Spontaneous and auditory-evoked activity of medial agranular cortex as a function of arousal state in the freely moving rat: interaction with locus coeruleus activity

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**Abstract**

To characterize the electrophysiological properties of neurons in the medial agranular frontal cortex (Fr2) with respect to arousal level and locus coeruleus (LC) activity, we recorded spontaneous and auditory-evoked single unit activity in these areas simultaneously during different states of arousal in the rat. In the low-arousal state, as determined by EEG, 14/56 Fr2 neurons showed a tonic increase in spontaneous firing rate and 9/56 presented a clear inhibitory response to tone (onset latency 37 ms, duration 200 ms). The inhibitory response was not clear during the high-arousal state. Cross-correlation analysis of pairs of Fr2 and LC units, excluding activity during the actual tone, also showed a negative correlation from 120 ms before, to 170 ms after, Fr2 discharge in 5/63 pairs, only during low arousal. Significantly, 4/5 of the Fr2 neurons having this negative correlation with LC were included in that population which showed a tonic increase in spontaneous firing rate and inhibited to tone during low arousal. LC neurons, on the other hand, all showed excitation to tone stimulation (peak latency 30 ms), and response amplitude was not affected by changes in arousal level. The negative correlation in spontaneous activity, as well as their differential responses to tone, suggests an interaction between a select population of Fr2 neurons and the LC during the low-arousal state. Future studies with lesion or pharmacological manipulations would be necessary to confirm the presence of this interaction. © 2000 Elsevier Science B.V. All rights reserved.

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**1. Introduction**

Anatomical studies have suggested that the medial agranular frontal cortex (Fr2) of the rat contains the homologue of the primate frontal eye field with extensive cortico-cortical connections [16,17] and subcortical projections to brain stem visuomotor centers [23]. Lesion studies show that unilateral damage of Fr2 in the rat is followed by neglect of the contralateral side [8] and appearance of ipsilateral response bias in a spatial reaction-time task [4]. There are relatively few studies on electrophysiological properties of Fr2 neurons in awake animals, with the exception of studies by Pirch et al. [14,18] showing both excitatory and inhibitory conditioned responses in this region in awake rats. Based on these observations, it has been proposed that the Fr2 is a frontal association cortex of the rat and is engaged in cognitive functions including attention.

The locus coeruleus (LC) is a brain stem noradrenergic nucleus that is also involved in attention [1,27] with diffuse projection to widely-distributed areas of the brain [24]. Administration of drugs which inhibit LC activity or impair noradrenergic function such as clonidine and 6-hydroxydopamine produces a decrease in novelty-seeking behavior and electrodermal response to orienting stimuli indicating the disturbance of attention [22,26]. Electrophysiologically, LC neurons show an increase in firing
when arousal level is elevated [1], and are activated in a burst in response to sensory stimuli when they are novel [21] or task-relevant [2,20,25].

Luppi et al. [10] have revealed by anatomical tracing methods that the Fr2 is a cortical afferent to LC in the rat. Furthermore, the Fr2 is the only region, among multiple frontal areas investigated, that is antidromically activated by LC stimulation [19]. Suppression of neuronal activity in the Fr2 increases LC firing in anesthetized rats [19] and electrical stimulation of Fr2 elicits excitatory, inhibitory or mixed excitatory-inhibitory responses in LC [3,7,12].

These studies suggest a functional relationship between Fr2 and LC and this might be involved in modulation of attention. Simultaneous recording of unit activity in the two regions allows further evaluation of this functional relationship through analysis of correlation in the neuronal activity. In anesthetized rats, correlative activity between the Fr2 and LC has already been described using single unit recording [19]. Further analysis of these data by cross-correlograms revealed a consistent phase difference between the Fr2 and LC activity [9]. However, the presence of slow oscillations, perhaps induced by anesthesia, limits the functional interpretation of these data. The present study, therefore, was designed to investigate the interaction between spontaneous Fr2 and LC neuronal activity in the awake, freely-moving rat, as well as to compare the electrophysiological properties of Fr2 neurons to LC neurons in response to sensory stimulation during different states of arousal or attention.

