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Report

Emotional and Social Behaviors Elicited by Electrical Stimulation of the Insula in the Macaque Monkey

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Summary

Evidence from a large number of brain imaging studies has shown that, in humans, the insula, and especially its anterior part, is involved in emotions and emotion recognition [1–8]. Typically, however, these studies revealed that, besides the insula, a variety of other cortical and subcortical areas are also active. Brain imaging studies are correlative in nature, and, as such, they cannot give indications about the necessary contribution of the different centers involved in emotions. In the present study, we aimed to define more clearly the role of the insula in emotional and social behavior of the monkey by stimulating it electrically. Using this technique, one may determine whether direct activation of the insula can produce specific emotional or social behaviors and exactly which parts of this structure are responsible for these behaviors. The results showed that two emotional behaviors, a basic one (disgust) and a social one (affiliative state), were easily elicited by electrical stimulation of specific parts of the insula. Both behaviors were characterized by specific motor and vegetative responses and by a dramatic change in the monkey's responsiveness to external stimuli.

Results and Discussion

Emotions are particular brain states that allow individuals to cope with the challenges of their physical and social environment. One of the centers involved in emotion processing is the insula (see [9]). Studies in monkeys have shown that the insula receives interoceptive, nociceptive, and other somatosensory inputs. It also receives information from the main subcortical nodes of the emotional network, as well as inputs from higher-order visual and acoustical cortical areas [1, 10, 11]. A series of recent human brain imaging data supported the notion that the insula is involved in emotion [3, 12]. However, given the correlative nature of brain imaging studies, these data could not elucidate whether the insula might have a causal role in emotion production. Attempts to clarify it have been carried out both in humans (neurosurgery patients) and monkeys using electrical stimulation [13–18]. However, these attempts have not solved the issue because of severe methodological limitations: short duration of the experiments in patients and use of anesthesia in monkeys. In the case of monkey studies in particular, the data showed motor and visceromotor responses, but not fully fledged emotional behaviors.

The present experiments were carried out on two behaving macaque monkeys (*Macaca mulatta*, M1 and M2) habituated to interaction with the experimenters. Intracortical microstimulation (ICMS) was carried out through tungsten microelectrodes. One hundred thirty-three penetrations were performed in the left insula of M1, and 71 penetrations were performed in that of M2. For each penetration, stimulations were performed every 500 μm. As stimulation paradigm, we adopted a behavioral timescale ICMS strategy, previously used to study complex behaviors such as goal-related actions, feeding, and mating behaviors [19–21]. The stimulation parameters used to assess the presence of emotional responses to ICMS were the following: trains of biphasic pulses with duration 3 s and intensity 4 mA. Stimulation current threshold was determined by gradually decreasing the current intensity while the duration of pulse trains was maintained constant (3 s). Stimulation time threshold was assessed by gradually decreasing the duration of pulse trains while the current intensity was maintained constant (4 mA). The thresholds at which the emotional behavior could still be evoked were determined by two observers. The experiments were videotaped. EKG was also recorded (see Supplemental Experimental Procedures available online).

Figure 1A and Figure 2A show an unfolded view of the insula of monkey M1 and the locations of the microelectrode penetrations. ICMS elicited two types of emotional behaviors: disgust and affiliative behavior. The elicited behavior depended on the stimulation location (see Figure 1A, blue dots and Figure 2A, yellow dots). Other responses evoked by ICMS were difficult to account for in emotional terms, possibly because of the absence of an appropriate context or the limitation of the approach used.

Disgust was characterized by facial grimace, autonomic responses, and a characteristic behavior in response to stimulus presentation. The facial grimace was comparable to that naturally occurring during the presentation of unpleasant stimuli. It was characterized by the curling of the upper lip and the wrinkling of the nose (Figure 1C). The autonomic response (analysis of the instantaneous heart frequency, see Supplemental Experimental Procedures) consisted of a bradycardia that reached its peak after a delay (Figure 1B). Although bradycardia is associated with a wide range of behavioral states, our finding is in agreement with previous data showing that disgust, unlike other negative emotions, is typically associated with a decrease in heart rate [22].

