5 seconds. Note that this difference reflects only the effect of both rats working as a dyad (see below for comparison of latencies during training and testing).

In addition to the neuronal transfer from the encoder to the decoder rats, feedback information, related to the decoder rats' performance, was sent back to the encoder animal. This feedback provided an additional reward to the encoder rat every time the decoder rat performed a trial correctly. Under these conditions, the encoder rats' response latency decreased after the decoder rat made an error (after correct response: 20.67 ± 1.665 seconds and after an incorrect response = 15.26 ± 2.031 seconds; Mann Whitney $U = 13570$; $P < 0.0001$). Furthermore, an analysis of the variation in Z-scores, demonstrated that the signal to noise ratio of the neural activity extracted from the encoder rat's M1 increased after the decoder rat

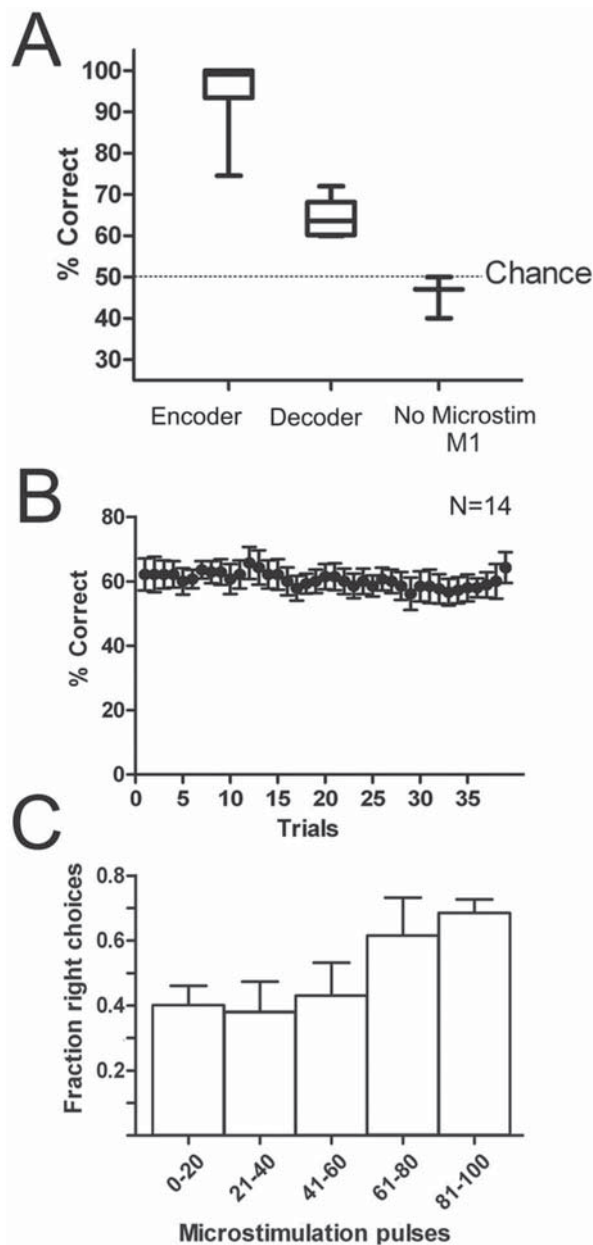


Figure 2 | Behavioral performance using a BTBI for transferring cortical motor signals. A) Performance of encoder and decoder animals during transfer of motor information via a BTBI. The performance of the encoder animals was above 90% in all but one session. The BTBI allowed the decoder animals to repeatedly perform significantly above chance. This performance immediately dropped to chance levels when the cable was disconnected but the system remained fully functional. B) The performance of the decoder animals across a session is presented with a moving average of 10 trials. C) The panel depicts the fraction of right lever presses after different microstimulation patterns were delivered to the decoder's M1. As the number of microstimulation pulses increased, a higher fraction of right lever presses occurred. The microstimulation threshold for response in most animals was situated between 41 and 60 pulses.

committed an error (Chi Square = 4.08, $df=1$; $P = 0.0434$). Thus, both the behavior and neuronal modulations of the encoder rat became dependent on the trial by trial behavioral performance of its dyad partner, the decoder rat.

In experiment 2, we tested whether a BTBI could enable a real-time transfer of tactile information between a pair of rats' brains

(Figure 4). Encoder rats ($N = 2$) were trained to discriminate the diameter of an aperture width with their whiskers¹⁷. If the aperture was narrow, rats were required to nose poke on the left side of the chamber, otherwise they had to poke on the right side of the chamber. Decoder rats ($N = 5$) were trained to poke on the left water port (narrow aperture) in the presence of ICMS and on the right water port (wide aperture) in the absence of ICMS. Similar to experiment 1, the difference between the S1 neuronal ensemble activity, recorded while the encoder rat examined the aperture with its whiskers in each trial, and an average template obtained previously, was utilized to create ICMS patterns applied to the decoder rat's S1. We named these ICMS patterns *virtual narrow* and *virtual wide*. A total of 120 units and 223 multiunits were recorded in experiment 2.

The BTBI accuracy for tactile information transfer was similar to that observed in experiment 1 (Figure 5 A–B). While encoder rats performed at $96.06 \pm 1.14\%$ correct, decoder animals performed somewhat worse but significantly above chance (Percent correct: $62.34 \pm 0.59\%$, range 60 – 64.58%; Binomial test: $P < 0.05$ in all sessions) (Figure 5 A–B and Figure 6 A–D). In this second experiment, the response latency of encoder rats was 2.66 ± 0.1 seconds, while in decoders the latency was 2.68 ± 0.09 seconds.

To further demonstrate that the accuracy of the decoder rats' performance was based on the ICMS patterns, which in turn were triggered by larger number of spikes produced by S1 neuronal ensembles, we compared the fraction of Virtual Narrow choices with the number of ICMS pulses delivered to the decoder's S1 cortex. Increases in the number of ICMS pulses delivered to the decoder's S1 were associated with a higher fraction of Virtual Narrow choices (≤ 25 pulses: 0.3966 ± 0.04476 correct; >25 pulses: 0.5433 ± 0.02991 correct; Paired samples t-test = 2.321, $df = 16$, $P = 0.0338$) (see Figure 5 C and Figure 6 A–D). Since ICMS patterns were directly derived, through a transfer function, from the neural ensemble activity recorded from the encoder animal's S1 cortex in each trial, this result demonstrates that the decoder rat's correct choices relied on the accuracy of the ICMS pattern in reproducing the number of action potentials generated by the real tactile stimulus information presented to the encoder rat. Feedback information, providing an additional reward to the encoder rat every time the decoder rat performed a trial correctly, also induced changes in the neural activity of the encoder rat. The encoder's latency of response was similar after correct and incorrect trials (After correct = 2.6 ± 0.1 secs; After incorrect = 2.7 ± 0.2 secs; Mann Whitney U = 19790, $P = 0.49$). However, similarly to the effects observed in experiment 1, the signal to noise ratio of neural activity in S1 also increased after an incorrect trial (Chi Square = 4.2, $df=1$; $P = 0.0404$).

