Similar Inhibitory Processes Dominate the Responses of Cat Lateral Amygdaloid Projection Neurons to Their Various Afferents

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Lang, E. J. and D. Paré. Similar inhibitory processes dominate Bechara et al. 1995) . The lateral amygdaloid (LAT) nucleus, a major recipient of cortical and thalamic sensory pathways to the amygdala (Amaral et al. 1992; LeDoux et al. 1985; Romanski and LeDoux 1992; Russchen 1982b; Turner et al. 1980) and source of afferents to other amygdaloid nuclei (Krettek and Price 1978; Pitkänen et al. 1995; Smith and Paré 1994; Stefanacci et al. 1992), appears to be a necessary link for the development of auditory conditioned fear responses (LeDoux et al. 1986, 1990). Characterization of the mechanisms governing LAT neuronal activity therefore is required for understanding the neuronal basis of these responses and more generally of emotional expression.

Recent evidence suggests that local inhibitory processes are major determinants of LAT neuronal activity and are central to its normal functioning as, for example, a decrease in GABAergic neurons within this structure is correlated with the development of kindled seizures (Callahan et al. 1991). In single unit studies, LAT neurons were found to have very low firing rates (Ben-Ari et al. 1974; Bordi et al. 1993), with the majority of projection neurons being virtually silent unless presented with a specific sensory stimulus (Gaudreau and Paré 1996). Moreover, in vitro studies have shown that synaptic responses in the basolateral amygdaloid (BL) complex are characterized by large inhibitory postsynaptic potentials (IPSPs) (Rainnie et al. 1991b; Sugita et al. 1993; Takagi and Yamamoto 1981; Washburn and Moises 1992). In agreement with these physiological findings, synaptic boutons immunoreactive for glutamic acid decarboxylase (GAD) are concentrated strategically around the soma, proximal dendrites and axonal initial segment of amygdaloid projection cells (Carlsen 1988).

Inhibition within the LAT nucleus must arise largely from GABAergic neurons located within the LAT nucleus itself, as lesions deafferenting the BL complex lead to minor decreases in GAD levels (Le Gal La Salle et al. 1978). Moreover, although other amygdaloid nuclei contain GABAergic interneurons (McDonald 1985; Nitecka and Ben-Ari 1987; Paré and Smith 1993), internuclear projections linking the different nuclei of the BL complex appear to consist only of excitatory projections (Paré et al. 1995c; Smith and Paré 1994). An intriguing finding, therefore, was that both spontaneous and evoked γ-aminobutyric acid-A (GABA_A) and GABA_B responses could occur independently in LAT neurons recorded in vitro (Sugita et al. 1992), suggesting that the LAT nucleus contains two distinct GABAergic neuronal populations, each having access to different types of GABAergic receptors.

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INTRODUCTION
The amygdala is critical for imbuing sensory stimuli with the appropriate emotional significance (Adolphs et al. 1995; Bechara et al. 1995). The lateral amygdaloid (LAT) nucleus, a major recipient of cortical and thalamic sensory pathways to the amygdala (Amaral et al. 1992; LeDoux et al. 1985; Romanski and LeDoux 1992; Russchen 1982b; Turner et al. 1980) and source of afferents to other amygdaloid nuclei (Krettek and Price 1978; Pitkänen et al. 1995; Smith and Paré 1994; Stefanacci et al. 1992), appears to be a necessary link for the development of auditory conditioned fear responses (LeDoux et al. 1986, 1990). Characterization of the mechanisms governing LAT neuronal activity therefore is required for understanding the neuronal basis of these responses and more generally of emotional expression.

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Inhibition within the LAT nucleus must arise largely from GABAergic neurons located within the LAT nucleus itself, as lesions deafferenting the BL complex lead to minor decreases in GAD levels (Le Gal La Salle et al. 1978). Moreover, although other amygdaloid nuclei contain GABAergic interneurons (McDonald 1985; Nitecka and Ben-Ari 1987; Paré and Smith 1993), internuclear projections linking the different nuclei of the BL complex appear to consist only of excitatory projections (Paré et al. 1995c; Smith and Paré 1994). An intriguing finding, therefore, was that both spontaneous and evoked γ-aminobutyric acid-A (GABA_A) and GABA_B responses could occur independently in LAT neurons recorded in vitro (Sugita et al. 1992), suggesting that the LAT nucleus contains two distinct GABAergic neuronal populations, each having access to different types of GABAergic receptors.

