Inhibition and Synchronization of Basal Amygdala Principal Neuron Spiking by Parvalbumin-Positive Interneurons

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In the cortex and hippocampus, GABAergic interneurons are local circuit inhibitory cells that exhibit diverse morphological, biochemical, molecular, and synaptic properties (Freund and Buzsaki 1996; Markram et al. 2004; McBain and Fisahn 2001; Somogyi and Klausberger 2005). Although these neurons show a variety of firing properties, their functional roles are largely determined by the postsynaptic domain over which they make their synaptic connections. Thus interneurons targeting perisomatic regions are thought to affect somatic action potential generation (Cobb et al. 1995; Miles et al. 1996), whereas those terminating more distally may modulate the induction of synaptic plasticity (Miles et al. 1996), prevent dendritic action potentials or gate the transfer of information along the somatodendritic axis (Buzsaki et al. 1996). These diverse types of interneurons can be divided into distinct populations based on their firing properties and on the complement of peptides and calcium binding proteins that they express (Gupta et al. 2000). Moreover, a clear correlation exists between the complement of neurochemical markers expressed by an interneuron and the domain of the postsynaptic neuron it targets (Gupta et al. 2000).

In the basolateral amygdala, four populations of interneurons are currently recognized: those expressing parvalbumin (McDonald and Betette 2001), those expressing somatostatin (McDonald and Mascagni 2002), those expressing cholecystokinin and either calretinin or vasoactive intestinal peptide (Mascagni and McDonald 2003), and those expressing only cholecystokinin (Mascagni and McDonald 2003). Of these, parvalbumin-containing neurons form ~50% of the interneuronal population and show significant co-localization with calbindin (McDonald and Betette 2001). Principal neurons within the basolateral amygdala are well known to have a low spontaneous firing rate in the absence of sensory stimulation (Pare and Gaudreau 1996; Rosenkranz and Grace 1999). This low firing rate is largely due to the dominant role of inhibition in the amygdala, as suggested by the presence of large amplitude IPSPs in projection neurons during both spontaneous and evoked synaptic activity (Lang and Pare 1997). By making paired recordings in mice where EGFP was expressed under the control of the parvalbumin promoter, we have recently completed a detailed analysis of the firing properties and connectivity of parvalbumin expressing interneurons in the basolateral amygdala (Woodruff and Sah 2007). Here we address the functional implications of local inhibition provided by these cells by investigating the ability of interneuron activity to alter the firing probability of BLA projection neurons. We show that appropriately timed activation of these interneurons can not only prevent spiking in postsynaptic principal neurons but can also subsequently synchronize principal neuron spiking.

METHODS

Coronal brain slices containing basolateral amygdala were cut from 16- to 25-day-old mice that expressed EGFP under the control of the parvalbumin promoter as previously described (Woodruff et al. 2006). After halothane anesthesia, animals were decapitated, and the brain was rapidly removed and placed in ice-cold, oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM) 118 NaCl, 2.5 KCl, 2.5 NaHCO3, 10 glucose, 1.3 MgCl2, 2.5 CaCl2, and 1.2 NaH2PO4. Slices were cut (300 μm) and incubated at 32°C for 30 min before being allowed to equilibrate at room temperature for at least a further 30 min. All procedures were conducted with approval from the University of Queensland Animal Ethics Committee.

During recording, slices were perfused with heated ACSF (34 ± 2°C). Recording pipettes (3–5 MΩ) fabricated from borosilicate glass were filled with a solution containing (in mM) 135 KMeSO4, 8 NaCl, 10 HEPES, 2 Mg2ATP, 0.3 Na2GTP, 0.1 spermine, 7 phosphocreatine, and 0.3 EGTA. Neurons were visualized using an upright microscope (Olympus BX51WI, Olympus Optical, Tokyo, Japan) equipped with a fluorescence attachment. Paired whole cell recordings were made using Axopatch 1D and Axopatch 200B amplifiers (Molecular Devices, Sunnyvale, CA), filtered at 5 kHz and digitized at 10 kHz using an ITC-16 board (InstruTech, Port Washington, NY) and Axograph (Axograph X, Sydney, Australia).