Disgust-related responses were elicited by stimulation of rostral agranular/disgranular insula (see Figure 1A and Figure S1). They were observed in 84 out of 158 stimulated sites in 18 penetrations in M1 and in 29 out of 77 stimulated sites in 7 penetrations in M2 (for more details, see Table 1).

The average current threshold measured as described above was 1.9 ± 0.8 mA (2.0 ± 0.6 mA and 1.9 ± 0.8 mA in M1 and M2, respectively). The average time threshold was 395 ± 337 ms (437 ± 353 ms and 242 ± 218 ms in M1 and M2, respectively). Retching was also observed (24 sites in 5 penetrations in M1, 3 sites in 1 penetration in M2). In these sites, retching was systematically evoked without habituating. The average current

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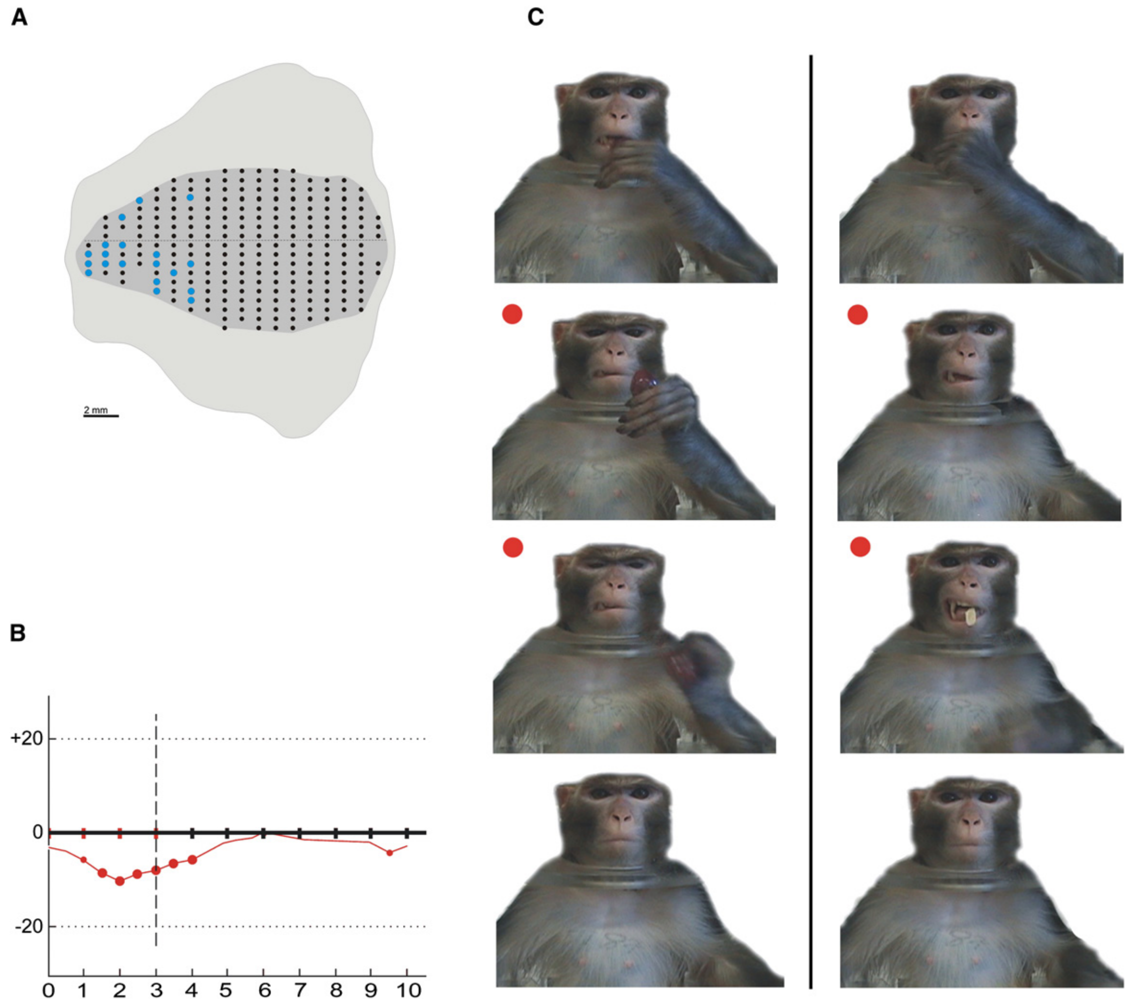