Given the importance of inhibitory processes in the functioning of the amygdala, and to test the possibility that separate interneuronal populations are accessed differentially by
particular amygdaloid afferents, we sought to characterize the IPSPs of intracellually recorded LAT projection neurons in vivo. Our results demonstrate that powerful IPSPs regulate the response of LAT neurons to their synaptic inputs and that their response pattern is relatively constant irrespective of the stimulation site. Further, evidence was obtained that feedback inhibition within the LAT nucleus is mediated by similar IPSPs. This uniformity suggests that distinct GABA-ergic subpopulations cannot be independently accessed in vivo. Instead, the degree of activation within a particular afferent pathway was found to be critical in shaping the synaptic responses of LAT neurons.

METHODS

Surgery

Intracellular recordings were obtained from adult cats (2.5–3.5 kg) anesthetized with sodium pentobarbital (Somnotol, 40 mg/kg ip), paralyzed with gallamine triethiodide (33 mg/kg iv), and ventilated artificially. The level of anesthesia was determined by continuously monitoring the electroencephalograph (EEG). And supplemental doses of Somnotol (5–7 mg/kg iv) were given as needed to maintain a synchronized EEG pattern. Lidocaine (2%) was applied to all skin incisions. End tidal CO2 concentration was kept at 3.7 ± 0.2% (mean ± SE), and the rectal temperature was maintained at 37–38°C with a heating pad. To ensure recording stability, the cisterna magna was drained, the cat suspended, and a bilateral pneumothorax performed.

The bone and dura mater overlying the parietal and temporal cortices were removed, allowing a lateral approach to the amygdala and a dorsal approach to the perirhinal (PRH) and entorhinal (ENT) cortices. Pairs of tungsten electrodes (0.5 MΩ) whose tips were separated by 1.5 mm in the vertical axis, or concentric bipolar tungsten electrodes were used to record the PRH and ENT EEG activity and to apply local electrical stimuli (100–200 μs pulses, 100–1,500 μA, at 0.2–1 Hz). The tungsten electrodes were implanted stereotactically into the PRH and ENT cortices with one electrode of each pair in the superficial (II) and deep cortical layers (IV-V), respectively. The exact dorsoventral coordinate was obtained by lowering the ENT electrodes until they contacted the temporal bone and then raising them to their correct position using the EEG criteria of positive or negative going sharp potentials as indicative of superficial or deep cortical layers, respectively (Paré et al. 1995a). The dorsoventral position of the electrodes in the PRH cortex was corrected as a function of the difference between the stereotaxically predicted (Berman and Jones 1982) and actual positions of the ENT cortex. The ENT electrodes were placed into the ventromedial ENT area.

Recording procedure

Intracellular recording electrodes consisted of glass capillary tubes pulled to a tip diameter of ≈0.5 μm (35–45 MΩ). The electrodes were filled with K+-acetate (4 M) and Neurobiotin (14 mg/mL; Vector Labs). They were lowered obliquely 6 mm through the posterior sylvian gyrus to the border of the LAT nucleus using a piezoelectric manipulator. The exposed cortical surfaces then were covered with agar to improve recording stability.

Intracellular recordings were made using a high impedance amplifier with active bridge circuitry (Neurodata, NY). Typically, cells were recorded for 30 min to 2 h. After penetration and stabilization of impalements, spontaneous and evoked synaptic potentials were recorded at different membrane potentials (Vm,s) induced by DC current injection. The intracellular and EEG signals were monitored using a digital oscilloscope, printed out on a chart recorder, and stored on VCR tape for off-line analysis using IGOR (Wave-metrics, OR) data analysis software. For each cell, measurement of IPSP amplitudes were performed at the same time for each potential. This time corresponded to when the IPSP reached its maximal amplitude at the most depolarized tested level. The IPSP reversal potential was then determined by plotting the IPSP amplitude as a function of Vm. The input resistance (Rm) before and during an IPSP was estimated by calculation of the slope resistance (the reciprocal of the slope conductance) (Johnston and Wu 1995). Here the change in voltage from the resting Vm was plotted against the DC current level, and the slope of the fitted line was used to estimate the Rm of the cell. Although I-V curves showed deviations from linearity, the comparison of the slope resistance before and during IPSPs remained useful for estimating the impact of IPSPs. Linear fits were performed with the least-squares method as calculated by the computer program IGOR (WaveMetrics, OR).

RESULTS

Stable intracellular recordings were obtained from 74 LAT neurons with stable resting potentials greater than or equal to −65 mV (−72.6 ± 1.3 mV; mean ± SE; n = 74), and spike amplitudes ranging between 60 and 90 mV. Eighteen percent of them were identified formally as projection neurons using morphological and/or physiological criteria (Fig. 1). In agreement with previous Golgi studies (for review, McDonald 1992), neurobiotin-filled neurons were considered as projection cells when they had spiny dendrites with a stellate or a modified pyramidal somatodendritic morphology. An example of a morphologically identified LAT neuron is shown in Fig. 1A1, along with a high-power photomicrograph of a spiny dendritic segment (Fig. 1A2).