It could be argued that the results reported here could have been obtained if prerecorded signals from encoder rats had been used to guide the behavior of the decoder rats. Qualitative and quantitative observation of the behavior of the animals reveals that this is not at all the case. In both motor and tactile BTBI sessions we observed drastic changes in the behavior of encoder and decoder rats as soon as they started to work as part of a dyad. Both encoder and decoder animals either made quick attempts to respond earlier or, conversely, they reduced their response rate or even stopped performing according to the dyad behavior. Thus, response latencies during motor BTBI sessions were largely increased for encoder animals (encoder training: 14.77 ± 0.9 seconds; encoder BTBI session: 20.06 ± 1.0 seconds; $t = 3.975$, $df = 1170$, $P < 0.0001$) and decreased in decoder rats (decoder training: 16.29 ± 0.6 seconds; decoder BTBI sessions: 13.59 ± 0.5 seconds; $t = 3.559$, $df = 1636$, $P = 0.0004$). During the tactile BTBI sessions the responses latency was reduced in both encoder (encoder training: 5.40 ± 0.6 seconds; encoder BTBI sessions: 2.66 ± 0.1 seconds; Mann-Whitney U = 13960, $P < 0.0001$) and decoder animals (decoder training: 4.632 ± 0.6 seconds; decoder BTBI sessions: 2.68 ± 0.09 seconds; $t = 4.638$, $df = 12$, $P = 0.0006$) as they

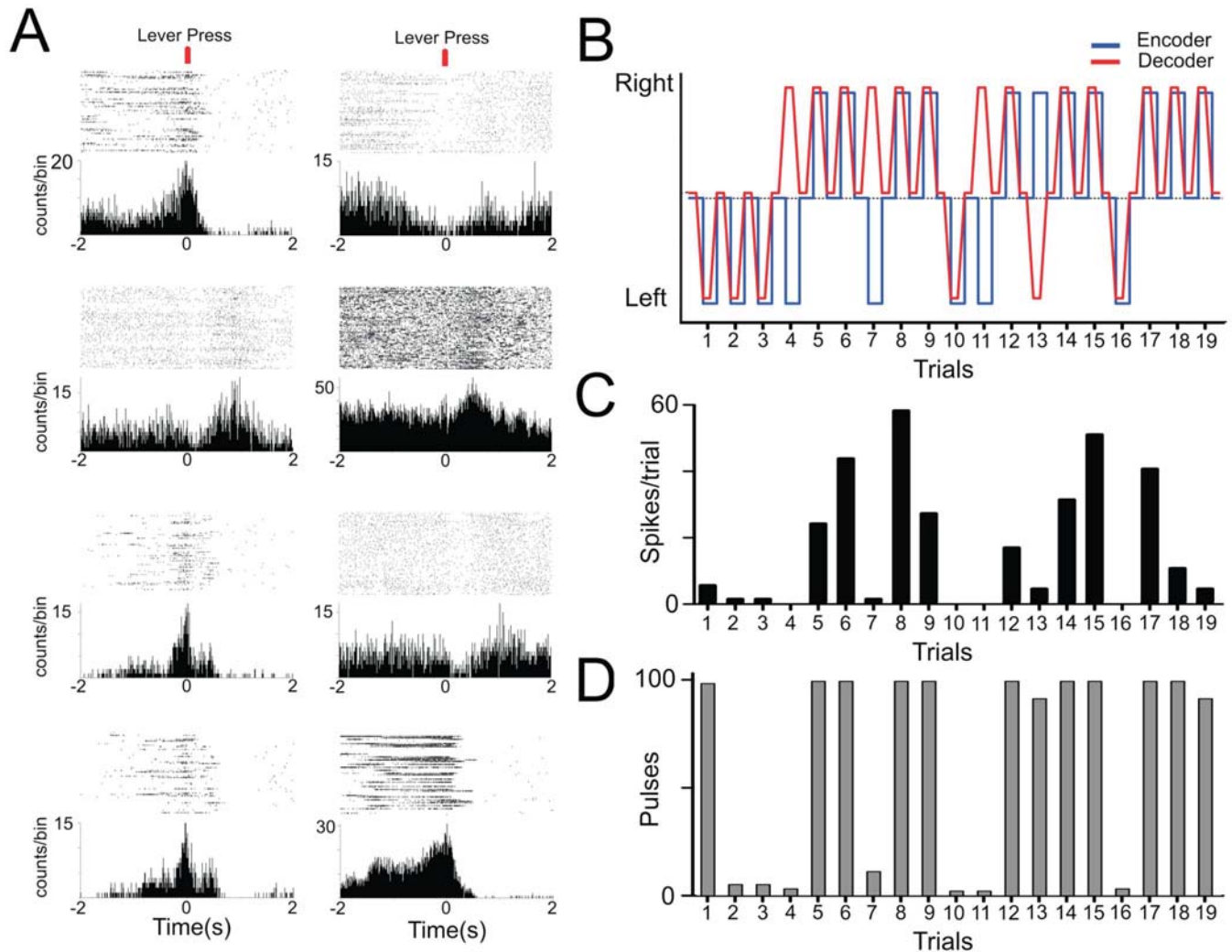


Figure 3 | Trial examples of a BTBI for transferring cortical motor signals. A) Examples of M1 neurons recorded while the encoder rat performed the task. Time = 0 corresponds to the lever press. Very different patterns of increased and decreased activity were observed before and after the lever press, suggesting that multiple task parameters were encoded by this M1 ensemble. B) Sample of trial by trial choices of the rat dyad (encoder and decoder) during execution of the motor task. The encoder's performance is depicted by a blue line, while a red line indicates the decoder's choices in the same trials. In trials 4,7,11 and 13 the behavioral response of the decoder rat did not match the one of the encoder. The overall performance of the decoder rat in this session was 69% correct. C) The bars represent the number of encoder's M1 neuronal spikes recorded during each trial. The neuronal ensemble used in this session encoded very accurately each of the behavioral responses. D) Number of ICMS pulses delivered to the decoder's M1 that resulted from the comparison of each trial in C to the template.

started to work as a dyad. Therefore, the dyad performance depended on the nature of the task performed jointly by the animal pair. Likely the increased latencies observed in the motor task reflect the fact that pressing a lever is a learned artificial behavior, while the exploratory nose poking necessary for the tactile task is part of the rats' natural behavioral repertoire. These overall changes in the dyad behavior, irrespective of their direction (e.g. increased or decreased latency), are a clear indicator that a fundamentally more complex system emerged from the operation of the BTBI; one which required considerable adaptation from the participant animals so that they could jointly perform the sensorimotor tasks.

