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Electrophysiological properties of neurons in the rat subiculum in vitro

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Abstract. The present study determined the membrane and synaptic properties of neurons in the rat subiculum. Using the in vitro hippocampal slice preparation, intracellular recordings were obtained from 91 subicular neurons. Membrane properties and morphological characteristics were similar to those reported for hippocampal pyramidal neurons. Two categories of subicular neurons were distinguished based on their response to a depolarizing current pulse. One type of neuron showed bursting behavior and the second type was characterized as regular firing. Analysis of the charging functions during hyperpolarizing current pulses yielded a mean τ₀ and τᵢ for subicular neurons of about 13 ms and 0.60 ms, respectively. Using the model of an equivalent cylinder, the mean dendrite-to-soma conductance ratio (p) was estimated at 6.0 and electrotonic length constant (L) at 0.7. There was no difference in these values between bursting and regular firing neurons. Tetrodotoxin-resistant potentials (presumed calcium hump/spike) were evoked from bursting subicular neurons at lower current intensities than CA1 pyramidal neurons. Calcium humps could only be evoked from about half the regular firing subicular neurons. Subicular cells showed an excitatory/inhibitory postsynaptic potential (EPSP/IPSP) sequence in response to electrical stimulation in different layers of the CA1 area. An EPSP could also be evoked from stimulation of the superficial or deep layers of the presubiculum and was attributed to activation of entorhinal fibers of passage. At high stimulation intensity, an antidromic spike was often evoked following stimulation in the presubiculum area or CA1 alveus. The evoked EPSPs were blocked by addition of 6-cyano-7-nitroquinazoline-2,3-dione (CNQX) to the bathing medium. In magnesium-free, CNQX bathing solution, a longer lasting depolarization was recorded; this response was blocked by application of a N-methyl-D-aspartate (NMDA) receptor antagonist (AP5). Ionophoretic application of glutamate or quisqualate (10 mM) along the soma-dendritic axis of subicular neurons leads to either a short-latency depolarization or a burst of action potentials. Application of 10 mM GABA near the recording site usually produced a hyperpolarization, which, at times, was mixed with a depolarization. Mixed hyperpolarizing/depolarizing responses were observed when GABA was applied to the basal or apical dendritic areas. There were no significant differences in the synaptic properties or responses to drug application between bursting and regular firing neurons. These results indicate that subicular neurons (1) are composed of a heterogeneous population of cell types, (2) have similar electrical properties to other hippocampal principal neurons, (3) receive glutaminergic synapses from CA1 and entorhinal cortical neurons, (4) project to the presubiculum area and fornix (via the alveus), (5) are inhibited by local circuit neurons, and (6) display complex responses to GABA.

Key words: Hippocampus – Subiculum – Electrophysiology – Glutamate – GABA – Rat

Introduction

The electrophysiological properties of neurons in the mammalian hippocampus have been well-characterized and detailed descriptions have been reported for the three major cell types: dentate granule cells, CA3 pyramidal neurons, and CA1 pyramidal neurons (Schwartzkroin 1975; 1977; Barnes and McNaughton 1980; Brown et al. 1981a; Johnston 1981; Frick and Prince 1984). Many properties have also been described for interneurons within these regions (Lacaille et al. 1989; Scharfman and Schwartzkroin 1988; Lacaille and Williams 1990). Most neuronal communication from the hippocampus to the cortex occurs through the subicular complex. Three major areas comprise the subicular complex: subiculum, presubiculum, and parasubiculum. The subiculum receives its major input from CA1 pyramidal

1 Some investigators recognize the postsubiculum as a fourth distinct area of the subicular complex. The postsubiculum comprises the dorsal portion of the presubiculum.
cells, but also receives afferents from the entorhinal cortex (Swanson and Cowan 1977; Witter et al. 1989). The subiculum, in turn, projects to the presubiculum, parasubiculum, and back to the entorhinal cortex (Sorenson and Shipley 1979; Kohler 1985; Van Groen et al. 1986).

Recent studies have begun to determine the behavioral correlates of cells from this region. Taube et al. (1990) stated that cells in the dorsal presubiculum (an area which receives a major subiculum projection) discharge as a function of the animal's head direction in its environment, independent of the animal's location and behavior. Similarly, other studies have reported location-specific firing in cells in the subiculum (Barnes et al. 1990) and in a preliminary report, Sharp et al. (1990) found head direction cells in the subiculum. In addition to these behavioral/spatial correlates, interest in the properties and function of the subicular complex has arisen following pathological descriptions of neurons in this region. For example, Davies and colleagues (1988) reported a high concentration of senile plaques in the subiculum from patients with Alzheimer's disease. Walther et al. (1986) found epileptiform activity in the entorhinal area when the connectivity with the subiculum was intact, and Uemura (1985) noted age-related changes in the dendritic branching pattern of subicular neurons.

Although much is known about the electrophysiological properties of cells in the hippocampus, little is known about the membrane and synaptic properties of subicular neurons. Previous physiological studies in the subiculum have mostly been confined to extracellular recordings (Bartesaghi and Gessi 1986) and verifying the anatomical connections using physiological criteria (Finch and Babb 1980; Finch et al. 1986; Van Groen and Lopes da Silva 1986). There have been no studies on the intracellular properties of subicular neurons. Using the in vitro slice preparation and intracellular recording techniques, the present study examined the electrophysiological properties of subicular neurons. A preliminary report concerning some of these findings has previously been presented (Taube and Cotman 1991).

