Morphological and Electrophysiological Properties of Principal Neurons in the Rat Lateral Amygdala In Vitro

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Received 8 June 2000; accepted in final form 24 October 2000

Faber, E.S.L., R. J. Callister, and P. Sah. Morphological and electrophysiological properties of principal neurons in the rat lateral amygdala in vitro. J Neurophysiol 85: 714–723, 2001. In this study, we characterize the electrophysiological and morphological properties of spiny principal neurons in the rat lateral amygdala using whole cell recordings in acute brain slices. These neurons exhibited a range of firing properties in response to prolonged current injection. Responses varied from cells that showed full spike frequency adaptation, spiking three to five times, to those that showed no adaptation. The differences in firing patterns were largely explained by the amplitude of the afterhyperpolarization (AHP) that followed spike trains. Cells that showed full spike frequency adaptation had large amplitude slow AHPs, whereas cells that discharged tonically had slow AHPs of much smaller amplitude. During spike trains, all cells showed a similar broadening of their action potentials. Biocytin-filled neurons showed a range of pyramidal-like morphologies, differed in dendritic complexity, had spiny dendrites, and differed in the degree to which they clearly exhibited apical versus basal dendrites. Quantitative analysis revealed no association between cell morphology and firing properties. We conclude that the discharge properties of neurons in the lateral nucleus, in response to somatic current injections, are determined by the differential distribution of ionic conductances rather than through mechanisms that rely on cell morphology.

INTRODUCTION

The amygdala has been implicated in a variety of functions including memory and attention and in placing emotional significance to sensory input to produce the appropriate physiological response (Adolphs et al. 1994; Aggleton 1993; Kluver and Bucy 1939; LeDoux 1995; Rogan and LeDoux 1996). The amygdala has also been shown to be involved in the generation of temporal lobe epilepsy (Callahan et al. 1991; Danober and Pape 1998) and to be important in auditory fear conditioning (LeDoux 1995). Anatomically, the amygdaloid complex is divided into a number of nuclei including the basolateral complex (BL), which is made up of the lateral (LA), basolateral (BLA), and basomedial nuclei (Brodal 1947; Pitkänen et al. 1997). These nuclei can then be further divided into subdivisions that have extensive interconnections (Krettek and Price 1978; Pitkänen et al. 1997). The amygdala receives input from both cortical and thalamic regions (McDonald 1999; Turner and Herkenham 1991). The LA is particularly important in mediating fear conditioning as sensory inputs involved in this process have been proposed to enter the amygdaloid complex via this nucleus (LeDoux 1995; Pitkänen et al. 1997; Romanski and LeDoux 1993).

A complete understanding of information processing in the amygdala requires detailed information about the properties of neurons comprising the various nuclei as well as their connections. Golgi studies have identified two types of neurons in the LA and BLA (McDonald 1984; Millhouse and DeOlmos 1983; Price et al. 1987). The principal cell type has three to five primary dendrites that are invested with a moderate density of spines and resembles cortical pyramidal neurons. These cells are thought to be projection neurons and use glutamate as their neurotransmitter (Carslen 1988; Smith and Paré 1994). The other cell type has dendrites that lack spines and are thought to be local circuit cells that use GABA as their transmitter (McDonald 1984; Paré and Smith 1993).

