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Functional synergism between putative γ-aminobutyrate-containing neurons and pyramidal neurons in prefrontal cortex

(fast spike/monkey/memory/interneurons/vision)

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ABSTRACT **The responses of putative γ-aminobutyratergic interneurons (fast-spiking) and pyramidal (regular-spiking) cell pairs were compared in monkeys performing visual and memory-guided oculomotor tasks. Both fast- and regular-spiking neurons had similar receptive fields, indicating that γ-aminobutyratergic interneurons carry a specific informational signal, as opposed to providing nonspecific modulation. However, the responses of the pairs were inverted and the timing of excitatory and inhibitory responses appeared to be phased, a property consistent with γ-aminobutyrate-mediated shaping of receptive fields. These observations (*i*) provide evidence that interneurons and pyramidal cells can be differentiated *in vivo* and (*ii*) begin to elucidate the role of γ-aminobutyratergic mechanisms in cognition.**

γ-Aminobutyric acid (GABA) is an inhibitory neurotransmitter crucial for the stimulus selectivity of receptive fields in visual and somatosensory cortices (1–3). Infusions of bicuculine, an antagonist of GABA receptors, transform the stimulus selectivity into nonspecific responsiveness, suggesting that GABAergic inhibitory interneurons shape receptive fields. However, the properties of GABAergic interneurons (2) are largely unknown, and this lack of information impedes the analysis of local circuit operations underlying receptive-field properties and therefore sensory, motor, and cognitive function. *In vitro* intracellular studies have, however, physiologically identified a type of neuron possessing smooth dendrites characteristic of interneurons and possessing the immunohistochemical signature of GABA (4, 5). These fast-spiking (FS) interneurons have brief action potentials and high firing rates distinguishable from those of pyramidal (RS) neurons (4–11). The objective of the present study was to determine the functional relationship, if any, between FS and RS neurons recorded in the prefrontal cortex of monkeys performing oculomotor tasks.

METHODS

Animals. Rhesus monkeys (*Macaca mulatta*) were used in accordance with the Yale University Animal Care Committee.

Electrophysiology and Data Collection. Standard extracellular techniques (12, 13) were used to sample prefrontal regions receiving direct inputs from prestriate and visual association cortex (14, 15). Recordings were made with a single microelectrode. The amplitude and time course of action potentials were measured on a Nicolet 12-bit oscilloscope. Spikes were isolated with a discriminator by setting voltage and time criteria to determine spike occurrence (resolution, 4 msec). Data on each neuron were recorded sequentially.

At the beginning of each experiment a microelectrode was advanced into the brain looking for the activity of single neurons while the monkey performed the behavioral tasks. When a stable neuronal recording was obtained, data were collected until all tasks had been completed (if possible) in order to characterize each neuron's functional properties. The discriminator was then adjusted to isolate a different neuron at the same site or the electrode was advanced. Typically, 5–10 neurons were recorded in a pass through the cortex.

Behavioral Tasks. Two monkeys were trained to fixate a central point on a video monitor while a stimulus was presented either on the fovea or in a peripheral location to map receptive fields. Eye position was monitored with a scleral search coil. Following initial fixation (0.5 sec), a stimulus was presented for 1 sec. Juice was delivered 0.5 sec after stimulus offset if fixation was maintained. Monkeys performed four tasks requiring sustained fixation. Each task had seven to eight different stimulus types, and 8–10 presentations of each type were obtained. (*i*) The RF task was used to identify receptive fields in peripheral visual space: a stimulus (subtending 0.5°) was presented at one of eight locations at 13° eccentricity, with 45° of angular separation between them. (*ii*) The Sac task was used to identify neurons with directional saccadic correlates: a stimulus appeared at one of eight locations simultaneous with the disappearance of the fixation point. The monkey made a saccade to and fixated the stimulus for 1 sec. (*iii*) The Pic task was used to identify neurons with foveal receptive fields which required complex, textured patterns (subtending 10°), which were digitized photographs of laboratory objects. (*iv*) One of the monkeys performed an oculomotor delayed response (ODR) task, a test of spatial working memory requiring maintained fixation while a cue (0.5 sec duration) is presented at one of eight locations around the point of fixation. The memory for the cue location had to be retained for 2.5 sec. At the end of the delay the fixation point disappeared and a saccade was made to the remembered location of the cue.