Figure 1. Disgust-Related Behavior

(A) Unfolded view of the lateral sulcus of the left hemisphere of M1. Dark gray area indicates the insula (dorsal and ventral aspects above and below the horizontal line, respectively); light gray area indicates the frontoparietal and temporal opercula of the lateral sulcus. Sites in which disgust-related behavior was evoked from M1 are shown in blue. Small black dots indicate penetrations from which no responses or other types of responses (not described here) were evoked. Each dot in the dorsal aspect of the insula indicates the electrode entrance point, and each dot in the ventral aspect indicates the electrode exit point. In all penetrations, several sites were stimulated every 500 μm below the entrance point and above the exit point. A coronal section showing an example of penetration reconstruction is present in Figure S1B.

(B) Mean cardiac instantaneous frequency averaged on the stimulations aligned with the onset of intracortical microstimulation (ICMS). Abscissae denote time (s); dashed vertical line denotes end of ICMS. Small dots: $p < 0.01$; large dots: $p < 0.001$.

(C) Disgust-related behaviors shown by video frames. Left: if stimulation (red dots) started when the monkey had food in its hand, the animal threw the food away. Right: if stimulation started (red dots) when the food was already inside its mouth, the monkey spat it out.

threshold to evoke retching was 2.3 ± 0.9 mA (2.4 ± 0.9 mA and 1.5 ± 0.5 mA in M1 and M2, respectively). The average time threshold to evoke retching was 1320 ± 1090 ms (1650 ± 1029 ms and 200 ± 0 ms in M1 and M2, respectively).

The effect of ICMS was also tested during two different moments of spontaneous feeding: while the monkey was bringing food to the mouth, and during chewing. In both instances, the monkey refused the food. In the first one—the stimulation starting while food was in its hand—the monkey threw the food away immediately, even if the food was the one the monkey liked most (Figure 1C, left; see also Supplemental Experimental Procedures). In the latter—the stimulation starting with the food already inside the mouth—the monkey spat it out (Figure 1C, right; see also Supplemental Experimental Procedures).

The other emotional behavior elicited by ICMS was an affiliative behavior. It was characterized by motor and autonomic responses and by specific behaviors occurring during social interaction. The motor response consisted of repetitive up-down movements of the jaw, with the lips also repetitively opening and closing. This behavior ("lip smacking") is interpreted as having a reassuring function (lowering the tendency to flee), as well as an attracting function (raising the tendency to approach) [23]. At the end of the ICMS-evoked lip smacking, the monkey occasionally showed chewing movements, but the presence, or absence, of this effect was not dependent on the site stimulated. Interestingly, chewing is considered to be part of the monkey behavioral repertoire associated with lip smacking [23]. Lip smacking site stimulation never resulted in any other behavior.