Electrophysiologically, neurons within the BLA have been classified into three groups with distinct morphologies (Paré et al. 1995; Rainnie et al. 1993; Washburn and Moises 1992). In contrast, there have been few extensive studies of the physiological properties of neurons in the LA. In one study using microelectrode recordings from rat brain slices, three classes of cell were identified based on the fast afterhyperpolarization that followed single action potentials and their responses to applied agonists (Sugita et al. 1993). Other studies have revealed two cell types in the LA based on the properties of their action potentials. One type has relatively broad action potentials, shows spike frequency adaptation, and has been classified as pyramidal cells. The other type has faster action potentials and shows no spike frequency adaptation in response to prolonged current injection. The latter cells have been classified as interneurons (Mahanty and Sah 1998; Paré et al. 1995). In the LA, in vivo and in vitro recordings from projection (noninterneuronal) neurons from cat and guinea pig have shown that these cells generate two types of intrinsic membrane potential oscillations in response to somatic current injection (Pape et al. 1998; Paré et al. 1995). A small proportion of cells did not generate these oscillations, suggesting that there may be two types of pyramidal neurons in the LA. In a recent in vitro study in the rat (Faulkner and Brown 1999), only neurons bordering the external capsule in the LA were studied. In this study, noninterneuronal cells were divided cells into four categories.
(see Discussion). However, a detailed analysis of the repetitive firing properties of neurons in the LA has not been performed. In the present study, we have characterized the noninterneuronal neurons in the rat LA in vitro using electrophysiological and quantitative morphological techniques. We find that these cells show a range of repetitive firing properties that are best explained by their afterhyperpolarizations (AHPs) and by the fact that their morphology cannot predict their discharge properties.

METHODS

Tissue preparation

All experiments were performed on rat brain slices maintained in vitro. Wistar rats (of either sex, 17- to 20-days old) were anesthetized with intraperitoneal pentobarbital (50 mg/kg) and decapitated. These procedures were in accordance with the guidelines of the Institutional Animal Ethics guidelines. Brains were rapidly removed and placed in ice cold artificial cerebral spinal fluid (ACSF) containing (in mM) 118 NaCl, 2.5 KCl, 25 NaHCO₃, 10 glucose, 1.3 MgCl₂, 2.5 CaCl₂, and 1.2 NaHPO₄. Coronal slices (400 mm thick) containing the amygdala (see Fig. 1) were cut using a microslicer (Dosaka, DTK-1000). Slices were allowed to recover in oxygenated (95% O₂ -5% CO₂) ACSF at 30°C for 30 min, then kept at room temperature for a further 30 min before experiments were performed. Slices were transferred to the recording chamber as required and were continuously perfused with oxygenated ACSF maintained at 28–30°C.

Electrophysiological analysis

Whole cell recordings were made from neurons in the LA using the “blind” or infrared differential interference contrast (IR/DIC) technique. Patch pipettes (3–6 MΩ) were fabricated from borosilicate hematocrit glass and filled with a solution containing (in mM) 135 KMeSO₄, 8 NaCl, 10 HEPES, 2 Mg₂⁺ ATP, and 0.3 Na₃ GTP (pH 7.3 adjusted with KOH, osmolarity 300 mOsm). On some occasions biocytin (0.1%) was included in the internal solution. Access resistance was 5–30 MΩ and was monitored throughout the experiment. Signals were recorded with an Axopatch 1D amplifier (Axon Instruments, Foster City, CA). Responses were filtered at 5–10 kHz and digitized at 10 kHz (Instrutech, Greatneck, NY, ITC-16). All data were acquired, stored, and analyzed on a Power Macintosh 7500/100 using Axograph (Axon Instruments).

To investigate the firing properties of neurons, six to eight current injection steps (600 ms in duration) were delivered in 100 pA increments. AHPs were evoked in current clamp by applying a 50-mv, 400 pA current step from a holding potential of −70 mV. Currents underlying the AHP (I_AHP) were evoked in voltage clamp by applying a 50-mv, 50-mV depolarizing step from a holding potential of −50 mV. To compare AHPs across cells, their properties were quantified by measuring the area under the curve from baseline over the first 2 s of the response. Spike amplitudes were measured from resting potential. The threshold for spike initiation was taken as the beginning of the upstroke of the action potential.

Tissue processing and histology

Following physiological recording slices were fixed overnight in 4% phosphate buffered formalin (0.1 M, pH 7.4) at 4°C. The slices were rinsed in phosphate-buffered saline (0.1 M, pH 7.4; 3 changes, 20 min each) and permeabilized by overnight exposure to 0.5% Triton-X in phosphate buffered saline. The slices were then soaked overnight in avidin-horseradish peroxidase (Vectastain ABC Elite Kit, Vector Laboratories, Burlingame, CA). After washing in Tris buffer (0.1 M, pH 7.4), filled cells were visualized using the diaminobenzidine (DAB) procedure (Adams 1977). Slices were then mounted on albumin coated slides, dried overnight, dehydrated in an ascending series of alcohols, cleared in xylene, and coverslipped in Permount.

