Firing properties and connectivity of neurons in the rat lateral central nucleus of the amygdala.

Mikel Lopez de Armentia and Pankaj Sah.

Division of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra ACT 2601, Australia. and Queensland Brain Institute, University of Queensland, St Lucia, QLD 4072 Australia

Corresponding author:

Dr. Pankaj Sah,
Queensland Brain Institute
University of Queensland
St Lucia, QLD 4072
Australia.
Telephone: +61 7 33468815
Fax: +61 7 3346 8837
e-mail: pankaj.sah@uq.edu.au
ABSTRACT

Using whole cell recordings from acute slices of the rat amygdala we have examined the physiological properties of and synaptic connectivity to neurons in the lateral sector of the central amygdala (CeA). Based on their response to depolarizing current injections, CeA neurons could be divided into three types. Adapting neurons fired action potentials at the start of the current injections at high frequency and then showed complete spike frequency adaptation with only 6-7 action potentials evoked with suprathreshold current injections. Late firing neurons fired action potentials with a prolonged delay at threshold, but then discharged continuously with larger current injections. Repetitive firers discharged at the start of the current injection at threshold and then discharged continuously with larger current injections. All three cells showed prolonged afterhyperpolarisations (AHPs) that followed trains of action potentials. The AHP was longer lasting with a larger slow component in adapting neurons. The AHP in all cell types contained a fast component that was inhibited by the SK channel blocker UCL1848. The slow component, not blocked by UCL1848 was blocked by isoprenaline and was significantly larger in adapting neurons. Blockade of SK channels increased the discharge frequency in late firers and regular spiking neurons but had no effect on adapting neurons. Blockade of the slow AHP with isoprenaline had no effect on any cell type. All cells received a mixed glutamatergic and GABAergic input from a medial pathway. Electrical stimulation of the lateral and basolateral nuclei evoked a large monosynaptic glutamatergic response followed by a disynaptic IPSC. Activation of neurons in the LA and BLA by puffer application of glutamate evoked a small monosynaptic response in 13 of 55 CeA neurons. Local application of glutamate to the CeL evoked a GABAergic response in all cells. These results show that at least three types of neurons are present in the CeA that can be distinguished on their firing properties. The firing frequency of two of these cell types is determined by activation of SK channels. Cells receive a small input from the LA and BLA but may receive inputs that course through these nuclei en route to the CeA.
INTRODUCTION

The amygdaloid complex is a structure located within the medial temporal lobe that is intimately involved in emotional behaviour, particularly in the generation of anxiety and conditioned fear (Davis and Whalen 2001; LeDoux 2000). Anatomically, the amygdaloid complex can be divided into more than 13 nuclei that have extensive local and extraamygdaloid connections (Sah et al. 2003). Sensory information from cortical and thalamic structures reaches the amygdala at the level of the lateral (LA) and basolateral (BLA) nuclei. Here information is processed locally and then transmitted, either directly and via the basal nucleus, to the central nucleus (CeA). Projections from the CeA to brainstem and hypothalamic areas mediate the behavioural, hormonal and autonomic features of fear conditioning (Davis and Whalen 2001; LeDoux 2000).

The CeA is subdivided in four sectors: capsular, lateral, intermediate and medial (Cassell et al. 1986). All subdivisions of the CeA receive inputs from other amygdaloid nuclei as well as from extramygdaloid sources (Krettek and Price 1978; McDonald 1998; Pitkänen 2000). Within the CeA, the capsular, lateral and intermediate sector project to the medial sector (Jolkkonen and Pitkänen 1998), however, the medial division does not appear to provide reciprocal connections but has prominent connections to hypothalamic and brain stem regions. Consistent with its autonomic connections, electrical stimulation of the CeA in rats, produces physiological fear responses with increase in heart and blood pressure, defecation, vocalization and a potentiated startle response (Davis et al. 1994). Thus, the central nucleus CeA is often considered to be an output station of the amygdaloid complex. However, given the extensive intranuclear connections within the central nucleus, local processing of information arriving at the CeA seems likely.