Data Analysis. Graphical displays of neuronal activity were plotted off-line. For quantitative analysis, the onset/offset latencies of the neuronal responses were first determined by using cumulative sum histograms (16), and these values were used to identify a neuronal response epoch from which the firing rate was calculated. Data on firing rate for each trial was entered into a computer-based spreadsheet and subjected to a two-way analysis of variance (Systat, Evanston, IL) to compare stimulus-elicited responses (range, 100 msec to 1 sec) with a 1-sec pretrial control period and to determine tuning of the responses to different stimuli.

We were particularly interested in comparing the responses of pairs of FS and RS neurons that were recorded within 400 μm of each other. Anatomical studies have shown

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Abbreviations: FS, fast-spiking; RS, regular-spiking; GABA, γ-aminobutyric acid.

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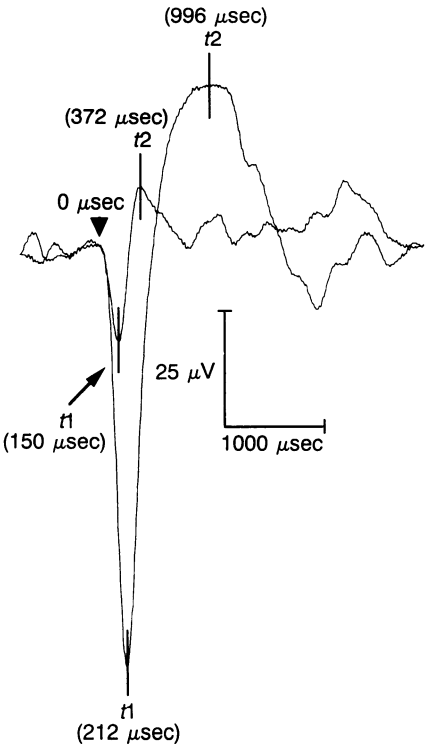

FIG. 1. Action potentials of a FS/RS pair recorded at the same electrode site. The fast spike is smaller and of briefer duration than the regular spike. *t1* and *t2* are times at which amplitude peaks occur.

that the axons of prefrontal GABAergic chandelier cells (areas 9 and 46) project a vertical distance of 400 μm from the cell body (17). Thus, FS neurons may synapse on the soma and proximal dendrites of pyramidal cells located within 400 μm or less. Cells pairs conforming to this criterion were quantitatively analyzed by determining neuronal response epochs suitable for both neurons.

RESULTS

FS neurons were identified by the brief time difference (*t1* − *t2*) between waveform peaks (Fig. 1; Table 1). They had relatively low-amplitude, biphasic action potentials, high firing rates, and characteristic sound on an audio monitor. They were less stable during recording and frequently obscured by larger, longer-duration regular spikes (Fig. 1).

Table 1. Properties of FS and RS neurons

Cells	Firing rate, spikes per sec	*t1*, μsec	*t2*, μsec
FS	17 ± 10.8	167 ± 31.5	408 ± 60.3
RS	5 ± 5.5	224 ± 56.2	717 ± 171.0

Spontaneous firing rate (spikes/sec) was measured in 1-sec bins in the intertrial interval. Of FS cells, 72% had firing rates greater than 10 spikes per sec, compared with 13% of RS cells. *t1* and *t2* are the averaged times to the first and second amplitude peaks from the onset of the action potential (see Fig. 1). Standard deviations are given.