Morphological analysis

Biocytin-filled cells were drawn at a final magnification of ×650 using a camera lucida mounted on an Olympus BX50 microscope. These drawings were used for morphological analysis. All measurements were made from the drawings using a Bioquant Image Analysis system. Soma size was taken as the area enclosed by a smooth line made on the drawings (Culheim et al. 1987; Kernell and Zwaagstra 1981). This line closely followed the soma’s border but excluded emerging dendritic processes. The length of each dendritic segment was measured and corrected for its trajectory in the z plane by using the depth values from the fine focus of the microscope and the nominal section thickness (400 μm) and applying Pythagoras’ theorem. Using this approach, we were able to account for shrinkage due to tissue processing in the z plane. No allowance was made for shrinkage in the x and y planes, so dendritic dimensions in these planes will have been slightly underestimated. The extensive investment of dendrites with spines often caused the surface of dendrites to appear blurred when viewed under the light microscope. For this reason, we were unable to accurately measure the diameters of dendritic segments. Emerging processes were classified as axons if they showed no evidence of tapering, possessed “en passant” boutons and lacked spines.

For analysis of dendritic branching patterns, we have used the terminology originally proposed by Percherson (1979) and subsequently modified by Larkman et al. (Larkman and Mason 1990) for cortical pyramidal cells. According to this scheme, dendritic trees branch at “branch points” and the portion of the dendrite between each branch point is called a “dendritic segment.” The segment originating from the soma is called the “stem segment” and the final dendritic segment terminates at a “dendritic tip.” Dendritic segments can be further classified as first order (the stem segment), second order, and so forth.

Results are expressed as means ± SE. Student’s t-tests were used for statistical comparisons between groups. Tetrodotoxin was obtained from Alomone and biocytin from Vector Laboratories.
RESULTS

All recordings were made from dorsolateral, ventrolateral, or medial portions of the lateral amygdaloid nucleus (Fig. 1).

Electrophysiology

A total of 252 neurons were recorded from the LA that reached the minimal criteria for health and stability. These cells had a resting membrane potential more negative than $-55 \text{ mV}$ and access resistance less than 15 MΩ. Of these, four were classified as interneurons and are not included in this study. The remaining cells were classified as projection neurons based on their action potential half width and varying degrees of spike frequency adaptation in response to a 600 ms depolarizing current injection. These cells had a range of firing properties that varied from those that showed complete spike frequency adaptation, firing at most a few action potentials, to those that fired repetitively throughout the depolarizing current step with little or no spike frequency adaptation. In a population of 73 cells recorded using the blind method, 81% (59/73) of cells spiked only two to five times, whereas 19% of cells (14/73) generated more spikes in response to a 600-ms current injection. For comparison, in a population of 66 neurons recorded using IR-DIC techniques, 79% (52/66) fired only two to five spikes, whereas 21% (14/66) fired more than five spikes. These results indicate that the differences in firing properties are unlikely to be due to removal of dendritic processes in cells close to the surface of the slice. Three representative examples of the extremes and middle of this continuum are shown in Fig. 2. There were no clear differences in the passive membrane properties in cells showing different degrees of spike frequency adaptation. These measurements have therefore been lumped together. The average resting membrane potential was $-66 \pm 0.8 \text{ mV}$, input resistance was $150 \pm 9 \text{ MΩ}$, and membrane time constant was $29 \pm 1 \text{ ms} (n = 66)$. Application of the sodium channel blocker tetrodotoxin (TTX, 1 μM) completely blocked action potentials in all cells (Fig. 3A, 1 and 2). In the presence of TTX, depolarizing current injections did not initiate calcium spikes but revealed a sustained outward rectification in all cells at potentials more depolarized than $-50 \text{ mV}$ (Fig. 3B).