To understand information processing in the amygdala it is important to have an understanding of the properties of neurons containing in amygdaloid nuclei as well as their connectivity. The physiological properties of neurons within the LA and BLA, and their synaptic inputs have been described in much detail (see refs in (Sah et al. 2003)). For the CeA, the electrophysiological properties of the neurons have been described in three species: guinea pig, rat and cat (Dumont et al. 2002; Schiess et al. 1999). In one study using sharp microelectrodes in rat slices, two types of neuron were described which were clearly separated on their firing properties and type of afterhyperpolarisation (AHP) that followed trains of action potentials (Schiess et al. 1999). In contrast, using
whole cell recordings from rat, cat and guinea pig (Dumont et al. 2002), four types of neuron were described which had varying degrees of AHP. Both cat and rat show similar electrophysiological properties in CeA neurons, whereas guinea pig CeA neurons differ from those in the cat and rat (Dumont et al. 2002). It has been suggested that these differences between guinea pigs and rats or cats could underlie the distinct behavioural fear responses observed in those species (Dumont et al. 2002). The organization of inputs to CeA neurons have been studied in the guinea pig (Royer et al. 1999). However, while the pharmacological properties of some inputs have been examined in the rat (Delaney and Sah 2001; Lopez de Armentia and Sah 2001), little is known about the organization of these afferents. Here we investigate the electrophysiological properties and organization of synaptic inputs to neurons in the lateral sector of the central amygdala (CeL) of the rat.

MATERIAL AND METHODS

Experiments were performed on rat brain slices in vitro. All procedures were in accordance with the Institutional Animal Care and Ethics Committee guidelines. Wistar rats (21-27 days old) were anaesthetised with intraperitoneal pentobarbitone (50 mg/kg), and coronal slices (400 µm) were prepared using standard methods. After a recovery period of 30 minutes at 32°C, slices were maintained at room temperature in oxygenated (95% O₂-5% CO₂). Ringer solution containing (in mM) NaCl 118, KCl 2.5, NaHCO₃ 25, NaH₂PO₄ 1.2, MgCl₂ 1.3, CaCl₂ 2.5 and glucose 10. For recordings slices were transferred to the recording chamber and superfused with Ringer at 32-35 °C.

Whole cell recordings were made from neurons in the lateral division of the central amygdala using the ‘blind’ approach. Electrodes were filled with intracellular solution containing either (mM): CsGluconate, 117.5, CsCl 17.5, NaCl 8, HEPES 10, Mg₂ATP 2, Na₃GTP 0.2, BAPTA 10 and spermine 0.1 (pH 7.3 osmolarity 290 mOsm/kg) or KMeSO₄ 135, NaCl 8, HEPES 10, Mg₂ATP 2, Na₃GTP 0.2 and spermine 0.1 (pH 7.3 osmolarity 290 mOsm/kg). Access resistance was 7-25 MΩ and was monitored throughout the experiment. In some experiments recordings were made with sharp microelectrodes (70-100 MΩ) filled with 0.5 M KCl. Signals were recorded using an Axopatch 1-C or Axoclamp 2B amplifier (Axon Instruments, Foster City, CA, USA) filtered at 5-10 kHz and digitised at 10-20 kHz (Instrutech, ITC 16, Long Island, NY,
USA). All cells described in this study had a membrane potential more negative than -50 mV. Data were recorded and analysed using Axograph 4.8 (Axon Instruments).

Synaptic responses from the basolateral complex were evoked electrically using an array of 8 stainless steel electrodes (FHC Inc, Bowdoinham, ME, USA). In each case stimulation was applied through two adjacent electrodes allowing the stimulus location to be moved along the basolateral nuclei (Royer et al. 1999). A bipolar stainless steel stimulating electrode was also placed in the medial pathway, just ventral to the central nucleus. Stimuli (50 µs, 10-30 V) were applied by a constant–voltage isolated stimulator (Digitimer, DS2A, Hertfordshire, UK). In some experiments glutamate 0.1 mM was applied locally by a pressure ejection of 5-10 ms using a Picospritzer II (Parker Instrumentation, Fairfield, NJ, USA).

All values are expressed as mean ± SEM, and statistical comparisons were done using the two tailed paired t test and single factor ANOVA test. Drugs used were 6-cyano-7-nitroquinoxilane-2,3-dione (CNQX), 2-amino-5-phosphonovalerate (D-APV, Tocris Cookson, Bristol, UK), bicuculline methiodide (RBI Research Chemicals, Natick, MA, USA) kynurenic acid, picrotoxin, L-glutamate (Sigma-Aldrich, Australia) and tetrodotoxin (TTX, Alamone Laboratories, Jerusalem, Israel).

RESULTS

In coronal slices, axons bundles delimiting different nuclei of the amygdala could be identified easily using transillumination (Fig 1). Recordings were made from the lateral sector of the central amygdala which is separated from the basolateral complex by the intermediate capsule.

Firing properties of CeL neurons.