As the ICMS cues were delivered to primary cortical areas that are commonly involved in processing motor and somatosensory information in intact animals, we further asked how the decoder rat's S1 cortex represented both real tactile stimuli, generated by mechanical stimulation of its own facial whiskers, and ICMS signals representing the encoder rat's whisker stimulation, during operation of a BTBI. To measure this, we tested pairs of encoder and decoder rats during passive transmission of tactile information via a BTBI, while

the whiskers of the encoder and decoder rats were mechanically stimulated. This experiment consisted of two parts: first, the encoder animal was lightly anesthetized and head fixed to an automated whisker stimulator that accurately reproduces the movement and speed at which the whiskers interact with the bars in the active tactile discrimination task (see Methods). The animal's S1 neural activity following each movement of the bars was analyzed in real time and delivered, as an ICMS pattern, to the decoder rat's S1. Meanwhile, the decoder rat remained in an open field in a different room while its S1 neural activity was recorded. After this phase was completed, the decoder animal was also lightly anesthetized and placed in the automated whisker stimulator. This allowed us to determine how the decoder rat's S1 neuronal sample, that responded via the BTBI to the tactile stimuli delivered to the encoder's whiskers, responded to tactile stimuli elicited by passive whisker stimulation of their own vibrissae.

Passive whisker stimulation, in either the encoder or decoder rats, induced significant firing modulations in the decoder rat's S1. These were characterized by clear increases of firing activity occurring

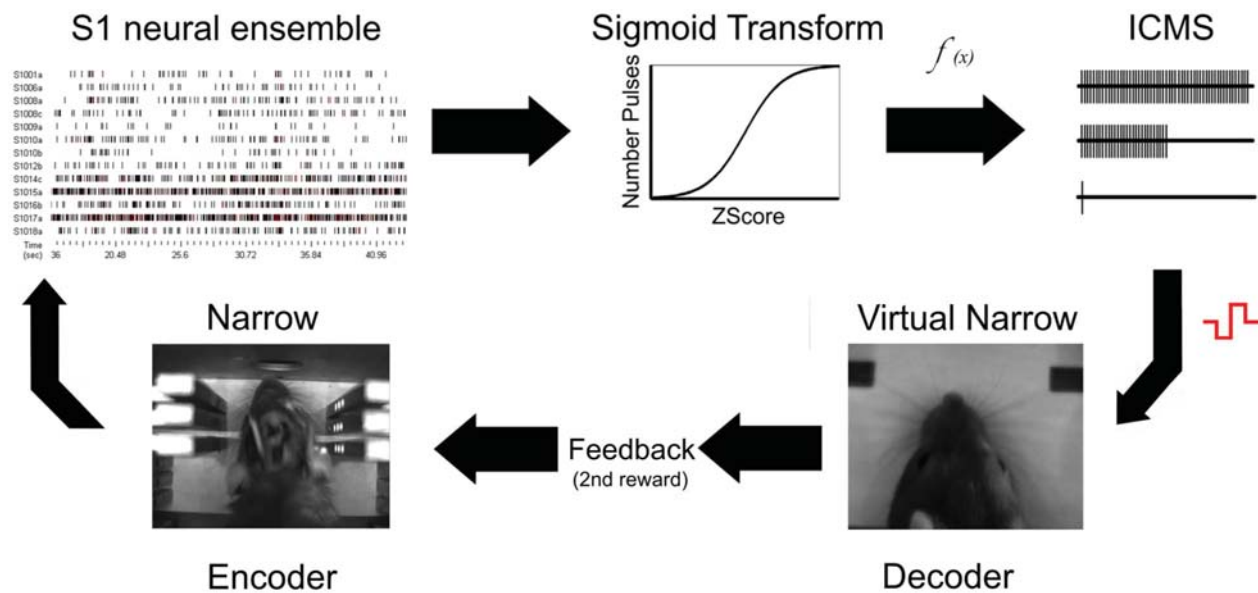


Figure 4 | Experimental apparatus scheme of a BTBI for transferring cortical tactile information. A) In the tactile discrimination task, the encoder animal was required to sample a variable width aperture using its facial whiskers. The width could be “Narrow” as shown in the left photograph, or “Wide”. After sampling, the encoder rat’s S1, which were used to deliver ICMS patterns through the BTBI, and in 93.06% (67/72 multiunits) of the microwires from which S1 neuronal activity was recorded from decoder rats (see Figure 7 B and C). The magnitude of the S1 tactile responses elicited by mechanical stimulation of the decoder rat’s facial whiskers was 4.82 ± 0.4 spikes/trial and the duration was 111.4 ± 11 ms. During the BTBI transmission, ICMS of the decoder’s S1 induced a significant increase of S1 neurons firing activity lasting for 119.7 ± 20 ms. Due to the microstimulation artifact in the recordings, we focused our analysis on the firing activity increases occurring after the last pulse of microstimulation was transmitted (see red traces Figure 7 C).

immediately after the moving bars touched the whiskers of each animal (see Figure 7 A and B). These significant S1 neuronal responses occurred in 70.91% (39/55 multiunits) of the microwires implanted in the decoder rat’s S1, which were used to deliver ICMS patterns through the BTBI, and in 93.06% (67/72 multiunits) of the microwires from which S1 neuronal activity was recorded from decoder rats (see Figure 7 B and C). The magnitude of the S1 tactile responses elicited by mechanical stimulation of the decoder rat’s facial whiskers was 4.82 ± 0.4 spikes/trial and the duration was 111.4 ± 11 ms. During the BTBI transmission, ICMS of the decoder’s S1 induced a significant increase of S1 neurons firing activity lasting for 119.7 ± 20 ms. Due to the microstimulation artifact in the recordings, we focused our analysis on the firing activity increases occurring after the last pulse of microstimulation was transmitted (see red traces Figure 7 C).

