Localized microstimulation of primate pregenual cingulate cortex induces negative decision-making

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The pregenual anterior cingulate cortex (pACC) has been implicated in human anxiety disorders and depression, but the circuit-level mechanisms underlying these disorders are unclear. In healthy individuals, the pACC is involved in cost-benefit evaluation. We developed a macaque version of an approach-avoidance decision task used to evaluate anxiety and depression in humans and, with multi-electrode recording and cortical microstimulation, we probed pACC function as monkeys performed this task. We found that the macaque pACC has an opponent process-like organization of neurons representing motivationally positive and negative subjective value. Spatial distribution of these two neuronal populations overlapped in the pACC, except in one subzone, where neurons with negative coding were more numerous. Notably, microstimulation in this subzone, but not elsewhere in the pACC, increased negative decision-making, and this negative biasing was blocked by anti-anxiety drug treatment. This cortical zone could be critical for regulating negative emotional valence and anxiety in decision-making.

Many of the decisions that we make in daily life involve weighing benefits against the costs that they entail. Even these everyday decisions can evoke anxiety, and, in clinical conditions such as anxiety disorders and depression, such decision-making can be especially difficult. For example, people suffering from anxiety make more plans based on expected negative consequences and people suffering from depression make fewer plans based on expected rewards. Neuropsychological tasks requiring such cost-benefit evaluation, known as approach-avoidance conflict tasks, have been designed to characterize these clinical and psychological states and to quantify anxiolytic drug effects in animal models of anxiety. Drugs that can increase approach decisions in approach-avoidance tasks are promising candidate anxiolytics, suggesting that the emotional state of anxiety is a crucial factor in approach-avoidance decision-making. This suggests a functional link between potentiated anxiety states characteristic of neuropsychiatric conditions and the type of cost-benefit decision-making embodied in approach-avoidance tasks.

At the core of such approach-avoidance decision-making are judgments about the subjective value of the ‘good’ and ‘bad’ alternatives that are simultaneously confronted. An optimistic view leads to more approach decisions; a pessimistic view leads to more avoidance decisions. Decision variables are widely represented in the frontal cortex of macaque monkey. Especially for the dorsal part of the anterior cingulate cortex (ACC), many recording studies have shown that the ACC neurons can encode the gain or cost associated with expected outcomes, as well as prediction errors and loss of reward. Consistent with these physiological studies, anatomical work has suggested that the pACC has predominant connectivity with limbic and prefrontal regions. We recorded in these regions, but focused on the ventral bank of the anterior cingulate sulcus because this region has been implicated in humans in cost-benefit decisions and in emotional responses triggered by conflict situations, and has anatomical connections with striosomes of the caudate nucleus.

The fact that the pACC is involved in both cost-benefit decision-making and emotional regulation suggests a potential function for the pACC in controlling emotional state. Consistent with this view, evidence of a correlation between abnormal pACC activity and anxiety-related disorders has emerged in human neuropsychiatric studies. The pACC has been implicated in anxiety-related disorders, including obsessive-compulsive disorder, post-traumatic stress disorder, addiction and major depression. In particular, activity in the pACC has been found to predict clinical response to antidepressant treatments, and it is thought to be affected indirectly by deep brain stimulation of the subgenual ACC applied as a therapy for depression. However, the neuronal mechanisms operating in the pACC to affect emotional anxiety and emotional decision-making remain largely unknown.

To approach this issue, we developed a cost-benefit decision-making task for macaque monkeys modeled after the approach-avoidance protocols used in human cognitive science to quantify the degree of anxiety about the outcome of decision-making. Using multi-electrode recording, cortical microstimulation and anxiolytic treatment, we sought to identify characteristics of the pACC that could underlie a functional interaction between anxiety-related emotional processes and decision-making.

RESULTS

Decision-making in the approach-avoidance task

We first recorded behavior and neural spike activity in the pACC in two macaque monkeys as they performed an approach-avoidance (Ap-Av) task in which they made decisions to approach or to avoid combinations of positive (food) and negative (airpuff)
To the right, a schematic diagram of the pACC is shown with cingulate sulcus (Cgs).

Figure 1  Task procedures and recording regions. (a) Task flow diagram of the Ap-Av task. The task started when the monkey put its hand on the home position. After a 1.5-s fixation period, two bars appeared on the screen as a visual cue. The lengths of the red and yellow bars indicated the amount of liquefied food and airpuff delivered after approach choice, respectively. After the 1.5-s cue period, the monkey could move the joystick to choose one target. Cross target indicated approach and square target indicated avoidance. The locations of two targets were randomized across trials. After approach decisions, both airpuff and food were delivered in the indicated amounts. After avoidance decision, the monkey did not receive the indicated airpuff and food. (b) Task flow diagram of the Ap-Ap task. The lengths of red and yellow bars corresponded to the amount of reward that the monkey could obtain after choosing cross and square targets, respectively. (c) Stimulation procedure. Daily sessions consisted of stimulation-off and stimulation-on blocks, each with 250 trials. In stimulation-on trials, microstimulation (a train of biphasic pulses; frequency, 200 Hz; current amplitude, 70–80 µA) was applied for 1 s, starting at the onset of the visual cue. (d) Sagittal (left) and coronal (right) magnetic resonance images of the pregenual recording region. Red lines indicate estimated tracks of recording and stimulation electrodes ranging from interaural anterior-posterior (AP) 32 to AP36. To the right, a schematic diagram of the pACC is shown with cingulate sulcus (Cgs).