A total of 69 cells were recorded in whole cell configuration, and were classified based on their firing properties in response to a 600 ms depolarising current injection. Neurons were classified into three types: adapting, late firing and regular spiking. In adapting neurons (25/69; 36%), cells fired several spikes at high frequency at the start of the current injection and showed complete spike frequency adaptation (Fig 2A). These neurons fired at most 6 or 7 action potentials in response to suprathreshold current injections. In (20/69; 29%) neurons there was a noticeable delay between the start of the
depolarising pulse and the first AP, and were classified as late firing (Fig 2B). Finally, regular spiking neurons (24/69; 35%) discharged at high frequency at the start of the current injection with no delay but fired repetitively with a small amount of spike frequency adaptation (Fig 2C). The passive membrane properties of the three types of cell were generally similar (Table 1) but notably late firing neurons showed a more hyperpolarized resting membrane potential than the other groups. Adapting or regular spiking neurons did not display a late-firing response when they were hyperpolarized by current injection (data not shown) showing that the lack of delay was in these cells is not due their more depolarized membrane potentials.

Role of Ca$^{2+}$ dependent K$^{+}$ currents

In many neurons, trains of action potentials are followed by a slow afterhyperpolarisation (AHP) due to activation of Ca$^{2+}$ activated K$^{+}$ currents. The AHP controls the excitability of the cell during trains of action potentials and is largely responsible for setting the firing frequency and spike frequency adaptation (Sah 1996). Action potentials in CeL neurons were followed by a slow afterhyperpolarisation that lasted a variable duration between one and several seconds that was slower and larger in adapting neurons (Fig 3). Under voltage clamp, at a holding potential of $–50 \text{ mV}$, depolarising voltage step (100 ms) to $+10 \text{ mV}$ generated outward tail currents with fast and slow components. Consistent with the duration of the AHP, the slow component of the outward current was larger in adapting neurons. The ratio of the amplitude of the slow component (measured at 400 ms after the end of the voltage step) to that of the fast current, measured immediately after the voltage step, was 0.33 ± 0.05 (n=12) in adapting neurons, significantly larger (P < 0.01) than that measured in late firing (0.19 ± 0.05; n=5) and regular spiking (0.12 ± 0.02, n=10) neurons.

As the AHP is generally due to activation of calcium dependent potassium currents we next tested the calcium sensitivity of the AHP. In all cell types application of tetrodotoxin blocked action potential generation and revealed the presence of a depolarising hump at the beginning of the current injection (Fig 4). This depolarising hump was abolished when voltage dependent calcium channels were blocked with cadmium (250 µM) showing that all cell types express voltage gated calcium currents. Blockade of calcium currents with cadmium (250 µM, n = 9, Fig 4) or loading cells with
the calcium chelator BAPTA (10 mM; n = 3) abolished the slow AHP showing that it was due to activation of calcium activated potassium conductances.

Two types of slow calcium activated potassium currents $I_{AHP}$ and $sI_{AHP}$ contribute to the slow AHP that follows action potentials (Sah and Faber 2002). SK channels are responsible for $I_{AHP}$ which has rapid rise and decay kinetics and is blocked by the selective blockers apamin and UCL1848 (Sah and Faber 2002; Shah and Haylett 2000). In contrast, $sI_{AHP}$ has significantly slower kinetics, is insensitive to SK channel blockers, but is modulated by a range of neurotransmitters. As these currents play a significant role in neuronal firing properties we tested for the presence of these two conductances and their roles in determining the firing properties of CeL neurons. In response to a 100 ms depolarising voltage step from a holding potential of −50 mV, the fast component of the outward tail current was selectively blocked by UCL1848 (100 nM; Fig 5). The decay time constant of the $I_{AHP}$ current, measured as the UCL1848 sensitive component of the current by digital subtraction was 169 ± 35 ms in late firing neurons (n=5), 175 ± 69 ms in regular spiking neurons (n=4) and 126 ± 18 ms in adapting neurons (n=4). The similarity in the decay time constants of $I_{AHP}$ indicates that the calcium buffering capacity of these neurons are similar (Sah 1992). The slow component of the tail current had a slow rising phase and decayed much slower and was similar in the three cells with an average time constant of 1.6 ± 0.2 s (n=8) (Fig 5B). These kinetic features are consistent with the underlying currents being $I_{AHP}$ and $sI_{AHP}$. As expected for $sI_{AHP}$ currents, the slow component was selectively blocked by the beta catecholamine receptor agonist isoprenaline (n= 3; Fig 5) or muscarine (n=3; not shown). Consistent with the roles of these currents in mediating the two components of the AHP, the fast, rapidly rising component of the AHP was selectively blocked by UCL1848, while the slow component was selectively blocked by isoprenaline (Fig 5). The absence of any effect of isoprenaline on the AHP in non adapting neurons (Fig 5D) is consistent with virtual absence of the slow current in these cells.