Analysis of the data obtained during passive BTBI communication further demonstrated that S1 neurons in the decoder’s brain responded differently for each of the virtual tactile stimuli. More than half of the S1 multiunits recorded presented differential firing rates for Virtual Wide and Virtual Narrow stimuli ($28/44 = 63.64\%$ multiunits). Also, the Virtual Narrow stimulus was characterized by higher neuronal response magnitudes (Virtual Narrow: 3.861 ± 0.6229 spikes/trial; Virtual Wide: 2.200 ± 1.079 spikes/trial; Wilcoxon sum of ranks = 197; $P = 0.0182$) and durations (Virtual Narrow: 102.7 ± 16.28 ms; Virtual Wide: 31.54 ± 14.85 ms; Wilcoxon sum of ranks = 200; $P = 0.0074$). To measure whether the differences in firing rates were due to discrimination or due to an ‘upstate’ related to the repeated microstimulation, we also compared which S1 multiunits exhibited different firing rates for Virtual Narrow and Virtual Wide. From the total of S1 multiunits that displayed differences in firing rates for the discrimination period, we found that 35.7% (10/28 multiunits) had no significant differences in the baseline firing rate. Thus, more than one third of the S1 multiunits recorded showed no signs of an ‘upstate’ in their baseline due to repeated microstimulation. This supports the hypothesis that after

the decoder rats learned to use the BTBI, via ICMS cues, their S1 became capable of accurately representing, processing, storing and recalling information about both the tactile stimuli delivered to its own whiskers, as well as mechanical displacements of the encoders’ facial vibrissae.

Finally, to further demonstrate the range of potential operation of our BTBI preparation, we tested whether a long-distance communication of a rat dyad, with the encoder rat performing the tactile discrimination task at the IIN-ELS (Natal, Brazil) and the decoder rat receiving patterns of microstimulation and responding at Duke University (Durham, USA), would be capable of performing the same task. For this, neural activity recorded from S1 of the encoder rat performing the tactile discrimination task was sent via an internet connection and delivered, as an ICMS pattern, to the decoder rat S1 (Figure 8). Even under these extreme conditions, the BTBI was also able to transfer in real-time behaviorally meaningful neuronal information. Although the mean time of data transmission observed in this long-distance BTBI was increased from 20 ms (during transmission in our Duke lab) to 232 ± 217.5 ms, a similar number of correct responses was found (short distance transmission: $62.34 \pm 0.59\%$; long distance transmission: $62.25\% \pm 0.71$) in 26.5 ± 0.5 trials in the decoder animals.

Discussion

The present study demonstrates for the first time that tactile and motor information, extracted in real time from simultaneously recorded populations of cortical neurons from a rat’s brain, can be transmitted directly into another subject’s cortex through the utilization of a real-time BTBI. Operation of a BTBI by an encoder-decoder rat dyad allowed decoders to rely exclusively on neural patterns donated by encoders in order to reproduce the encoder’s behavioral choice. ICMS patterns reflecting the number of action potentials recorded from either the encoder rat’s M1 or S1 during a single trial were sufficient for decoder rats to repeatedly perform two different tasks, significantly above chance levels, in real-time.

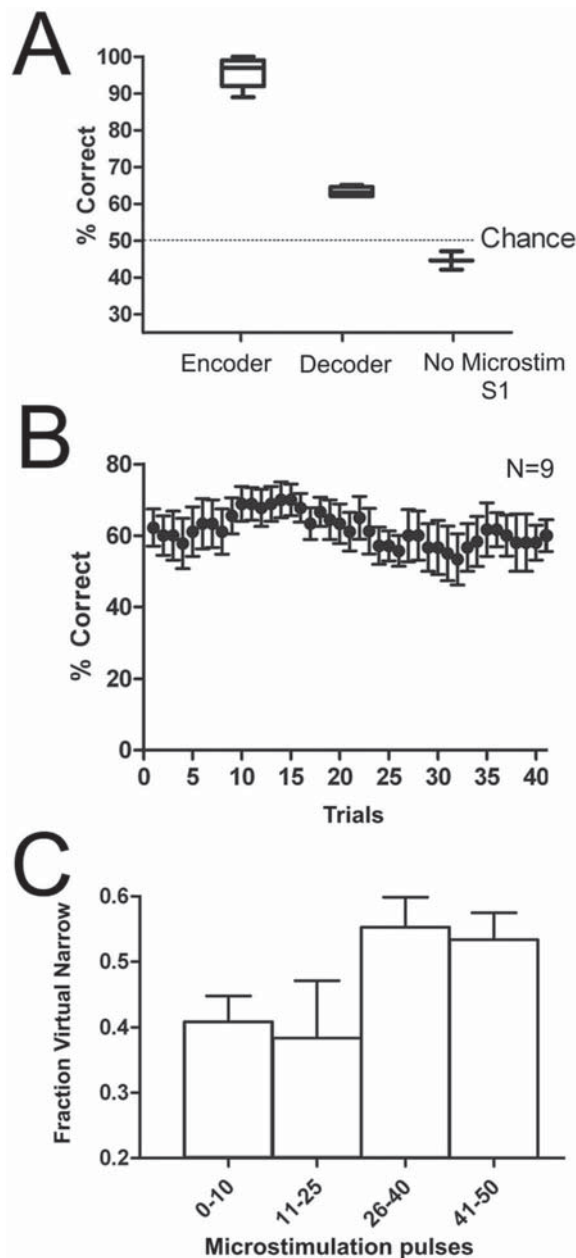


Figure 5 | Behavioral performance using a brain-to-brain interface to transfer cortical tactile information. A) Performance of encoder and decoder animals during operation of a BTBI for tactile information sharing. Notice that the performance of the encoder animals was above 85% in all sessions. The performance of the decoder animals was above 60% in all sessions presented and immediately dropped to chance levels when the cable was disconnected but the system remained fully functional. B) Performance of all decoder animals analyzed with a moving average of 10 trials. C) The panel depicts the fraction of the decoder's responses in the Narrow reward port after different patterns of microstimulation were delivered. As the number of microstimulation pulses increased a higher fraction of responses was observed in the Narrow reward port (Virtual Narrow choice), suggesting that the microstimulation threshold of response for decoder animals was situated between 26-40 pulses.

Interestingly, half of the number of pulses used to stimulate the decoder's M1 were sufficient to successfully deliver a message to the decoder's S1 cortex, suggesting that primary sensory cortical areas may have a lower threshold to operate a BTBI.

We also demonstrated that operation of a BTBI induced differential patterns of activation in the decoder rat's S1. Thus, the same S1 neurons that responded to the mechanical stimulation of the decoder rat's own whiskers were capable of representing information derived from stimulation of the encoder rat's whiskers via the BTBI.

Additionally, continuous operation of the BTBI also affected the behavior and neural activity of the encoder rat, which was able to reduce its response latency and increase the signal/noise ratio of its S1/M1 neuronal activity in response to an error by the decoder rat. As far as we can tell, these findings demonstrate for the first time that a direct channel for behavioral information exchange can be established between two animal's brains without the use of the animal's regular forms of communication. Essentially, our results indicate that animal brain dyads or even brain networks could allow animal groups to synchronize their behaviors following neuronal-based cues.