We identified 121 (15%) FS neurons out of a total of 804 cells examined in Walker's areas 12 and 45. Sixty-one of these 121 FS neurons had visual receptive fields, driven either by foveation of the fixation point, by foveal presentation of complex patterns, or by peripherally presented spots of light contralateral to the recorded hemisphere. The responses of 36 of the 121 FS cells had visuomotor and/or auditory correlates, and 24 cells were unresponsive. The receptive fields of both FS and RS spiking neurons were similar in their selectivity and size, representing the fovea and/or contralateral visual space (18–20). In this respect, FS and RS neurons could not be distinguished when recorded on the same vertical penetration through prefrontal cortex, as is the case for smooth nonpyramidal and spiny pyramidal neurons in striate cortex (2).

However, there were major differences between FS/RS pairs with respect to the time course of their responses. Thirty-six FS cells were compared with RS cells at the same or adjacent locations. Although FS/RS pairs responded to the same visual stimuli, the directions (excitation, inhibition) of their responses were often inverted. Furthermore, there were marked differences in the onset of the responses between pairs of neurons.

Fig. 2*A* illustrates a FS/RS pair that responded to stimuli presented on the fovea. The responses were inverted, with the FS cell responding with an increase in firing and the RS cell with a decrease in firing. Fig. 2*B* shows the responses of another pair of neurons with peripheral receptive fields. The FS cell responded with a decrease in firing rate to the stimulus, whereas the RS cell responded with an increase in firing. The cessation of firing of the FS neuron preceded the increase in firing of the RS cell (latencies of 110 and 145 msec; Fig. 2 *B1* and *B2*).

Of 36 FS/RS pairs, 19 (53%) showed inversions of activity for their optimal stimuli (Fig. 3*A*), satisfying two criteria: (*i*) that each cell had a significant ($P < 0.05$) difference in firing between the stimulus-elicited response with a 1-sec pretrial

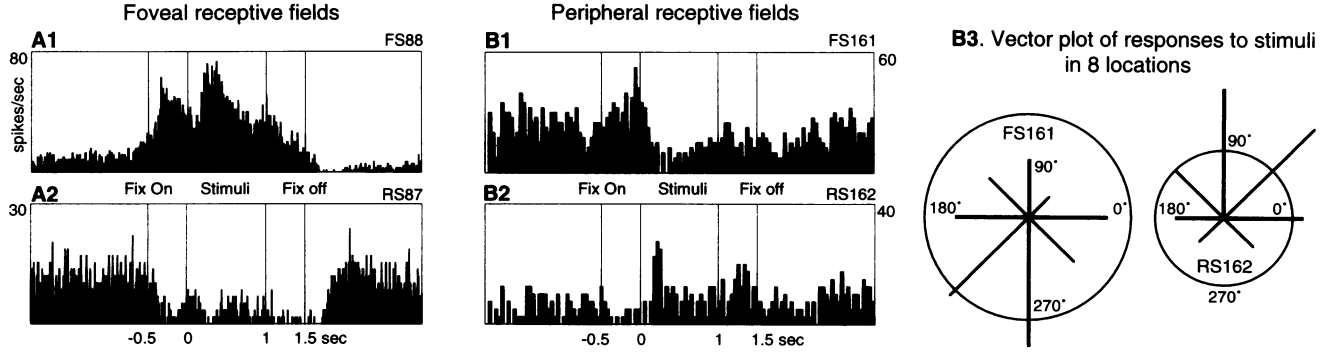

FIG. 2. (*A1* and *A2*) Inverted responses of a FS/RS pair (50 μm apart) during the Pic task (see *Methods*). Histogram bin width, 12 msec; 16 trials per histogram. (*B1* and *B2*) Inverted responses of a FS/RS pair (200 μm apart) in the RF task. These neurons responded maximally to stimuli 13° above (RS162) or 9° right and 9° above (FS161) the fixation point. Bin width, 40 msec; 10 trials per histogram. (*B3*) Plots illustrating the overall tuning of the pair-graded increases in the RS cell firing correspond to graded decreases in the FS cell, and vice versa. Each vector represents response magnitude plotted relative to a stimulus location. Firing rates are normalized so that the maximum vector length is 100%. The circles represent spontaneous firing rates.