outcomes cued in advance by visual stimuli during a decision period (Fig. 1a and Online Methods). The monkeys looked at a cue composed of contiguous red and yellow bars whose lengths linearly corresponded to the amount of food (red bar) and the strength of the airpuff (yellow bar) that would be delivered at the end of the trial. They could then choose to approach or to avoid the combinations by moving a cursor. After approach decisions, both food and airpuff were delivered in the indicated amounts. After avoidance decisions, the monkeys did not receive the indicated food and airpuff, but only a standard minimum food amount, which was necessary to maintain motivation to perform the task.

The monkeys systematically varied their decisions to approach or to avoid depending on the relative sizes of the food rewards and airpuffs indicated by the cues (Fig. 2a,b). On the basis of Bayesian information criteria (Supplementary Fig. 1), we adopted the logistic behavioral model that could best characterize the behavioral choice of monkey. With this model, the decision boundary between approach and avoidance decisions was linear (Fig. 2a,b). The fact that mean reaction times increased along the decision boundary indicated conflict in the monkey’s decision-making, particularly for low values of indicated reward (Fig. 2c).

To test whether these approach–avoidance decisions could be influenced by drugs that relieve anxiety in humans, we administered the anxiolytic drug diazepam9 to two monkeys (monkeys S and P) during 14 sessions (Supplementary Fig. 2). Diazepam treatment increased the monkey’s approach decisions in the Ap-Av task by an average of 7%, as measured by the size of significant change in decision in the decision matrix (that is, the matrix of decision relative to offered reward and airpuff, see Online Methods), and these effects were dose dependent. Saline treatment was without effect, yielding significant differences between the diazepam and saline treatments (t test, P < 0.001). These results suggest that the behavioral response induced in the monkeys by approach–avoidance decision-making could be regulated by an anxiolytic drug9, which could mean that in monkeys, as in humans, the task tapped into some anxiety-like state.

As a control decision-making task, we designed an approach–approach (Ap-Ap) task that was identical to the Ap-Av task except that the red and the yellow bars both signaled potential reward amounts (Fig. 1b). The rewards offered by the red bar and the yellow bar were obtained, respectively, by choosing cross and square targets. To have the decision boundaries be similar to those in the Ap-Av task, we adjusted the reward amount offered per unit length of the red bar to be double the reward amount offered by the equivalent length of the yellow bar (Fig. 2d,e). Reaction times increased around the decision boundaries, particularly when the monkeys were offered smaller amounts of reward than that they obtained on average (Fig. 2f). In sharp contrast with decision-making in the Ap-Av task, decisions in the Ap-Ap task were not affected by treatment with diazepam at any of the doses that we used (Supplementary Fig. 2). Thus, the observed behaviors of the monkeys were comparable in the Ap-Av and Ap-Ap tasks, but the pharmacologic treatment suggests that the monkeys could have been subject to different types of subjective state while performing the two different decision-making tasks.

Neural activity during the decision-making tasks
We recorded the activity of 1,065 well-isolated single units in the pACC and adjoining medial prefrontal cortex of monkeys S and A (Fig. 1d) with chronically implanted electrodes as they performed alternating 150-trial blocks of the Ap-Av and Ap-Ap tasks (Supplementary Fig. 3). We focused on analyzing the activities of
Figure 2. Behavioral patterns. (a) Avoidance (red square) and approach (blue cross) choice made by monkey S in a single session of the Ap-Av task. Black line indicates the decision boundary calculated by logistic regression. Light blue and orange lines indicate 90% and 10% probability, respectively, of choosing approach estimated by the regression model. (b,c) Decision (b; red, approach; blue, avoidance) and reaction times (c; red, slow; blue, fast) averaged over all experiments for the Ap-Av task and plotted according to pseudocolor scales at right. Dotted lines indicate decision boundaries calculated based on all the accumulated data. (d) Choice of square (red square) and cross (blue cross) targets in a single session of the Ap-Ap task by monkey S. Black, light blue and orange lines represent 50%, 90% and 10% probabilities of choosing cross target, respectively, calculated by logistic regression. (e,f) Target choice (e) and reaction times (f) averaged over all Ap-Ap experiments. Dotted lines indicate decision boundary.

