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Activity of Primate Subgenual Cingulate Cortex Neurons Is Related to Sleep

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Rolls, Edmund T., Kazuo Inoue, and Andrew Browning. Activity of primate subgenual cingulate cortex neurons is related to sleep. *J Neurophysiol* 90: 134–142, 2003; 10.1152/jn.00770.2002. The most frequent type of neuronal response found in the subgenual cingulate cortex (area 25) of the rhesus macaque was a highly significant increase of firing rate when the monkey fell asleep (median rate = 1.6 spikes/s) compared with the awake state (median rate = 0.1 spikes/s). On average, the firing rate of the neurons when awake was 23% of that when the monkeys were asleep. Neurons were not found in this region with responses related to taste, olfactory, and visual stimuli including faces or related to movement. These results are relevant to understanding the function of this region in humans, in which it has been suggested that activation may be related to disengagement from tasks and to induced sadness, both of which we note lead to a more passive or resting behavior. A decrease in the activation of this area in humans has been observed during the recovery from depression, which we note leads to a more active state of behavior.

INTRODUCTION

The cortex located in primates under the genu of the corpus callosum in the midline, the subgenual cingulate cortex, Brodmann's area 25, has connections with the amygdala, orbitofrontal cortex (medial parts such as area 32 and also parts of the lateral orbitofrontal cortex area 12), and temporal pole cortex (Barbas 1993; Carmichael and Price 1995, 1996; Ongur and Price 2000; Van Hoesen et al. 1993; Vogt et al. 1987; Vogt and Pandya 1987). Area 25 has descending connections in the rat to autonomic effector regions—such as the nucleus of the solitary tract, dorsal vagal nucleus, magnocellular neurosecretory cell groups in the hypothalamus, ventrolateral medulla, or intermediolateral column of the spinal cord (Gabbott and Busby 1997, 2000; Hurley et al. 1991). In the monkey, descending projections from area 25 do reach structures with strategic roles in autonomic function, such as the bed nucleus of the stria terminalis, perifornical, and anterior hypothalamus, periaqueductal gray, dorsal raphe, and lateral parabrachial nucleus (Freedman et al. 2000). The anterior cingulate cortex (which includes also areas 24a and 24b) has output projections to the periaqueductal gray in the midbrain (which is implicated in pain processing), to the nucleus of the solitary tract and dorsal motor nucleus of the vagus (through which autonomic effects can be elicited), and to the ventral striatum and caudate nucleus (through which behavioral responses could be produced).

The subgenual cingulate cortex has been implicated in depression in humans in that the recovery of depression associated with fluoxetine treatment is associated with a decrease of glucose metabolism [as indicated by fluorodeoxyglucose positron emission tomography (PET)] in the ventral (subgenual) cingulate area 25, while the induction of a mood of sadness in normal subjects increased glucose metabolism in the same area (see Mayberg 1997; Mayberg et al. 1997). On the other hand, Drevets et al. (1997) showed that PET measures of regional blood flow and glucose metabolism and magnetic resonance imaging (MRI) based measures of gray matter volume are abnormally reduced in the "subgenual prefrontal cortex" in depressed subjects with familial major depressive disorder ("unipolar depression") and bipolar disorder ("manic-depressive illness").

Raichle (1998) has noted that when humans become engaged in tasks such as word reading and verb generation, the activity observed in neuroimaging studies along the midline in the orbitofrontal cortex decreases, whereas when the humans are not actively involved in such tasks, but merely passively viewing the words, the activity increases.

Given that the subgenual cingulate cortex has been implicated in depression, we performed the single neuron recording investigation described here to provide fundamental evidence on the types of input it might receive that would activate neurons in it. Because the subgenual cingulate cortex has connections with areas such as the amygdala and orbitofrontal cortex in which the stimuli that activate neurons have been analyzed (Rolls 1999, 2000a,b), we performed the experiments described here using similar stimuli and testing methods. In particular, we investigated whether neurons in the pregenual cingulate cortex are activated by taste, olfactory, oral somatosensory, or visual stimuli. The visual stimuli were given during a visual discrimination reversal task, as this has been shown to activate orbitofrontal cortex neurons (Rolls et al. 1996a; Thorpe et al. 1983), and included faces, as these activate a small proportion of orbitofrontal cortex neurons (Rolls et al. 2003). We were also able to measure whether the neurons had activity related to movements, in that during the Go/NoGo visual and olfactory discrimination tasks, the monkeys had to initiate the response of licking a tube to obtain fruit juice when some olfactory or visual stimuli were shown, and to withhold licking when other visual or olfactory stimuli were shown to avoid the taste of aversive saline (Rolls et al. 1996a).

These are the first recordings we know from the primate subgenual cingulate cortex.

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METHODS

Recordings

Recordings were made from single neurons in the subgenual cingulate cortex, area 25. The subjects were two rhesus macaques (*Macaca mulatta*) weighing 2.5–3.5 kg. Neurophysiological methods were the same as described previously (Rolls et al. 1990; Rolls and Baylis 1994; Scott et al. 1986a,b; Yaxley et al. 1990). All procedures, including preparative and subsequent ones, were carried out in accordance with the "Policy on the use of animals in neuroscience research" of the Society for Neuroscience and were licensed under the UK Animals (Scientific Procedures) Act 1986. The monkey was fed during the experiments and on return to its home cage was allowed ad libitum access to water. Glass-coated tungsten microelectrodes were constructed in the manner of Merrill and Ainsworth (1972) without the platinum plating. A computer (Pentium) with real-time digital and analogue data acquisition collected spike arrival times and displayed on-line summary statistics or a peristimulus time histogram and rastergram. To ensure that the recordings were made from single cells, the interspike interval was continuously monitored to make sure that intervals of <2 ms were not seen, and also the waveform of the recorded action potentials was continuously monitored using an analogue delay line.