Nomenclature

The cytoarchitecture of the subiculum has been described in several reports (Lorente de No 1934; Blackstad 1956; Amaral 1987; Witter et al. 1989; Lopes da Silva et al. 1990), not all of which are consistent with one another. For the purposes of this paper, the subiculum will be characterized as containing three principal lamina (going from superficial to deep): (1) an external plexiform, or molecular, layer, which is continuous with strata lacunosum-molecular and radiatum of CA1; (2) an expanded pyramidal cell layer containing the cell bodies of principal neurons; and (3) a deep polymorphic layer. Although the subicular pyramidal cell layer is continuous with the CA1 pyramidal layer, the cell packing is considerably less dense in subiculum than CA1. Deeper to the deep polymorphic layer is the alveus, which contains axons from the hippocampus and parahippocampal regions.

Material and methods

Electrophysiology

The methods used for intracellular recording in hippocampal slices are substantially the same as previously described for investigating the properties of hippocampal neurons (Taube and Schwartzkroin 1986; 1987; 1988). Transverse hippocampal slices, 400 μm thick, were prepared from 60 to 90 day-old Sprague-Dawley rats and maintained in vitro at 35°C. The tissue sections were prepared in a way which included a large area of the subicular complex (and sometimes a portion of the entorhinal cortex). The slices were kept at a liquid-gas interface and their undersurfaces perfused with an oxygenated artificial cerebrospinal fluid (CSF) solution containing: NaCl 127 mM, KCl 2.0 mM, CaCl₂ 2.0 mM, MgSO₄ 2.0 mM, KH₂PO₄ 1.25 mM, NaHCO₃ 26 mM, D-glucose 10 mM at pH 7.4. Somatic intracellular recordings were obtained with glass micropipettes (tip resistance approximately 80 MΩ filled with 4 M potassium acetate and 0.01 M KCl). Stimulating electrodes were constructed from a pair of tungsten wires, insulated except at the tips, and spaced 0.5 mm apart. A high input resistance intracellular amplifier with internal bridge circuit (Axon Instruments) was used for intracellular recording and for intracellular stimulation via constant current pulse injection. Bridge balance was continuously monitored and adjusted when needed. Amplified voltage signals were displayed on an oscilloscope and stored on a VCR tape recorder (Neurodata Instruments) for later off-line analysis.

Synaptic properties were assessed by electrically stimulating (50 μs pulse) different locations of the hippocampal slice. In order to determine the underlying neurotransmitters contributing to the synaptic responses, some slices were perfused with 20 μM 6-cyano-7-nitroquinolinol-2,3-dione (CNQX; Tocris Neuramin); and/or 100 μM 2-amino-5-phosphonovaleric acid (AP5; Sigma) added to the bathing medium. Subicular recordings were also monitored following iontophoretic application of 10 mM glutamate, 10 mM quisqualate, or 10 mM gamma-aminobutyric acid (GABA; Sigma). These drugs were applied through a multi-barrel micropipette positioned at different locations along the soma-dendritic axis in the subiculum. Microelectrodes were visually positioned into the subiculum by first selecting tissue slices where the CA1 pyramidal cell layer was clearly visible and then placing the microelectrode at least 500 μm laterally from the end of the visible CA1 pyramidal cell layer. For five cases, the recording site was marked by making a small hole in the tissue after completion of recording. The tissue was then fixed in 10% formalin for 48 h and placed into a 20% sucrose solution for 24 h. The slices were sectioned into 40 μm sections, mounted on microscope slides, and stained with cresyl violet. In each case, examination at the light microscope level of the marked area clearly showed that the recording sites were located in the pyramidal cell layer of the subiculum.

Data analysis

All analysis was done off-line using the computer software package pClamp (Axon Instruments). When described, all values are expressed as mean ± the standard error of the mean (SEM). Action potential threshold was defined as the amount of current necessary to elicit an action potential 50% of the time during a 100 ms depolarizing current pulse. The magnitude of the afterhyperpolarization (AHP), which follows a train of action potentials evoked by a depolarizing current pulse, was defined as the peak voltage deflection measured after the offset of the current pulse. The threshold for eliciting a "calcium spike" (tetrodotoxin- (TTX) resistant potential; see Taube and Schwartzkroin 1986) was defined as the amount of current necessary to evoke a depolarizing response which was 5 mV greater than the final steady state membrane potential for a 100 ms depolarizing current pulse (see Fig. 3A).
Cable properties

The membrane time constants and passive membrane cable properties were estimated from responses to small hyperpolarizing current pulses according to the methods of Rall (1969), Brown (1981a), and Turner (1984). Hyperpolarizing responses were transformed to a charging function \( V(t) = -V \) and expressed as a sum of exponentials:

\[ V_0 - V = C_i e^{-\tau_i t} + C_0 e^{-\tau_0 t} + \ldots \]

where \( V \) = membrane voltage at time \( t \), \( V_0 \) = final steady-state value of \( V \), \( \tau_0 \) = 1st order time constant, \( \tau_i \) = second order time constant, \( C_0 \) = first order coefficient, and \( C_i \) = 2nd order coefficient.

With the bridge carefully balanced, two or three responses to a \( -0.1 \) nA, 100 ms current pulse were averaged and the initial portion of the averaged curve (15-20 ms) was selected for curve fitting. Estimates for \( \tau_0 \), \( \tau_i \), \( C_0 \), and \( C_i \) were calculated using a curve-fitting program (pClamp, Axon Instruments) using two time constants.

