Prolonged Synaptic Integration in Perirhinal Cortical Neurons

JOHN M. BEGGS,[1] JAMES R. MOYER, JR.,[1] JOHN P. MCGANN,[2] AND THOMAS H. BROWN[1–3]
[1]*Department of Psychology,* [2]*Interdepartmental Neuroscience Program, and* [3]*Department of Cellular and Molecular Physiology, Yale University, New Haven, Connecticut 06520*

Beggs, John M., James R. Moyer, Jr., John P. McGann, and Thomas H. Brown. Prolonged synaptic integration in perirhinal cortical neurons. *J Neurophysiol* 83: 3294–3298, 2000. Layer II/III of rat perirhinal cortex (PR) contains numerous late-spiking (LS) pyramidal neurons. When injected with a depolarizing current step, these LS cells typically *delay spiking* for one or more seconds from the onset of the current step and then *sustain firing* for the duration of the step. This pattern of delayed and sustained firing suggested a specific computational role for LS cells in temporal learning. This hypothesis predicts and requires that some layer II/III neurons should also exhibit delayed and sustained spiking in response to a train of excitatory *synaptic* inputs. Here we tested this prediction using visually guided, whole cell recordings from rat PR brain slices. Most LS cells (19 of 26) exhibited *delayed spiking* to synaptic stimulation (>1 s latency from the train onset), and the majority of these cells (13 of 19) also showed *sustained firing* that persisted for the duration of the synaptic train (5–10 s duration). Delayed and sustained firing in response to long synaptic trains has not been previously reported in vertebrate neurons. The data are consistent with our model that a circuit containing late spiking neurons can be used for encoding long time intervals during associative learning.

INTRODUCTION

Previous work on perirhinal cortex (PR) identified pyramidal neurons in layer II/III that generated *long delays* to initiate spiking when injected with depolarizing current steps (Faulkner and Brown 1999). Following the initial delay, these late spiking (LS) neurons also exhibit *sustained firing* during the current step, in contrast to the strong accommodation more commonly seen in regular spiking (RS) cortical cells (McCormick et al. 1985). The intrinsic firing properties of LS neurons in PR, combined with their apparent circuit-level organization, gave rise to a model of how these cells might be used in cortical circuits to process temporal information (Tieu et al. 1999). In particular, the cellular anatomy and neurophysiology suggested that LS neurons might be organized into delay lines that are capable of encoding intervals of seconds to tens of seconds.

An untested prediction of our model is that these LS neurons can also exhibit *delayed and sustained firing* in response to *synaptic* inputs. Several factors could preclude delayed and sustained firing of LS neurons in response to a train of synaptic inputs. For example, conventional feed-forward or feedback synaptic inhibition, activity-dependent synaptic depression, and the presence of certain voltage- or calcium-dependent conductances on the postsynaptic dendrites (cf. Magee 1998) could act to prevent both delayed and sustained firing to synaptic inputs. Here we used visually guided, whole cell recordings from rat PR layer II/III, which is known to contain numerous LS pyramidal neurons (Faulkner and Brown 1999), while trains of synaptic stimuli were delivered to layer I afferents. The experimental question was whether layer II/III pyramidal neurons can exhibit delayed and sustained firing to trains of synaptic inputs produced by repetitive electrical stimulation of layer I. Preliminary results have been presented in abstract form (Beggs et al. 1997).

METHODS

Brain slices from 12- to 32-day-old Sprague-Dawley rats were prepared and maintained as previously described (Moyer and Brown 1998). Whole cell recordings were made from layer II/III pyramidal neurons in horizontal slices (300–400 μm) containing perirhinal cortex (corresponding to plates 98 to 100 of Paxinos and Watson 1998) limited by the rostral and caudal extent of the lateral amygdala (approximately −2.4 to −4.8 mm posterior to bregma). Layer II/III PR pyramidal neurons were visualized and identified with infrared-filtered, video-enhanced DIC optics (Moyer and Brown 1998; Xiang and Brown 1998).