Localization of recordings

X-radiography was used to determine the position of the microelectrode after each recording track relative to permanent reference electrodes and to the anterior sphenoidal process. This is a bony landmark whose position is relatively invariant with respect to deep brain structures (Aggleton and Passingham 1981). Microlesions made through the tip of the recording electrode during the final tracks were used to mark the location of typical units. These microlesions together with the associated X-radiographs allowed the position of all cells to be reconstructed in the 50-μm brain sections with the methods described in Feigenbaum and Rolls (1991). The subgenual cingulate cortex was as defined in the studies of Carmichael and Price (1994, 1995); Freedman et al. (2000); Van Hoesen et al. (1993); Vogt et al. (1987); Vogt and Pandya (1987); and where these sources differ, we used the borders shown in the standard laboratory atlas for macaques of Paxinos et al. (2000), which uses Nissl and other histochemical staining techniques to define cortical areas.

Screening of neurons

TASTE. The testing methods used were those described by Rolls et al. (1990, 1996b) and shown to activate orbitofrontal cortex neurons. The gustatory stimuli included 1.0 M glucose (G), 0.1 M NaCl (N), 0.01 M HCl (H), 0.001 M QHCl (Q), and 0.1 M monosodium glutamate (M). The concentrations of most of the tastants were chosen because of their comparability with our previous studies, and because they are in a sensitive part of the dose-response curve. The monkey's mouth was rinsed with distilled water during the inter-trial interval (which lasted $\geq$30 s, or until neuronal activity returned to baseline levels) between taste stimuli. The stimuli within a set were delivered in random sequence. The stimuli were delivered orally in quantities of 0.2 ml with a hand-held 1 ml syringe. For chronic recording in monkeys, this manual method for stimulus delivery is used because it allows for repeated stimulation of a large receptive surface despite different mouth and tongue positions adopted by the monkeys (Scott et al. 1986a,b). The firing rates were measured in a 3-s poststimulus delivery period, as this is the period in which taste neurons, and the neurons described here, were found to have their main responses. For additional comparisons, the neuronal responses were also tested to a range of foods including banana, orange, apple juice, milk, and 20% blackcurrant juice.

ORAL SOMATOSENSORY STIMULI INCLUDING FAT. The testing methods used were those described by Rolls et al. (1999) and shown to activate orbitofrontal cortex neurons. To test for the oral effects of fat on neuronal activity, a set of fat and fat-related stimuli was delivered in the same way with a pseudorandom sequence. The fat stimuli included "single" cream (cream) (18% fat), "double" cream (47.5% fat), triolein, groundnut oil, and half-fat milk (milk) (1.8% fat). Triolein (glyceryl trioleate) was used as a pure fat. Vegetable oil (59.5% monounsaturates, 34% polyunsaturates, and 6.5% saturates) and groundnut oil were used as other natural high-fat stimuli. To investigate whether the neurons responsive to cream were in some way responding to the somatosensory sensations elicited by the fat, stimuli with a similar mouth feel but nonfat chemical composition were used. These stimuli included paraffin oil (pure hydrocarbon) and silicone oil $[Si(CH_3)_2O)_n]$. To control for specificity of the somatosensory input which could activate these neurons, other nonfat-related, oral somatosensory or motor responsiveness of neurons was screened for by allowing the monkey to chew on a short length of plastic tubing. Due to the tenacious nature of the oral coating resulting from the delivery of cream or oil, the interstimulus interval was prolonged (usually more than 2 min) and repeated rinses with water were given during this period.

OLFACTORY STIMULI. Responses to odorants were determined using either a perfumer strip method or an olfactory discrimination task (see Critchley and Rolls 1996a,b; Rolls et al. 1996a). The criteria for olfactory responsiveness were a significant elevation of cellular firing above the spontaneous firing rate to an odorant (measured during a 5-s period of presentation in front of the monkey's nose of a cotton bud/perfumer strip saturated in odor vapor), and no response to an odorless cotton bud was used as a control. The olfactory discrimination task involved the randomized delivery of odorant-saturated air via a computer-driven olfactometer (Critchley and Rolls 1996a). A cue tone preceded the delivery, following which the monkey was required to sample each odor to identify odors as part of a Go/NoGo task. A lick response to a rewarded odorant was rewarded with the delivery of a sweet aspartame solution from the lick tube; a lick response on the NoGo trials was associated with the delivery of a mildly aversive saline solution. On-line rastergrams and statistics enabled the determination of olfactory responsiveness. An air extraction apparatus was located above the monkey's head to remove odor (see Critchley and Rolls 1996a).

VISUAL STIMULI. Responses to visual stimuli were determined using a visual discrimination task to present images, views of objects, and faces on a video monitor (see Rolls et al. 1996a, 2003; Rolls and Deco 2002), and by presenting real objects and faces through a large aperture (5 cm) shutter (Thorpe et al. 1983). The visual discrimination task involved the randomized presentation on a video monitor of one stimulus per trial in a Go/NoGo paradigm. A 500-ms cue tone preceded the visual stimulus. A lick response to a rewarded visual stimulus was rewarded with the delivery of a fruit juice solution from the lick tube; a lick response on the NoGo trials was associated with the delivery of a mildly aversive saline solution. The task was typically run with two visual stimuli, one of which was rewarded and the other was not, or with six visual stimuli, three of which were rewarded and three of which were not. The actual stimuli being used could easily be changed, so that a comparison of the effects of novel and already familiar stimuli could be compared. The stimuli could include faces (cf. Rolls and Tovee 1995). On-line rastergrams and statistics enabled the determination of visual responsiveness.