Rall's model of a lumped soma and single dendritic cylinder can be used to estimate the values for the dendrite to soma conductance ratio (\( \rho \)) and electrotonic length (\( L \); Rall 1969). Four important assumptions underlie this model: (1) the membrane is passive (i.e., no active conductances), (2) the dendrites can be represented as an equivalent uniform cylinder, (3) the extracellular space has infinite conductivity, and (4) there is continuity of current and voltage at dendritic branch points and at the soma-dendritic junction. To satisfy the first assumption, values for membrane voltages were obtained from the linear portion of the current-voltage (I-V) curve using 0.1-nA hyperpolarizing current pulses. Active membrane conductances do not appear to be major contributors to the voltage deflection when the membrane potential is at rest and small hyperpolarizing currents are injected into the cell. The validity of the remaining assumptions has been previously discussed (see Rall 1969; Brown et al. 1981a). Although these assumptions remain questionable, the model provides a reasonable first estimate for \( \rho \) and \( L \), and the values calculated for subicular neurons can be compared with values calculated in a similar manner from hippocampal neurons.

Using the methods of Brown et al. (1981a), the dendrite-to-soma conductance ratio was obtained:

\[ \rho = \frac{C_0}{C_0 + C_i} \]

The electrotonic length was estimated according to Rall (1969):

\[ L \approx \frac{\pi \rho (\rho + 1)^{1/2}}{(\tau_0/\tau_i)^{1/2} - 1} \]

Intracellular staining with lucifer yellow

For five neurons, the micropipette was filled with 3% lucifer yellow (LY) dissolved in 1 M LiCl. Subicular neurons were impaled and following electrophysiological analysis the neurons were filled with LY by injecting 1 nA of tonic hyperpolarizing current for 10–20 min. The slices were allowed to remain in the chamber for 5 min after the injection and then transferred to a petri dish containing 10% buffered formalin. After 24 h the slices were dehydrated and placed on a subbed slide. A drop of methyl salicylate was applied to the slide and when the slice became transparent the slide was covered slipped with methyl salicylate and examined using a fluorescence microscope (Olympus BH-2) with a fluorescein isothiocyanate (FITC) filter set. Cells were then photographed using Kodak 800 ASA film for later analysis.

Results

Electrotonic properties

Stable intracellular recordings were obtained from 91 subicular neurons (58 tissue slices from 37 animals). Subicular neurons could be characterized into two groups based on their response to a 0.5 nA, 100 ms depolarizing current pulse. One type of neuron responded with a brief burst of action potentials during the initial 40 ms of the current pulse (Fig. 1A). Each burst contained 3–5 action potentials embedded in a depolarizing envelope and was followed by a 20 to 30 ms period in which the neuron did not discharge. This pause in firing was associated with hyperpolarization of the membrane (and is referred to as the hyperpolarizing afterpotential, in order to distinguish it from the AHP which occurs at the termination of the depolarizing current pulse). During the final phase of the current pulse (30–40 ms) the neurons discharged single action potentials at a lower rate. This subicular cell type showed little spontaneous activity at resting membrane potential. The second type of subicular neuron responded to the depolarizing current pulse with firing of single action potentials throughout the current pulse; four to seven action potentials were discharged, but there was no initial burst of firing and there was no apparent membrane hyperpolarization during the current pulse (Fig. 1B). These cells are referred to as regular firing neurons.

Of the 91 subicular neurons tested, 63 were classified as bursting and 28 as regular firing. The membrane properties for both types of subicular neurons are shown in Table 1. There was no significant difference between bursting and regular firing neurons for these membrane properties. Both bursting and regular firing neurons produced an AHP following the depolarizing current pulse (Fig. 1). Mean values for the peak amplitude and latency to peak of the AHP are also listed in Table 1 for both types of neurons. Figure 2A shows the response of a representative bursting subicular neuron to 0.1 nA steps of hyperpolarizing current. As with hippocampal pyramidal neurons, a sag in the membrane potential occurred at higher levels of current intensity before reaching a steady state. I-V curves were constructed for 73 neurons and the input resistance was calculated from the slope of the best-fit line for the linear portion of the curve. The mean input resistance was 34.02 ± 1.24 MΩ for bursting neurons and 34.67 ± 0.83 MΩ for regular firing neurons; there was no significant difference between these values (P > 0.05; t-test). Figure 2B shows the mean steady-state voltage deflections following current injections into bursting and regular firing neurons.

Passive cable properties

The cable properties of both bursting and regular firing neurons were analyzed and are shown in Table 2. As with the other membrane properties described above, there was no significant difference between the mean values for \( \tau_0 \) and \( \tau_i \), \( \rho \), and electrotonic length \( L \) for bursting and
regular firing neurons ($P > 0.05$, t-test). In general, the values for $p$ and $L$ were higher and lower, respectively, than values reported for hippocampal pyramidal neurons by Brown et al. (1981a) and Turner (1984). Our low values for $L$ suggest that subicular neurons are more electrically compact than hippocampal neurons.

**Calcium potentials**

The depolarizing envelope and burst of action potentials in response to a depolarizing current pulse resembled responses observed in bursting CA3 pyramidal cells (Wong and Prince 1978). The depolarizing envelope in CA3 cells is attributed to the activation of voltage-sensitive calcium channels. In contrast to CA3 neurons, CA1 pyramidal neurons do not burst under normal conditions. However, a “calcium spike” can be evoked in CA1 neurons when a sufficient amount of depolarizing current is injected into the neuron under conditions where voltage-dependent sodium channels are blocked with TTX (Schwartzkroin and Slawsky 1977; Taube and Schwartzkroin 1986). In order to determine the threshold level for activation of calcium spikes, TTX-resistant potentials were examined in subicular neurons and compared with CA1 neurons after exposure to a microdrop of 100 μM TTX.