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Cells included in this study were restricted to those exhibiting an initial resting membrane potential more hyperpolarized than \(-55\) mV. Immediately after breakthrough, cells were injected with 600-ms current pulses (\(-100–700\) pA, 50-pA increments) to confirm interneuron or principal neuron identity. To assess the ability of a single interneuron to inhibit principal neuron spiking, a sinusoidal current (peak amplitude: 20 pA) was injected into the postsynaptic principal neuron. This waveform had a period of 283 ms (frequency: 3.53 Hz). Presynaptic interneurons were activated by injection of 2-nA, 2-ms current delivered via the patch pipette. The timing of this current pulse was initially set to occur 20 ms prior to the positive peak of the second oscillation cycle recorded in the postsynaptic neuron (\(t = 480\) ms from sweep onset, \(t = 3,000\) ms for traces of extended duration, as in Fig. 3). For experiments investigating the importance of appropriately timed inhibition, interneuron activation was negatively shifted by 100 or 200 ms. In some cases, trains of action potentials (3 pulses at 40 Hz) were evoked to enhance the inhibitory effect of a single cell. Extracellular stimulation was achieved via a theta-glass electrode filled with ACSF and placed locally within the basal nucleus of the amygdala. Stimuli were 1–40 V in amplitude and 50 \(\mu\)s in duration. Significance for all data were assessed using Student’s \(t\)-test. Values are expressed as means \(\pm\) SE.

**RESULTS**

We have previously shown that parvalbumin-expressing interneurons make GABAergic connections with local principal neurons (Woodruff and Sah 2007; Woodruff et al. 2006). To investigate the physiological impact of interneuron firing, principal neurons were depolarized by a steady current injection to spike at 3–4 Hz while the presynaptic interneuron was driven to spike by short (2 ms) suprathereshold current injection. Analysis was restricted to inhibitory connections that were \(\geq 20\) pA in amplitude when recorded with principal cells voltage clamped at \(-40\) mV (Fig. 1A) and represented \(-60\%\) of all inhibitory connections (42/71). With the principal neuron spiking, a single action potential in the connected interneuron was able to block action potentials for \(-50–150\) ms (Fig. 1B, left). As previously described in hippocampal neurons (Cobb et al. 1995), inhibition could also synchronize spiking of the principal neurons (Fig. 1B, right). However, unlike in hippocampal neurons, this effect was rarely observed possibly because constant depolarization did not typically result in rhythmic spiking. Parvalbumin-expressing interneurons can be divided into four distinct categories based on a variety of electrophysiological and circuit properties (Woodruff and Sah 2007). However, we did not observe any differences in action potential inhibition evoked by different interneuron types.

Recordings from basolateral amygdala (BLA) principal neurons in vivo demonstrate periodic fluctuations in membrane potential (Pape et al. 1998) that occur over a range of frequencies and are capable of generating spikes on the depolarizing phase of the oscillation (Pape et al. 1998). Such oscillatory activity is common in cortical networks (Buzsaki and Draguhn 2004), and activity of perisomatic targeting interneurons has been suggested to synchronize this activity in both the cortex and hippocampus (Bartos et al. 2002; Buzsaki and Draguhn 2004; Cobb et al. 1995). We therefore tested if parvalbumin-expressing interneurons could also synchronize activity in the basal amygdala. Principal neurons recorded in acute slices are largely silent. To mimic the spiking pattern of projection neurons in vivo, we injected an oscillatory current waveform into principal neurons to initiate regular spiking (see METHODS). The magnitude of steady-state depolarization was adjusted such that the oscillatory input evoked spikes with a probability of \(\sim 0.5\), calculated across the duration of the sweep (see METHODS). Under these conditions, it can be seen that the probability of the principal neuron spiking at the start of current injection is high but then reduces to a steady level (Fig. 1C). This reduction in spike probability recapitulates spike frequency adaptation as seen in lateral amygdala neurons (Faber et al. 2001). Under these conditions, a single action potential in the connected interneuron, evoked 20 ms before the peak of the oscillation in the principal neuron, could strongly inhibit postsynaptic principal neuron firing during one cycle with a rebound excitation in the following cycle (Fig. 1D). Here the cycle on which the presynaptic interneuron fired is designated as cycle 0. It can be seen (Fig. 1D) that not only are spikes abolished on cycle 0, but a rebound increase in spiking occurs on the following cycle (designated +1). Summary data from 10 cells are shown in Fig. 1E (graph on left), in which a clear decrease in spike probability is seen on cycle 0 (control, 0.74 \(\pm\) 0.06; with interneuron spike, 0.26 \(\pm\) 0.06, \(P < 0.001\)), followed by a significantly increased probability on cycles +1 (control, 0.53 \(\pm\) 0.05; with interneuron spike, 0.76 \(\pm\) 0.06, \(P < 0.01\) and +2 (control, 0.48 \(\pm\) 0.04; interneuron spike, 0.56 \(\pm\) 0.04, \(P < 0.05\)).