Physiological identification of LAT projection cells rested on eliciting antidromic responses by PRH or ENT stimulation (Fig. 1B). All neurons described in this study displayed similar electroresponsive properties known to be characteristic of LAT projection cells including lack of spontaneous discharges at rest, time- and voltage-dependent rectification, and generation of voltage-dependent oscillations in the 4–10 Hz range upon steady membrane depolarization to around −65 mV (Pape and Paré 1995; Paré et al. 1995b).

Recordings of spontaneous activity revealed synaptic events typically consisting of truncated excitatory postsynaptic potentials (EPSPs) followed by large (5–15 mV) hyperpolarizing potentials. These intracellular events were often related to spontaneous EEG waves in the PRH and ENT cortices, termed sharp potentials (SPs) (Paré et al. 1995a). For brevity, we will henceforth refer to evoked and SP-related hyperpolarizing potentials as IPSPs; however, we demonstrate in the accompanying paper that they actually
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are generated by a combination of synaptic and synaptically-activated intrinsic conductances (Lang and Paré 1997).

An example is shown in Fig. 2B1 where the intracellular trace (INTRA) displays numerous IPSPs that occurred in association with SPs (Fig. 2B1, top; ENT) or in response to a PRH stimulus (arrowhead). Depolarization to ~60 mV with +0.6 nA led to sustained spiking (Fig. 2A1), which was interrupted by large SP-related IPSPs (curved arrows) lasting ~500 ms. The IPSPs could occur in relation to either simple (monophasic, Fig. 2C1) or complex (triphasic, Fig. 2C2) SPs, as demonstrated by peri-event averages of intracellular events using the negative peak (arrows in Fig. 2C) of SPs as a temporal reference.

Cortically evoked responses were similar to SP-related intracellular potentials in amplitude and duration (Fig. 2B2). Like SP-related IPSPs, cortically evoked IPSPs often had small postsynaptic potentials (PSPs) embedded within them (Fig. 2D, arrowheads).

Cortical and intra-amygdaloid stimuli evoke similar IPSPs

To test whether different afferents generate distinct IPSPs in LAT neurons, synaptic responses were evoked from several brain regions connected with this nucleus, including the PRH and ENT cortices as well as the basomedial (BM) nucleus. The latter was stimulated in an attempt to evoke recurrent inhibition, as this nucleus receives a massive input from the LAT nucleus but does not reciprocate this projection (Krettek and Price 1978). In addition, the LAT nucleus itself was stimulated to assess the influence of intrinsic connections. Histological controls confirmed the electrode placements in these regions (Fig. 3, A–D). In the PRH cortex, a series of four electrodes was placed along its rostro-caudal extent as shown schematically in Fig. 3A1.

Cortical stimuli (PRH, Fig. 3A2; ENT, Fig. 3B) evoked similar responses with respect to the PSP sequence, IPSP amplitude, and reversal potential. The latency of cortically evoked IPSPs (from the site eliciting the shortest latency response) was 4.8 ± 0.5 ms (range, 2.8–7.3 ms; n = 8), whereas the BM-evoked IPSPs had a slightly longer latency of 6.6 ± 0.9 ms (range, 2.1–10.8 ms; n = 8). These differences did not reach significance (P < 0.1).

The monophasic IPSPs evoked from cortical and BM sites typically had reversal potentials ranging from ~80 to ~90 mV (cortex, ~84.7 ± 1.3 mV, n = 17; BM, ~82.1 ± 0.9 mV, n = 4) and were not significantly different (P < 0.2). In particular, IPSPs evoked in the same cells by PRH and BM stimulation had closely matched reversal potentials. In the cell of Fig. 4, A and B, for example, PRH-evoked IPSPs (Fig. 4A1) reversed at ~83.1 mV, whereas BM-evoked IPSPs (Fig. 4A2) reversed at ~81.9 mV, nearly the same potential (Fig. 4A3). In this cell, BM stimulation evoked IPSPs of lower amplitude that were associated with a smaller drop in RIN (55%, Fig. 4B2) than PRH-evoked IPSPs (83%, Fig. 4B1). However, there were large variations between cells in the RIN drops related to IPSPs.