SLEEP. If the monkey was not being delivered stimuli, he sometimes fell asleep. Because some of the subgenual cingulate neurons altered their activity when this occurred, we now define the criteria used to judge that the monkey was asleep. The criteria included the onset of smooth (in contrast to saccadic) eye movements, closing of the eyes, and being behaviorally less responsive, in that sometimes a loud noise or a touch was required to awaken the monkey. When the

monkey was awakened, it typically took a few seconds for the monkey to become fully alert, as judged by the eyes being fully open, the monkey opening his mouth to receive fruit juice offered, and starting to perform a visual discrimination task if this was available. To confirm that these behavioral criteria were adequate to define sleep, the electrocorticogram (ECG) was measured on a number of occasions. The recordings were made between a large electrode touching the dorsal parietal cortex and a large reference ground wire attached to the head. The potentials were low-pass-filtered to include the frequency range 0–50 Hz and sampled at 100 Hz. When the monkey was awake by the behavioral criteria, the ECG showed low-voltage fast activity. When the monkey was asleep by the behavioral criteria, large-voltage slow waves appeared in the ECG. Analysis of the power spectrum as described in the results indicated that when the monkey was judged to be asleep by the behavioral criteria (described further below), the ECG showed slow waves (low-frequency activity), indicating that the type of sleep was slow-wave sleep.

RESULTS

The data described here were obtained during 109 recording tracks in two monkeys (76 in bk and 33 in be), in which 152 (93 + 59) neurons were recorded in the subgenual cingulate cortex. No neurons were found that responded to taste or olfactory stimuli. A small number of neurons responded to novel visual stimuli, as described later. However, we discovered that some of the neurons increased their firing rate when the monkey fell asleep from a baseline level of activity which was typically zero spikes per second when the monkeys were awake. This was the most frequent type of neuronal response found in this region and is described next.

The activity of a neuron that had a baseline firing rate of 0 spikes/s when the monkey was awake and performing a visual discrimination task, or being tested with taste and olfactory stimuli, is shown in Fig. 1. Each point represents the firing rate measured over a 2-s period. At the start of the period of recording shown, the monkey was awake, having just been tested with taste, olfactory, and visual stimuli. After a period without testing and interaction with the experimenter, the monkey fell asleep at 26 s after the start of the recording period shown. Sleep was assessed by the experimenter by the monkey adopting a relaxed position in which the arms and legs remained still, the eyes closed, and only a loud noise from the experimenter would arouse the monkey to perform the task if it was re-enabled. In this period the eyes showed the typical slow drift typical of drowsiness and slow-wave sleep. Rapid eye movements were not observed, and indeed in the relatively short periods of sleep that took place during the recordings, given that the monkey was squatting in a chair, and given that the monkeys were not sleep deprived, it is very unlikely that the monkeys entered paradoxical sleep from slow-wave sleep. At the time when the monkey became drowsy and fell asleep at 26 s in Fig. 1, the subgenual cingulate neuron increased its firing rate to approximately 4 spikes/s. As the neuron had previously been so silent, this was a major change in its activity. The neuron continued to fire for approximately 110 s, and then it stopped firing and the monkey woke up. The increase of firing rate when the monkey was asleep was thus repeatable and highly statistically significant (*t*-test, awake versus asleep, $P = 7 \times 10^{-17}$). No other stimuli or event was found that altered the firing rate of the neuron, including performance of the visual discrimination task. The decrease of firing rate as the sleep period progressed was found for this particular neuron, but was not characteristic of the population as a whole. Most of the neurons did not show a trend of firing rate during the period while the monkey was asleep. The 10-s decrease in the firing rate of the neuron during the second sleep epoch shown in Fig. 1 (around time 230 s) was probably related to a short epoch of ECG activation, which often occurs during slow-wave sleep (Steriade 1996a,b, 2000).

The behavioral criteria for sleep were complemented by ECG recordings as described in the Methods. The changes in the ECG from the low-voltage fast activity when the monkey was behaviorally awake to the large-voltage slow waves found when the monkey was asleep were measured quantitatively by calculating the power spectra of the ECG when awake and asleep, using 40 samples each 1 s long to provide low errors in the spectral calculation performed by fast fourier transform (FFT) methods (Press et al. 1992). These power spectra are shown in Fig. 2. It is clear that when asleep, there was much more power in the low-frequency range (5–15 Hz) than when awake. This is the typical signature of slow-wave sleep and