Figure 3. Classification of units recorded in the pACC region. (a) Results of stepwise regression analysis for the Ap-Av task. Regression variables are offered reward (Rew), offered airpuff (Ave), choice (Cho), chosen reward (Cho × Rew), chosen airpuff (Cho × Ave), reaction times (RT), expected utility (Eutil) and conflict in decision (Conf). The y axis indicates the number of recorded units best characterized by one or a combination of the variables identified in the matrix below. Black squares in the matrix along the x axis indicate the variable or variables selected by the stepwise regression procedure. Many units (397 units) were characterized by the single variable that we chose. These units were further classified by whether the activity was positively (red) or negatively (blue) correlated with the variable. Another 159 units were characterized by particular combinations of variables indicated by black squares in the matrix. (b) Results of multidimensional scaling performed on the basis of the correlation distance of the firing patterns of each type of unit. The x axis shows the principal feature dimension and the y axis shows the secondary feature dimension extracted by this procedure. Types of units located closely to each other indicate that their firing patterns are similar. Blue circles indicate the locations of units with activity that was negatively correlated with the indicated variable. Red circles indicate the locations of units with activity that was positively correlated with the indicated variable. Units were classified by the similarity derived in the primary feature, N type (light blue region) and P type (light red region). (c) Cortical distribution of N-type (blue circle) and P-type (red circle) units. In the ventral bank of the cingulate sulcus (blue shading), N-type units significantly outnumbered P-type units (Fisher’s exact test, P < 0.05).
had activity characterized by option utility. Second, we considered whether the pACC was involved in monitoring conflict, especially when decisions had to be made between two options with similar offered values. To search for pACC units encoding such ‘conflict in decision’, we introduced the calculated information entropy of decision-making as an indicator of decision conflict and used this as an explanatory variable.

Stepwise regression analyses (Supplementary Note) indicated that, among 875 task-related units, many (556 units, 63.5%) had activity during the decision period of the Ap-Av task that was significantly well characterized by linear combinations of these eight factors ($F$ test, $P < 0.05$; Fig. 3a). Among these, the majority (397 units, 71.4%) had activity patterns that were characterized by one of these variables. Because many of these units were uniquely characterized by a single variable, but not by a combination of these variables, the majority of units encoded, within the detection limits of the regression model, a single factor specifically characterized by the variable. We focused on the 397 units that had cue-period activity that was accounted for by single variables (Supplementary Fig. 5).

Classification of pACC unit activity

We next asked whether there was a property of their response patterns during the decision period that was shared across the different types of units. We applied multidimensional scaling (MDS) to quantify the similarity of their firing patterns and categorized them on the basis of their similarity (Fig. 3b). Two principal features derived by the MDS accounted for nearly all of the variability across different types of units, and most of the features were accounted for by the first principal dimension (Supplementary Fig. 6). Moreover, in the first-feature dimension (Fig. 3b), nearly all unit types fell into two clear clusters: the units that were positively correlated with the regression variables and the units that were negatively correlated with the regression variables. Thus, we categorized the pACC unit populations as either being P type or N type (Fig. 3b and Supplementary Fig. 7). The two outliers in this first principle feature analysis were accounted for by the second principal feature: units with activity that was positively or negatively correlated to the offered airpuff (Ave) were almost maximally distant on the dimension defined by the second principal feature (Fig. 3b).

P-type units exhibited spike activity that was correlated with variables that preceded or followed approach decisions. They were activated when the monkeys were offered large reward (high Rew), expected high value for the outcome (high Eutil), were about to decide to approach (Cho = 1), or were ready to receive reward (high Cho × Rew) and airpuff (high Cho × Ave). These variables therefore appeared to be consistently related to the causes or effects of positive motivational states. We refer to the variables that activates P-type units as ‘motivationally positive’ variables. Conversely, N-type units consisted of units with activities that were correlated with variables that led to or followed avoidance decisions. They were activated when the monkeys were offered small reward (low Rew), expected low value for the outcome (low Eutil) or were about to decide to avoid (Cho = 0). They were also activated when the decision was variable, indicating potentially subjective conflict (high Conf) and when the reaction times were prolonged (high RT). These behavioral variables appeared to be related to negative motivational states. We refer to the variables that activate N-type units as ‘motivationally negative’ variables.

These results suggested that many units in the pACC could be divided into distinct populations of neurons related to cost-benefit...
Firing patterns of N-type and P-type units

To characterize the representative properties of these two distinct populations of pACC neurons, we examined their activity profiles during the cue period (Fig. 4). In the Ap-Av task, the N-type population responded to visual cues that were subsequently followed by an avoidance decision, and they responded maximally to visual cues offering low food and low airpuff (Fig. 4a). The responses of N-type unit population were task dependent; in the Ap-Ap task, they responded only to visual cues offering low food rewards (Fig. 4b). Under these conditions, both the frequency of omission errors (Supplementary Fig. 8) and the monkeys’ reaction times (Fig. 2c,f) increased, suggesting that they might have been relatively less motivated, experiencing conflict or possible frustration with the low yields cued. In contrast, the P-type population responded to cues that were followed by approach in the Ap-Av task, and fired maximally to the cues that offered large reward and weak airpuff (Fig. 4f). Similar to the N-type population, neurons in the P-type population changed their activity depending on the task version. In the Ap-Ap task, they responded to long red and yellow bars offering large amounts of reward (Fig. 4g).