We next tested the role of the two AHPs in the firing properties of CeL neurons. Blockade of $I_{AHP}$ with UCL 1848 (100 nM) increased the discharge frequency and the number of APs triggered by depolarising current pulses both in late firing neurons (n=5) and regular spiking neurons (n=5; Fig 6). In the presence of UCL 1848, the number of action potentials evoked by a twice threshold current injection changed from 7.6 ± 1.5 to
12.5 ± 3.1 spikes in late firing neurons (n = 6, Fig 6B) and from 11.0 ± 2.1 to 17.7 ± 4.2 in regular spiking neurons cells (n = 4, Fig 6C). In contrast, blockade of SK channels had no effect on the firing properties of adapting CeL neurons. The number of action potentials evoked by a twice threshold current injection was 3.7 ± 1.2 in control Ringer and 3.5 ± 0.5 in the presence of UCL 1848 (n=4; Fig 6A). As shown above, the sI\text{AHP} current in adapting neurons is larger than that present in the other two cell types. This current is thought to be a major determinant of spike frequency adaptation in a number of neurons (Sah 1996). However, no specific blockers of this current are currently available. The sI\text{AHP} current is the target for modulation by a range of transmitter systems (Sah 1996). Application of isoprenaline and muscarine, which blocked the slow AHP also caused other actions in CeL neurons which precluded us using this as a test for the role of the slow current. However when recordings were made with electrodes filled with a K\text{gluconate} based internal solution (n = 18), when the amplitude of sI\text{AHP} is much reduced (Zhang et al. 1994) or when cells were loaded with high concentrations of BAPTA (n=4), no adapting cells were found. Thus, the adaptation seen in these cells likely results from the presence of the sI\text{AHP} current.

**Synaptic inputs to CeL neurons**

*Connections from the medial pathway*

We have shown previously that stimulation of fibers medial and ventral to the CeL evokes a robust input to CeL neurons (Delaney and Sah 2001; Lopez de Armentia and Sah 2003). This pathway is thought to contain afferents originating in the lateral parabrachial area (Bernard et al. 1993; Neugebauer et al. 2003). Electrical stimulation of the medial pathway elicited a biphasic response in all CeL neurons. When recordings were made in current-clamp stimulation of this pathway generated an excitatory postsynaptic potential (EPSP) followed by an inhibitory postsynaptic potential (IPSP) (Fig 7A\textsubscript{1}). Under voltage-clamp at depolarized membrane potentials, stimulation evoked a fast inward current followed by a slower outward current (Fig. 7A\textsubscript{2}). Application of the non specific glutamatergic blocker kynurenic acid (3 mM) abolished the fast inward current (n= 17) showing that the excitatory input was mediated by glutamate receptors (Fig 7A\textsubscript{2}). The outward current was little affected by kynurenic acid but could be blocked by the GABA\textsubscript{A} antagonist picrotoxin (100 µM, n=10; Fig 7A\textsubscript{2}). Thus, inputs
arriving into the CeL through the medial pathway contain both glutamatergic and GABAergic afferents.

Connections from the BLA complex

Electrical activation within the BLA complex also elicited a biphasic response in CeL neurons (Fig 7B). Application of the nonspecific glutamate receptor antagonist kynurenic acid (3 mM) blocked both the excitatory and inhibitory components of the response (n=17, showing that the inhibitory response in this pathway is polysynaptic in nature. Stimulation from all regions from the dorsal part of the lateral amygdala to the basal nucleus (Fig 1) evoked a similar response in CeL neurons. We were unable to detect any significant differences in the amplitudes of the evoked synaptic response as the stimulus was moved ventrally thought the basolateral complex. An example from one cell is illustrated in Fig 7C that shows responses in one cell in response to stimulation from the most dorsal and most ventral pair of electrodes (Fig 1). Neurons in the CeL are well known to receive feedforward inhibition from the intercalated cell masses that are located on the border of the basolateral complex and the central nucleus (Delaney and Sah 2001; Royer et al. 1999). Consistent with this, when stimulation electrodes were moved to the intermediated capsule, an IPSC could be consistently evoked in the presence of kynurenic acid that was abolished by bath application of 100 µM picrotoxin (n= 6; Fig 7D). Thus, CeL neurons receive glutamatergic inputs from the basolateral complex and a GABAergic feedforward projection from the intercalated cell masses.