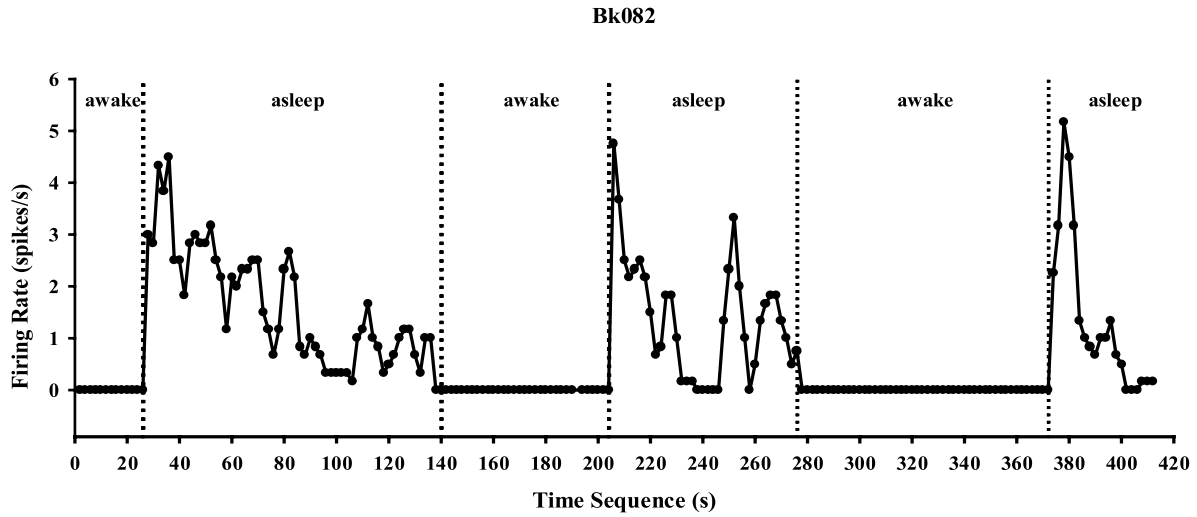

FIG. 1. The firing rate of subgenual cingulate neuron bk082 during repeated periods of waking and sleeping. The mean firing rates over successive 2-s periods (with a three-point filter applied) are shown.

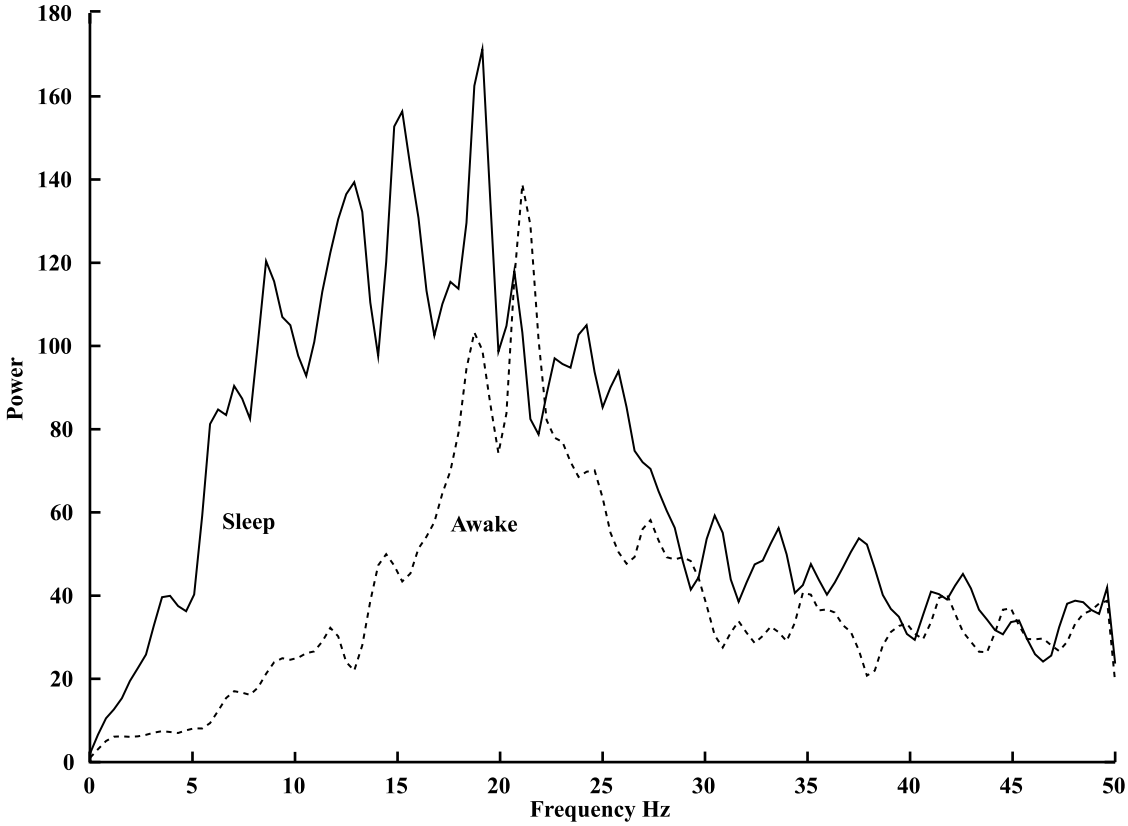

FIG. 2. Power spectra of the electrocorticogram when the monkey was awake versus asleep. The increase in power in the low-frequency (5–15 Hz) range when the monkey was rated as asleep confirms that the monkey was asleep and identifies the sleep as slow-wave sleep.

shows that in the periods when the monkey was behaviorally identified as in sleep, then the ECG signs confirmed this and showed that the sleep was slow-wave sleep. The power spectra shown in Fig. 2 show that, although low frequencies (<15 Hz) are drastically reduced during waking, fast frequencies (from approximately 18 to 50 Hz) survive during sleep and are almost similar to fast activities during waking. This is due to the fact that the depolarizing phase of the slow sleep oscillation is crowned by fast activities, which are not only evident in anesthetized animals (Steriade et al. 1996) but also by using intracellular recordings of neocortical neurons in naturally sleeping animals (Steriade et al. 2001).

Twelve of the sample of 93 neurons (12%) recorded in the subgenual cingulate cortex had responses that were similar to those of the neuron shown in Fig. 1 (apart from the trend to gradually decrease toward the end of the sleep period). Although typically the neurons recorded in the subgenual cingulate cortex with sleep-related activity increased their firing rate when the monkey fell asleep, one neuron had a decrease from 7.5 to 5 spikes/s when the monkey fell asleep. The spontaneous firing rates of this population of 12 neurons, and the changes they showed when the monkeys fell asleep, are shown in Table 1. The changes were highly statistically significant, as shown. The very low firing peak rates of these neurons were very notable, as shown in Table 1. The firing pattern of the neurons was quite regular when they were firing, and the interspike intervals of a typical neuron are shown in Fig. 3. It is of interest that these subgenual cortex neurons not only increase their activity during slow-wave sleep, but also do not show bursting patterns (which would be reflected in the interspike interval histogram by many intervals shorter than 100 ms) during sleep. Both of these properties are different from those of the majority of neurons in other cortical areas (Steriade 1996a,b, 2000).