This pattern of results suggests that expected utility might provide a more comprehensive construct to account for the firing patterns34,35. We therefore performed correlation analyses between expected utility and the activity of N-type and P-type units in the Ap-Av and Ap-Ap tasks. For each N-type unit (Fig. 4c,d), we calculated the correlation coefficient between the cue-period firing rate in the Ap-Av task and the expected utility calculated for the Ap-Av task (Fig. 4e). The mean of the distribution of the correlation coefficients (r(Eutil, Ap-Av)) was significantly negative (two-tailed t test, P < 10^{-56}). For the same individual units, we calculated the correlation coefficients between the cue-period activity in the Ap-Ap task and the expected utility for the Ap-Ap task (Fig. 4e). Notably, the mean of the correlation distribution (r(Eutil, Ap-Ap)) was also significantly negative (two-tailed t test, P < 10^{-17}). The N-type population included 45 single units with activities that were significantly correlated with the expected utility in both task versions (Pearson’s correlation coefficients, P < 0.05). Moreover, the correlation distributions for the Ap-Av task and the Ap-Ap task were themselves significantly correlated (r = 0.49, P < 10^{-12}; Fig. 4e).

Similarly, for each P-type unit (Fig. 4h–j), we calculated the correlation distributions between cue-period activity and expected utility in both tasks: r(Eutil, Ap-Av) and r(Eutil, Ap-Ap). The mean of the correlation distribution in the Ap-Av task (r(Eutil, Ap-Av)) was significantly positive (two-tailed t test, P < 10^{-42}), as was the mean of the correlation distribution in the Ap-Ap task (r(Eutil, Ap-Ap); two-tailed t test, P < 10^{-22}). There were 53 units in the P-type population that had activities that were significantly correlated with the expected utility in both task versions (P < 0.05). The correlation distributions for the population activity in the two populations were again significantly correlated with each other (r = 0.56, P < 10^{-12}). Together, these findings suggest that the populations of N-type and P-type pACC units are activated in relation to the subjective value of the chosen outcome, but with contrasting negative and positive coding schemes that are robustly expressed across the two tasks.

Distribution of negative and positive unit-coding

If the pACC did contain a decision mechanism based on complementary positive and negative representations of expected utility, then the border between the subjective cost and benefit of the decisions could be set by the balance of activity of the N-type and P-type units40. In most of the pACC regions that we sampled, N-type and P-type units were apparently evenly intermixed, but N-type units predominated over P-type units in the ventral bank of the cingulate sulcus (Fig. 3c).

To determine whether this biased distribution toward negatively correlated units in the ventral bank region was produced by units with one primary behavioral correlate or by units with activity correlated with multiple behavioral variables, we performed a series of correlation analyses between unit activity and each of the behavioral variables used in the regression analyses. For each variable, we tested whether there was a predominance of ventral bank units with activity either positively or negatively correlated with the given variable. We
examined the distributions of units with significant correlations (Pearson’s correlation coefficients, \( P < 0.05; \) Fig. 5). Negatively correlated units predominated positive ones in the ventral bank (Fisher’s exact test, \( P < 0.05 \)) for offered reward (Rew; Fig. 5a), expected utility (Eutil; Fig. 5c) and chosen reward amount (Cho × Rew; Fig. 5e).

For conflict in decision (Conf; Fig. 5g) and reaction time (Fig. 5h), positively correlated units dominated negative ones. For decision (Cho; Fig. 5d), units coding avoidance (Cho = 0) dominated units coding approach (Cho = 1). Thus, except for offered and chosen airpuff strength (Ave and Cho × Ave; Fig. 5b, f), units responding to multiple different variables (low Rew, low Eutil, low Cho × Rew; high Conf and high RT; Cho = 0) predominated over their counterparts in the ventral bank region. These variables corresponded to the motivationally negative variables that activated N-type units.

### Microstimulation alters approach-avoidance decision-making

On the basis of these biased distributions, we reasoned that microstimulation in this ventral bank zone might bias the monkeys’ decision-making toward avoidance decisions, whereas stimulation elsewhere in the sampled pACC region might have limited effect because of the balance between the N-type and P-type populations.

To test this prediction, we microstimulated at 97 sites in the pACC of monkeys S and A, using 1-s-long trains of biphasic pulses that started at the onset of the visual cues (Fig. 1c). Each site was stimulated in successive daily Ap-Av \( (n = 97) \) and Ap-Ap \( (n = 31) \) sessions. Within individual sessions, we alternated stimulation-off and stimulation-on trials in blocks of 250 trials.