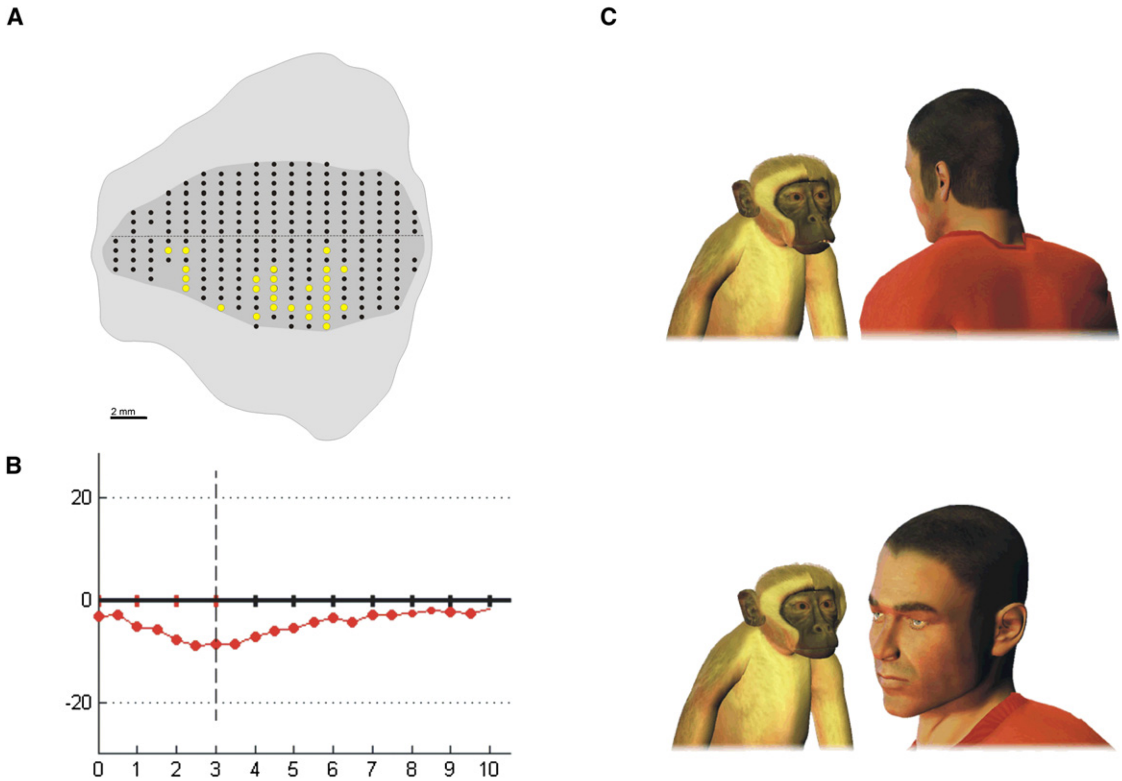

Figure 2. Affiliative Behavior

(A) Unfolded view of the lateral sulcus of the left hemisphere of M1. Dark gray area indicates the insula (dorsal and ventral aspects above and below the horizontal line, respectively); light gray area indicates the frontoparietal and temporal opercula of the lateral sulcus. Sites in which affiliative behavior was evoked from M1 are shown in yellow. Small black dots indicate penetrations from which no responses or other types of responses (not described here) were evoked.

(B) Mean cardiac instantaneous frequency averaged on the stimulations aligned with the onset of ICMS. Abscissae denote time (s); dashed vertical line denotes end of ICMS. Small dots: $p < 0.01$; large dots: $p < 0.001$.

(C) The two panels illustrate the two conditions during which ICMS was performed. Top: eye contact condition. Stimulation was delivered during eye contact between the monkey and the experimenter. Bottom: no eye contact condition. Stimulation was delivered while the experimenter was visible to the monkey but the eye contact was lacking. For other conventions, see Figure 1.

Unlike disgust, wherein the motor responses (e.g., face grimace) were constantly observed following stimulation, affiliative responses did not occur in the absence of eye contact between the monkey and the experimenter (Supplemental Experimental Procedures). The experimental procedure was the following. The experimenter stood in front of the monkey at a distance of about 80 cm, with his face turned away (no eye contact condition). Then the experimenter turned his face toward the monkey, staring at it in the eyes (eye contact condition). Typically, this procedure did not determine any reaction from the monkey in the absence of electrical stimulation. When this procedure was repeated during insula stimulation, no response was present with the experimenter gazing away from the monkey (no eye contact condition; Figure 2C, bottom). However, when the stimulation was carried out with the experimenter staring at the monkey (eye contact condition), an immediate affiliative response occurred (Figure 2C, top). This procedure (stimulation both with and without eye contact) was performed in 43% of penetrations (20 penetrations out of 46; 12 out of 28 in M1, 8 out of 18 in M2).