To investigate whether disengagement from the task that the monkeys performed was sufficient to activate these neurons, their firing rate was measured in the inter-trial interval, which was normally 8 s. This was not sufficient for any of the neurons with sleep-related activity to increase their firing rate. However, to increase the disengagement from the task much more in the inter-trial interval, the inter-trial interval was lengthened

TABLE 1. *Subgenual cingulate cortex*

Cell	Awake	Asleep	*P*	% of Asleep
bk086	0.0	0.3	1.8E−06	0.0
bk085	0.1	0.5	3.1E−04	19.8
bk084	0.0	1.0	3.3E−11	2.5
bk083	0.0	0.4	2.4E−13	1.3
bk082	0.0	1.5	7.1E−17	0.0
bk081	0.5	21.8	1.4E−10	2.2
bk065	21.4	34.6	5.6E−07	61.9
bk060	0.3	1.3	4.9E−10	24.0
bk059	0.0	0.9	8.6E−03	0.0
bk058	7.5	5.0	4.0E−04	148.1
bk053	0.4	2.2	6.7E−09	16.5
bk047	0.0	1.7	6.7E−12	0.0
Average				23.0
SD				43.3
SE				12.5

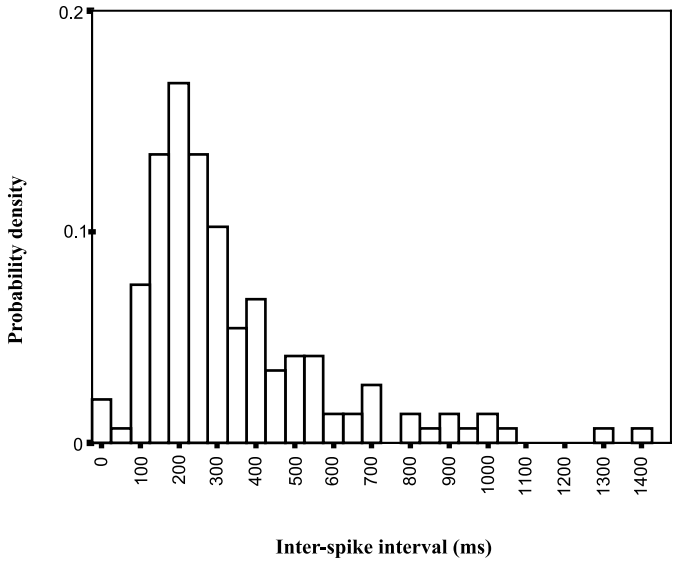

FIG. 3. Inter-spike interval histogram of one of the subgenual cingulate neurons (bk082) when the monkey was asleep. When the monkey was awake, the neuron was not firing.

to periods of ≤60 s. No sleep-related neurons increased their firing rate with these long inter-trial intervals if the monkey remained awake. For two other neurons, not classified as sleep-related, the neuronal response was generally fast during the inter-trial interval and decreased only during the trial. An example of one such neuron is shown in Fig. 4. The visual stimulus was shown at *time 0* and was preceded by a 500-ms warning tone cue. The neuron decreased its firing rate approximately 100 ms after the visual stimulus was shown and remained low until approximately the time when the trial ended (which occurred 0.5 s after the 2-s discriminative visual stimulus was turned off). The other neuron (bk036) which fired when the monkey was disengaged in this way from the task had activity that was similar to that illustrated for neuron bk022 in Fig. 4. These two neurons did not alter their activity further when the monkey became drowsy and fell asleep.

It was found that some subgenual cingulate neurons increased their firing rates only when novel visual stimuli were shown. An example is provided in Fig. 5. The neuron had no responses to familiar visual stimuli, including three faces associated with fruit juice reward and three faces associated with saline in the visual discrimination task. However, when a completely novel visual stimulus was shown on the screen (in the visual discrimination task), the neuron increased its firing rate (to 4 spikes/s). Figure 5 shows that over subsequent presentations of these novel visual stimuli (interleaved with others), the neuronal response decreased, reaching half-maximum over three to four trials. For the other neuron (bk063), the mean firing rate to novel stimuli was 3.6 spikes/s, and the mean firing rate to familiar stimuli was 3.0 spikes/s, with no further decrease found with increasing familiarity.

The recording sites of the neurons described here are shown in Fig. 6. A summary of the proportions of each neuron type is shown in Table 2.

In addition, we explored beyond the subgenual cingulate cortex to investigate whether any neurons in nearby areas had activity related to sleep. We found a population of 4 neurons of 177 neurons tested in the orbitofrontal cortex that increased their activity during sleep. Data on these neurons are shown in Table 3. The recording sites of these four cells in the orbitofrontal cortex are shown in Fig. 7.