The distribution of firing properties formed a continuum and is plotted as the number of action potentials fired in response to a 400 pA, 600 ms current injection in Fig. 4. It can be seen that the most common cell type (151/248 cells; 61%) fired only one to five action potentials in response to increasing amplitudes of current injection. The remaining cells fired between 6 and 30 action potentials during the current injection. Of these cells, it was notable that some (70/248; 28%) showed clear spike
frequency adaptation during the first 5–10 action potentials (e.g., Fig. 2B), while others (27/248; 11%) fired repetitively throughout the current injection with little or no spike frequency adaptation (e.g., Fig. 2C).

In the BLA, pyramidal neurons have been classified into two types that resemble the fully adapting and repetitively firing cells observed in this study (Rainnie et al. 1993; Washburn and Moises 1992). Neurons that showed little spike frequency adaptation in the BLA were also described as late firing. These cells showed a clear delay in the initiation of the first action potential when it was evoked from negative membrane potentials (Washburn and Moises 1992). In the LA, we did not find any late firing neurons, and hyperpolarization of the membrane potential did not delay the time to first spike initiation in either fully accommodating cells or those that fired repetitively (data not shown).

In many cell types, the AHP that follows trains of action potentials is largely responsible for spike frequency adaptation (Sah 1996). We therefore tested if the differences in spike frequency adaptation was correlated with differences in the amplitude and time course of the AHP following a train of action potentials. In neurons that accommodated fully (Fig. 2A), action potentials evoked by a 100-ms depolarizing current injection evoked a large slow AHP (Fig. 5A, left) that had a mean integral of 4.3 ± 1 mV.s (n = 42). In contrast, cells that fired repetitively throughout the current injection (Fig. 2C) had a significantly smaller slow AHP with a mean integral of 2.3 ± 0.6 mV.s (P < 0.05, n = 12, Fig. 5A, right). The differences in the integral of the AHP were also reflected in the amplitude of the current underlying the slow AHP (Fig. 5B). In cells that showed complete spike frequency adaptation, the peak amplitude of the slow AHP current (measured at 500 ms after the voltage step) was 108 ± 13 pA (n = 40) compared with 34 ± 7 pA (n = 10) in cells that fired repetitively. These results suggest that the differential firing properties in these cells may be due to differences in the amplitude of the slow AHP. In agreement with this, in neurons that showed full spike frequency adaptation, blockade of the AHP with cadmium (250 μM) evoked repetitive spikes in response to the same current injection (Fig. 6A).

FIG. 3. Outward rectification in LA neurons. A1: responses from 2 cells to a series of current injections from −100 to +400 pA on 100-pA steps. Left: cell showed full spike frequency adaptation; right: cell fired repetitively. Application of TTX (1 μM) blocks action potential’s in both cells (A2). B: the current-voltage relations for these 2 types of cell in the absence of action potentials. The data are averages from 12 and 4 cells, respectively. Note the clear outward rectification seen in both cell types in response to depolarization beyond −50 mV.

FIG. 4. Distribution of firing properties of pyramidal cells in the LA. The histogram is based on the response to an injected current pulse (600 ms, 400 pA) in 252 neurons. The firing properties of each cell were recorded as described in Fig. 2. The most common cell type are those represented in Fig. 2A, firing 1–5 action potentials in response to a current injection. The remaining cells showed varying degrees of adaptation, firing between 6 and 30 times during a similar current injection.

