Supporting Information

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SI Methods

Stimulation Parameters. The stimulation parameters used in the present study were trains of biphasic pulses of 200 μ s, duration of 3s, frequency of 50 Hz, and intensity of 4 mA. These parameters are similar to the parameters of intracortical microstimulation (ICMS) experiments on the human insular cortex (1–4). In particular, note that, unlike in most neurophysiological studies in monkeys, the used frequency in the studies of human insula is typically 50 Hz.

It is well-established that long train stimulation can evoke meaningful behaviors in both subcortical structures (5-7) and the cerebral cortex (8-10). In a recent study, Tolias et al. (11) performed ICMS with biphasic pulses (pulse width = $200 \ \mu s$) and currents up to 2 mA delivered at 100 Hz and lasting 4 s on V1 macaque cortex during MRI to assess the spread of cortical activation by analyzing blood oxygenation level-dependent (BOLD) activity. Results showed that current spread around the electrode tip was bigger than expected from previous calculations based on passive spread of current (12-15), most likely because of horizontal projections. Most interestingly, however, the stimulation induced a BOLD response within a hodologically connected network of areas. These data show that parameters very similar to the ones used in the present experiment could be used to probe cortical functions by means of a physiological activation of a cortical network.

To evaluate the validity of spatial localization of the elicited behavior in our study, we assessed the degree of spatial clustering of evoked responses by using cumulative distributions of the Euclidean distances between stimulation sites evoking a given behavior and stimulation sites evoking either the same (distribution within) or different (distribution between) behaviors. The results confirmed the segregation of different behavior elicited by ICMS.

Instantaneous Heart-Rate Frequency Analysis. Electrocardiographic activity was collected with surface electrodes (Medtronic). Electrocardiogram (EKG) signal was amplified (CED 1902; Cambridge

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Electronic Design) and sent to a computer by an analog-digital converter (ADC) (Power 1401; Cambridge Electronic Design). EKG data and stimulation signals were monitored online and stored by a computer program (Spike2; Cambridge Electronic Design). Raw signal was filtered and automatically analyzed for QRS complexes. Instantaneous heart rate frequency (HR) was then calculated as the inversed temporal difference between every two consequent QRSs: HR[t] = 1/(QRS[t] - QRS[t - 1]). Baseline HR was calculated as the mean HR in a 60-s period immediately preceding the stimulation.

ICMS effect on the HR was assessed within a 10-s period starting at stimulation onset. For this purpose, relative-to-baseline HR profiles consisting of 21 epochs were first calculated by interpolation. For every epoch and behavior outcome, average relative HRs were calculated and confronted against zero with two-tail *t* tests. Significance level was fixed at P < 0.05, and false discovery rate (FDR) corrected for multiple corrections (16). Significance is indicated in HR plots with dots overlaid on the relative HR profiles (black, significant; white, nonsignificant). Peak deviation per outcome and corresponding significance level are reported in the text.

Histological Control. At the end of the experiment, the animal was anesthetized with ketamine hydrochloride (15 mg/kg i.m.) followed by an i.v. lethal injection of sodium thiopental that was perfused through the left cardiac ventricle with saline, 3.5-4% paraformaldehyde, and 5% glycerol in that order. All solutions were prepared in phosphate buffer (0.1 M, pH 7.4). Each brain was then blocked coronally on a stereotaxic apparatus, removed from the skull, photographed, and placed in 10% buffered glycerol for 3 d and 20% buffered glycerol for 4 d. Finally, it was cut when frozen into 60-µm-thick coronal sections. The sections were stained by using the Nissl method. Outer and inner cortical contours, the locations of the electrolytic lesions, and the electrode tracks were assessed, plotted, digitalized by a computer program, and used for the reconstruction of the penetration sites.

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Fig. S1. Spatial clustering of evoked behaviors as shown by cumulative distributions of the Euclidean distances between stimulation sites evoking a given behavior and stimulation sites evoking either the same (distribution within) or different (distribution between) behaviors. *Upper Left* shows the results for monkey 1 (M1). *Lower Left* shows the results for M2. *Left* displays the overall distributions. Distances within (M1: average = 3.4 mm, SD = 2.5, n = 171,502; M2: average = 4.9, SD = 3.5, n = 68,847) are much smaller than distances between (M1: average = 6.2 mm, SD = 3.6, n = 976,828; M2: average = 8.4, SD = 4.4, n = 311,810). In other words, similar behaviors are evoked by adjacent point. *Right* presents the distributions separately for each evoked behavior. Note that the calculations were performed only on stimulus-evoked behavior and that the points from which no behavior was elicited were not included in the database.



Fig. S2. Unfolded view of the lateral sulcus of the left hemisphere of M2 depicting the upper bank of the lateral sulcus (UBLS), the insula, and the lower bank of the lateral sulcus (LBLS). Each dot indicates the entrance point of the electrode in that region according to specific AP coordinates. Black arrows indicate the AP positions of the central sulcus (C) and the intraparietal sulcus (IP). In all penetrations, several sites were stimulated every 500 mm below the entrance point and above the exit point. (A) Posterior dorsal field. Red dots, mouth movements; yellow dots, hand movements; green dots, face movements; gray dots, lower Legend continued on following page

and upper limbs. (B) Anterior field. Red dots, ingestive behavior; blue dots, disgust behavior; green dots, movement inhibition. (C) Ventral field. Yellow dots, affiliative behavior. (D) Miscellaneous responses. Gray dots, discomfort reactions; purple dots, gaze-trunk contralateral displacement; yellow dots, twitch of the chest; red dots, tremors. (E) Unresponsive sites. (F) Percentage of sites per each category. Blue, sensorimotor sites; green, ingestive sites; yellow, disgust sites; orange, affiliative sites; red, movement inhibition sites; tan, miscellaneous responses sites; gray, unresponsive sites.



Fig. S3. Instantaneous HR for each category of behavioral responses. Black lines, mean of the instantaneous HR for both monkeys; dark gray, M1; light gray, M2; abscissa, time in seconds; time 0, stimulation onset; ordinates, percentage of deviation from baseline (*SI Methods*). The number of stimulations is shown under each panel (black, M1 plus M2; dark gray, M1; light gray, M2).



Movie S1. Hand movements. Effects of ICMS of the sensorimotor sector of the insula. Red light on the primate chair indicates the stimulation time. In the first couple of video clips, ICMS elicits the rotation of the wrist and the finger closure. In the second couple of video clips, ICMS elicits visual explorations of the hand, likely because of some somatosensory feeling.

Movie S1



Movie S2. Ingestive behavior. Effects of ICMS of the ingestive sector of the insular region. Different types of chewing and mouthing movements are presented. Conventions are as in Movie S1.

Movie S2



Movie S3. Ingestive behavior. Effects of ICMS of the ingestive sector of the insular region. Examples of context-dependent licking are presented. Conventions are as in Movie S1.

Movie S3



Movie 54. Disgust-related behavior. Effects of ICMS of the disgust-related sector of the insular region. Grimace responses and food refusals are presented. Conventions are as in Movie S1.

Movie S4



Movie S5. Disgust-related behavior. Effects of ICMS of the disgust-related sector of the insular region. Retching responses. Conventions are as in Movie S1.

Movie S5



Movie S6. Affiliative behavior. Effects of ICMS of the affiliative sites of the insular region. Note the change from aggressive to affiliative behavior after stimulation. Conventions are as in Movie S1.

Movie S6