DISCUSSION

The most frequent type of neuron found in the primate subgenual cingulate cortex had a very low spontaneous activity during waking and increased the firing rate to a steady though low discharge rate during sleep. The changes of firing rate were extremely statistically significant. When the neurons were firing, the ECG was showing that the monkey was in slow-wave sleep. We checked whether simply disengaging from a task such as a visual discrimination in the inter-trial interval was sufficient to produce an increase in the firing rate of these neurons, and in most cases it was not. A longer period of 1–2 min was typically needed in which the experimenter was not running a task, or interacting with the monkey by offering food, etc., before these neurons increased their firing rates, and the increase in firing rate occurred as the monkey was falling asleep and continued while the monkey was sleeping. In this sense, the best correlate of the firing of the majority of these neurons was being asleep, rather than just being disengaged from the task. However, two neurons in the subgenual cingulate cortex did increase their firing rates when the inter-trial interval was in the range of 8–30 s, and the monkey was disengaged from the task but not asleep. Engagement in the task again produced by the 500-ms tone cue that signaled the start of a trial of the visual discrimination task resulted in the monkey alerting again toward the video monitor and preparing to perform a lick response in the limited time available (1.5 s)

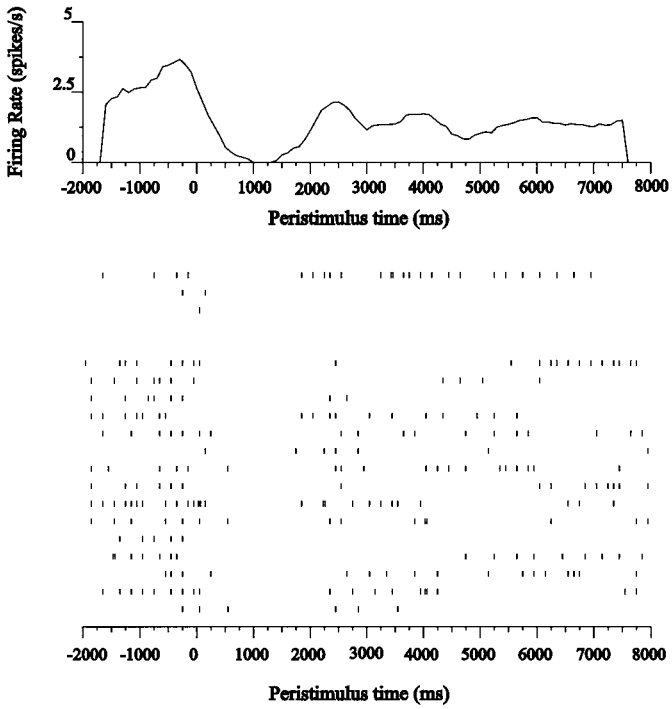

FIG. 4. The firing shown as peristimulus rastergrams and a firing rate histogram of the activity of a neuron (bk022) that decreased its firing rate, while a stimulus was shown from *time 0* until poststimulus time 2,000 ms in a Go/NoGo visual discrimination task. A 500-ms tone preceded the visual stimulus. Six visual stimuli were being used, with 3 associated with fruit juice rewards, and 3 with saline.

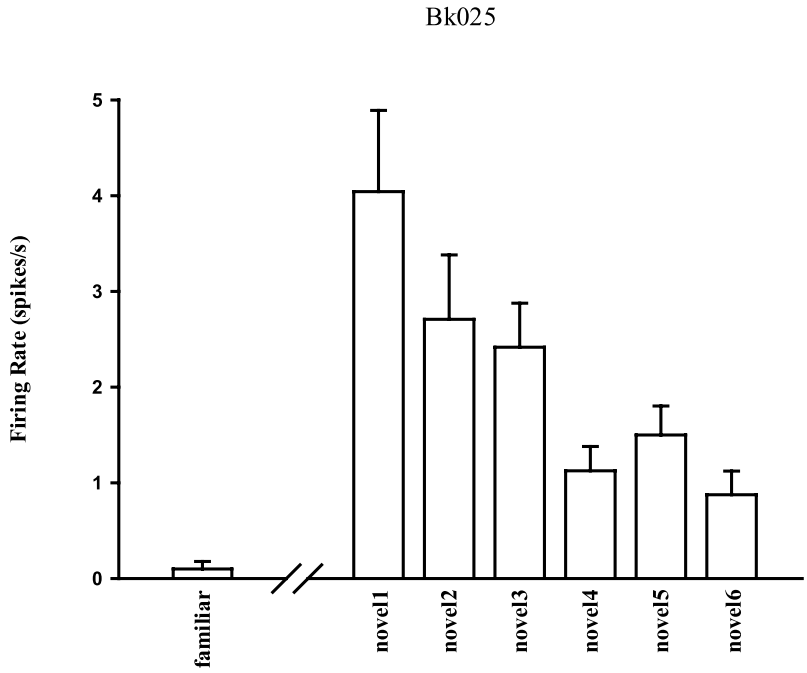

FIG. 5. Novel visual stimuli neuron, bk025. The firing rate (mean ± SE) of the neuron to a set of 6 familiar visual stimuli, and to a set of 12 novel visual stimuli shown in a sequence of 6 successive interleaved presentations (the mean over the set of 12 stimuli for the first novel presentation is shown as novel 1, for the second presentation as novel 2, etc.).

if the Go visual stimulus was shown. Thus overall, although disengagement from a task for a period of perhaps 10 s was sufficient to activate a few of the neurons, the best correlate for the majority of subgenual cingulate neurons was actually being asleep.