For CA1 neurons in the presence of TTX, the first response to appear as the level of injected current is increased is a hump-like potential ("calcium hump"; see Fig. 3B). With further increases in current injection, the hump-like potential changes to a broad spike. Calcium-like potentials were evoked in 9 of 10 bursting and 4 of 8 regular firing subicular neurons. In contrast to CA1 neu-
brane potential was measured at the arrow and I-V plots constructed for each neuron. Solid bar shows current onset. Calibration bars 6 mV, 20 ms. B Mean I-V plots for 53 bursting and 20 regular firing subicular neurons. The slope of the best-fit line through the points indicates the mean input resistance for each neuron type.

Table 2. Cable properties of subicular neurons

<table>
<thead>
<tr>
<th>Neurons</th>
<th>( \tau_0 ) (ms)</th>
<th>( \tau_1 ) (ms)</th>
<th>( C_0 )</th>
<th>( C_1 )</th>
<th>( \rho )</th>
<th>( L )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bursting</td>
<td>22</td>
<td>13.77 ± 1.34</td>
<td>0.94 ± 0.13</td>
<td>4.58 ± 0.34</td>
<td>1.27 ± 0.16</td>
<td>5.00 ± 0.78</td>
</tr>
<tr>
<td>Regular firing</td>
<td>11</td>
<td>12.34 ± 1.71</td>
<td>0.74 ± 0.12</td>
<td>4.26 ± 0.73</td>
<td>1.99 ± 0.44</td>
<td>7.23 ± 1.40</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM.

\( \tau_0 \), 1st order time constant; \( \tau_1 \), 2nd order time constant; \( C_0 \), 1st order coefficient; \( C_1 \), 2nd order coefficient; \( \rho \), dendrite-to-soma conductance ratio; \( L \), electrotonic length.

Bursting and regular firing subicular neurons were characterized more by a "hump-like" response than the prominent broad spike observed in CA1 neurons (Fig. 3A, C1, E2). Surprisingly, it was difficult to elicit a CA1-like calcium spike from bursting subicular neurons, even at high levels of injected current (Fig. 3C2); one of the two exceptions is shown in Fig. 3D. For regular firing neurons it was often difficult to evoke a significant calcium hump (Fig. 3E1). It was also noteworthy that the calcium hump observed in subicular neurons was not followed by a hyperpolarization, although this hyperpolarizing afterpotential was a prominent characteristic of the response under conditions when the cells were not exposed to TTX. However, the hyperpolarizing afterpotential during the current pulse was observed in two bursting cells when the TTX-resistant potential contained a broad spike (Fig. 3D); this finding was also true for CA1 pyramidal neurons (Fig. 3B).

The mean current level to evoke a calcium hump in five CA1 neurons was 1.15 ± 0.09 nA (range 0.9-1.4). In contrast, a calcium hump was evoked at a significantly lower current level in bursting subicular neurons, where the mean threshold level was 0.82 ± 0.07 nA (range 0.4-1.6; \( P < 0.05 \), t-test). For the four regular firing neurons in which a calcium potential could be evoked, the mean threshold for activation of the calcium hump was 0.975 ± 0.246 nA (range 0.5-1.2). There was no significant difference between bursting and regular firing subicular neurons (\( P > 0.05 \), t-test).

Response to electrical stimulation

Electrical stimulation from several different areas of the hippocampal slice evoked synaptic responses in the two types of subicular neurons. Because no differences were observed in synaptic responses between bursting and regular firing neurons, both neuron types are grouped together for description of their responses. Stimulation of different areas within CA1 (stratum radiatum, stratum pyramidale, stratum oriens, alveus) evoked an EPSP in subicular neurons (Fig. 4A). The evoked EPSP was often followed by a longer-lasting IPSP (Fig. 4B), which had a reversal potential of \(-89.3 ± 1.4\) mV (range -86.1 to -93.7, \( n = 7 \)). In five neurons, the IPSP was biphasic, with an early and late component (Fig. 4C). In some cells an IPSP was evoked without the presence of an EPSP. Increased levels of stimulation in any of the CA1 areas would elicit one or more action potentials rising from the EPSP. Multiple action potentials embedded in a depolarizing envelope were often triggered at high levels of stimulation in bursting neurons (Fig. 4E). The shape and time
course of the depolarizing envelope corresponded with the shape and time course of the depolarizing envelope observed during intracellular current injection (see above). An antidromic spike was driven when the stimulating electrodes were positioned in the alveus and the stimulation intensity was increased (Fig. 4F).

Stimulation of the presubiculum area evoked either an EPSP or an antidromic spike at higher levels of stimulation. Either response could also be elicited when the stimulating electrodes were positioned in the superficial or deep layers of the presubiculum. When the stimulating electrodes were placed in the CA3 area (stratum radiatum or pyramidal), electrical stimulation sometimes evoked a small depolarizing response in the subicular neuron (Fig. 4D). No evoked responses were observed in the subicular cells when the stimulating electrodes were placed in the dentate area.