The effects of the microstimulation on the monkeys’ decision-making were markedly selective. Stimulation was effective almost exclusively during performance of the Ap-Av task; it almost exclusively produced an increase in avoidance decisions and it produced this effect almost exclusively for stimulation applied to the ventral bank of the cingulate sulcus (Figs. 6 and 7). Compared with the stimulation-off trials (Fig. 6a), the slope of the decision boundary during the stimulation-on trials was shifted rightward and the number of avoidance decisions was increased (Fig. 6b). To quantify the effect of the stimulation, we introduced a spatial smoothing method and used Fisher’s exact probability test (Online Methods). We defined effective sites as those for which stimulation significantly changed the monkey’s decisions \( (P < 0.05) \) for at least 5% of all combinations of the two cues. Microstimulation in the ventral bank of the cingulate sulcus significantly increased avoidance choices for 16.6% of all cue combinations, most strongly for those indicating high airpuff strengths (Fig. 6c). Identically applied stimulation at the same site during Ap-Ap task performance did not induce any change in decision (Fig. 6d–f).

Of the 97 sites that we examined in the medial wall cortex, 15 sites were effective, 13 of which (86.7%) produced an increase in avoidance and were in the ventral bank of the cingulate sulcus (Fig. 7a and Supplementary Fig. 9). To characterize the stimulation effects at these 13 effective sites, we accumulated all of the behavioral data (Supplementary Fig. 10) and, with the accumulated data, expressed the difference in decision-making between stimulation-on and stimulation-off trials as \( t \) scores. The tendency for increased avoidance in the Ap-Av task was positively correlated with the strength of the aversive airpuff indicated by the visual cues (Fig. 7b), but was unchanged in the Ap-Ap task (Fig. 7c). Reaction times were affected in both monkeys, most strongly in monkey S, and were, in both, larger for high-conflict decisions than for low-conflict decisions (Supplementary Fig. 11). In some experiments, we ran triple-session...
experiments with successive days of Ap-Av, Ap-Ap, and Ap-Av tasks and confirmed that the differential results of the two tasks were consistently observed (Supplementary Fig. 12a). For two sites at which we found increased approach on stimulation, the effects were about 5% (Fig. 7a).

Cumulative effects of microstimulation on decision-making

What could account for this highly selective biasing in decisions toward avoidance? Classical electrical stimulation studies showed that electrical stimulation of the rostral ACC evokes changes in autonomic signs, including skin conductance\(^{41}\). However, we found no changes in skin conductance induced by the microstimulation (Supplementary Fig. 12b,c), suggesting that the microstimulation that we used was too weak to induce strong fear or pain. We did note an increased incidence of ‘coo’ vocalization\(^{41}\) in some sessions, consistent with this pACC region’s known importance for vocalization and social communication\(^{42}\).

A clue to the origin of the increased avoidance behavior came from another set of experiments in which we randomly alternated the stimulation-on and stimulation-off trials in a given session. We found little difference between the stimulation-on and stimulation-off decisions (Fig. 7d). However, when we compared the averaged decision boundaries for random stimulation experiments to the decision boundaries calculated in the prior normal sessions from pooled stimulation-off trials (Fig. 7b), we found that the boundaries for the random experiments were biased toward avoidance. This unexpected result suggests that the effect of the randomized stimulation persisted to affect the following stimulation-off trials. Using the standard block-design, we found that the stimulation effects were cumulative (Fig. 7e). These accumulating effects raise the possibility that pACC stimulation could bring about a tonic, persistent state affecting the relative evaluation of cost and benefit.

Blockade of induced negative decisions by anxiolytic

These results suggest that such a persistent state could be influenced by anxiolytic treatment. We tested this possibility in another set of experiments by asking whether anxiolytics could reduce the stimulation effects (Fig. 8a). We divided Ap-Av sessions into three successive 200-trial blocks: stimulation-off, stimulation-on, and stimulation-off after drug administration. Between the second and third blocks, we administered diazepam intramuscularly (0.25 mg per kg of body weight). We represented the effect of microstimulation by the change in the decision matrix between the first and second blocks.
Our findings suggest that neurons in the macaque pACC can be divided into populations representing two poles of an opponent-process evaluation mechanism. Depending on whether their cue-period responses were greater for cost-related variables or for benefit-related variables in the Ap-Av task, the pACC neurons could be separated into two broad groups, the N-type and P-type neuronal populations. Those neurons encoding motivationally negative and positive variables were widely distributed in the pACC and were apparently intermixed with one another, as would be appropriate for components of a decision-making neuronal mechanism. However, there was one exception to this symmetric distribution of N-type and P-type units; in the pregenual zone in the ventral bank of the cingulate sulcus, there were significantly more neurons that, across nearly the full range of variables examined, represented negative motivational variables more than positive ones. It was in this ventral bank zone alone, of all of the pACC sites explored, that electrical microstimulation altered the approach-avoidance decisions made by the monkeys, specifically biasing them toward avoidance of the anticipated outcome. This effect of the microstimulation accumulated over time, suggesting that the effect represented a persistent state variable. Anxiolytic drug treatment blocked the effects of such microstimulation, suggesting that the stimulation could have induced an anxiety-like state. Collectively, these findings suggest that this localized ventral bank region of the pACC is differentially involved in weighing cost against benefit in situations requiring approach-avoidance decision-making, and suggest that excessive activation of this region can induce pessimistic evaluation of offered outcomes.