To confirm that electrical stimulation in the basolateral complex activated local neurons, rather than fibers of passage, glutamate (100 µM) was applied locally by puffer application to the lateral and basolateral amygdala cortex. In each case, the glutamate application pipette was moved over the entire lateral and basolateral nuclei to test for inputs from different regions of the lateral and basolateral amygdala. Responses were observed only in one of 17 CeL neurons when glutamate was applied in standard physiological Ringer. In 4 of these cells, when responses were not observed in control Ringer, application of picrotoxin revealed the presence of a response (Fig 8A). When inhibition was blocked either by picrotoxin or bicuculline, local application of glutamate to the basolateral complex produced a synaptic response in 13 of 55 CeL neurons. Of the 55 cells, 26 were recorded with a potassium based internal solution and in these cells 2
neurons that responded were late firers and 2 were regular spikers. In other cases, recordings were made with a cesium based internal and we could not determine the cells firing properties. When recordings were made from pyramidal cells in the basolateral complex, locally applied glutamate led to a depolarisation and generation of action potentials that were curtailed due to activation of the IPSP. Blockade of GABAergic inhibition with picrotoxin increased the number of action potentials evoked (Fig 8B). These results indicate that neurons in the lateral and basolateral amygdala project to CeL and that the BLA complex is under a strong inhibition by local interneurons. In most cells the response to puffer application of glutamate was small. Indeed, the response was little larger than the size of the spontaneous miniature synaptic potentials recorded in the same neurons (Fig 8C). At a holding potential of \(-70\) mV, the average amplitude the spontaneous miniature synaptic current was \(17 \pm 2\) pA while the response to puffer application of glutamate in the basolateral complex was \(42 \pm 8\) pA (\(n = 13\)). This result indicates that at least in a coronal section, projection neurons in the basolateral complex make contacts with CeL neurons that have at most two or three release sites. Depolarisation of the postsynaptic cell revealed a slow component to the synaptic current consistent with the presence of dual component glutamatergic synapses at these inputs (Lopez de Armentia and Sah 2003). It was notable that in some cells, responses could be obtained in a single cell from several sites within the basolateral complex indicating that there is some convergence of inputs to CeL neurons.

Puffer application of glutamate in the medial sector did not produce any responses in CeL neurons (\(n = 5\)) while application of glutamate within the CeL consistently activated GABAergic responses in CeL neurons (\(n = 15\); Fig 8E). These results are consistent with the lack of projections from the medial to the lateral sector (Jolkkonen and Pitkänen 1998) and suggest that there are extensive local connection within the largely GABAergic neurons of the lateral division (Sun and Cassell 1993).

**DISCUSSION**

In this study, using whole cell recordings in acute brain slices we have examined the physiological properties and synaptic connectivity of neurons in the lateral division of the rat central amygdala. We find that these neurons can be separated into at least three types that can be distinguished on their firing properties. In response to sustained
depolarising current injection, one class of cell (adapting neurons) fires only few action potentials and then shows complete spike frequency adaptation. A second class of cell, regular firing neurons, fires a train of action potentials during the current injection. This train shows some spike frequency adaptation at the start of the action potential train but continues to spike during the current injection. The third type of cell (late firer) is similar to regular spiking neurons except for the finding that hyperpolarising these neurons leads to a noticeable delay before the first action potential.

There have been two previous studies examining the electrophysiological properties of CeL neurons (Dumont et al. 2002; Martina et al. 1999; Schiess et al. 1999). In the first study, using sharp microelectrode recordings from rat CeA neurons (Schiess et al. 1999) two cell types (called type A and B) were described. The properties of these neurons are compatible to those described here as repetitive firers and adapting cells respectively. Type B cells formed 37% of the cell population close to our estimate of 36% for adapting neurons. Late firing neurons described by us were not described in the Schiess study. However, in response to suprathreshold current injections, these cells behave similarly to repetitive firers and together (late + repetitive firers) constitute 63% of the total cell population, similar to the 63% described for type A cells by Schiess et al (1999). In another set of experiments we made intracellular recordings with sharp microelectrodes from 46 CeL neurons (Table 1). Only two types of cell were encountered when recording in this modality: adapting and regular spiking. Ten (22%) exhibited full adaptation while the other 36 neurons (78%) were similar to the repetitive firers. No single or late firing neurons were found with intracellular microelectrodes. These values are very similar to those reported by Schiess et al. and indicate that the differences in firing properties arise from the different recording modes in the two studies.

More recently, whole cell recordings have been made from both lateral and medial sectors of the CeA from rat, cat and guinea pig (Dumont et al. 2002; Martina et al. 1999). In this study, three main cell types were described and called low threshold bursters, regular spiking and late firing neurons. The late firing and regular spiking neurons are similar to those described by us. However, no adapting neurons were described. Furthermore, for the CeL in the rat, regular spiking neurons formed 65% of the cell population while the late firers accounted for only 9%. In our study we estimated late
firing neurons form 29% of the total population, significantly different from the 9% described by Dumont et al (2000). What accounts for these differences in firing properties?

Dumont et al made whole cell recordings using potassium gluconate based internal solutions. In contrast, in the current study, we have used potassium methlysulphate based internal solutions. The difference in cell properties in the two studies likely stems from this difference in internals solutions. This effect of gluconate in downregulating the calcium activated $K^+$ currents has been previously described (Zhang et al. 1994). To confirm this result we have also made recordings with Kgluconate based internals ($n = 18$) and confirmed the lack of the slow AHP and the absence of fully adapting neurons.