It is noteworthy that if the monkey was exhibiting, at the moment of stimulation, a spontaneous threatening or submissive expression directed to the experimenter, the stimulation interrupted this behavior and replaced it with lip smacking. Immediately after the end of stimulation, the monkey resumed the previous expression (Supplemental Experimental Procedures).

Analysis of the instantaneous heart frequency showed that ICMS evoked a bradycardic effect starting during the stimulation and lasting a few seconds after its cessation (Figure 2B). The bradycardic effect is congruent with parasympathetic activity, normally associated with approach behavior and group-oriented affiliative emotions [1, 24]. The fact that a bradycardic effect was evoked during the stimulation of both the disgust and affiliative sites is in line with the view that the left insula controls several emotions whose expression is accompanied by a parasympathetic activation [25].

An affiliative state was elicited from a large region of the ventral aspect of the disgranular insula (see Figure 2A and Figure S1). In M1, 85 out of 243 sites in 28 penetrations gave affiliative responses, and in M2, 71 out of 187 sites in 18 penetrations gave affiliative responses (for more details, see Table 2). The average current threshold measured in this fashion was 2.3 ± 0.5 mA (2.4 ± 0.6 mA in 25 sites in M1 and 2.3 ± 0.5 mA in 40 sites in M2). The average time threshold measured was 335 ± 168 ms (344 ± 225 ms in 19 sites in M1 and 327 ± 103 ms in 22 sites in M2).

The presence of motor responses following insula stimulation is in agreement with early stimulation studies, which showed motor and autonomic responses evoked by

Table 1. Disgust-Related Behavior

Disgust Behavior	M1	M2	Total
Penetrations	18	7	25
Stimulated sites	158	77	235
Sites with response	140 (88.6%)	68 (88.3%)	208 (88.5%)
Sites without response	18 (11.4%)	9 (11.7%)	27 (11.5%)
Sites with disgust	84 (60.0%)	29 (42.6%)	113 (54.3%)
Sites with other responses	56 (40.0%)	39 (57.4%)	95 (45.7%)

macrostimulation of monkey insula [15–18]. However, those classical studies were performed in deeply anesthetized monkeys; as a consequence, the obtained responses could not be correlated with specific emotional behaviors, in particular with the affiliative one.

The present study has overcome this problem by applying ICMS in behaving monkeys and employing behavioral time-scale stimulation in order to have reliable responses. The results show that two emotional behaviors may be elicited by stimulation of the insula: disgust and affiliative behavior. This does not imply, of course, that the insula is not involved in other emotions. It is quite possible that by using either a stronger or more prolonged stimulation, other emotions, like pain, could be evoked from those insular sites from which we did not obtain clear responses. Similarly, the presentation of specific stimuli, in parallel with ICMS, could possibly reveal other emotional behaviors in which the insula is involved. Future studies will show whether this is indeed the case.

The insula is not considered to be one of the "core structures" of emotion, such as periaqueductal gray, the hypothalamus, and the centromedial nuclei of the amygdala, but rather it is seen as one of the centers, also called "intermediate structures," that are involved in a more complex elaboration of emotional stimuli and in the production of appropriate responses [9]. The present data support this anatomical taxonomy. They show that the insula is responsible both for immediate overt motor and autonomic responses and for internal state modifications that radically change the individual responsiveness to specific stimuli. The immediate responses consisted of changes in heart rate and in facial expressions. The internal modifications are demonstrated by the fact that the responses to the same stimuli changed according to the stimulated site and the corresponding induced emotion. Thus, the monkey's response to the face of the experimenter expressing threat, following specific site stimulation, turns from a previous menacing or submissive attitude into an affiliative one. Similarly, following disgust site stimulation, the monkey's attitude toward food radically changed: a favorite food was refused or spat out.

The refusal to bring food to the mouth, evoked by ICMS, is particularly interesting because it permits one to overcome the possible objection that the monkey refused the food because the stimulation evoked unpleasant or painful sensations. In fact, although this hypothesis could be an objection to our interpretation when the monkey spat out the food already inside the mouth, the same hypothesis is very unlikely for the case in which the monkey threw away the food that it was bringing toward the mouth. Note also that the refusal to bring food to the mouth was accompanied by its previous careful examination (Supplemental Experimental Procedures). Moreover, it was observed that the rate of food acceptance between stimulation trials was not altered with respect to normal behavior. Despite repeated experiences of disgust, the monkey remained willing to reach for and accept food even immediately after ICMS. This suggests that the effect of ICMS was time locked with the stimulation. Taken together, this suggests that the monkey refused the food because of the presence of a stimulation-induced disgust state rather than because of some unpleasant or painful sensations.