Successful BTBI operation required four simultaneous conditions to be present: first, the encoder animals had to achieve a very high level of performance in both tasks. As proof, only one successful BTBI session was obtained when the encoder rat's performance was below 80%. Second, recordings from the encoder's cortex had to yield stable neural ensemble activity which was highly correlated to the behavior that needs to be encoded by the BTBI. Note that successful BTBI operation was achieved using information collected from random ensembles of neurons dispersed within each cortical area. This finding indicates that information was not anatomically segregated either in S1 or in M1. Third, the midpoint of the sigmoid transfer function (which was set at the beginning of the session) had to closely match the midpoint of the neural function that represented the two stimuli/actions. We found that such a midpoint tended to be the same for each cortical region, suggesting that groups of neurons with similar physiological profiles were recorded in most cases. Fourth, our results showed that both encoder and decoder rats changed their behavior according to the dyad performance. This observation suggests that operation of a BTBI induces the establishment of a highly complex system, formed by a pair of interconnected brains. As such, this brain dyad behaved in a way that could not be predicted if only pre-recorded neural signals had been used for encoding purposes. We speculate that the description of the complex system generated by the dyad transferring information and collaborating in real time, will reveal fundamental properties about the neural basis of communication and social interactions^{18,19}.

Although we have shown accurate transfer of brain-derived motor and sensory information through a BTBI, it remains to be explained how the brain simultaneously integrates information generated by direct ICMS and by natural stimuli (e.g. real whisker stimulation). Previous studies in rhesus monkeys have shown that the brain is able to decode highly complex ICMS patterns in a single trial⁷. Specifically, it has been shown that a brain-machine-brain control loop allows for continuous update of information in the S1 cortex, while a monkey explores a virtual tactile stimulus. The effects of a neuroprosthetic's operation on cortical neuronal responses have also been studied in the representation of the rat forelimb sensorimotor cortex, where it was shown that information flow can be altered by S1 microstimulation²⁰⁻²². Lastly, a recent study has shown that the ability to use a BMI is mediated by the striatum in mice⁴. Altogether, this body of evidence supports the notion that continuous use of ICMS to deliver information to the brain is associated with plastic changes in neuronal ensemble responses in cortical and subcortical regions. The data obtained here during passive BTBI operation supports this conclusion by showing that as animals learned to use the microstimulation cues, differential patterns of S1 neuronal responses emerged for each of the virtual tactile stimuli. This finding is consistent with our previous observation that S1 neurons undergo significant functional plasticity during the period in which rats learn a tactile discrimination task²³. Accordingly, our results further suggest that successful

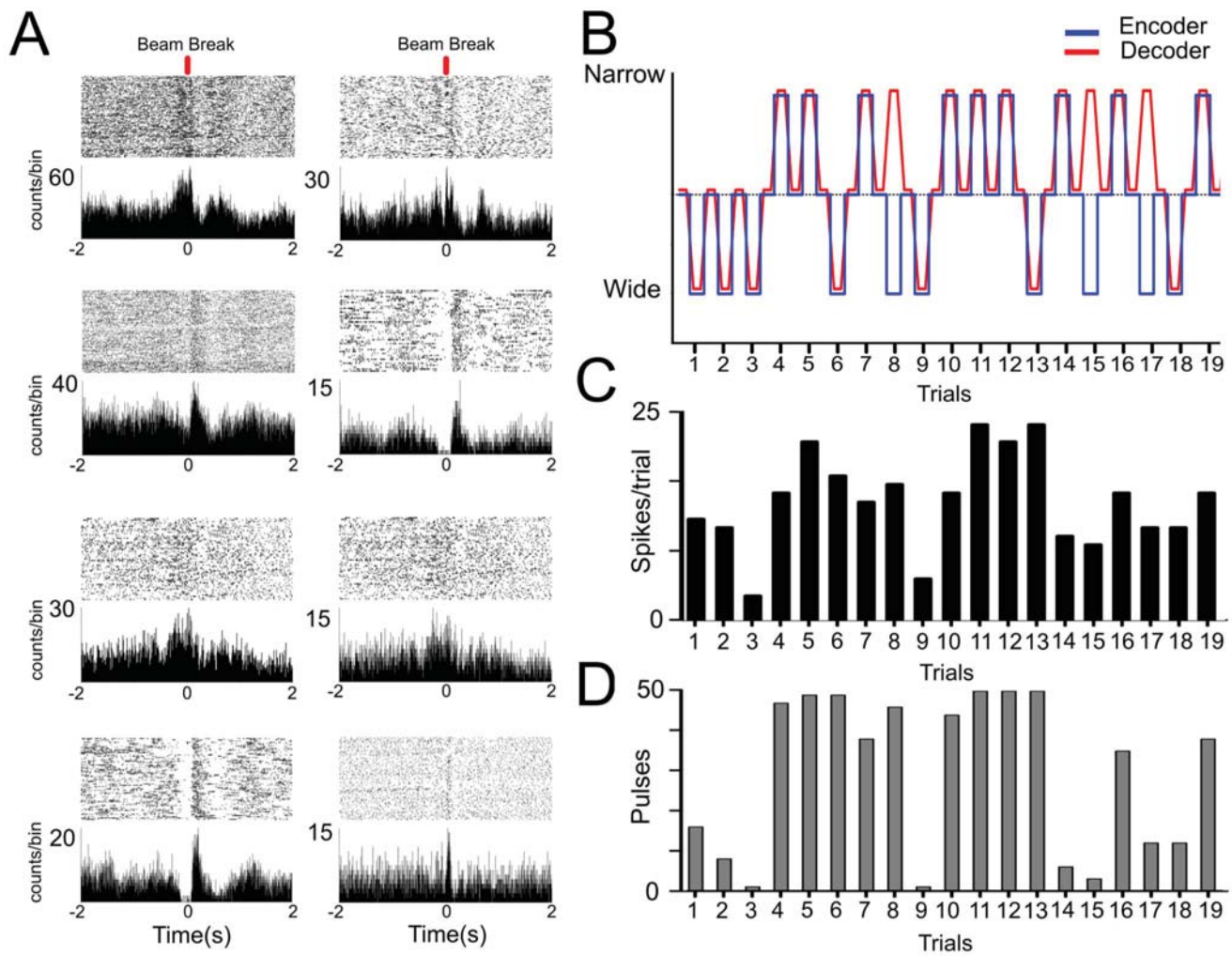


Figure 6 | Trial examples of a BTBI for transferring cortical tactile signals. **A**) Examples of S1 neurons recorded while an encoder rat performed the aperture discrimination task. Time = 0 corresponds to the moment the animal breaks the photo beam in front of the discrimination bars. **B**) Blue lines represent the choices of the encoder rat and red line represents the choices of the decoder rat. In trials 8, 15 and 17 the decoder rat selected the incorrect reward port. **C**) Number of action potentials recorded from 3 S1 neurons in each trial after the whiskers sampled the discriminanda. Typically, a higher spike count was found for narrow trials, when compared to wide trials. **D**) Number of pulses delivered to the S1 cortex of the decoder rat in each trial. The number of pulses delivered to the S1 cortex of the decoder rat was directly derived from the number of spikes present in the encoder animal in each trial. The overall performance achieved by the rat dyad in this session was 64% correct trials.