The EPSPs evoked by CA1 and presubicular stimulation were mediated by excitatory amino acid receptors because the EPSP was substantially reduced, and sometimes blocked, with addition of 20 μM CNQX in the bathing medium (n = 23; Fig. 5A). Sometimes in CNQX, a longer-lasting EPSP could also be evoked at higher stimulation intensities (Fig. 5B1). This response was always blocked with the addition of the N-methyl-D-aspartate (NMDA) antagonist AP5 (50 μM) into the bathing medium (n = 9; Fig. 5B2), suggesting that the residual response following CNQX treatment was mediated by NMDA receptors. In some cases an IPSP was uncovered in the presence of CNQX (Fig. 5C). Because of the diffi-
Fig. 4A–F. Synaptic responses in subicular neurons following stimulation of the hippocampus. A An excitatory postsynaptic potential (EPSP) is evoked following stimulation of CA1 stratum radiatum. B Response in a different subicular neuron showing an EPSP followed by a monophasic inhibitory postsynaptic potential (IPSP) with stimulation in CA1 stratum radiatum. C CA1 stratum radiatum stimulation evoked an EPSP followed by a biphasic IPSP in this neuron. D Stimulation of CA3 stratum radiatum evoked a small depolarizing response. E Response of a bursting neuron to suprathreshold stimulation of CA1 stratum radiatum. Note the burst of action potentials embedded in a large depolarizing envelope. F Response of a regular firing neuron following stimulation of CA1 alveus. Note the depolarization following the antidromic action potential. Arrows denote onset of stimulation. Calibration bars: A 4 mV, 20 ms; B,D 3 mV, 20 ms; C 2 mV, 40 ms; E 7 mV, 20 ms; F 10 mV, 20 ms.

culty in eliciting an NMDA component, 17 neurons were recorded in a Mg²⁺ free bathing solution containing CNQX in order to facilitate the activation of NMDA receptors. Under these conditions, the longer-lasting depolarization was usually elicited (Fig. 5D1). When CNQX was washed out of the bathing medium for 30 min, a shorter latency, larger amplitude EPSP returned (Fig. 5D2). In three cases, the NMDA response could only be observed when the stimulating electrodes were positioned in CA1 pyramidal or alveus, but not when the stimulation was applied to CA1 radiatum. Finally, application of AP5 alone to subicular neurons either had no effect or slightly reduced the EPSP amplitude (n = 3).

Iontophoretic studies

Responses to glutamate. Glutamate (10 mM) was applied iontophoretically to three different areas within the subiculum and responses monitored in 18 neurons. Application of glutamate near the recording site, presumably the soma region, produced a short-latency depolarization in all 5 cells tested. Application of glutamate to the apical and basal dendritic layers produced similar depolarizations in 12 of 15 and 8 of 12 neurons, respectively (Fig. 6A). A burst of action potentials followed by a hyperpolarizing afterpotential was often elicited when increased levels of glutamate were applied to the soma or dendritic regions of bursting type neurons (Fig. 6C). Single action potentials were evoked from regular firing neurons with increased levels of glutamate application (Fig. 6D). Sometimes a small, longer duration hyperpolarization was observed, instead of the depolarizing response, when glutamate was applied to the apical dendritic region (Fig. 6E). When this response occurred, lowering the drug pipette further into the tissue usually uncovered a depolarizing response. To determine whether
glutamate was acting via the quisqualate/AMPA (R,S)-
α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)
type of excitatory amino acid receptors, quisqualate was
applied to the apical dendritic layers of three neurons
from the second barrel of a multi-barrel micropipette. In
two of nine cases, the application of quisqualate produced a
depolarization similar to the response to glutamate
(Fig. 6B).

Responses to GABA. Ten neurons were monitored for
their response to iontophoretically applied GABA
(10 mM) at different locations within the subiculum. All
neurons tested, except one, were classified as bursting. In
general, the response to GABA was complex and partially
dependent on the location of the ejection site. Application
of GABA to various locations near the recording
site (i.e., near the soma region) for eight neurons usually
produced some form of hyperpolarization. The hyperpo-
larization could be (1) monophasic (Fig. 7A), (2) followed
by a depolarization (not shown), or (3) embedded in a
longer-lasting depolarization (Fig. 7B). At one injection
site, a small depolarization was embedded in a hyperpo-
larization. There were only two instances where GABA
application near the recording site produced only a depo-
larization; in both cases, movement of the drug pipette to
a different location near the soma produced a response
containing some form of hyperpolarization. In some cases,
lowering or raising of the drug pipette by 50 μm
changed the response type dramatically (Fig. 7C). The
hyperpolarizing responses could be reversed with tonic
current injection, although the reversal potentials varied
considerably among the five neurons tested. The mean
reversal potential was −82.2 mV (range −69 to −99
mV, n = 5). Figure 7D,E shows the responses from two
neurons to GABA application near the recording site at
different membrane potentials. Note the prominent, slow
depolarizing response which is uncovered when the neu-
Fig. 6A–E. Responses to iontophoretic application of 10 mM glutamate or 10 mM quisqualate. A Response of a subicular neuron to glutamate applied to the basal dendritic region. B Depolarizing response following application of 10 mM quisqualate to the subicular molecular layer (apical dendritic region). C, D Responses to glutamate applied to the subicular molecular layer in a bursting neuron (C) and regular firing neuron (D). Glutamate application triggered three bursts in the bursting neuron; each burst contained three to four action potentials and was followed by a hyperpolarization afterpotential. In the regular firing neuron, glutamate application led to a burst of single action potentials embedded in a depolarizing envelope. Note the absence of the hyperpolarizing afterpotentials observed in C, E. In this neuron, a small, longer duration hyperpolarizing response was observed following application of glutamate to the subicular molecular layer. Arrows denote onset of 30-ms drug application. Calibration bars: A 3 mV, 200 ms; B, E 2 mV, 200 ms; C 8 mV, 200 ms; D 7 mV, 200 ms.

The neuron in Fig. 7E is hyperpolarized. This depolarization is presumably due to the activation of a different GABA receptor subtype which was "unmasked" following hyperpolarization of the neuron.