FIG. 5. Afterhyperpolarization (AHP) amplitude largely explains the firing properties of LA neurons. The AHP and the underlying current are shown recorded from the 2 cells shown in Fig. 2, A and C. A: in cells that adapt fully injection of a 100-ms, 400-pA depolarizing current injection (bottom) caused the cells to fire 1 or more action potentials followed by an AHP (middle). Note that the slow AHP in the cell on the right (a cell that shows no accommodation) is smaller despite twice as many action potentials being evoked. B: currents underlying the AHP in the cells shown in A in response to a 50-ms depolarizing voltage step from −50 to 0 mV.
Broadening of action potentials during spike trains has been reported in a number of cell types (Agmon and Connors 1992; Kasper et al. 1994; McCormick et al. 1985; Shao et al. 1999). Since calcium influx largely occurs during the repolarizing phase of the action potential (Llinás et al. 1982), broadening of the action potential would increase calcium influx leading to more activation of calcium-dependent ion channels (Jackson et al. 1991). We therefore examined whether the differences in AHP amplitude in the different cell types could be due to a contrast in spike broadening. In LA neurons, action potentials during a train showed a clear change in the rate of repolarization and half-width. Recordings from two cells with distinct firing properties are shown in Fig. 7. Clearly spike broadening is similar in both cell types. In both cases, the first action potential had a mean amplitude of $90.1 \pm 0.6$ mV ($n = 66$), a mean half-width of $1.4 \pm 0.02$ ms ($n = 230$), and a spike threshold of $-41 \pm 1$ mV ($n = 66$). The second and subsequent action potentials had the same peak amplitude but a significantly ($P < 0.001$) wider half-width of $1.9 \pm 0.04$ ms ($n = 230$; Fig. 6, B and D).

Apart from the firing properties described thus far, 7 of the 252 neurons (3%) spiked only once (Fig. 8A) despite attempts to raise their excitability by either increasing the amplitude of depolarizing current injection or by holding the cell at more depolarized membrane potentials. These neurons had a mean resting membrane potential of $-56 \pm 1$ mV, a mean input resistance of $158 \pm 30$ MΩ, and a mean time constant of $24.7 \pm 4$ ms ($n = 7$). No AHP could be evoked in any of these cells either in current clamp (Fig. 9B) or voltage clamp (Fig. 8C).

**Morphology**

All cells characterized in this study had their somata located at least 100 μm below the surface of the slice (Table 1). These criteria ensured that much of a cell’s complete dendritic arbor was represented in our drawings. However, despite these measures, more than 30% of the dendritic processes traveled out of the slice (see Table 1). Thus the data presented here underestimate the true size of LA neurons. However, this caveat will not affect comparisons between the morphology of different neurons because it likely applies equally to all types.

One possible explanation for the extremes in electrophysiological response described in the preceding text is that projection cells in the LA fall into distinct morphological classes as...
has been suggested in the BLA (Washburn and Moises 1992). With this in mind, for morphological analysis, we divided cells into those that showed marked spike frequency adaptation and fired one to five spikes and those that showed little or no adaptation (more than 10 spikes in the spike train). These will be referred to as type 1 and type 2, respectively. Eighteen electrophysiologically characterized neurons were selected for detailed morphological analysis. An example of a recovered biocytin filled cell is shown in Fig. 9. Selection for detailed analysis was based on the following criteria: cells were located deep in the slice (see preceding text), were deemed to be well filled, had complete electrophysiological data sets, and had dendritic processes that traveled to both the top and bottom of the slice. Of these cells, nine were classed as type 1 and the other nine as type 2 (see Fig. 10).

At first glance, our recovered cells appeared to form two classes based on the shape of their somata; those with a clear pyramidal morphology and those that appeared multipolar or stellate. On close examination, however, it became clear that cells oriented predominantly in the horizontal plane appeared to have rounded or stellate cell bodies, whereas cells that lay in the plane of section, i.e., coronal, could be described as pyramidal (see Fig. 9). In spite of the apparent differences in the shape of their somata, when viewed in two dimensions, all cells featured a dominant dendrite that had a greater number of branch points and dendritic membrane. These qualitative observations were confirmed by our detailed quantitative data (see Table 1). In all cells examined, one dendrite contained more dendritic segments and branch points and represented over 30% of the cell’s total dendritic length. This dendrite was classed as the apical dendrite. For these reasons, we have classified all our cells as pyramidal-like.