BTBI operation is fundamentally linked to the ability of S1 ensembles to undergo plastic reorganization in response to microstimulation patterns²⁴.

Altogether, the results described here indicate that the channel capacity (amount and precision of information, bandwidth) and the dynamic properties of cortical neuronal ensembles are the two major determinants of the amount and quality of information that can be transferred between animal brains via a BTBI. Thus, beyond the neurobiological challenge of understanding how the brain integrates natural and virtual stimuli, a second class of problems directly related to the characteristics of the BTBI as a channel for information transfer must be addressed. In general terms, the BTBI can be described as a discrete noisy channel, meaning “a system whereby a sequence of choices from a finite set of elementary symbols $S_1; \dots; S_n$ can be transmitted from one point to another”²⁵. The limit for the amount of information that can be transferred by unit of time (i.e. capacity)²⁵ is currently unknown for a BTBI channel. In the tasks used here, the minimum and maximum inputs depended on the range of the firing rate in the neurons used, while the output depended mostly on the electrical microstimulation threshold of cortical ensembles in the decoder’s brain. However, channel capacity

can be increased while still using the rationale described in Figure 1 (neural data - transfer function - stimulation delivery). For example, it will be important to test in the future the effect of other types of inputs (e.g. larger neuronal ensembles; Local Field Potentials), transfer functions (e.g. exponential, linear, step functions) and outputs (e.g. one versus several pairs of microelectrodes used for ICMS, disposed in 2D or 3D cortical space, delivering photostimulation instead of electrical current) on the overall dyad performance. In this context, we expect that the use of newly introduced microelectrode cubes, created in our laboratory, that spread across 3D cortical space, to deliver spatiotemporal patterns of information from the encoder’s brain to the decoder’s will provide a significant increase in BTBI bandwidth, likely leading to a substantial improvement in the overall animal dyad performance.

Lastly, it is important to stress that the topology of BTBI does not need to be restricted to one encoder and one decoder subjects. Instead, we have already proposed that, in theory, channel accuracy can be increased if instead of a dyad a whole grid of multiple reciprocally interconnected brains are employed. Such a computing structure could define the first example of an organic computer capable of solving heuristic problems that would be deemed non-computable