It is of interest to compare the responses of subgenual cortex neurons with those of neurons in the head of the caudate nucleus, for the comparison underscores that fact that whereas the head of caudate nucleus neurons become engaged in firing on a trial-by-trial basis, the subgenual neurons have a tonic change which lasts throughout the period in which the monkey is asleep and do not generally switch on tonically just while the monkey waits between trials for the next trial to start a few seconds later. In this sense the subgenual cingulate neurons are not just the converse of the large population of neurons in the head of the primate caudate nucleus, which are very closely related to the monkey being involved in each trial of a task. These head-of-caudate neurons start to respond when a 500-ms warning cue such as a tone or a light, which indicates that a trial of the visual discrimination task used here is starting. They continue to respond throughout the trial; that is, they stop firing on reward trials when the reward-related discriminative visual stimulus disappears and fruit juice is no longer available and stop responding on saline trials within approximately 200 of the onset of the visual stimulus which informs the monkey that licks should not be made; otherwise aversive saline will be obtained (Rolls et al. 1983; Rolls and Treves 1998; Chapter 9; Rolls 1999; Chapter 6). Some neurons in this population respond to environmental cues such as the door opening, or the experimenter reaching toward the place where food that may be given to the monkey during testing is located. This population of neurons in the head of the caudate thus respond between them to a wide range of stimuli which have in common the property that they engage the monkey in the testing situation by indicating that an event such as a reward stimulus which will require an action or a nonreward stimulus is shown. In contrast, the primate subgenual cingulate neurons described here do not respond in a clear time-locked way to the engagement in a trial and the stimuli that initiate this, but instead are much more closely related to actually sleeping.

It was surprising in view of the connections between the orbitofrontal cortex and the subgenual cingulate cortex that subgenual cingulate neurons did not in some cases have responses to taste, olfactory, and most visual stimuli, even though the testing methods, including the tasks used, were the same for both areas. One type of response found in the subgenual cingulate cortex was, however, similar to those of a population of neurons in the anterior orbitofrontal cortex that responds to novel visual stimuli (in preparation).

The subgenual cingulate cortex neurons recorded were probably pyramidal cells and not interneurons in that the spikes of these neurons were large and wide, which is typical of cortical pyramidal cells.

The findings on the subgenual cingulate cortex indicate that the most frequent correlate of neuronal firing was the state of sleep, with high firing rates during slow-wave sleep. Neurons that show large increases in their firing rates during sleep are unusual, and indeed, are not characteristic of any other cortical area we know apart from callosal neurons linking precentral motor areas (see Steriade 2000 and Steriade et al. 1974). To obtain further evidence on whether there is any similar set of neurons in nearby, and connected, brain areas, we also recorded from orbitofrontal cortex neurons in one of the same monkeys with the same protocol. Of quite a large sample of orbitofrontal cortex neurons (177), we found four that did increase their firing rates during slow-wave sleep. This shows that such neurons are not found only in the subgenual cingulate cortex. However, the orbitofrontal cortex neurons were different from those in the subgenual cingulate cortex, in that the increases of rate were small, from a mean of 0.1 spikes/s when awake to a mean of 1.0 spikes/s when asleep. Moreover, in contrast to the subgenual cingulate neurons, the orbitofrontal cortex sleep-related neurons were intermixed with neurons that had responses to many other types of stimuli and events,

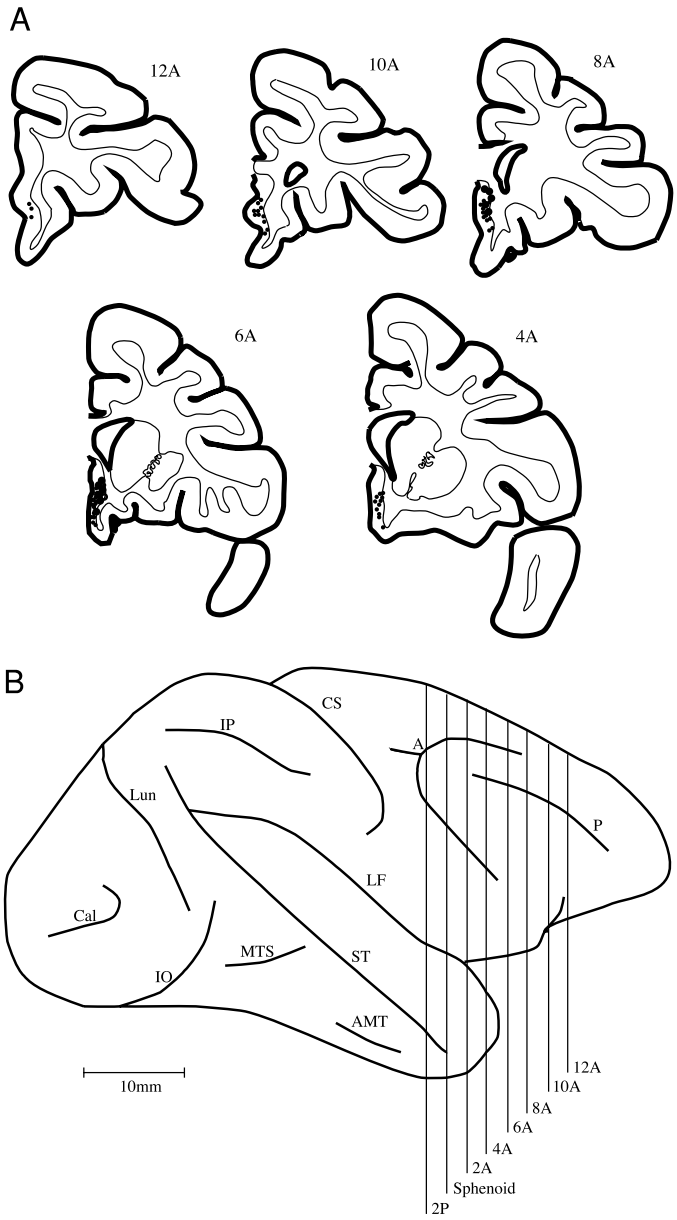

FIG. 6. *A*: recording sites of the cingulate neurons recorded in this investigation. The large circles show the sleep-related neurons, and the small dots show the recording sites at which nonresponsive neurons were recorded. There are fewer dots than neurons recorded just because some of the dots in Fig. 6 overlap (the sites are based on the X-ray data for every track and a standard atlas calibrated in X-ray coordinates). *B*: a lateral view of the macaque brain showing the levels with respect to sphenoid of the coronal sections shown in *A*.

including taste, olfactory, and visual reward-related stimuli (see Rolls 1999, 2000a,b).