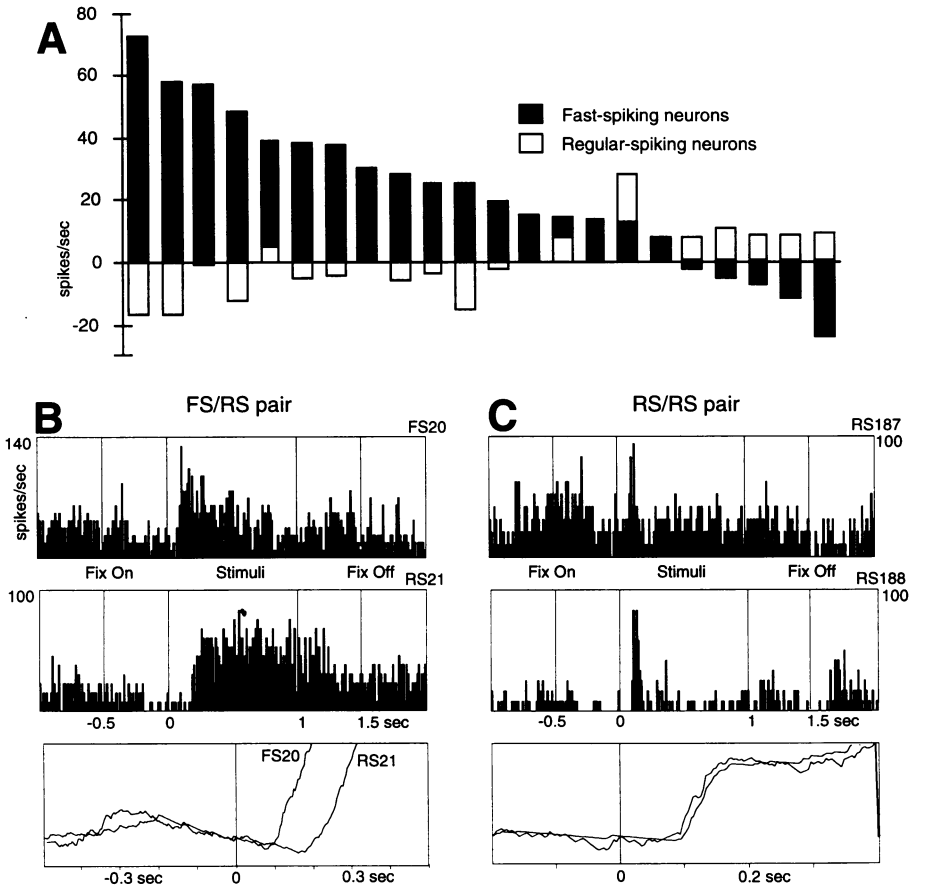

FIG. 3. (*A*) Response directions and magnitudes of 22 FS/RS pairs from monkey G. Each bar represents the response of a FS cell to the most effective stimulus and the corresponding discharge for the RS cell. These values are increases or decreases in firing relative to each neuron's spontaneous firing rate (0 on the ordinate). (*B*) Latency differences of a FS/RS pair (400 μm apart) during the RF task. The optimal stimuli were located 13° right along the horizontal meridian (FS20) and at 9° right and 9° below (RS21). The time base of the cumulative sum histogram (*Bottom*) is shorter than the histogram to illustrate the latency differentials. (*C*) Responses of two RS neurons (265 μm apart) in the RF task. Both cells respond with the same latency and direction to the stimulus, located 9° above and 9° right from the fixation point. The time base of the cumulative sum histogram (*Bottom*) is shorter than the histogram to illustrate the similarity of the latencies.

control period and (*ii*) that one cell responded with an increase in firing and the other cell with a decrease in firing. The majority of FS cells responded with increases in firing rate, with a concomitant decrease in firing for RS cells for the same time period. Only one FS/RS pair responded significantly in the same direction to the stimuli.