Application of GABA to the apical or basal dendrites also produced complex and different types of responses. GABA application to the basal dendritic region lead to a depolarizing response in four of five neurons (Fig. 8A). One of these four neurons responded with a hyperpolarization embedded in a depolarization (Fig. 8B). For one neuron, GABA application in the basal dendrites did not evoke a response, although responses were observed at injection sites in the molecular layer and near the recording site. When GABA was applied to the apical dendritic region (n = 6), neurons responded with (1) a long duration hyperpolarization (Fig. 8C), (2) a monophasic, long-duration depolarization (not shown), or (3) a mixed response containing both a hyperpolarization and a depolarization (Fig. 8D). In three neurons which had a prominent hyperpolarizing response, the mean reversal potential was −80.0 mV (range −78 to −82 mV).

Morphology

Five subicular neurons, four bursting and one regular firing, were injected with LY. No difference in morphology was observed between the two cell types. Each neuron was pyramidal in shape and contained a set of short basal dendrites which were confined within 250 μm from the soma and one long apical dendrite which extended into the molecular layer before dividing into several branches. The apical dendrite usually extended to the hippocampal fissure. An example of a representative neuron stained with LY is shown in Fig. 9.
Fig. 7A–E. Responses of subicular neurons to iontophoretic application of 10 mM GABA near the recording site (soma region). A Response is composed of a monophasic hyperpolarization. B Mixed response in a second neuron containing a hyperpolarization embedded in a longer-lasting depolarization. C Responses in another neuron to GABA application near the recording site when the micropipette was positioned at different depths within the tissue. At position 1 the response was composed of three components: a, a short duration, small amplitude depolarization, b, a large-amplitude hyperpolarization; and c, a long-duration, small-amplitude depolarization. The components of this response changed when the micropipette was lowered 50 μm into the tissue. At position 2 a short duration depolarization (open arrowhead) was embedded in the hyperpolarizing response. The onset of the hyperpolarization was faster, but the peak response was delayed. In addition, there was an increase in the amplitude of the long duration depolarizing component (solid arrowhead). As the GABA micropipette was lowered deeper into the tissue (position 3), the onset of the depolarization embedded in the hyperpolarization was delayed while its amplitude increased. D,E GABA responses evoked at different membrane potentials for two subicular neurons. The neuron in D had a resting membrane potential of −60 mV. The hyperpolarizing GABA response "reversed" at about −95 mV. For the neuron in E, the reversal potential for the hyperpolarizing GABA response was about −91 mV. Note the long, prominent depolarization which appeared when the neuron was hyperpolarized. Resting potential was −73 mV. For D and E, the action potentials have been chopped and the open arrowheads denote traces at resting membrane potential. Solid arrows denote onset of 30 ms GABA application. Calibration bars: A 2 mV, 200 ms; B,C 3 mV, 200 ms; D 7 mV, 200 ms; E 5 mV, 200 ms.

Discussion

The results show that there are at least two physiological types of pyramidal cells in the subiculum. These two neuronal types can be differentiated on the basis of their response to (1) a pulse of depolarizing current and (2) orthodromic stimulation. One type of subicular neuron had similar properties to CA3 and layer IV/V entorhinal neurons, in that they showed a burst of action potentials embedded in a depolarizing envelope and followed by a hyperpolarization in response to an intracellular pulse of depolarizing current or orthodromic stimulation (Wong and Prince 1978; Jones and Heinemann 1988). The hyperpolarizing afterpotential resembled the AHP which usually follows termination of a depolarizing current pulse (Hotson and Prince 1980). In contrast to CA3 cells, the bursting behavior of subicular neurons was usually not observed spontaneously at resting membrane potential. Instead, the burst was only observed when it was evoked by injecting the cell with depolarizing current or
Fig. 8A–D. Responses to iontophoretic application of GABA in the basal and apical dendritic regions. A, B Responses to GABA applied to basal dendrites in different neurons. In A the response is primarily composed of a short duration depolarization, although a small amplitude hyperpolarization terminates the response. In B, the response is composed of four phases: (1) an initial depolarization, (2) a short-duration hyperpolarization, (3) a large-amplitude, short-duration depolarization, and (4) a small-amplitude, long-duration depolarization. C, D) Responses in two different neurons following application of GABA in the subicular molecular layer (apical dendritic region). In C the response is a biphasic hyperpolarization; in D, the response is a depolarization followed by a small-amplitude hyperpolarization. Arrows denote onset of GABA application for 30 ms. Calibration bars: A 4 mV, 100 ms; B 2 mV, 300 ms; C 4 mV, 400 ms; D 3 mV, 200 ms

with synaptic stimulation. The second type of subicular neuron shares properties in common with CA1 pyramidal cells in that it shows regular firing (see Schwartzkroin 1975 1977). However, regular firing subicular neurons also differ from CA1 cells. For example, regular firing subicular neurons usually do not show the prominent calcium spikes so characteristic of CA1 cells (Schwartzkroin and Slawsky 1977; Taube and Schwartzkroin 1986). Furthermore, in about half of the regular firing neurons, it was not possible to elicit the calcium hump-like potential.

The results also show that subicular neurons share many of the same electrophysiological and morphological properties as hippocampal pyramidal cells. For example, the membrane properties of subicular neurons, such as resting membrane potential, action potential amplitude, action potential threshold, input resistance, AHP, and I-V curves, were similar to those observed for hippocampal neurons (Schwartzkroin 1975; 1977; Brown et al. 1981a; cf. Spruston and Johnston 1992 reported different values for hippocampal neurons using patch clamp techniques). For CA3 neurons, the depolarization underlying the burst and the hyperpolarization following the burst appear to be calcium-dependent (Wong and Prince 1978, 1981). Although experimental manipulations with calcium antagonists were not conducted in the present experiments, the finding that bursting subicular neurons displayed depolarizations which were TTX-resistant and occurred at low thresholds of activation (presumed calcium humps) would indicate that the depolarizing envelope and hyperpolarizing afterpotential in subicular neurons may also be calcium-dependent.