Lastly, a cell type called low threshold bursters was also described (Dumont et al. 2002; Martina et al. 1999). This cell type fired bursts of action potentials due to activation of low threshold calcium currents. We have also seen such neurons in the CeL. However, in our study these cells formed a subset of adapting neurons and showed a clear slow AHP. Thus, we have not separated them as a distinct population.

The firing properties of neurons are determined in large by the complement of ion channels that they express (Hille 1992). In particular, slow calcium activated potassium currents are key determinants of the repetitive firing properties (Sah and Faber 2002). We find that CeL neurons express both types of slow calcium activated potassium currents. The apamin and UCL1848 sensitive $I_{AHP}$ current is present in all three cell types and makes a major contribution to setting the spiking frequency of repetitive firers and late firers. However, while clearly present in adapting cells, this current appears to have little impact on the firing properties. While the $sI_{AHP}$ current is present in all three cell types, its amplitude is much larger in fully adapting neurons. The $sI_{AHP}$ current has been proposed to be the major determinant of spike frequency adaptation (Sah 1996). The lack of fully adapting neurons under conditions where the slow AHP was much reduced is consistent with the presence of a larger $sI_{AHP}$ current in adapting neurons. Late firing neurons are similar to repetitive firers in that they do not show marked spike frequency adaptation. These neurons have a more hyperpolarized resting membrane potential. The slow ramp to action potential initiation has been previously been described and has been shown to be due to activation of the slowly inactivating, voltage dependent potassium current $I_D$ (Martina et al. 1999). In agreement with this, as shown by Martina et al (1999) low concentrations of 4-AP (30 $\mu$M) that block $I_D$ (Storm 1988) was able to block the delayed response (data not shown).
The central amygdala receives amygdaloid as well as extraamygdaloid projections. We find that all neurons in the lateral division of the CeA receive mixed excitatory/inhibitory inputs from the medial pathway, perhaps containing inputs from the parabrachial nucleus (Bernard et al. 1993; Neugebauer et al. 2003). This is a robust input in all cells and has been proposed to mediate the inputs containing pain related information. However, stimulation in this region also consistently evoked a monosynaptic GABAergic inhibitory input to these cells. The source of this input is not clear, but may represent inputs from the bed nucleus of the stria terminalis (Dong et al. 2001).

Electrical stimulation in the basolateral complex evoked excitatory responses in all CeL neurons. As the stimulus was moved dorsally from the LA to the basal nucleus equally strong inputs could be evoked in CeL neurons suggesting that neurons in the lateral and basolateral amygdala project to the CeL. Similar findings have been reported for CeL neurons in the guinea pig (Royer et al. 1999) and is consistent with tract tracing studies that show projections from these areas to the CeL (Paré et al. 1995; Pitkänen et al. 1995; Smith and Pare 1994). However, direct excitation of pyramidal neurons in the basolateral complex with glutamate was less effective than electrical stimulation and the responses to glutamatergic activation of basolateral neurons were generally small. Thus, it seems likely that innervation of CeL neurons by neurons in the basolateral complex is relatively weak. This result is consistent with the finding that stimulation of the lateral amygdala leads to spread of excitation into the basal regions but not the central amygdala (Wang et al. 2001). It was notable that activation of the neurons in the basolateral complex was much more robust when inhibition was blocked showing that local inhibition is very strong within these nuclei (Lang and Pare 1997). This difference between electrical stimulation and direct activation of neurons in basolateral nuclei suggests that there are fibers of passage coursing through the lateral, basolateral and basal nuclei that innervate neurons in the lateral division of CeA. Indeed in preliminary experiments we have found that puffer application of glutamate to the adjacent perirhinal or endopiriform cortex can also evoke excitatory responses in CeL neurons. The axons of these cells are likely to course through the basolateral complex on their way to the CeA and be excited by electrical stimulation in the basolateral complex (Fig 1). As shown previously (Royer et al. 1999), stimulation in the basolateral complex evoked a
disynaptic IPSP in CeL neurons and is thought to be due to excitation of the intercalated cells that lie between the basolateral and central nuclei (Delaney and Sah 2001; Royer et al. 1999).

The CeL receives extensive projections from cortical, brainstem and thalamic regions (McDonald 1998; Pitkänen 2000). While the intraamygdaloid projections are relatively sparse, neurons within the lateral division do receive a large inhibitory projection from the intercalated cell masses (Delaney and Sah 2001; Paré and Smith 1993) which in turn receive excitatory inputs from the lateral, basolateral and basal nucleus (Royer et al., 1999). Studies on fear conditioning have concentrated at changes in glutamatergic inputs in the lateral amygdala (Davis and Whalen 2001; LeDoux 2000). The extensive convergence of inputs into the central amygdala coupled to the fact that the physiological measures in fear conditioning are initiated by outputs of this nucleus suggests that changes in synaptic transmission within the central nucleus is another potential site for regulation of fear conditioning.