The stimulation effect that we selectively observed in the Ap-Av task should reflect its special characteristics. In the Ap-Av task, approach and avoidance options were directly associated with the task should reflect its special characteristics. In the Ap-Av task, approach and avoidance options were directly associated with the negatively evaluated outcomes. This view suggests that the cost-benefit ratio in the Ap-Av task did not affect decision-making in the Ap-Ap task. However, despite the absence of behavioral change, the N-type and P-type populations were activated in the Ap-Ap task. These activities suggest that, irrespective of the task type, these units generally evaluate whether the consequence of an upcoming decision is evaluated as being ‘good’ or ‘bad’ relative to one’s expectation. This view suggests that the cost-benefit boundary could have been set by balanced activity of N-type and P-type populations, flexibly changing depending on the average offers in each task.

The proposal that the pACC is involved in cost-benefit evaluation rather than specifically in option selection is also supported by the types of units that we observed in the pACC. With stepwise regression, we characterized pACC units representing offered cost (Ave), offered benefit (Rew) and chosen value (Eutil), suggesting that the pACC contains units involved in cost-benefit integration to derive motivational value. Notably, those units were intermingled with units representing consequential motivational responses such as anticipation of each outcome (Cho × Rew and Cho × Ave), emotional conflict (Conf) and modulation of reaction time. Because units that uniquely encode targeted selection were not dominant, the function of the pACC might be characterized by cost-benefit integration necessary to derive a subjective value that could control multiple motivational processes.

We note that, to derive utility, we employed the conditional logit model and approximated the choice behavior. Although the model could account for the behavior with substantial accuracy (Supplementary Note), the utility was not directly derived from the behavior. Further, because we did not vary the reward amount for avoidance option, we could not express utility in terms of primary reward values. Thus, the utility that we derived was specific to the task-context of our experiments.

One finding that emerged from our recording and stimulation maps is that, in the broad pACC zone explored, neurons encoding negative motivational variables were differentially concentrated in the ventral bank of the cingulate sulcus, the region in which we found microstimulation to induce an increase in avoidance decisions. This biased distribution suggests one possible answer to questions of why the stimulation only induced avoidance and why the stimulation was effective selectively in the ventral bank. According to this view,
stimulation of the ventral bank pACC zone over-activated pACC neurons representing negative motivational values, leading to a pessimistic evaluation of future outcome and to increase avoidance. Of the 97 pACC sites that were stimulated, we found increases in approach decisions only at two sites. These rare instances suggest that there could be pACC or other nearby zones in which stimulation would push the balance of decision-making toward approach decisions.

However, the neuronal mechanisms underlying the stimulation-induced changes are still unclear. The effects could depend not only on circuits in the pACC, but also in the brain regions downstream of the effective pACC stimulation zone. The effective zone lies in the rostral part of area 24 and the dorsal part of area 32. These are interconnected with other cortical regions that have been implicated in decision-making, emotion and motivation\(^{19,43}\), including the dorsal part of the dorsolateral prefrontal cortex, which has been implicated in cognitive and motivational planning\(^{44}\), and the orbitofrontal cortex, which has been implicated in stimulus evaluation\(^{34}\). The late sulcus also shares connectivity patterns with the amygdala\(^{21}\), indirectly via the lateral habenula\(^{46,47}\), itself a brain site that is related to reward\(^{31,32}\), in major depression\(^{31,32}\) and stress disorder\(^{29}\), as well as in addictive states\(^{30}\). Notably, increased activity in the genual ACC\(^{33}\), in the orbitofrontal cortex during saccade countermanding\(^{33}\), and the amygdala\(^{33}\), in the anterior cingulate cortex during saccade countermanding. The authors declare no competing financial interests.

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**AUTHOR CONTRIBUTIONS**

K.A. and A.M.G. designed the experiments and performed the surgeries. K.A. collected the data. K.A. and A.M.G. analyzed the data and wrote the manuscript.

**COMPETING FINANCIAL INTERESTS**

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ONLINE METHODS

Subjects. Three female Macaca mulatta monkeys (A, 6.8 kg; S, 7.5 kg; P, 6.3 kg) were studied in experiments conducted in accordance with the Guide for Care and Use of Laboratory Animals of United States National Research Council and the guidelines of the Committee on Animal Care of the Massachusetts Institute of Technology. Before training, the monkeys were habituated to sitting in a monkey chair and then sterile surgery was performed, with anesthesia induced by intramuscular atropine (0.05 mg per kg) and ketamine (10 mg per kg), followed by inhalation of 1–2.5% sevoflurane with 2 l of O2, to implant over the occipital bone a titanium headpost, secured by titanium screws and bone cement. For all surgeries, the monkeys were maintained on analgesics postoperatively, and prophylactic antibiotics were injected intramuscularly both on the day of surgery and daily thereafter for 1 week.