In the hippocampus, different classes of interneuron fire at stereotyped times during extracellularly recorded oscillations (Klausberger et al. 2003, 2004). To examine the impact of interneuron spiking at different phases of principal neuron firing, we varied the timing of interneuron spikes relative to the phase of principal neurons oscillation. When moved to near the negative peak of the oscillation (120 ms before the peak of the oscillation in the principal neuron), interneuron spiking was considerably less effective in preventing suprathreshold principal neuron activity. Although there is a small reduction in spiking at cycle 0 (control, 0.69 \(\pm\) 0.05; interneuron spike, 0.53 \(\pm\) 0.09, \(n = 9\); Fig. 1E, middle graph), this effect is not significantly different (\(P = 0.10\)). Finally, when the interneuron action potential is evoked a further 100 ms earlier, on the hyperpolarizing phase of cycle \(-1\), no effect on PN spiking was seen (Fig. 1E, right). This experiment shows that inhibition of projection neuron firing is most effective when the interneuron spikes on the ascending phase, shortly prior to the peak membrane depolarization of principal cell firing. Thus during imposed oscillatory activity, principal cell spiking is inhibited by single presynaptic interneurons when their spikes are time locked to the excitation of the postsynaptic neuron. More interestingly, this inhibitory control leads to increased principal neuron spiking on immediately subsequent cycles.

These results show that when inhibitory connections are large, single action potentials in the interneuron can block spiking in the principal neuron with rebound excitation. However, in many connections, the amplitude of the inhibitory postsynaptic potential (IPSP) is too small to inhibit spiking. We thus tested if these weak inhibitory connections have an effect on the timing of principal cell spiking. For this, we analyzed trials in which interneuron activation did not prevent postsynaptic spiking and compared the location of the spike to its location in the immediately preceding control sweep in which the interneuron remained silent. Only pairs of sweeps in which a postsynaptic action potential was present under both control and interneuron spike conditions were analyzed. The
location of the spike was measured from the trough of the preceding oscillation cycle. We found that interneurons reliably and robustly delayed postsynaptic spiking (100/115 trials from 10 pairs; control 144.1 ± 2.8 ms; interneuron spike 163.6 ± 3.8 ms, n = 10 pairs, P < 0.01, Fig. 2). Thus in addition to being capable of preventing and subsequently synchronizing postsynaptic action potentials, single parvalbumin-positive interneurons in the basal amygdala can effectively determine the output timing of principal neuron activity.