Recordings were done at room temperature (~24°C) in physiological saline containing (in mM) 124 NaCl, 2 KCl, 2 $CaCl_2$, 2 $MgSO_4$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 D-glucose, pH 7.4, 290 mosmol. The electrophysiological methods are described in detail elsewhere (Moyer and Brown 1998). Patch pipettes (~4 MΩ), were filled with (in mM) 120 K-gluconate, 10 HEPES, 1.0 EGTA, 20 KCl, 2.0 $MgCl_2$, 2.0 Na_2·ATP, 0.25 Na_3·GTP·$2H_2O$, pH 7.3, 280–290 mosmol. Electrical signals were recorded using an EPC-7 or AxoPatch 1D amplifier, filtered at 3 kHz, digitized at 44 kHz, stored on VCR tape, and analyzed using custom software written for Igor Pro. All voltages were corrected for a +10-mV liquid junction potential between the bath and the gluconate-based patch pipette solution (Neher 1992).

The responses of perirhinal layer II/III pyramidal neurons to both somatic current injection and trains of synaptic inputs were evaluated. With current injection, neuronal firing characteristics and subthreshold membrane responses were examined. Current-voltage (*I-V*) relationships were constructed by injecting small hyperpolarizing and depolarizing current pulses (<25 pA) that resulted in small voltage excursions (<10 mV) from the resting membrane potential. Responses within this restricted range of linear and symmetrical voltage excursions were used to obtain the neuronal input resistance (R_N) and the time constant of the membrane voltage response (τ_m), The value of R_N was calculated from the slope of the best-fitting linear regression equation (least-squares criterion). The time course of the membrane voltage response to small current steps could always be well approximated by an exponential function with a single time constant (least-squares criterion). Values of τ_m were taken from either single pulses or averages of 10–20 pulses.

Cells were studied only if they had a healthy visual appearance (Moyer and Brown 1998), an uncorrected resting membrane potential

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of −60 mV or more negative, an input resistance >120 MΩ, and overshooting action potentials. Layer II/III pyramidal neurons were first determined to be LS or RS based on their response to depolarizing current steps (Faulkner and Brown 1999). In response to suprathreshold current steps, LS neurons in PR commonly delay the onset of their spike trains for ~1 s or longer and continue firing for the duration of the current step. In contrast, RS cells fire relatively soon after onset of the current step and often exhibit strong accommodation that terminates firing in spite of maintained depolarization. LS neurons tend to exhibit less accommodation and can even show "anti-accommodation" (Faulkner and Brown 1999), a progressive acceleration in the firing rate, during the early part of a current step.

Synaptic inputs were evoked using a concentric stimulating electrode whose 250-μm diameter tip was positioned into PR layer I, which contains afferents to layer II/III pyramids. Before studying the effects of synaptic trains, we first explored the responses to individual synaptic inputs (under current-clamp conditions) to get a *baseline stimulation intensity* (using monophasic current pulses of 0.2 ms duration). The stimulation intensity was gradually increased until an excitatory postsynaptic potential (EPSP) of ~2 mV amplitude was evoked.

The response of the postsynaptic neurons to long sequences (5–10 s) of EPSPs was examined by passing a train of 100–200 monophasic current pulses (0.2 ms duration/pulse) through the stimulating electrode at 20 or 25 Hz. These trains were repeated at 30-s intervals, each time increasing the stimulation from the baseline intensity until at least one action potential was produced in the postsynaptic neuron, thereby determining the threshold for synaptically produced orthodromic spiking in response to a long train. Once the threshold was found, the stimulation intensity was gradually increased to determine whether trains of EPSPs could produce sustained repetitive spiking in the postsynaptic neurons.

RESULTS

Whole cell recordings were made under current-clamp conditions from 61 layer II/III pyramidal neurons as previously described (Moyer and Brown 1998). Slightly more than half of the neurons were LS cells (33 of 61; Table 1). Of these 61 neurons, a subset of 42 neurons was subjected to an extensive experimental protocol that included stimulation of synaptic inputs to the neuron.