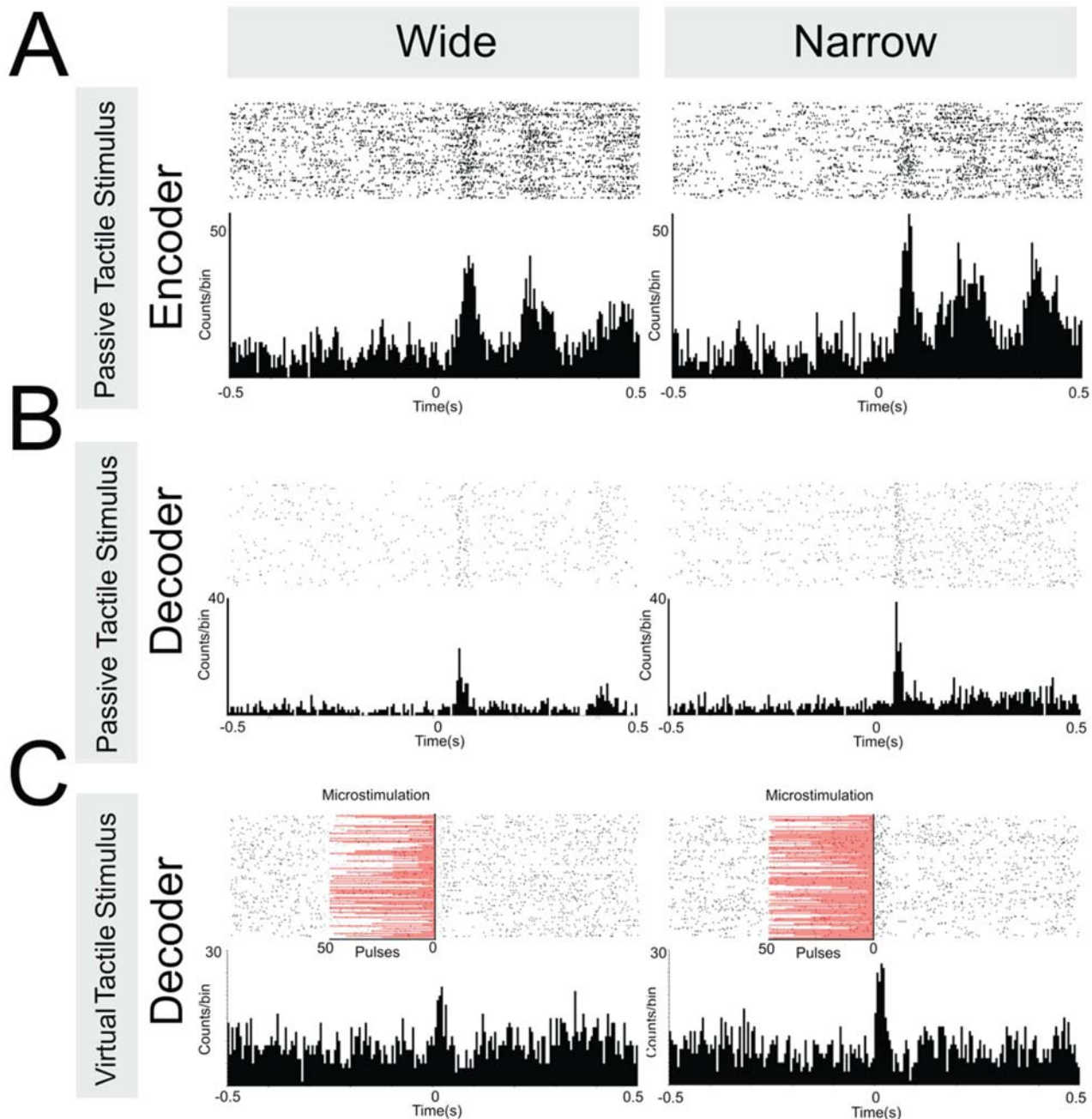


Figure 7 | Neural activity in the decoder brain discriminates stimuli applied to the encoder's whiskers. PSTHs on the left panels show S1 neuronal responses during the wide tactile stimulus whereas PSTHs on the right panels depict narrow tactile stimulus. The top and middle panels show S1 activity recorded in anesthetized encoder and decoder rats while their facial whiskers were passively stimulated by a set of moving bars. The moving bars generate a tactile stimulus exactly like the one produced during the tactile discrimination task. The lower panels represent the decoder rat's S1 activity while receiving ICMS (red traces) via a BTBI that transmitted tactile information from an anesthetized encoder rat which was having its whiskers passively stimulated. Time zero in all panels corresponds either to the tactile stimulus or the last microstimulation pulse. **A**) A clear peak of S1 activity can be observed immediately after the encoder's whiskers contacted the bars (other peaks occurred due to rebounding of the moving bars). Increased counts of action potentials were typically associated with the narrow stimulus (compare peaks in left versus right panels). **B**) Like encoder rats, when the decoder rats' whiskers were passively stimulated by the moving bars, clear peaks of S1 activity with different heights can be observed (see left versus right panels). **C**) When the encoder rats' whiskers were passively stimulated (shown in **A**) and the BTBI was used to transfer tactile information in real time (shown in **C**), clear increases in activity were observed in the decoder's S1 cortex after time 0. These S1 firing modulations were larger when the narrow stimulus was applied to the encoders' whiskers when compared to the wide stimulus (see left versus right panels) and were observed in the same S1 neuronal ensembles that responded to natural whisker stimuli (shown in **B**). Thus, the S1 neuronal responses observed in the decoder rat demonstrate that it learned to use the BTBI and that a representation of the tactile stimuli applied to the encoders' whiskers could be superimposed on the preexisting representation depicting tactile stimuli applied to its own facial whiskers.

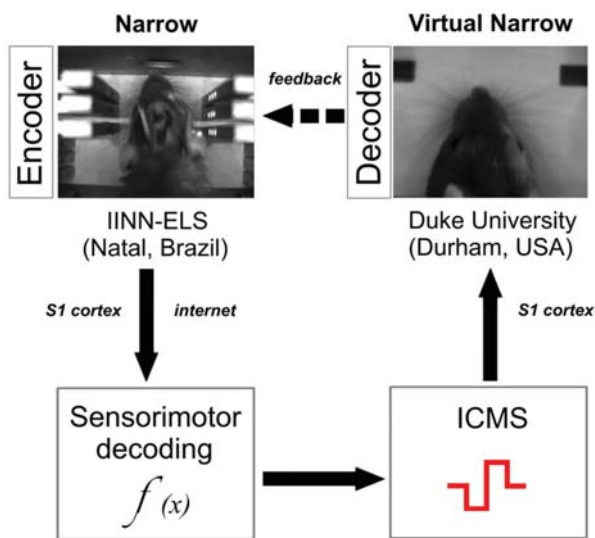


Figure 8 | Intercontinental brain-to-brain interface to transfer cortical tactile information. To test the full potentialities of the BTBI, a brain-to-brain interface to transfer cortical tactile information was established between our laboratory at the IINN-ELS in Brazil and our laboratory at Duke University in the USA. An encoder rat performed a tactile discrimination task at the IINN-ELS. Meanwhile its neuronal activity in S1 was recorded and sent over the internet to our laboratory at Duke University. The sigmoid transformation algorithm was used to transfer the number of action potentials into microstimulation patterns that were then delivered to the decoder rat's S1 cortex. As the decoder rat made a behavioral response, feedback was sent over the internet to the encoders' chamber back at the IINN-ELS.

by a general Turing-machine. Future works will elucidate in detail the characteristics of this multi-brain system, its computational capabilities, and how it compares to other non-Turing computational architectures²⁶.

Methods

All animal procedures were performed in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals and were approved by the Duke University Institutional Animal Care and Use Committee. Long Evans rats weighing between 250–350 g were used in all experiments.

Motor brain-to-brain interface. The behavioral motor task consisted of a dark operant chamber equipped with two levers, one LED (Light Emitting Diode) above each lever and, on the opposite wall, a water reward port. Animals were trained to press one of two levers, cued by an LED turned on at the beginning of each trial. A correct choice opened the reward port and allowed brief access to water (300 ms). When animals reached stable performances above 80% correct choices they were assigned either to an encoder or decoder group. The operant chamber configuration remained similar in both the encoder and the decoder groups. Animals assigned to the encoder group were implanted with recording arrays of 32 microelectrodes in the primary motor cortex and after recovery resumed the initial training scheme. Animals assigned to the decoder group were implanted with arrays of 4 to 6 microstimulation electrodes in the primary motor cortex and were further trained to associate the presence of electrical microstimulation pulses with the correct lever press. Extra training followed, with a sequence of 60 to 100 pulses indicating a correct choice in the right lever while the absence of microstimulation pulses (1 pulse) indicated a correct left lever choice. During the electrical microstimulation training phase a trial started with a brief period of white noise, followed by the electrical microstimulation cue. Immediately after this cue both LEDs were turned on. If a correct choice was made the reward port would open and the animal was allowed a brief period of access to water (300 ms), otherwise both LEDs were turned off and the intertrial interval started.

When decoder animals reached stable performances above chance, brain-to-brain interface (BTBI) sessions with motor activity were performed. These sessions were composed of three different phases: 1) identification of cells accurately encoding the motor action performed, 2) data collection for template trials during a rat-to-brain mode and, 3) brain-to-brain interface using real-time analysis followed by electrical microstimulation. There were no setup differences between the rat-to-brain mode and the brain-to-brain interface. The only difference between these two conditions

was that in the rat-to-brain mode the patterns of microstimulation depended on the behavior of the encoder animal, while during the brain-to-brain interface mode the patterns of microstimulation depended solely on the neural activity of the encoder rat.

Neural activity was first studied in encoder animals performing the behavioral task to identify units that accurately encoded for the motor activity associated with each lever press. During the rat-to-brain mode the operant chambers remained as in the training phase, however the presence of a correct choice in the encoder operant chamber activated the pattern of electrical microstimulation cue corresponding to the same lever on the decoder animal operant chamber. A correct choice by the decoder rat was signaled by a tone and both encoder and decoder animals were allowed a brief period of access to water. The goal of this phase was to create a template trial based on the neural activity of the encoder rat pressing one of the levers in several trials.

During the brain-to-brain interface mode the number of action potentials in each trial was compared to the template trial and the Zscore of the difference between them was used to determine the number of pulses present in the pattern of microstimulation. A sigmoid function was used to transfer the Zscore value to the number of pulses present in the microstimulation pattern. A higher Zscore was associated with an increased number of pulses in the microstimulation cue (i.e. right lever) while a lower Zscore was associated with a decreased number of pulses in the microstimulation cue (e.g. left lever).