In contrast to FS/RS pairs, inverted firing patterns were relatively uncommon (15 of 153; 10%) for RS/RS pairs. Commonly, RS/RS pairs responded in the same direction (50 of 153; 33%) compared with 1 of 36 FS/RS pairs (3%); in 54 of 153 pairs (35%), one of the neurons did not respond to the stimuli; 34 (22%) pairs were unresponsive. Fig. 3*C* illustrates the responses of a RS pair to the same stimulus; the time course of the responses is very similar, in contrast to the differing latencies of FS/RS pairs. These data are consistent with studies showing that FS neurons induce hyperpolarization in RS neurons, whereas RS neurons induce depolarization in ES neurons (11).

Although the inverted responses of certain pairs were sustained, other responses were relatively transient (mean, 350 msec). Fig. 3*B* shows a FS neuron with a transient response at an onset latency of 100 msec, ≈70 msec before the RS cell. The FS response decays as the RS neuron starts a sustained discharge. The different time courses of these responses are illustrated with cumulative sum histograms (Fig. 3*B Bottom*). The latency differences in the responses of FS/RS pairs may reflect transient phases of interactive inhibition and excitation.

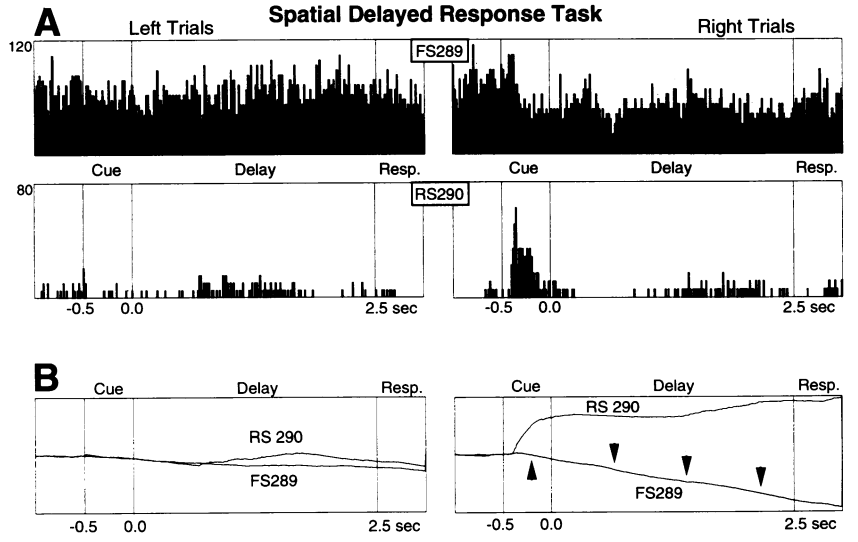

FIG. 4. Responses of a FS/RS pair during the oculomotor delayed response (ODR) task. (*A*) During this task, both neurons responded selectively to stimuli located 13° right of fixation, with increases (RS290: left and right stimuli, 2 versus 21 spikes per sec, respectively) or decreases (FS289: left and right stimuli, 58 versus 48 spikes per sec, respectively) in firing rate. (*B*) In addition to the responses to peripheral visual cues, the FS neuron responded differentially during the delay period, less on the right than on the left (47 versus 62 spikes per sec, $P < 0.01$). The slope of the cumulative sum histograms shows a decrease in firing to the peripheral stimulus which was sustained throughout the delay (see arrowheads).

In one monkey, we recorded FS/RS pairs that encoded information about the spatial location of visual cues and the retention of this information during the delay period of delayed response tasks (12, 21). Fig. 4 illustrates the inverted responses of a FS/RS pair. Both neurons responded selectively to visual cues located 13° right of fixation, with increases (RS290) or decreases (FS289) in firing rate. Moreover, the decrease in firing of the FS neuron continued during the delay period, significantly less on the right than on the left ($P < 0.01$).