Because a single interneuron contacts many postsynaptic neurons, this rebound increase in principal neuron spiking is likely to occur in a number of cells, and therefore may act as a means of transiently synchronizing principal neuron output (Cobb et al. 1995). To test if interneurons in the basal amygdala could also synchronize activity paired recordings were made from two principal neurons in the presence of the glutamatergic antagonists NBQX (10 μM) and d-APV (30 μM). Both neurons were depolarized to near-threshold potentials and injected with identical sinusoidal current waveforms to evoke spiking with a probability of ~0.5. A theta-glass stimulating electrode, filled with aCSF, was placed locally within the basal nucleus and a single pulse of afferent stimulation was given on

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**FIG. 1.** Inhibition and subsequent enhancement of principal neuron firing by single presynaptic interneurons. Paired recordings were made between parvalbumin-expressing (EGFP positive) interneurons and principal neurons. A: unitary inhibitory postsynaptic current (IPSC) recorded in a basolateral principal neuron, evoked by an action potential in the presynaptic interneuron. Insets: firing properties of the recorded cells. B, left: panels show the effect of unitary IPSPs on principal neurons held via current injection at subthreshold (middle) and threshold (bottom) membrane potentials. Right: synchronizing ability of IPSPs. Note the clustering of action potentials at the offset of the IPSP. Ten sweeps have been superimposed in each case. C and D: response of principal neurons to sinusoidal somatic current injections (middle), in the presence (C) and absence (D) of interneuron activation 20 ms before the peak of the oscillation in cycle 0 (top). Spike counts for the cell pair illustrated are shown in the histogram at bottom. E: varying the timing of interneuron spiking by −100 ms (middle) or −200 ms (right) diminishes the inhibitory and synchronizing effect. *P < 0.05, **P < 0.01; error bars represent SE. Insets: injected sine wave and the timing of the interneuron spike.
the rising phase of the 11th oscillation cycle after firing probability had stabilized. The magnitude of the resultant IPSP was adjusted to be between 2–3 mV when recorded at just sub-threshold potentials, to mimic a moderately large unitary IPSP (Fig. 1). As shown with paired recordings (Fig. 1), principal neuron spiking was abolished on cycle 0 (control, 0.43 ± 0.05; stim, 0.01 ± 0.01, n = 10 cells, P < 0.001) and increased on cycle +1 (control, 0.43 ± 0.06; stim, 0.60 ± 0.04, P < 0.01) by GABAergic stimulation (Fig. 3A,B). We then calculated the probability that both principal neurons would fire a spike on the cycle following GABAergic activation (cycle +1), and found it to be twice as likely as in the absence of activation (control, 0.19 ± 0.06; stim, 0.37 ± 0.05, n = 5, P < 0.05, Fig. 3C). We conclude that during oscillatory membrane fluctuations, GABAergic activation can silence principal neuron output and subsequently increase their synchronous firing.

**DISCUSSION**

Parvalbumin-positive interneurons in the basal amygdala can be divided into four distinct types based on their electrophysiological properties, the nature of the inhibitory connections they make, and the excitatory connections they receive (Woodruff and Sah 2007). In agreement with ultrastructural studies (Muller et al. 2006; Smith et al. 1998), we have suggested that one type of interneuron (designated the accommodating, AC type) preferentially innervates more distal sites on principal neurons (Woodruff and Sah 2007). In the current study, we have shown that single parvalbumin-expressing interneurons in the basal amygdala can prevent action potentials in postsynaptic principal neurons. All interneuron types produced similar inhibition of action potentials in principal neurons. This observation appears at odds with the view that dendritic targeting interneurons may not be effective at preventing sodium action potentials. However, somatically recorded IPSCs evoked by dendritically targeting interneurons are not significantly smaller than those evoked by interneurons making somatic synapses (Woodruff and Sah 2007). Thus it is perhaps not surprising that AC interneurons were also capable of preventing somatic action potentials. However, we cannot exclude the possibility that the higher conductance state of neurons in vivo (Desteche et al. 2003) may serve to better isolate the putative dendritic inhibition arising from these AC interneurons, thus reducing their overall impact on excitability. Additionally, the higher in vivo conductance state may require that even for perisomatic synapses, multiple interneurons may need to be simultaneously active to prevent principal neuron action potentials. Such synchronous activation of interneurons is likely to be facilitated by the recently described gap-junctional communication between similar interneuron subtypes (Woodruff and Sah 2007).