2. Materials and methods

2.1. Animals and surgery

Four rats of Sprague–Dawley strain, obtained from IFFA CREDO, were used (316–430 g at the time of surgery). They were housed individually in Plexiglas cages in a temperature controlled vivarium with a 12 h light–dark cycle. They were handled daily, before and after surgery. All recordings and behavioral observations were carried out in the middle of the light cycle.

Under pentobarbital anesthesia (60 mg/kg), the skull was fixed with the ear bars in a stereotaxic apparatus with the upper jaw level being 7.3 mm below the inter-aural line.

2.2. Micro-drive for electrode manipulation

The micro-drive apparatus was made in our laboratory using two metal screws (about 10 mm long) attached in parallel to an Amphenol strip (about 5×5 mm) to which a stainless steel microelectrode (2–5 MΩ) was glued for unit recording. The shape of the screw tip was designed as to stay firm but movable in the resin after being cemented on the rat skull. The width of the screw groove was 400 μm, and each full rotation of the screw advanced the electrode 400 μm. During the recording sessions, the electrode was moved by increments of 50 μm to find a stable unit firing activity.

Two stainless microelectrodes attached to the micro-drive apparatus were implanted in the Fr2 (Bregma +3.0, L 1.25–1.5) and in the LC (Lambda −3.9, L 1.15), respectively. The electrode tip in the Fr2 was set at the surface of the cortex. The LC electrode was lowered to a depth of 5.0–6.0 mm from the cerebellar surface, and was held at the depth where the recorded cells were identified as of LC with the following criteria: localization just below the fourth ventricle, situation just medial to the mesencephalic trigeminal cells that respond to jaw movement, low spontaneous discharge (below 2 Hz during surgery under anesthesia), broad spikes (above 0.7 ms), biphasic excitatory–inhibitory response to paw pinch [5]. At the end of each experiment, the recording site was confirmed histologically by passing current through the electrode (Fig. 1A). The alpha 2 receptor agonist, clonidine (15 μg/kg, i.p.) was injected following some LC recording sessions to

![Fig. 1. (A) A histological picture showing the recording site of LC firing. The arrow indicates the lesion at the dorsal part of LC produced by passing current to the electrode at the end of experiment. The LC is situated medial to the mesencephalic nucleus of trigeminal nerve (Me5). The position of the motor nucleus of trigeminal nerve (M5) is also presented. (B) Effect of clonidine injection (15 μg/kg, i.p.) on the spontaneous firing rate of a LC neuron. The arrow indicates the time of clonidine injection and each bar represents the number of LC firing during 10 s period. Suppression of the firing activity was observed following clonidine injection.](https://example.com/fig1.png)
pharmacologically control for the noradrenergic nature of the cell from which the recording was made (Fig. 1B).

During the surgery, two stainless screw electrodes were also placed on the frontal (Bregma +3.0, L 1.0) and occipital (Bregma −8.0, L 1.0) areas of the cortical surface for EEG recording. The lead wires from the microelectrodes and the screw electrodes were connected to a socket. Then the micro-drives, screw electrodes, and the socket were firmly attached to the skull with resin cement.