Active tactile brain-to-brain interface. Mildly water deprived animals were trained to perform a behavioral discrimination task as previously described¹⁷. Briefly, this task required animals to discriminate between a wide or narrow aperture in order to receive a water reward. The animal was placed in the behavioral box compartment where it waited for the central door to open and allow access to the second compartment, the inner chamber. After the animal entered the inner chamber, it had to pass through the variable width discrimination bars and then nose poke the center of the front wall. The nose poke in the inner chamber opened two water reward pokes located in the outer chamber from which the animal had to select one. The reward poke on the right corresponded to the wide aperture, whereas the poke on the left corresponded to the narrow aperture. As the animal chose from one of the reward pokes, the door separating the inner and outer chambers closed. Correct responses were rewarded by 50 μ l water rewards. Incorrect responses were followed by immediate closing of the reward pokes. The percent of trials performed correctly was used as a measure of tactile discrimination.

Animals were then evenly assigned to encoder or decoder groups. Encoder animals ($n = 2$) were implanted with recording arrays of 32 microelectrodes in the right S1 and after recovery resumed the initial training scheme. Animals assigned to the decoder group were implanted with arrays of six microstimulation electrodes in the right S1 ($n = 7$). In six decoder animals, recording electrode arrays were also implanted either in the right ($N = 1$) or in the left S1 ($N = 5$). After recovering from surgery decoder animals were further trained to associate the presence of electrical microstimulation pulses with the correct lever press. Extensive training followed, with a sequence of 50 pulses indicating a correct choice in the left reward poke while the absence of microstimulation pulses (1 pulse) indicated a correct choice in the right reward poke. Decoder animals were required to identify the microstimulation cue and associate it with a behavioral response in one of the reward pokes. A brief tone indicated the beginning of the trial immediately followed by the microstimulation cue. After a period of 500 ms both reward pokes would open and the rat was required to make a response in one of the photo beams. A correct choice was followed by a brief tone and access to water. When decoder animals reached stable performances of $> 65\%$ correct trials for 3 consecutive sessions, tactile BTBI sessions began.

Neural activity was first studied in encoder animals performing the behavioral task to identify units that accurately encoded for the tactile stimuli associated with sampling the width between bars. During the rat-to-brain mode the operant chambers remained as in the training phase, however the microstimulation cue presented to the decoder animal always matched the stimulus presented to the encoder animal. After a correct response by the encoder rat, a brief tone followed by a microstimulation cue of 1 or 50 pulses was sent to the decoder animal and both reward ports in the second chamber would open. If the decoder rat accurately discriminated the microstimulation cue both rats were rewarded. During the brain-to-brain interface mode the neural activity of the encoder rat was analyzed from the moment that the rat broke the discrimination bars photo beam to the moment that the rat broke the photo beam in the center poke. The number of action potentials found in this interval was then counted and compared to the distribution of the Zscores relative to the spikes present in all the previous Wide trials. A Zscore was determined and transferred using a sigmoid function, into the number of pulses present in the pattern of microstimulation.

Passive tactile brain-to-brain interface. The encoder animal was anesthetized and remained head fixed in one room, while the decoder rat was in an open field in a different location with the neural activity also being recorded. The encoder rats' whiskers were then stimulated by a set of moving bars that accurately reproduce the dynamics observed in the active tactile width discrimination task¹⁴. The bars were set up for Wide or Narrow widths and, for each width, the head fixed animal was stimulated for approximately 6 minutes at 0.3 Hz. Neural activity was first analyzed in real time and units with clear whisker related activity were used for the session. To establish a baseline distribution; an initial group of 100 wide stimulus trials was recorded. Then the passive brain-to-brain interface mode started. The encoders' whiskers were stimulated by the moving aperture corresponding to a Wide stimulus. Meanwhile the number of action potentials recorded from the encoder animal was



counted. The number of action potentials was compared to the distribution of action potentials found at the baseline at the beginning of the session and a Zscore was calculated. This Zscore was transferred into the number of pulses to be used in the microstimulation using a sigmoid function. The decoder rat then received the pattern of microstimulation derived from the sigmoid function. Immediately after the encoder animal had been passively stimulated with both Wide and Narrow widths, the decoder animal was anesthetized, head fixed, and its whiskers were passively stimulated with the same Wide and Narrow stimuli as the encoder animal.

Surgery for microelectrode array implantation. Fixed or movable microelectrode bundles or arrays of electrodes were implanted in the M1 and S1 of rats. Craniotomies were made and arrays lowered at the following stereotaxic coordinates for each area: S1 [(AP) -3.0 mm, (ML), +5.5 mm (DV) -0.7 mm], M1 [(AP) +2.0 mm, (ML) +2.0 mm, (DV) -1.5 mm].

Electrophysiological recordings. A Multineuronal Acquisition Processor (64 channels, Plexon Inc, Dallas, TX) was used to record neuronal spikes, as previously described²⁷. Briefly, differentiated neural signals were amplified (20000–32,000×) and digitized at 40 kHz. Up to four single neurons per recording channel were sorted online (Sort client 2002, Plexon inc, Dallas, TX). Online sorting was validated offline using Offline Sorter 2.8.8 (Plexon Inc, Dallas, TX).

Intracortical electrical microstimulation. Intracortical electrical microstimulation cues were generated by an electrical microstimulator (Master 8, AMPI, Jerusalem, Israel) controlled by custom Matlab script (Nattick, USA) receiving information from a Plexon system over the internet. Patterns of 1–100 (bipolar, biphasic, charge balanced; 200 µsec) pulses at 400 Hz (motor BTBI) or 250 Hz (tactile BTBI) were delivered to the cortical structures of interest (M1 and S1 respectively). Current intensity varied from 38–200 µA (motor BTBI) and 30–240 µA (tactile BTBI).

Data analysis. For both behavioral tasks the number of correct responses was used as a measure of behavioral performance. We also analyzed the animals' response latency as a measure of independency between the performance of each animal alone or in a dyad.

Neuronal data were processed and analyzed using Neuroexplorer (version 3.266, NEX Technologies) and custom scripts written in Matlab (7.9.0, Mathworks, Natick, MA). Statistical significance of neural responses was evaluated using a method based on cumulative-summed spike counts^{28,29}. Comparisons of characteristics of neural responses for different conditions were performed using non-parametric tests (Mann-Whitney-Wilcoxon or Kruskal-Wallis). Signal-to-noise ratio of neural responses was calculated as the proportion of responses, occurring after a correct or incorrect decoder response, that presented Zscore absolute values above 0.3 standard deviations. This specific value was used because it corresponded to the midpoint of the sigmoid curve). Statistical significance was determined using a chi square test for proportions.

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Author contributions

M.P.V., M.A.L. and M.A.L.N. designed experiments, analyzed data and wrote the paper; M.P.V., C.K. and J.W. conducted experiments.

Additional information

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>

Competing financial interests: The authors declare no competing financial interests.

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