After inhibition, the return of the membrane potential to resting values was often associated with a period of rebound excitability. During oscillatory fluctuations in principal neuron membrane potential, this rebound increase in principal neuron spike probability after inhibition could provide an additional means to synchronize their output. Although we have not examined the mechanisms underlying this rebound, it likely

**FIG. 2.** Weak inhibitory inputs alter the phase of principal neuron firing. **A.** top: traces (red) show episodes in which a sinusoidal current injection into the postsynaptic principal neuron (post) evokes variable spikes. Bottom: traces (black) show episodes in which an interneuron spike (top traces) was evoked 20 ms before the peak of current injection into the principal neurons (post). Note that interneuron action potentials do not prevent spiking but delay the firing of principal neurons B: plot of spike timing in pairs of sweeps from the cell illustrated in A. Location is measured from the trough of the preceding oscillation cycle, as shown in A. C: distribution of all principal neuron spikes occurring on cycle +1 after interneuron activation (red bars) across 10 cells, and the timing relative to spikes occurring on the immediately preceding control sweep (black bars). Only pairs of sweeps in which spikes occurred under both “control” and “interneuron spike” conditions are plotted. D: summary data show the average shift in principal neuron spiking after weak inhibitory inputs. ***P < 0.001; error bars represent SE.
results from deinactivation of resting voltage-dependent currents in these interneurons such as low-threshold calcium current or $I_h$ as occurs in some thalamic neurons (Destexhe and Sejnowski 2002). It is notable that although some exceptions exist (Carter and Regehr 2002; Csicsvari et al. 1998; Destexhe et al. 2003; Szabadics et al. 2006), the majority of unitary excitatory synaptic connections are too weak to reliably fire connected cells. Consequently, multiple synaptic inputs must temporally summate to trigger action potentials in the postsynaptic cell, requiring the synchronous firing of many neurons. This is most likely achieved by synchronized membrane potential oscillations (Paz et al. 2006) that provide windows of depolarization that enhance the probability of neuronal firing. We have shown that in the basolateral amygdala, inhibition from parvalbumin-positive interneurons enhances the synchronizing effect of oscillations. This effect was highly dependent on the timing of inhibition relative to the phase of the oscillation. Such enhanced synchrony of BLA principal neurons has recently been shown to facilitate communication between perirhinal and entorhinal cortices, an effect proposed to be involved in the emotional enhancement of memory (Pare et al. 2002). Given the suggested involvement of BLA oscillations in promoting information transfer between neocortical and temporal lobe memory structures (Pare et al. 2002), the inhibition-evoked enhanced firing of principal neurons that we describe may be important for ensuring emotionally salient events are well remembered.

In those instances where inhibition was too weak to prevent firing of principal neurons, it nevertheless had a robust effect on their output, delaying the phase on which they spiked. The precise timing of spikes during oscillatory fluctuations in membrane potential is thought to encode information, such as odor concentration in the olfactory bulb (Cang and Isaacson 2003) and animal location in hippocampal place cells (O’Keefe and Recce 1993). In the basolateral amygdala, although the relative timing of principal neuron and interneuron output has been assessed during both delta (1–4 Hz) and theta (4–7 Hz) oscillations (O’Keefe and Recce 1993; Paz et al. 2006), the functional significance of the timing of interneuron activity with a particular phase of firing of the principal neurons is not known. However, the importance of synchronous BLA output in facilitating rhinal information transfer (Pare and Gaudreau 1996; Pare et al. 2002) suggests that weak inhibition of a subpopulation of oscillating principal neurons could serve a vetoing effect, creating asynchronous output and decrease the effective depolarization of downstream rhinal targets.

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**Figure 3** Inhibitory inputs can synchronize activity in principle neurons. 

**A**: response of 2 simultaneously recorded principal neurons to sinusoidal current injections in the absence (control) and presence (Stim) of local GABAergic stimulation. Traces in which both principal neurons fired on cycle $+1$ are drawn in black. 

**B**: Averaged data showing the probability of spiking of individually analyzed cells. GABAergic stimulation enhances the probability that both principal neurons will spike on cycle $+1$. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; error bars represent SE.
REFERENCES