Pyramidal-like cells have a dominant or apical dendrite and two to five basal dendrites emerging from the base of the cell somata (Table 1). Cells in either physiological class showed no preferred orientation or location within the LA (see Fig. 1), which contrasts with pyramidal neurons in other brain regions such as cerebral cortex (Colonnier 1981). Of the type 1 neurons, 4/9 had their long axis orientated chiefly in the horizontal plane and the remaining five cells in the coronal plane. Of the type 2 neurons, 6/9 were orientated primarily in the horizontal plane and the other 3 in the coronal plane. In some cells, a number of dendritic processes traveled into and even crossed the external capsule as previously described (Millhouse and DeOlmos 1983). The dendrites of all cells in both classes were invested with spines (see Fig. 9). These were located on all regions of the dendrites except on the initial portion where they emerged from the somata. Axons (see Fig. 9) were noted on 6/9 of type 1 neurons and 7/9 of type 2 neurons. In most cases, the axon originated from the somata (5/6 and 5/7 for type 1 and 2 neurons).
neurons, respectively). In the other cases, the axon originated from a basal dendrite.

Three examples of type 1 (left) and 2 (right) neurons and their dendritic trees are shown in Fig. 10. For each class, cells have been chosen according to increasing (top to bottom) complexity of their dendritic arbor. Dendrograms representing the branching pattern for each cell’s primary dendrites are shown on the left and right of each cell (Fig. 10). Examination of all the cells and their dendrograms revealed no obvious qualitative morphological differences between the cells types. A detailed quantitative analysis of a variety of dendritic characteristics supports this initial observation (Table 1). The dendritic morphology of the two cell types is strikingly similar according to the parameters presented in Table 1. No significant difference ($P > 0.05$) was found among the mean soma size, number of primary dendrites, the mean length of second, third, and fourth, etc. segments and number of dendritic tips of the two cell types. The total dendritic length, which provides a crude estimate of cell size, suggests that type 1 and type 2 neurons are similar in overall size. Furthermore the dendritic morphology of apical and basal dendrites in terms of the number of branch points and total dendritic length in cells belonging to the two classes was not significantly different ($P > 0.05$). However, within both classes there were a significantly ($P < 0.05$) greater number of branch points in the apical dendrite compared with the basal dendrites (8.3 vs. 4.6 for type 1 and 9.2 vs. 3.6 for type 2 neurons). In both cell classes over 30% of the total dendritic tree length is contained within the apical dendrite (see Table 1).

In summary, there are no clear morphological distinctions between cells that differ markedly in their electrophysiological properties (as assessed by the response to a 600-ms current injection). In fact, the variation in dendritic complexity and the degree to which cells clearly exhibit apical versus basal dendrites is just as great within each physiological class.

**DISCUSSION**

The present experiments have categorized spiny noninterneuronal neurons within the LA based on their electrophysiological properties. Cells showed firing properties that varied in a continuum from those that had marked spike frequency adaptation to cells that fired repetitively with little or no spike frequency adaptation. However, the most common cell type were those that accommodated fully in response to depolarizing current injections. In spite of the clear differences in firing properties between cells, our morphological analysis shows that all cells are pyramidal in shape with spiny dendrites and could not be clearly separated in terms of cell soma area or dendritic structure into distinct classes.

These differences in spike frequency adaptation between pyramidal cells in the LA appear to be largely due to differences in the amplitude of the AHP that followed a short train of action potentials. Neurons that adapted fully have a large, long-lasting AHP, whereas neurons that showed little adaptation have a shorter duration AHP. The AHP that follows action potentials is mediated by activation of Ca$^{2+}$-activated potassium currents. Neurons are known to express two types of these currents that have been called $I_{AHP}$ and $sI_{AHP}$. $I_{AHP}$ underlies the medium AHP and is responsible for a reduction in spike frequency. In contrast, $sI_{AHP}$ underlies the slow AHP and its activation leads to spike frequency adaptation (Sah 1996). The relatively smaller amplitude of $sI_{AHP}$ in repetitively firing neurons would explain the lack of marked spike frequency adaptation observed in this cell type. Similar regulation of firing patterns by $sI_{AHP}$ has also been reported in neurons in the BLA (Washburn and Moises 1992), hippocampus (Madsen and Nicoll 1984) and neocortex (McCormick et al. 1985). These differences in the size of the AHP between pyramidal cells in the LA suggest that there are likely to be differential expression patterns of either voltage-dependent calcium channels or the potassium channels that underlie the AHP or both.
It is notable that in LA pyramidal cells recorded in vivo, calcium-activated potassium currents have also been suggested to be activated by subthreshold synaptic inputs (Lang and Paré 1997), suggesting that these neurons will also respond differently in response to afferent stimulation.