Table 2. Affiliative Behavior

Affiliative Behavior	M1	M2	Total
Penetrations	28	18	46
Stimulated sites	243	187	430
Sites with response	204 (84.0%)	136 (72.7%)	340 (79.1%)
Sites without response	39 (16.0%)	51 (27.3%)	90 (20.9%)
Sites with affiliative behavior	85 (41.7%)	71 (52.2%)	156 (45.9%)
Sites with other responses	119 (58.3%)	65 (47.8%)	184 (54.1%)

In conclusion, the present data bridge the gap between the human functional data showing a clear involvement of insula in emotion and the classic stimulation studies on nonhuman primates showing motor responses. They indicate that the insula plays an important role in social and communicative behavior, determining the external aspects of a given emotion and, most importantly, modifying the internal state of the individual in such a way as to induce responses to external stimuli congruent with the elicited emotional state.

Experimental Procedures

Subjects

Experiments were carried out on two adult male rhesus monkeys (*Macaca mulatta*) weighing 7–13 kg. Before the beginning of the experiments, the monkeys were trained to sit comfortably on a primate chair and to interact with the experimenter. When the training was completed, the monkeys were operated on, and a head holder and two recording chambers were implanted. All experimental protocols complied with the European law on human care and use of laboratory animals and were approved by the Veterinarian Animal Care and Use Committee of the University of Parma, as well as by the Italian Minister of Health. Surgical procedures were the same as previously used in our laboratory [26, 27].

Intracortical Microstimulation Procedures

Intracortical microstimulations were performed by means of tungsten microelectrodes with epoxylite insulation (FHC), with low impedance (<200 KΩ). Microelectrode penetrations were made perpendicularly to the lateral sulcus, spaced at 1–2 mm intervals in the rostrocaudal axis and at 0.5–1 mm in the mediolateral axis. The microdrive was attached to a stereotaxic arm and fixed to the monkey head holder. The electrodes were inserted through the dura, which was left intact, and were moved by a hydraulic micromanipulator.

Extracellular recording was performed as soon as the electrode entered the cortex in order to assess the functional properties of neurons of the entrance point. Neuronal activity was amplified (Bak Electronics) and monitored on an oscilloscope.

The position of the microelectrodes in the deep cortical regions was monitored by means of ultrasound technique (Logiq 400CL ProSeries, General Electric Medical System). As the target region was reached, ICMS was applied every 500 μm.

ICMS was applied by means of a Biphasic Pulse Generator (BAK Instruments) connected to an isolation unit (Stimulus Isolator, WPI). Stimulation was triggered by a hand-held button and consisted of a train of 200 μs biphasic pulses, cathodal pulse leading. Trains were delivered at 50 Hz with an intensity of 4 mA for 3 s. These parameters are similar to those commonly adopted in the insular cortex stimulation in patients undergoing presurgical evaluation of temporal lobe epilepsies [28]. The behavioral responses were included in the data set only when two observers recognized the elicited behavior and when this behavior could be evoked from more than 50% of ICMS trials.

The ICMS was performed as follows. For each site, the first ICMS was delivered after a period of 60 s, during which the monkey was quiet and its heart rate was around 120 bpm. EKG traces were recorded in each site during the first stimulation and for the following 10 s after stimulation. After the first stimulation, further ICMSs were applied, at least five times. Once the presence and the nature of a response were classified, current and time thresholds were assessed. The current threshold was assessed by gradually decreasing the current intensity while the train was maintained at 3 s; the time threshold was assessed by gradually decreasing the train while the current intensity was maintained at 4 mA.

Supplemental Information

Supplemental Information includes one figure and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2010.12.042.

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