2.3. Recording of unit activity and EEG

After several days for recovery, the recording session was initiated in a sound-attenuated chamber (25×25×40 cm) where the rat could move freely. Each lead wire from the electrode was connected to a pre-amplifier attached to the socket for impedance reduction, and then through a mercury commutator to an amplifier. The screw electrodes on the frontal and occipital cortices were used as the reference for unit recording in the Fr2 and LC, respectively. EEG was recorded bipartially between the frontal and occipital screw electrodes. A band-pass filter was set between 300 and 3000 Hz for unit and between 1 and 300 Hz for EEG recording. Unit firing was sought by advancing or retracting the microelectrode attached to the micro-drive apparatus. Neuronal activity was displayed on an oscilloscope and on an auditory monitor. Neuronal data were collected through the CED interface (1401plus, Cambridge Electronic Design) using Spike 2 software and stored on a computer. EEG activity was collected continuously on one channel. Two other Spike 2 channels collected the wave marks of neurons from the microelectrodes in Fr2 and LC. Individual units were discriminated by establishing templates of the wave forms of the units recorded from each electrode before each recording session. In addition, the wave marks of all neuronal events which passed a predetermined amplitude were stored as unidentifiable neurons for later off line evaluation. Movement artifacts were noted and later removed from analysis. Other Spike 2 channels recorded the individual tones or other events.

For each daily session, spontaneous firing was recorded for about 20 min followed by the recording with intermittent auditory stimulation for about 20 min, using 8 KHz tones of 40 ms duration, presented with the inter-stimulus interval of 2.5 s through a speaker situated on the ceiling of the recording chamber. The intensity of the tone was adjusted so that orienting or startle movements of the rat were not elicited in response to the tone.

In order to examine the relation to arousal level, the data were collected both during synchronization and desynchronization of EEG. These two states of arousal were identified by the difference in EEG voltage and power spectrum with FFT analysis using Spike2 software. The EEG with high voltage contained a larger amount of synchronized waves (Fig. 2B), and indicates that the arousal level was lower.

2.4. Data analysis

Using the Spike 2 software, off line analysis examined wave forms and spike duration to verify the accuracy of the separation of single units. Spontaneous firing rate and tone-evoked response for both Fr2 and LC unit recordings were analyzed. Cross-correlation analysis evaluated the relationship between the firing patterns of units in the two regions.

For analyzing the tone-evoked response, peri-stimulus time histograms were generated and the response amplitude was calculated as the difference between the firing rate during the response period and that during the baseline which was the 100 ms period before the tone onset. When the recording contained periods with synchronized and desynchronized EEG during tone stimulation, the effect of arousal level on the response amplitude was also analyzed by making separate histograms for each state.

Cross-correlograms were plotted as the distribution of

Fig. 2. (A) A pair of Fr2 and LC firings showing opposite relationships to arousal level as indicated by EEG (top). When EEG was synchronized (open horizontal bar), this Fr2 neuron showed a tonic increase accompanied by a decrease in firing of the LC neuron. When EEG was desynchronized (filled horizontal bar), the relation was opposite with a decrease in firing rate of the Fr2 neuron and an increase in LC activity. Bin width 1 s. (B) Power spectrum of the EEG when it is desynchronized (filled horizontal bar in A) and synchronized (open horizontal bar in A).
LC unit activity, with the trigger being the Fr2 spikes. The range of analysis was ±0.5 s from the Fr2 trigger and the bin width was 10 ms. When the tone was presented, the cross-correlation analysis was conducted for the period excluding the 200 ms period after the tone onset.

Wilcoxon’s non-parametric test was employed to statistically evaluate the difference between the data acquired during the periods with synchronized and desynchronized EEG.

3. Results

3.1. Unit activity as a function of EEG

A total of 56 Fr2 neurons were recorded from four rats. All the neurons had a wide spike duration (0.82±0.02 ms, mean±S.E.M.) and were most likely generated by pyramidal cells. They were located in the Fr2 area whose activation or inactivation changed the LC activity in previous studies [7,19]. The spontaneous firing rate was calculated for each neuron during the period with synchronized EEG and that with desynchronized EEG (Fig. 2A), and it was found that 25% (n=14) of the Fr2 firings exhibited an increase in firing rate during the period with synchronized EEG. For these 14 neurons, the firing rate during synchronized EEG was 8.4±2.4 Hz and was higher than that during the desynchronized EEG (4.8±1.7 Hz; Z=3.2958, P=0.001). The other 42 neurons did not manifest a clear relationship with EEG synchronization or desynchronization.