**Acknowledgements.** This work was supported by grants from the National Health and Medical Research Council of Australia. M. L. A. was supported by a grant from the Ministero de Educación, Cultura y Deporte (Spain). We thank Andrew Delaney for comments on the manuscript.

**REFERENCES**


afterhyperpolarization in hippocampal neurons: effects of internally applied anions.  

**Figure Legends**

**Figure 1.** Trans-illuminated slice from rat brain. (A) An example of acute coronal section 400 µm thick prepared for recording. The figure shows the region containing the basolateral complex and the central nucleus of the amygdala. These nuclei are delimited by bundles of axons that allow us to identify them with trans-illumination. In B the lateral and medial subdivisions of CeA are outlined as well as the disposition of the 8 stimulating electrodes array in the basolateral complex (white dots) and the two stimulating electrodes in the medial pathway (black dots). CeL and CeM, lateral and medial sector of the central amygdala respectively; LA, lateral amygdala; and BLA, basolateral amygdala.

**Figure 2.** Electrophysiological properties of three types of CeL neurons. Traces show responses recorded in current clamp to a series of 600 ms current injections (-100, 100, 300 pA) from adapting neurons (A) late firing neurons (B) and regular spiking neurons (C). Note that in the case of the late firing neuron the current pulses applied were: -100, 200 and 300 pA. In the lower panel the instantaneous frequency between action potentials is plotted against the spike number during the current injections illustrated above. Note that adapting neurons cease to fire after 6 or 7 action potentials while the other cell types continue to spike throughout the current injection.

**Figure 3.** The slow afterhyperpolarisation following action potential trains is different in different cell types. Voltage responses to a 600 ms current injections (100 pA) of the three types of neurons found in lateral division of the CeA. (A) adapting neurons (B) late firing neurons (C) regular spiking neurons. Action potential trains in response to a 100 ms current injection evoke a slow AHP that lasts up to several seconds (middle traces). The AHP lasts longer in adapting neurons. The current underlying the AHP evoked by a 100 ms depolarising voltage step to +10 mV from a holding potential.
of -50 mV, has fast and slow components. The slow component of the AHP current is more prominent in adapting neurons.

**Figure 4. The slow AHP is mediated by activation of Ca\(^{2+}\) depended K\(^+\) currents.** A depolarising current step (400 ms) generated a train of APs in, adapting (A) late firing neurons (B) and regular spiking (C). Application of tetrodotoxin (TTX, 1 µM) abolished action potentials and revealed a Ca\(^{2+}\) component that was eliminated by application of 250 µM Cd\(^{2+}\). The lower traces show the current underlying the AHP evoked by a 100 ms to +10 mV from a holding potential of −50 mV, before and after blockade of voltage dependent calcium channels with cadmium.

**Figure 5. The fast current underlying the AHP is due to activation of SK channels.** The current underlying the AHP (upper traces) and the AHP measured in current clamp (lower traces) in response to a short (100 ms) train of action potentials is shown in an adapting (A, B) and a regular spiking neuron (C, D). In adapting neurons, application of the SK channel blocker UCL1848 (100 nM) blocks the rapidly rising component of the AHP current and the medium AHP. The UCL sensitive current obtained by digital subtraction has rapid decay kinetics as shown in the inset (A). Application of isoprenaline blocks the slow component of the AHP and the underlying current (B). As shown in the inset, the isoprenaline sensitive current has a slow rise and decay indicative of the sI\(_{AHP}\) current. In regular spiking neurons, the AHP has as very small slow component and the current underlying the AHP is largely abolished by the SK channel blocker UCL1848 (C). Application of isoprenaline has no effect on the AHP or the underlying current in regular spiking neurons (D).