Fig. 9. Photomontage of bursting-type subicular neuron filled with lucifer yellow. CA1 hippocampus is on the right, presubiculum on the left. The neuron was located in the middle of the subicular pyramidal cell layer. Calibration bar 100 μm
The different types of TTX-resistant potentials (spike versus hump) observed in subicular neurons may be attributed to one of two possibilities. First, the calcium spike may simply be generated by a recruitment of more of the same type calcium channel contributing to the calcium hump. If this were the case, then many subicular neurons would contain a lower density of calcium channels than CA1 and CA3 neurons, because it was usually difficult to evoke a calcium spike in subicular neurons. Alternatively, different types of calcium channels may underlie the two response types and each type may be activated at different threshold levels. Previous studies have suggested that there are at least three types of voltage-sensitive calcium channels in mammalian central neurons (Miller 1987). At low current levels, only a long-duration, small-amplitude depolarization (calcium hump) was observed in bursting (and some-regular firing) subicular and CA1 neurons. At higher levels of current injection, the depolarization appeared as a large-amplitude, broad spike (calcium spike) in all CA1 neurons and in a few bursting subicular neurons. In addition, the hyperpolarizing afterpotential which followed the depolarizing envelope only appeared at high levels of current injection when a calcium spike was present, suggesting that a particular group of calcium channels must be activated in order to initiate this hyperpolarization. Robitaille and Charlton (1992) found evidence showing that calcium-dependent potassium channels are located in close proximity to voltage-dependent calcium channels at the frog neuromuscular junction. Taken together, these findings suggest that different types of voltage-sensitive calcium channels are being activated at different current levels, and that the low threshold calcium response may contribute to the depolarization underlying the burst response. Furthermore, the results also suggest that the high-threshold calcium channels, which generate the broad calcium spike in hippocampal neurons, lie in close proximity to the calcium-dependent potassium channels mediating the hyperpolarizing afterpotentials. However, these findings must also be considered in the context of observations reported by Schwartzkroin and Stafstrom (1980), who showed that the activation of the AHP may not be entirely attributed to the influx of calcium, because cells injected with the calcium chelator, ethylene glycol tetraacetic acid (EGTA), still contained a small AHP.

The function of burst firing in a subpopulation of subicular neurons is unclear. It may contribute to the initiation and spread of synchronized neuronal activity as has been suggested for hippocampal and neocortical bursting neurons (Schwartzkroin 1983; Chagnac-Amitai and Connors 1989). Brain areas which contain bursting neurons are often susceptible to the development of epilepsy (Wong et al. 1984), and Walther and colleagues (1986) have shown in combined entorhinal/subiculum slices the importance of these structures for the generation of epileptiform activity. Alternatively, bursting activity could be involved in signal amplification. McCormick and colleagues (McCormick et al. 1985; McCormick 1992) have shown that cortical neurons can also be classified as either bursting or regular firing, and both neuron types are present within a small cortical area. Thus, the heterogeneity of cell types in the subiculum is similar to cortex and contrasts with the homogeneity of cell types within CA1 (regular firing) and CA3 (bursting) hippocampal areas. It is also noteworthy that the proportion of bursting to regular firing neurons in the subiculum is opposite to those reported for cortical areas. The heterogeneity of cell types in the subiculum raises the possibility of selective activation of different cell populations, and has important implications for network properties. It will also be important to determine whether both cell types have similar synaptic inputs and neuronal targets.

Passive cable properties

$L$ was smaller and $p$ was larger for subicular neurons than values previously reported for hippocampal cells (Brown et al. 1981a; Turner 1984). These results suggest that subicular neurons are more electrically compact than hippocampal cells. Alternatively, this discrepancy may arise from the different techniques used for computing the values. In this report, the time constants were computed using a curve-fitting computer program rather than the “method of peeling.” Using this program, values obtained for the slow time constant ($\tau_s$) were similar to those previously reported for other hippocampal neurons, whereas the value for the fast time constant ($\tau_f$) was faster. A lower value for $\tau$ would yield the larger $p$ and smaller $L$ reported above. Another possibility is that the underlying assumptions for the equivalent cylinder model are not fully met. For example, the $3/2$ “Power Law,” which requires that the diameter of the parent dendritic segment raised to the $3/2$ power equal the sum of the diameter of the daughter dendritic segments, each raised to the same power, has not been verified. However, some subicular values for $p$ and $L$ fall within the range of values reported for neurons in other brain areas (see Brown et al. 1981b), suggesting that these differences are real and subicular neurons may have different cable properties than hippocampal cells. Finally, as with the other membrane properties, there was no difference for $p$ or $L$ between bursting and regular-firing subicular neurons.