Thirty seven LC neurons were recorded in the present study and all showed a lower firing rate during the period with synchronized EEG (0.8±0.2 Hz) than during the period with desynchronized EEG (3.5±0.7 Hz, Fig. 2A; Z=3.4078, P=0.001).

3.2. Responses to tone stimulation

There were two types of tone-evoked response in the Fr2 neurons. The first type was inhibitory with a decrease in firing rate between the period from 37.2±6.2 to 238.8±36.2 ms after the tone onset and was found in 16% (n=9) of the Fr2 units (Fig. 3, Fr2 A). The response amplitude during the inhibitory period was -4.9±2.1 Hz when the EEG was synchronized, and -0.7±1.1 Hz when desynchronized. The response was more prominent during the period with synchronized EEG (n=6, Z=1.9917, P=0.046).

The second type of Fr2 response, observed in 14% (n=8) of neurons was excitatory, with the response onset, peak and offset latencies being 11.9±1.2, 21.9±2.5, and 45.5±3.1 ms, respectively (Fig. 3, Fr2 B). The response amplitude during the interval between the onset and offset was 13.6±3.7 Hz when the EEG was synchronized, and was significantly larger than that when the EEG was desynchronized (9.0±2.1 Hz, n=5, Z=2.0226, P=0.043). Two units showed both excitatory and inhibitory responses (Fig. 5).

All the LC neurons showed an excitatory response with the onset, peak and offset latencies being 15.2±2.0, 29.7±3.9 and 89.1±6.7 ms, respectively (Fig. 3, LC). When the periods with synchronized EEG are compared with periods of desynchronized EEG, the response amplitude showed no statistical difference (10.4±1.9 Hz and 10.6±1.7 Hz, n=19, Z=0.4024, P=0.687). Ten LC cells also presented a second peak with the latency being 74.7±8.2 ms possibly reflecting the response to tone offset.
3.3. Relation between the Fr2 and LC activity

Cross-correlation analysis on 63 Fr2/LC neuronal pairs showed that in five pairs, the LC firing manifested a negative correlation from 117.4±48.6 ms before to 167.6±51.6 ms after the Fr2 discharge, but only during the period with synchronized EEG (Fig. 4). This negative correlation was not observed when EEG was desynchronized. Firing rates within the period of cross correlation analysis were further examined by measuring the ratio of the firing rate during the correlated period to the average firing rate during the period from −0.5 to −0.4 s and from 0.4 to 0.5 s after the Fr2 discharge. The ratio was 0.380±0.058 during the EEG synchronization which was significantly smaller than the ratio during the EEG desynchronization (0.962±0.058, \( Z=2.0226, P=0.043 \)). This ratio approaches 1 during desynchronized EEG which is merely a reflection of the fact that the correlogram is flat, as can be seen in Fig. 4. It should be noted that 4/5 Fr2 neurons having this negative correlation with LC discharge were included in the population which showed increase in spontaneous firing and inhibitory response to tone stimulation during low arousal i.e. synchronized EEG (Fig. 5).

3.4. Topographical distribution of Fr2 neurons

Fig. 5 presents the anatomical distribution of different types of Fr2 neurons described above. Among the data obtained with the four penetrations of electrodes in the present study, 8/9 inhibitory Fr2 neurons as well as the neurons manifesting a negative correlation with LC were found in the more medial region, although no marked regional localization was observed in the vertical direction. Neither Fr2 neurons showing an increase in spontaneous firing rate during low arousal, nor those with excitatory response presented noticeable topographical localization (Fig. 5).