Figure 1 compares some general features of LS and RS cells in response to depolarizing and hyperpolarizing current steps. By definition, RS cells fired early in response to a depolarizing current step, whereas LS cells fired late (Fig. 1*A*). On the other hand, most of the subthreshold electrophysiological properties of the two cell types were indistinguishable (summarized in Table 1). A notable exception is τ_m, which was slightly but significantly larger in the LS than the RS neurons ($t = 3.2$, df = 53, $P < 0.005$; unpaired). Figure 1*C* shows examples of the averaged responses to small (−10 pA) hyperpolarizing current steps and semilogarithmic plots that illustrate single-exponential fits to data from both a RS and a LS cell.

TABLE 1. *Summary of membrane properties of layer II/III pyramidal neurons in rat perirhinal cortex*

	Cell Classification		
	Total	LS neurons	RS neurons
Resting membrane potential, mV	−78.3 ± 0.5	−77.9 ± 0.8	−78.9 ± 0.7
Input resistance, MΩ	308.1 ± 17.5	326.0 ± 22.7	281.3 ± 27.2
τ_m, ms	69.3 ± 3.1	76.8 ± 4.1*	58.1 ± 3.6*
Spike threshold, mV	−49.8 ± 0.6	−49.3 ± 0.7	−50.6 ± 1.0
Spike overshoot, mV	28.5 ± 1.4	27.8 ± 2.1	29.6 ± 1.8

Values are means ± SE. Total number of cells is 55; number of LS neurons is 33 and RS neurons is 22. LS and RS refer to late spiking and regular spiking neurons; τ_m is the time constant of membrane voltage response. * τ_m is significantly larger in LS cells than in RS cells, $P < 0.005$.

Figure 1*D* highlights two key aspects of a LS neuronal response to a *prolonged* (60 s) depolarizing current step. First, the beginning of spiking is clearly *delayed* from the onset of the current step. In this case the delay is more than 2.3 s, and we have seen delays as long as 19 s. Second, once spiking is initiated, it is *sustained* for the duration of the current step, which lasted for 60 s in this example. Late spiking cells often exhibit anti-accommodation early in the spike train (slight tendency seen in Fig. 1*A*, *bottom right trace*) (see also Faulkner and Brown 1999), but mild accommodation is typically evident later in the spike train (Fig. 1*D*). By contrast, RS cells tend to show spike frequency accommodation throughout the spike train (Fig. 1*A*, *bottom left trace*).

When given a suprathreshold train of synaptic inputs, most LS cells (19 of 26) fired their first action potential more than 1 s after the onset of the synaptic train; that is they exhibited *delayed spiking* to synaptic stimulation. This delay is an order of magnitude longer than τ_m, the average value of which was 76 ms in LS cells (Table 1; see also Figs. 1, *A* and *D*, and 2*A*). An example of delayed firing to a long (7.5 s) synaptic train (150 pulses at 20 Hz) is illustrated in Fig. 2*A*. This set of traces shows the response of an LS cell to trains of progressively larger synaptic inputs. In the *bottom two traces*, the synaptic stimulation was subthreshold for eliciting spiking in the pyramidal neuron. In the *top trace* the synaptic stimulation elicited repetitive spiking that began 4.2 s after the onset of the train and continued for the duration of the train.

In contrast to the firing pattern observed in LS cells, RS neurons fired at a short latency from the onset of synaptic stimulation and then tended to show rapid accommodation (Fig. 2*B*). The *top* and *bottom* voltage traces in Fig. 2*B* show, respectively, the membrane response to supra- and sub-threshold trains of synaptic inputs. In both cases, the onset of the synaptic train caused an abrupt depolarization, but it was not sustained. The *onset* of synaptic stimulation also caused an abrupt depolarization in LS cells (Fig. 2*A*). A notable difference between cell types was that the depolarization in LS neurons was sustained for the duration of the synaptic train (Fig. 2*A*).