At first inspection, most of our biocytin-filled cells in the LA could be confidently classified as having pyramidal-like morphology (e.g., Fig. 9). However, the orientation of some cells in the slice made the distinction between the basal and apical region of the cell difficult (see also Danobor and Pape 1998; Millhouse and DeOlmos 1983; Paré et al. 1995). Thus LA pyramids differ from classical pyramidal neurons (Bannister and Larkman 1995; Larkman and Mason 1990) as there is no rigid orientation of the pyramids in any one plane. LA pyramids also differ from cortical pyramids in several other ways. First, there are very clear differences in the branching patterns of apical and basal dendrites in cortical pyramids. In LA pyramids, stem segment length (distance from soma to first branch point; see Table 1) is similar for both apical and basal dendrites. This contrasts with classical layer-5 cortical pyramids as well as hippocampal pyramidal neurons (Bannister and Larkman 1995; Larkman and Mason 1990) where the apical dendrite may extend for up to hundreds of micrometers before major branches emerge to form a terminal arbor. In addition, the apical dendrites of layer-5 cortical pyramids taper very little as they project toward the pial surface. In contrast, all dendrites in LA pyramids taper rapidly as they move away from the soma. In hippocampal and cortical pyramidal neurons, basal dendrites are easily distinguished from apical because they branch repeatedly close to the soma (Bannister and Larkman 1995; Larkman and Mason 1990). In LA pyramids, however, the distinction between apical and basal dendrites is less clear using the same criteria. Excluding orientation, we suggest that LA pyramids resemble most closely those of layer-2/3 visual cortex neurons, where the contribution of the apical dendrite accounts for about 40% of the total dendritic length (see Fig. 1 in Larkmann and Mason 1990 for comparison) compared with approximately 32% in LA pyramids. The classical layer-5 pyramids have a much greater proportion of their dendritic length (49%) and surface area (53%) in their apical dendrites. For these reasons, we propose that these spiny cells in the LA are perhaps better classified as pyramidal-like neurons rather than pyramidal to distinguish them from cortical type pyramidal neurons.

Previous morphological studies of the rat amygdaloid complex have shown that spiny projection neurons comprise the majority of the total neuronal population in the basal and lateral nuclei. The remaining cells are smaller aspiny cells that are thought to be local circuit interneurons (McDonald 1982; Millhouse and DeOlmos 1983; Price et al. 1987). In the BLA, electrophysiological studies have divided the spiny interneuronal cells into two distinct classes based on physiology and morphology (Rainnie et al. 1993; Washburn and Moises 1992). Thus a clear correlation was made between morphology and electrophysiology of these neurons in the BLA.

Our results in the LA failed to show such a correlation since all of the morphologically characterized cells were clearly pyramidal-like. The morphology of our recovered cells is consistent with that of neurons classified as pyramidal using Golgi techniques (McDonald 1982). This is also in agreement with in vivo studies, which, apart from fast firing interneurons, have seen no morphological differences between neurons with differing firing properties and AHPs in the LA (Paré et al. 1995). In the BLA, a small fraction of cells were described as having stellate or multipolar cell bodies (Washburn and Moises 1992). Such cells have also been described in Golgi studies of the rat BLA (McDonald 1982; Millhouse and DeOlmos 1983). We have not been able to identify cells that definitively fit into this category in the LA. Furthermore three-dimensional reconstructions of dendritic trees in studies on LA and BLA neurons (this study and others: Sah and Callister, unpublished observations) (Danobor and Pape 1998; Millhouse and DeOlmos 1983; Paré et al. 1995) have suggested that cells that appear stellate are likely to be pyramidal because their apical dendrite lies in the z plane of the section. The LA has been divided into several subdivisions (e.g., see figures in Pitkänen et al. 1997). It is clear from the dendritic arbors of filled cells (Paré et al. 1995; this paper), however, that pyramidal-like neurons in these subdivisions would likely extend their dendrites outside the proposed limits.