Task procedures. Monkeys were first trained to perform an approach-avoidance (Ap-Av) task (Fig. 1a). At task start, a white central fixation spot and a gray rectangular frame appeared simultaneously on a black screen. When the monkey put her hand on a designated position in front of a joystick (home position), an infrared photobeam sensor (FS-V31, Keyence, Japan) detected the hand placement, triggering the rectangular frame to turn white. The monkey was required to hold her hand in the home position for 1.5 s (fixation period). If contact was lost during this period, the rectangular frame became gray until the monkey again held her hand in the home position. After the fixation period, a compound visual cue consisting of red and yellow horizontal bars appeared at the center of the screen. The length of the red bar corresponded to the amount of reward (liquefied food; 0.1 ml at minimum, 2.0 ml at maximum) that the monkey could receive or reject later in the trial; the length of the yellow bar corresponded to the strength of the airpuff (0 psi at minimum, 50 psi at maximum) that it would simultaneously receive or reject. The lengths of the bars could vary independently over 101 steps, which were selected pseudorandomly. The cues remained on for 1.5 s (cue period) and the monkey had to maintain home-position contact with her hand during this period. If the monkey released the contact during the initial 1 s of the cue period, an airpuff pointed at her nose was delivered, and the trial was terminated (commission error). Otherwise, after the cue period, two target cues (a white cross and a white square) appeared above and below the two bars. The locations of the two targets alternated randomly. To obtain reward, the monkey had to use a joystick to move the cursor toward one of the two targets within 3 s (response period). If the monkey chose the square target, a sound signaling avoidance was played, and 500 ms later, a small and constant amount of reward (liquid food, 0.1 ml; equivalent to the minimum amount indicated by the red bar) was delivered to maintain the monkey’s motivation. If the monkey chose the cross target, a sound signaling approach occurred, and 500 ms later, an airpuff whose pressure was indicated by the yellow bar was delivered to her nose for 800 ms. Liquefied food reward was delivered 1 s later for 1.5 s. If the monkey failed to respond in a trial in the 3-s response period, an airpuff was delivered with the pressure indicated by the yellow bar, and the trial was terminated (omission error). After each trial, a 5-s intertrial interval began. The amount of reward was controlled by a computer and a pump attached to the food tube (L/S Masterflex, Cole-Parmer). The compressed air for the airpuffs was generated by an air compressor (20-A, Silent Air) and was controlled by a computer and an E/P transducer (900-EIA, Control Air).

The monkeys were also trained to perform an approach-approach (Ap-Ap) task (Fig. 1b). The procedure for this task was the same as that of the Ap-Av task during the fixation and cue periods, but was different after the response period. When the monkey chose the square target, a sound signaling approach occurred and, 500 ms later, food reward was delivered for 1.5 s at a flow rate indicated by the length of the yellow bar. When the monkey chose the cross target, a sound signaling approach was triggered and, 500 ms later, reward was delivered for 1.5 s at a flow rate indicated by the length of the red bar. Yellow bar lengths were linearly related to the amount of food ranging from 0.1 ml to 1.0 ml, but red bar lengths linearly corresponded to the amount of food ranging from 0.1 ml to 2.0 ml. Thus, the amount of reward per unit length of the red bar was twice as large as that of the yellow bar. After reward delivery, a 5-s intertrial interval began.

Recording setup. After behavioral training, a plastic recording chamber was implanted on two monkeys (A and S) in a second sterile surgery at stereotaxically determined coordinates for each monkey and secured by bone cement and ceramic screws. The chamber could be fitted with a plastic grid with holes spaced at 1-mm intervals to serve as guides allowing parallel microelectrode penetrations. Magnetic resonance images (T2-weighted turbo spin echo, 300 μm in resolution, 1-mm slice thickness) were then taken with the chamber and grid filled with saline to allow identification of the coordinates of each electrode track with an accuracy of 1 mm. Under anesthesia and sterile conditions, the dura matter overlying the frontal neo-cortex was incised. After recovery, a set of platinum-iridium electrodes (impedance, 0.8–1.5 MΩ, FHC) were implanted into the cortex. All electrodes were held by custom-made micromanipulators affixed to the grid. For a single implant, 12 electrodes were implanted (Fig. 1d). For monkey A and for the second implant of the monkey S, 20 additional electrodes were simultaneously implanted to target the dorsolateral prefrontal cortex and the striatum for another experimental purpose.

The recording and task control system consisted of five networked personal computers and other peripheral equipment. Eye positions were recorded by an infrared eye-movement camera system (Eyelink CI, SR Research). Two computers (one for operation and one for display) controlled the task, based on a NIMH CORTEX system developed by the National Institute of Mental Health. They collected data for eye position, joystick movement and the photobeam sensor that monitored hand-home position, and they sent event signals to the recording system. For recording, a digital data acquisition system (Digital Lynx, Neuralynx) collected all signals. Task events were also sent to another personal computer that ran Matlab (MathWorks) to control the microstimulation generated by the stimulator (Master-8, A.M.P.I.) and isolator (A365, WPI). Signals from the microelectrodes were amplified by the Digital Lynx system. Without pre-screening for task relatedness, neural signals were digitized and stored with Digital Lynx and were later classified into single-unit activities using Offline Sorter (Plexon). Sorted neuronal activity was analyzed using Matlab. During all recording and stimulation sessions, the monkey’s facial expression was monitored by an infrared camera.