The finding that the most frequent type of neuron found in the subgenual cingulate cortex increased the firing rate during sleep is of interest for a number of reasons. First, there are few brain regions known where the neurons show an overall increase in their firing rates very markedly during sleep. In the midbrain reticular-activating system, for example, neurons have low firing rates during sleep and increase their firing rates during wakefulness, being stimulated into activity by arousing stimuli such as somatosensory inputs, and once firing, by maintaining the evoked increase of firing rates for periods of often several minutes (Rolls 1971; Steriade 1996a,b).

TABLE 2. *Subgenual cingulate cortex*

	X	Percentage of *X*
Total cingulate neurons	93	
Sleep neurons	11	12
Awake neurons	1	1
Novelty neurons	2	2
Disengaged neurons	2	2
Onset-offset neuron	1	1

TABLE 3. *Orbitofrontal cortex*

Cell	Awake	Asleep	*P*	% of asleep
bk110-3	0.2	1.4	1.0E−03	16.4
bk112-1	0.1	1.4	1.5E−06	5.0
bk138-1	0.0	0.6	4.4E−04	0.0
bk139-1	0.1	0.7	2.4E−04	13.5
Average				8.7
SD				7.6
SE				3.8

Second, the neurophysiological findings reported here suggest that it would be interesting to extend the human neuroimaging observations described by Raichle (1998). He summarized a number of findings which indicated that in neuroimaging studies the activation of medial frontal lobe areas is increased when humans are not performing a task. The possibility that this activation reflected some disengagement from the tasks was discussed. In view of the findings described here, and to help understand these brain areas better in humans, we suggest that it would be of interest to investigate whether a better correlate of the activation of these medial frontal areas might be sleep. Indeed, some of the subjects in the investigations described by Raichle (1998) may have become drowsy when they were not required to perform a task, and it would be of interest to investigate whether it is the drowsiness or sleep that accounts for the activation here, rather than not actually being engaged in performing a task.

Third, the subgenual cingulate cortex may play a role as a

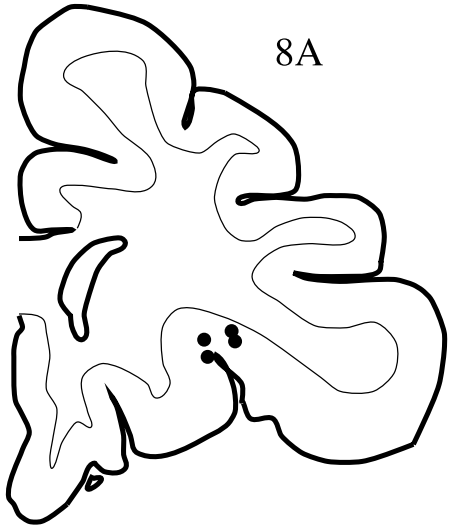

FIG. 7. The recording sites of the orbitofrontal cortex neurons with sleep-related activity recorded in this investigation. The large circles show the sleep-related neurons (the sites are based on the X-ray data for every track and a standard atlas calibrated in X-ray coordinates).

strategic cortical portal for regulation of the autonomic nervous system (e.g., Owens and Verberne 2001). This role is indicated by, for example, its descending connections in rats to brain stem autonomic effector regions such as the dorsal motor nucleus of the vagus (related to parasympathetic function) and the ventrolateral medulla (related to sympathetic function) (Gabbott and Busby 1997, 2000; Hurley et al. 1991). The connections in primates are to regions that may have similar functions and are in addition to the periaqueductal gray, dorsal raphe, parabrachial nucleus, and hypothalamus, as noted above. In this context, the increased activity in sleep in the subgenual cingulate cortex might be hypothesized to be involved in the altered control of gut and cardiopulmonary function that occurs during sleep.

Fourth, the findings reported here are of interest in relation to findings on the brain states related to depression (Drevets and Raichle 1992; Dolan 1997; Dolan et al. 1992). Mayberg and colleagues reported that the recovery of depression in humans associated with fluoxetine treatment is associated with a decrease of glucose metabolism (as indicated by fluorodeoxyglucose PET) in the ventral (subgenual) cingulate area 25, while the induction of a mood of sadness in normal subjects increased glucose metabolism in the same area (see Mayberg 1997; Mayberg et al. 1997). Now it might be expected that depressed patients would interact much less with their environment, while after recovery they would be much more alert and engaged in their environment. Inducing sadness might similarly produce less alertness and responsiveness to environmental activating stimuli. It would thus be of considerable interest to know whether simply reducing arousal and allowing sleep in humans would result in an increase in activation found by neuroimaging in the subgenual cingulate region. If so, the findings in this region in depressed patients might just reflect their altered state of alertness. Of course, it might also be the case that the activity in this region is a contributing causal factor to depression, one manifestation only of which might be altered alertness and engagement with the environment. In either case, it would help to interpret the findings in this brain region (and the reciprocal changes in more dorsal parts of the frontal cortex) of depressed patients if it were known whether this area did increase its activation in humans simply when they become less alert or fall asleep. Given the suggestions of a relation between depression and paradoxical sleep (Liscombe et al. 2002; Wichniak et al. 2000), it would also be of interest in future investigations to perform single-neuron recordings in monkeys from the subgenual cingulate cortex and to study this region using functional magnetic resonance imaging (fMRI) in humans, during paradoxical sleep.

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