4. Discussion

4.1. Spontaneous activity as a function of arousal state

In the present experiment, it was found that about one fourth of the Fr2 neurons presented an increase in firing rate during low-arousal state, defined by EEG. This variable relation to arousal level among Fr2 neurons is in contrast to LC activity, where all neurons show a low firing rate during low arousal state and an increase when the EEG becomes desynchronized. It is known that the effect of noradrenaline in the cortex released from terminals of the LC is principally inhibitory on the target cells [6,11], so there is a possibility that the low firing rate of Fr2 neurons during high arousal in the present study is related to LC activation during this state. However, the inhibitory effect of noradrenaline may not be sufficient to explain the relationship of Fr2 activity to arousal levels, since only a portion of the population of Fr2 cells are suppressed during LC activation (desynchronized EEG) in presence of diffuse release of noradrenaline in the region.
4.2. Evoked activity

The present study revealed that some of the neurons in the Fr2 cortex of the rat respond to auditory stimuli; both excitatory and inhibitory responses were observed as well as a majority of nonresponsive cells. This multiplicity of response types indicates that the Fr2 is not homogeneous regarding the processing of sensory information. As for the relation to arousal level, both inhibitory and excitatory responses were a function of the EEG, and were evoked significantly only during the low-arousal state. Furthermore, six of the nine Fr2 neurons showing inhibitory evoked responses belong to the group of cells showing an increase in spontaneous firing rate during the low arousal state (Fig. 5). These results concerning the auditory-evoked responses of Fr2 neurons in relation to arousal level, together with the arousal-related change in spontaneous firing rate, suggest that there is a group of neurons in the rat Fr2 whose activity depends on the low arousal level.

In contrast to this diversity of responses in Fr2 and the dependence upon arousal levels, the LC response to tone was homogeneous, excitatory in all cases, and all LC neurons responded to the tone. This response occurred under all levels of arousal.

4.3. Interaction between the Fr2 and LC

The correlograms revealed a decrease in LC firing from about 120 ms before to 170 ms after the Fr2 discharge, and indicated the presence of reciprocal firing pattern between the Fr2 and LC neurons. This relationship was conspicuous only during low arousal state, reminiscent of the tone-evoked inhibition and excitation in Fr2 neurons described above. However, the negative relationship between these areas should not be the result of common auditory input because the period of evoked response was excluded from the analysis. The proportion of Fr2 neurons negatively linked to LC unit activity (5/63=7.9%) is strikingly similar to the proportion of Fr2 cells found to be antidromically driven by stimulation of the LC in anesthetized rats (52/700=7.4%) [19]. Significantly, 4/5 of the Fr2 neurons having this negative correlation with LC were included in that population which showed a tonic increase in spontaneous firing rate during low arousal and inhibited to tone.

Thus, these observations in behaving rats are consistent with the previous finding by Sara and Hervé-Minvielle [19] suggesting the presence of reciprocal inhibitory influence between the LC and a small population of neurons in Fr2. A further similarity between the studies in
anesthetized rats and the present results lies in the fact that a relationship between the activities of neurons in the two structures is only found during a synchronized EEG. In the former studies the relationship was only discerned when both Fr2 and LC were firing in a synchronized, oscillating mode [9]. However, in order to confirm the presence of interaction between these two areas, it would be necessary to employ lesion or pharmacological manipulations in the future studies.

The functional significance of the present observations and their role in the putative attention functions attributed to both the Fr2 and the LC, as outlined in the introduction, remains to be elucidated. Other investigators have found that a small population of neurons in the same cortical region, show conditioning-related increase in inhibitory responses during auditory conditioning in both awake and urethane-anesthetized rats [14,15,18]. In the present experiments the inhibitory responses of Fr2 neurons to tone stimulation were present only during low arousal state, when LC neurons showed excitatory responses in spite of the low spontaneous activity. This suggests that the inhibitory response in Fr2 neurons projecting to the LC region might serve to promote or permit oriented and conditioned LC phasic responses, by blocking the Fr2 inhibitory influence. The critical question now is whether the population of Fr2 neurons inhibiting to tone are the same cells which have terminals in the LC region and have an inhibitory influence on LC activity. Future behavioral studies, in which unit activity is recorded simultaneously from both regions during discrimination learning, will further elucidate the role of interaction between these areas in control of attention.

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