Previous electrophysiological recordings from LA projection neurons both in vivo and in vitro have described intrinsic voltage-dependent oscillations in membrane potential near action potential threshold (Pape et al. 1998; Paré et al. 1995). We have not found these oscillations in recordings made in acute brain slices. Furthermore while the properties of all cells were not described in detail, these studies (Pape et al. 1998; Paré et al. 1995) have not reported the presence of fully adapting neurons. The reason for these differences is not immediately apparent. However, these studies were done using sharp microelectrodes at higher temperatures and different animal species (cat and guinea pig). It is therefore possible that these differences may be one reason for the different results. In addition, because of the use of sharp microelectrodes, the reported input resistance of the cells was much lower. This difference in the input resistance means that the impact of the AHP current activated by action potential trains would be greatly reduced and may explain the lack of accommodation.

In another recent investigation of cell physiology and morphology in both the LA and perirhinal cortex of rats in vitro, neurons were categorized into five groups based on their firing patterns in response to prolonged depolarizing current injection (Faulkner and Brown 1999). In the LA, neurons were only recorded from regions adjacent to the external capsule and characterized as regular spiking, fast spiking, or late spiking. The fast spiking neurons formed 7% of the total population and probably correspond to interneurones described previously (Lang and Paré 1998; Mahanty and Sah 1998). The majority of the other neurons were classed as regular firing. No fully accommodating neurons were described in the LA. However, all the recordings in the Faulkner and Brown study were performed at room temperature, with high concentrations of EGTA in the internal solution. Inclusion of EGTA would significantly buffer cytosolic calcium and thus calcium-activated potassium channels that contribute to action potential repolarization and the AHP would have been significantly less active. Furthermore differences in recording temperature are also likely to affect the firing properties of cells. Thus cell location and these two methodological factors are likely to explain the differences in cell types observed in this study versus those of Faulkner and Brown.

The less frequently encountered (7 of 252) type of neuron, which fired a single spike (Fig. 9), was not morphologically examined in the present study. However, Faulkner and Brown...
(1999) describe two neurons with electrophysiological properties similar to these cells. One of these cells was recovered and found to be pyramidal in shape. The similarity of the passive membrane properties of these neurons to the other pyramidal cells, together with the stability of these cells during recording, makes it unlikely that they are simply “sick cells” and supports the idea that they represent a distinct electrophysiological class. The precise role of this interesting electrophysiological class of cells is not clear and further studies are required to examine this more fully.

In summary, we have shown that the principal spiny cells within the LA fall into a continuum with extremes that can be separated according to their firing patterns. These different firing patterns are likely to be determined by differential expression patterns of ion channels in neurons. The morphological features of these cells place them in a single class consistent with previous morphological studies (McDonald 1982, 1984; Millhouse and DeOlmos 1983). The relative proportions of fully adapting cells compared with the other types suggests that the predominant role of neurons within the LA is to process sensory input by transforming tonic excitatory input into phasic output. This assertion is supported by the very low firing rates of LA neurons that have been recorded in vivo (Ben-Ari et al. 1974; Bordi et al. 1993; Paré and Gaudreau 1996). In contrast, neurons that have little spike frequency adaptation would turn a tonic input into sustained output. Thus inputs arriving at these two cell types would be differentially processed and suggest that they may be receiving afferent inputs from different sources.

Finally, in comparison to other reasonably well-understood brain regions such as the cerebral cortex, hippocampus, and cerebellum, the amygdaloid nuclei do not show a rigid organization of its neuronal elements. Cells are not organized into layers nor do they have the striking preferred orientation of their major output neurons as described in these other brain regions. Thus it is not surprising that input-output mechanisms in the LA are regulated by the various ionic conductances in the membranes of their major output neurons rather than through mechanisms that rely on architectonics and differences in cell morphology.

We thank J. Bekkers for comments on the manuscript. P. Sah is a Sylvia and Charles Viertel Senior Medical Research Fellow.

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