Synaptic potentials and iontophoresis

Subicular neurons were synaptically activated by stimulation of different layers within the CA1 area. No difference in response onset was detected by comparing stimulation from different strata of CA1. Several anatomical studies have shown direct pathways from CA1 and entorhinal cortex to subiculum (Swanson and Cowan 1977; Sorenson and Shipley 1979; Kohler 1985; Van Groen et al. 1986; Witter and Groenewegen 1990). Previous in vivo physiological studies have also shown that the subiculum is activated by stimulation of the entorhinal area (Van Groen and Lopes da Silva 1986). Taken together, these findings suggest that the short-latency response observed in subicular neurons following stimulation of different CA1 strata (excluding alveus) is probably due either to
activation of collateral fibers originating from the entorhinal cortex or to activation of the CA1-subiculum pathway. Alveus stimulation also produced a short-latency response in the subiculum, a finding consistent with previous studies showing that CA1 pyramidal axons project into the alveus and bifurcate, with one of the collaterals projecting toward the subiculum (Finch and Babb 1980; Knowles and Schwartzkroin 1981). In addition, stimulation of the presubicular area sometimes evoked an EPSP in subicular neurons. However, given the known anatomical connections of the parahippocampal region (Witter et al. 1989), the presubicular-evoked EPSP can be attributed to stimulation of passing fibers originating in entorhinal cortex.

Previous anatomical studies have indicated that CA3 neurons also project to the subiculum (Swanson et al. 1978), a finding consistent with the result that some subicular neurons could be synaptically activated following CA3 stimulation. Alternatively, the CA3-evoked subicular responses may be attributed to either activation of entorhinal fibers terminating in CA3 which contain collateral fibers synapsing in the subiculum, or to activation of the disynaptic CA3-CA1-subiculum pathway. It is noteworthy that only a fraction of subicular neurons were capable of being activated with CA3 stimulation. Although this finding suggests that only a subpopulation of subicular neurons receive CA3 input, a more parsimonious interpretation of this result is that many of the CA3-to-subicular projections traveled out of the plane of the recorded lamellar slice.

The findings that an antidromic spike often occurred following stimulation in the alveus or presubicular areas is consistent with the known anatomical projections of the subiculum (Kohler 1985; Van Groen et al. 1986) and previous in vivo electrophysiological studies (Finch et al. 1986; Van Groen and Lopes da Silva 1986). An antidromic spike was never evoked in subiculum following stimulation in the CA1 area (excluding the alveus). This result is not consistent with a finding by Berger and colleagues (1980) who reported a pathway from subiculum to the CA1 area based on a horseradish peroxidase (HRP) study.

Subicular neurons responded to glutamate in much the same manner as CA1 and CA3 pyramidal neurons (Nadal et al. 1976; Collingridge et al. 1983). The depolarizing responses were evoked at short latencies, had short durations, and could be evoked over a widespread area along the soma-dendritic axis. The additional finding that EPSPs were substantially reduced, if not entirely blocked, by CNQX suggests that CA1 and entorhinal neurons projecting to the subiculum use glutamate (or a glutamate-like substance) as a neurotransmitter at these synapses. The small residual response observed in the subicular EPSP following CNQX application is attributed to NMDA-receptor activation, because this residual response was blocked with AP5. This result is consistent with previous reports showing a high concentration of NMDA receptors in the subiculum (Monaghan and Cotman 1985). Finally, the results also showed that glutamate application in the apical dendritic region sometimes evoked a small hyperpolarizing response. A similar phenomenon has been reported in the CA1 area and was attributed to activation of inhibitory interneurons which synapsed onto the recorded pyramidal neuron (Taube and Schwartzkroin 1987). In a similar manner, the subicular hyperpolarizations may be due to glutamate activation of local circuit inhibitory interneurons in the subiculum.

The IPSPs observed for subicular neurons resembled those monitored in CA1 cells, including the biphasic IPSP (Alger and Nicoll 1979; Newberry and Nicoll 1984). The reversal potential for the monosynaptic IPSP and the early phase of the biphasic IPSP were similar to the reversal potential for most of the GABA responses, suggesting that these synaptic potentials are mediated by GABA. Although selective GABA agonists/antagonists were not applied in the present study, it remains to be determined whether the early and late phases of the biphasic IPSP are due to GABA$_A$ and GABA$_B$-receptor activation, as in other hippocampal areas (Thalman et al. 1981; Newberry and Nicoll 1985).

The complex GABA responses observed when GABA was applied to the soma and dendritic areas may reflect activation of different GABA receptor subtypes along different portions of the neuron (Andersen et al. 1980; Mueller et al. 1984). For hippocampal pyramidal neurons, a GABA$_B$-activated chloride channel is thought to mediate the fast hyperpolarization observed with GABA application near the cell soma (Nicoll et al. 1990). In contrast, the ionic conductances and receptor underlying the depolarizing GABA response in the hippocampus remain unclear. While some investigators suggest that a second type of GABA$_A$ receptor mediates the dendritic depolarizing GABA response and involves other ions in addition to chloride (Djorup et al. 1981; Wong and Watkins 1982; Mueller et al. 1984), other investigators have postulated that the depolarization arises from a differential intracellular chloride concentration between the dendritic and somatic regions (Nicoll et al. 1990). Despite this depolarizing action of GABA in the dendrites, the response may still have an inhibitory effect on the cell, through the shunting of excitatory currents generated in nearby dendrites (Staley and Mody 1992). For subicular neurons, hyperpolarizing responses were most commonly seen following GABA application near the recording site, although mixed responses containing both hyperpolarizing and depolarizing components were observed as well. The depolarizing component may have been due to GABA-activated receptors on nearby basal and apical dendrites, or to the inactivation of local inhibitory interneurons which in turn synapsed onto subicular cells. Further experiments in which synaptic transmission is blocked will be necessary to test this latter possibility.

In conclusion, this study identified the cellular and synaptic properties of subicular neurons. The subiculum is composed of a heterogeneous population of cell types and has similar electrical properties to other hippocampal principal neurons. Subicular neurons receive glutamimergic projections from CA1 and entorhinal cortical neurons and project to the presubiculum area and fornix. Finally, subicular neurons are inhibited by local circuit neurons and display complex responses to GABA.
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