**Figure 6. SK channels activation determines the firing frequency of neurons.** Responses of an adapting (A) late firing (B) and regular spiking (C) neuron to 600 ms current injection (375 pA) in control conditions. Blockade of SK channels with 100 nM UCL1848 increased the number of AP and their firing frequency in regular spiking neurons (B, C) but did not affect the firing properties of adapting neurons (A). The change in discharge frequency is plotted as the instantaneous frequency against spike number in the lower panels.
Figure 7. Electrical stimulation in the medial pathway evokes a monosynaptic excitatory and inhibitory input but and BLA complex evoked a biphasic response in CeA neurons. (A1) Stimulation of the medial input (see Fig 1) produced a depolarization followed by a hyperpolarization when the response was recorded in current clamp with the membrane potential at -45 mV. Similar response was recorded after activation of the BLA complex (B1). When the responses were recorded in voltage clamp at the same membrane potential, an inward current was followed by an outward current after stimulation of the two inputs (A2 and B2). Application of kynurenic acid (3 mM) blocks the excitatory inward current but leaves the outward current in the medial input (A2). Kynurenic acid blocks both excitatory and inhibitory inputs when stimulating the basolateral input. The inhibitory component in the medial input was blocked by 100 µM picrotoxin (A2). C, Inputs recorded in one cell when stimulation was applied in the lateral amygdala (left) and in the basolateral amygdala (right). D, stimulation of the intercalated cell masses in the presence of kynurenic acid evokes a pure inhibitory response that is blocked by the GABA antagonist picrotoxin.

Figure 8. Glutamate application in BLA evoked a response in CeL neurons after inhibition was blocked. (A1) Average of seven episodes recorded in a CeL neuron in voltage-clamp after puffing glutamate 100 mM (arrow) on BLA complex. No response was observed when recordings were made in normal physiological solution. (A2) Blockade of inhibition by picrotoxin now evoked an excitatory input in response to a glutamate puff in the BLA. (A3) application of tetrodotoxin (TTX 1 µM) abolished the responses recorded in CeL neurons in the presence of picrotoxin. (B) Application of glutamate elicited a single action potential in pyramidal neurons from BLA complex. Application of picrotoxin (100 µM) triggers more APs to the same application of glutamate. C, response in a neuron in the CeL to puffer application of glutamate (arrow) in the lateral amygdala. The response evoked by glutamate is shown on an expanded time scale in C2. The average miniature excitatory synaptic current recorded in the same cell is shown in C3. D, Average response (4 applications) in a CeL neuron to puffer application of glutamate in the basolateral amygdala recorded at -60 mV and +40 mV. Note the presence of the slow component at +40 mV. E, response recorded in a CeL neuron at -60 mV and -10 mV (upper traces) to a puffer application of glutamate in the CeL (arrow). Application of picrotoxin (100 µM) blocks the response to glutamate.
Table 1.- Electrophysiological properties of CeL neurons recorded with microelectrodes and with patch pipettes in whole cell configuration. Asterisk indicates P < 0.05.

<table>
<thead>
<tr>
<th>Type cell</th>
<th>n (%)</th>
<th>Vm (mV)</th>
<th>R_in (MΩ)</th>
<th>τ (ms)</th>
<th>Spike amplitude (mV)</th>
<th>Spike duration (ms)</th>
<th>Threshold (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole cell (KMe)</td>
<td>69</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adapting</td>
<td>25 (36)</td>
<td>-66±1.3</td>
<td>213±13.4</td>
<td>29±2.6</td>
<td>55±1.6</td>
<td>0.89±0.03</td>
<td>-32±2.7</td>
</tr>
<tr>
<td>Late firing</td>
<td>20 (29)</td>
<td>-73±1.4*</td>
<td>194±32.1</td>
<td>23±2.4</td>
<td>58±2.3</td>
<td>0.99±0.06</td>
<td>-33±1.2</td>
</tr>
<tr>
<td>Regular spiking</td>
<td>24 (35)</td>
<td>-65±1.8</td>
<td>220±29.7</td>
<td>32±3.3</td>
<td>54±1.9</td>
<td>0.98±0.03</td>
<td>-33±0.7</td>
</tr>
<tr>
<td>Whole cell (KGlu)</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late firing</td>
<td>9 (50)</td>
<td>-69±1.9</td>
<td>160±21.8</td>
<td>19±1.7</td>
<td>88±3.6</td>
<td>0.91±0.06</td>
<td>-35±1.3</td>
</tr>
<tr>
<td>Regular spiking</td>
<td>9 (50)</td>
<td>-68±1.9</td>
<td>166±20.4</td>
<td>21±2.1</td>
<td>82±2.9</td>
<td>0.97±0.06</td>
<td>-34±1.0</td>
</tr>
<tr>
<td>Microelectrodes</td>
<td>46</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adapting</td>
<td>10 (22)</td>
<td>-59±2.9</td>
<td>118±19.4</td>
<td>24±7.0</td>
<td>52±2.8</td>
<td>0.92±0.21</td>
<td>-40±1.9</td>
</tr>
<tr>
<td>Regular spiking</td>
<td>36 (78)</td>
<td>-60±1.3</td>
<td>169±11.7*</td>
<td>29±2.4</td>
<td>52±1.2</td>
<td>1.01±0.08</td>
<td>-41±1.1</td>
</tr>
</tbody>
</table>
Figure 5
Figure 6
Figure 7
Figure 8