In contrast to the rapid depolarization observed at the onset of synaptic stimulation, the *termination* of stimulation was followed by a *slow relaxation* back to the resting potential (Fig. 2*A*). This did not occur when a depolarizing current step was used to fire the cell. In the latter case, termination of the current step was followed by a rapid relaxation of the membrane potential back to the resting level (Fig. 1, *A* and *D*). In the LS cell illustrated in Fig. 2*A*, the decay time constant following a suprathreshold current step was 92 ms, whereas the decay time constant following synaptic stimulation was 504 ms. These differences were reflected in the group data. In 12 LS cells that showed delayed and sustained spiking to a synaptic train, the mean decay time constants following a suprathreshold current step and a synaptic train were 76.4 ± 6.6 and 405 ± 74.1

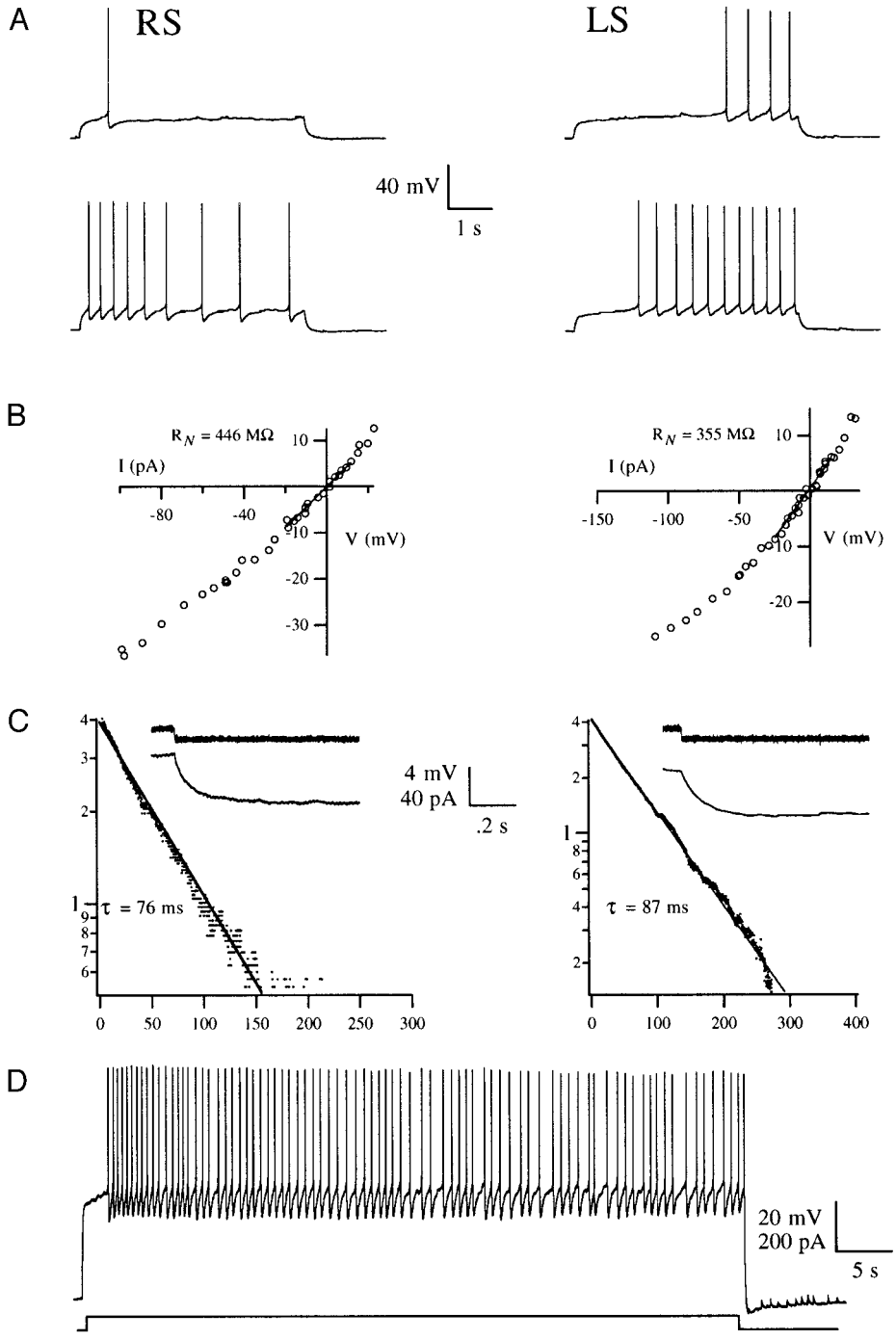

FIG. 1. Characteristics of regular spiking (RS) and late spiking (LS) neurons in layer II/III of rat perirhinal cortex. *A*: RS neurons typically begin firing at a short latency following onset of a depolarizing current step (*top*, 30 pA; *bottom*, 35 pA) and accommodate strongly. In contrast, LS neurons begin to fire at a long latency following onset of a depolarizing current step (*top*, 45 pA; *bottom*, 50 pA) and show sustained firing. Notice how the spikes tend to be added from left to right in the RS cell and from right to left in the LS cell as the size of the current step is increased. *B*: current-voltage (*I-V*) plots of RS and LS cells. Input resistance was calculated from the slope of the line where symmetrical voltage responses were elicited in response to small (<25 pA) hyperpolarizing and depolarizing current injections. *C*: time constant of membrane voltage response (τ_m) of RS and LS cells. An average of 20 voltage responses elicited using a 10-pA hyperpolarizing current injection was used to calculate τ_m. *D*: an LS cell that sustained firing for more than 55 s after an initial delay of 2.3 s following the onset of a depolarizing current step from a resting membrane potential of −90.7 mV. Data in *A–C* are from the same cells, membrane potentials: RS, −78 mV; LS, −81 mV.

(mean ± SE), respectively, a difference that was statistically significant ($t = 4.43$, df = 11, $P < 0.005$; paired comparisons).

DISCUSSION

This is the first demonstration of delayed and sustained spiking in response to trains of synaptic inputs in vertebrate neurons. The response of these cortical neurons to trains of synaptic inputs was similar in certain respects to their response to a depolarizing current step (compare Fig. 1, *A* and *D*, with Fig. 2*A*). The initial rapid depolarization was followed by a more gradual depolarizing ramp until the spike threshold was reached. Once above threshold, most LS neurons continued firing for the duration of the synaptic train or current step. Computer simulations have shown that this type of delayed and prolonged synaptic integration can theoretically furnish a convenient and robust platform for interesting forms of temporal encoding (McGann and Brown 2000; Tieu et al. 1999).

These interesting results naturally raise numerous questions regarding the ionic mechanisms underlying the late spiking firing pattern we observe. Recall that small current injections produced a rapid membrane voltage response that could be