Electrophysiological recording and microstimulation. Recordings were made while the monkeys performed each of the two (Ap-Av and Ap-Ap) tasks in alternating blocks of 150 trials. Between blocks, we inserted a 10-s inter-block interval, during which a white spot appeared at the center of the screen as an explicit signal of the block change. For the stimulation experiments, stimulation-off and stimulation-on trials were alternated in 250-trial blocks. No explicit signal was given at the block changes. The sequence of visual cues presented in the stimulation-off block was repeated in the following stimulation-on block. For both monkeys, we started stimulation experiments with the Ap-Av task (see below for control for session order). If we observed significant changes by comparing decisions made during stimulation-on and stimulation-off blocks in the Ap-Av task, then, in the next daily session, we performed stimulation during the Ap-Ap task without moving the electrodes. If we did not observe changes in the first Ap-Av task, then in the next session we went back to a recording or stimulation experiment in the Ap-Ap task, after advancing all electrodes. For stimulation experiments, either bipolar (36 sessions) or monopolar (51 sessions) stimulation was applied, and similar results were found by both methods. The stimulation train consisted of 200-µs pulses delivered at 200 Hz. Each pulse was biphasic and was balanced with the cathodal pulse leading the anodal pulse. The current magnitude was 70–80 μA (Fig. 1c).

Behavioral and neuronal analyses. Error trials, consisting of commission errors (premature termination of trial by cessation of fixation during the cue-period) and omission errors (failure to respond within response period), were removed from further analysis. To model the behavioral choice, we used Bayesian information criteria (BIC) and employed the logistic regression model that had the lowest BIC (Supplementary Fig. 1). A decision boundary was represented by the linear discriminant of approach and avoidance calculated by the regression. To calculate the behavioral differences observed with and without stimulation (Fig. 6c), we introduced the following spatial smoothing method. The length of each bar varied in 101 steps (0–100%). The datum of each trial was mapped to a reduced-size data matrix (M × M) and was convolved with a square smoothing window (size W%). Each statistical value was obtained at each element of the matrix. To calculate the difference between two stimulation conditions, we introduced Fisher’s exact probability test, which calculates precise probabilities when the number of samples is not sufficient to allow the use of normal approximation. We also calculated Wilcoxon’s rank sum tests and two-sample t tests (two-tailed), and used these to confirm the results. Although 1/M and W were in an inverse relationship to the spatial resolution of the statistical result, we needed a considerable
amount of 1/M and W to calculate proper statistics with a limited number of samples. By varying M (20, 25 and 30) and W (10, 20 and 30), we confirmed that the M and W values did not change any of the conclusions drawn (Supplementary Fig. 14). We then fixed M to 25 and W to 20.

To estimate the internal variables of monkey’s subjective decision-making, we made a statistical model of choice behavior based on the data obtained in a daily session (Supplementary Note and Supplementary Fig. 15). If there are two options (+ and □ targets), probability of choosing + targets can be written by logistic function as

\[
p_+ = \frac{\exp(U_+) \exp(U_u)}{\exp(U_+) + \exp(U_u)} \frac{1}{1 + \exp(-(U_+ - U_u))},
\]

where \(U_+\) and \(U_u\) are the subjective value or utility of each option. Then, \(U_+ - U_u = a_1 x + a_2 y + a_3\), where \(x\) is the length of red bar, \(y\) is the length of yellow bar, and \(a_1\), \(a_2\) and \(a_3\) are the coefficients determined by logistic regression. In the Ap-Av task, because avoidance decision always leads a small amount of reward (that is, \(U_u = U_{AV} = \text{constant}\)), we could model each utility as \(U_+ = U_{AP} = a_1 x + a_2 y\), and \(U_u = U_{AV} = -a_3\). In the Ap-Ap task, following BIC, we adopted the first-order approximation model without bias term. We modeled \(U_+ = a_1 x\) and \(U_u = -a_2 y\). The expected utility is defined by \(E(u) = p_+ U_+ + p_u U_u\). The degree of conflict in decision-making can be written by the entropy formula as \(H = -p_+ \log p_+ - p_u \log p_u\). To characterize the effect of microstimulation on the sensitivities to offered reward and offered airpuff (Fig. 8b), we defined the relative sensitivity as \(r = a_1 / a_2\). The percent change of \(r\) induced by the microstimulation becomes 100(\(r' / r - 1\)), where prime indicates the parameter derived from stimulation-on trials. Similarly, for the Ap-Ap task, the percent change of \(r\) becomes 100(\(r'/r - 1\)), where \(r = a_1 / a_2\).