DISCUSSION

Single-unit recording studies usually seek to establish the functional role of a particular brain region by examining correlations between an event and the firing rate of a neuron. An outstanding aspect of the behavior of single cells is their stimulus selectivity; i.e., their firing is selectively driven by specific stimuli or responses. Such selectivity is meaningful when changes in firing rate are lawfully related to changes of an event along a particular dimension. A major question for neurophysiology and brain function is how such stimulus selectivity arises. The seminal experiments of Sillito (1) strongly point to the involvement of GABAergic mechanisms in the generation of stimulus selectivity. The local circuit operations that result in stimulus selectivity are currently obscure, although Douglas and Martin (22) have provided a plausible model of a GABAergic mechanism for directional stimulus selectivity. One goal of the present study was to test the possibility that FS neurons are GABAergic, for such a relationship could be important for examining local circuit operations that give rise to stimulus selectivity.

In vitro intracellular techniques provide strong evidence that FS cells are GABAergic inhibitory interneurons. FS neurons have the dendritic morphology and chemical signature of GABAergic cells (4, 5); as a population, their spontaneous firing rate is high (5–8), as is their sustained response to depolarizing current (4, 5, 8–11), observations consistent with the hypothesis (23) that calcium-binding proteins colocalized in GABAergic neurons confer the ability to fire at high rates and the observation that the ultrastructure of GABAergic neurons is indicative of high metabolic activity (5). The present extracellular data provide less direct but compelling data for the hypothesis that FS cells are GABAergic inhibitory interneurons: the incidence of FS cells was 15%, close to (although lower than) the estimated 20–25% incidence of GABAergic neurons in cortex (24, 25); the firing rate of FS cells is high; their receptive-field properties are similar to those of RS cells and can demonstrate short latency responses to visual stimuli, as shown for striate cortical neurons with smooth versus spiny dendrites (2); the firing patterns of FS- and RS cells are frequently inverted, temporally staggered, and apparently synergistic, as expected from the hypothesis that FS cells are inhibitory interneurons. Finally, studies in visual and prefrontal cortex (26–28) have shown that the axons of FS neurons do not project to subcortical structures (although they receive incoming monosynaptic thalamic inputs), whereas RS neurons do so, consistent with the hypothesis that FS cells are intrinsic interneurons whose axons influence local circuits.

The present study shows that FS neurons, under certain conditions, can respond at shorter latencies to triggering events than adjacent pyramidal cells. A conventional view of interneurons is that they are interposed between input and output pyramidal cells and, simplistically, should respond at longer latencies. The latency differences in the responses of FS/RS pairs may reflect transient phases of interactive inhibition and excitation. There may be several underlying mechanisms for these latency differences. For example, afferents to GABAergic interneurons can synapse directly on the soma (5, 29), whereas pyramidal cells are primarily driven by dendritic inputs and thus may respond at longer latencies after integration of excitatory and inhibitory influences. Further, the threshold for depolarized spiking is lower for interneurons than for pyramidal cells (5, 8). Douglas and Martin (22) have proposed that GABAergic inhibition of pyramidal cells is conditional upon differences in latencies between interneurons and pyramidal cells. The latency differences between FS and RS neurons are consistent with this hypothesis (see also ref. 32).

Another finding of this study is that FS neurons are also subject to inhibition. Figs. 2*A* and 3*B* show FS neurons with primary excitatory responses; Figs. 2*B* and 4 show FS neurons with primary inhibitory responses. In fact, individual FS neurons can show both increases and decreases in firing depending on the stimulus, and Fig. 2*B3* shows that the direction of the response depends (in this case) upon the locus of the stimulus within the receptive field. Presumably, the ability to respond biphasically around a high spontaneous firing rate adds to the dynamic influence of GABAergic neurons on pyramidal cells and may extend the logic of local circuit operations. Consistent with these biphasic responses are observations that GABAergic synapses are found on GABAergic cell bodies, indicative of GABAergic inhibition of interneurons (29).

The accumulating evidence suggests that action-potential characteristics may be useful in functional studies of several neurotransmitter systems in behaving animals (13, 30, 31). As the receptive-field properties of FS cells are well defined, these putative GABAergic inhibitory neurons must carry a specific informational signal that contributes to the functional specialization of the prefrontal cortex, as opposed to providing a nonspecific modulation. Thus, interactions between FS and RS cells mediate visual processes in prefrontal cortex and contribute to the neural substrates of working memory.

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