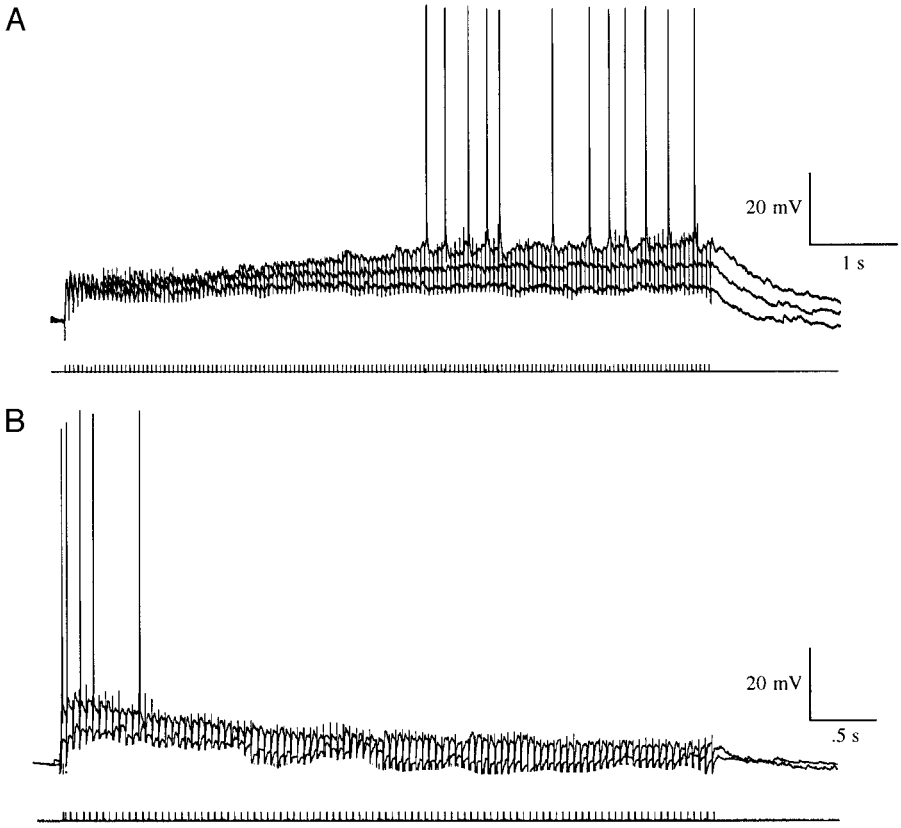

FIG. 2. Responses of LS and RS neurons to synaptic trains produced by electrical stimulation of layer I afferents. *A*: delayed response of an LS neuron to synaptic stimulation. Three different strengths of repetitive synaptic stimulation (150 pulses at 20 Hz; 7.5 s) are shown, one of which was just above spike threshold. Notice the long delay (>4 s) before the cell fired its first action potential and that once it began the cell continued firing for the duration of the synaptic train. *B*: typical RS neuron that quickly reached threshold and rapidly accommodated during the synaptic stimulation (100 pulses at 20 Hz; 5 s).

well fit by a single exponential (Fig. 1; Table 1). However, larger current injections seemed to recruit additional ionic conductances. The initial rapid membrane response in LS cells was followed by a gradual *depolarizing ramp* (Figs. 1*A* and 2*A*). In hippocampal and striatal neurons, a similar ramp has been suggested to result from a slowly inactivating potassium conductance that is blocked by 4-aminopyridine (4-AP) (Nisenbaum et al. 1994, 1996; Storm 1988).

The conductance mechanism could be similar in perirhinal LS cells, but there are two apparent differences. First, LS perirhinal cortical neurons do not require a strong hyperpolarizing prepulse to exhibit delayed spiking, in contrast to findings in hippocampus (Storm 1988). Second, the delays we see in LS perirhinal neurons are many times longer than those reported in striatal neurons (Nisenbaum et al. 1994). Previous voltage-clamp experiments performed on LS perirhinal neurons revealed a slowly developing inward current when the membrane potential was stepped from the resting potential to a just-subthreshold potential (Faulkner and Brown 1999). This inward relaxation, which has a time course similar to the depolarizing ramp mentioned above, could reflect a slowly inactivating potassium conductance. Although we have not yet fully studied the pharmacology of this ionic current, preliminary results indicate that bath application of 4-AP blocks both the depolarizing ramp and the delayed spiking in perirhinal LS neurons (Moyer et al. 2000).

These preliminary findings encourage a full investigation into the ionic mechanisms responsible for the firing properties of LS neurons in perirhinal cortex. To date, we have observed at least three different types of LS cells in rat perirhinal cortex. In addition to the LS layer II/III pyramids discussed here, there are also small LS "cone cells" in layer VI (Faulkner and Brown 1999) and large LS pyramids in layer V (Moyer and Brown, unpublished observations). These three cell types are morphologically quite distinct and are all contained within our standard horizontal brain slice of perirhinal cortex. It will be interesting and informative to compare quantitatively their firing properties and to examine possible similarities and differences in their pharmacology and ionic conductance mechanisms.

The presence of LS neurons in PR layer II/III, combined with their axonal projections (Faulkner and Brown 1999), have suggested some interesting computational possibilities. If groups of these cells were connected in series, they could form an array of delay lines capable of encoding and learning temporal relationships on the order of seconds to tens of seconds (McGann and Brown 2000; Tieu et al. 1999). Because LS cells can show sustained firing for tens of seconds, they might also play a role in maintaining temporary stimulus representations when incorporated into the appropriate recurrent circuit architecture (Tieu et al. 1999). Consistent with this possibility, single-unit recordings from rat PR have revealed neurons that are tonically active during the *delay period* of an odor-guided, delayed nonmatching-to-sample task (Young et al. 1997).

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Present address of J. M. Beggs: Laboratory of Systems Neuroscience, NIH,

Unit of Neural Network Physiology, Bldg. 36, Rm. 2D-30, Bethesda, MD 20892-4075.

Address for reprint requests: T. H. Brown, Dept. of Psychology, Yale University, PO Box 208205, 2 Hillhouse Ave., New Haven, CT 06520.

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