In a further attempt to reveal a second, distinct type of inhibitory response, IPSPs were evoked by direct stimulation of the LAT nucleus. However, placement of stimulation electrodes in the LAT nucleus altered the properties of the cells (higher RIN and more depolarized resting potentials and IPSP reversals). Consequently, this stimulation site was used in only two experiments. These IPSPs had a latency of 4 ± 0.5 ms (n = 3), consistently shorter than BM- or cortically evoked IPSPs. However, their reversal potentials (~80.4 ± 0.6 mV, n = 3) were similar to those of IPSPs elicited by cortical (~78.9 ± 0.7 mV, n = 3) and BM (~80.4 ± 0.2 mV, n = 3) stimulation in the same cells. Figure 4C illustrates LAT-evoked responses in a LAT projection cell. In this case, the IPSP reversed around ~79.6 mV (Fig. 4C1) and was associated with a 71% drop in RIN (Fig. 4C2). Similarly, ENT- and BM-evoked IPSPs reversed at ~77.5 and ~80.5 mV and produced 32 and 68% decreases in RIN, respectively.

SP-related IPSPs

To determine whether the uniform properties of the evoked IPSPs reflected the artificial nature of electrical stimuli, we compared them with synaptic events occurring spontaneously in relation to simple (monophasic) and complex (triphasic) SPs that occur in the ENT and PRH cortices under barbiturate anesthesia (Fig. 2) and during slow-wave sleep (Paré and Gaudreau 1996; Paré et al. 1995a). The
average reversal potential of SP-related IPSPs was $-83.8 \pm 2.7 \text{ mV}$ ($n = 7$), which was not statistically different from the reversal potential of IPSPs evoked by cortical and BM stimuli ($P > 0.50$). Furthermore, there was a high correlation between the reversal potential of evoked and SP-related IPSPs ($r = 0.87$, $P < 0.05$) in the same cells. Additionally, the reversal potentials of simple and complex SP-related IPSPs were compared and found to be near identical ($-86.3 \pm 5.9 \text{ mV}$, simple SP-related; $-86.4 \pm 5 \text{ mV}$, complex SP-related; $n = 3$).

Simple and complex SP-related IPSPs recorded at different $V_m$ in the same cell are shown in Fig. 5B. The IPSPs were aligned using the negative peak of the related SPs shown in Fig. 5A. In this cell, simple and complex SP-related IPSPs reversed at similar values of $-93.5$ and $-91 \text{ mV}$, respectively. ENT-evoked responses also reversed at a relatively negative value of $-87.5 \text{ mV}$.

It was found consistently that the amplitude of SP-related IPSPs was related to the size of the SP. This relationship was most clearly observed when comparing simple and complex SP-related IPSPs because of the large amplitude difference between the two SP types (Figs. 2, A and B, 5, and 6). As shown in the peri-SP averages of Fig. 2C, IPSPs related to simple SPs had a lower amplitude than those related to complex SPs (Simple, $2.2 \pm 0.2 \text{ mV}$; Complex, $6.9 \pm 5 \text{ mV}$, $n = 6$ at $-65 \text{ mV}$; $P < 0.00005$, paired $t$-test). In addition to their larger amplitude, complex SP-related IPSPs curtailed the initial EPSP more rapidly and had a longer duration. Superimposed traces of simple SP-related synaptic potentials (Fig. 6B) and complex SP-related ones (Fig. 6C) demonstrate these differences. Thus, while the initial EPSPs are similar in amplitude, the ones related to complex SPs are significantly narrowed (Complex, $21.5 \pm 10.4 \text{ ms}$; Simple, $59.3 \pm 15.2 \text{ ms}$, $n = 6$, $P < 0.02$) and have a steeper falling phase than the simple SP-related EPSPs (Fig. 6, D–B). Further, comparison of the averaged potentials shows the longer duration of the complex SP-related IPSP (Fig. 6D; Simple, $318.3 \pm 38.4 \text{ ms}$; Complex, $556.2 \pm 66.8 \text{ ms}$, $n = 6$, $P < 0.002$). This duration difference remained even after scaling the simple SP-related IPSP (Fig. 6E), suggesting that the complex SP triggers not only a larger amplitude potential, but also one that lasts longer.

**Synaptic responses of LAT neurons vary with stimulation intensity**

The parallel fluctuations between, on the one hand, the amplitude of SPs, and on the other, the nature of SP-related potentials, suggested that the balance between excitatory and inhibitory inputs converging on a LAT neuron might depend on the degree of activation in a LAT afferent pathway, additionally, so might the IPSP duration. To verify this, synaptic responses were evoked by PRH and BM stimulation at varying intensities, and a common response profile was observed ($n = 17$). At very low intensities, depolarizing responses typically were observed. However, with increasing intensi-
nuclei. Curved arrows point to tips of stimulating electrodes. Scale bar in dependent response profiles, IPSP reversal potentials, and A elicited at the higher intensities (Fig. 7) in the IPSP provided evidence that EPSPs continued to be polarizing responses; small depolarizing potentials embedded in A2, basomedial amygdaloid nucleus; CE, central lateral amygdaloid nucleus; DA, dentate gyrus; EC, external capsule; GP, globus pallidus; LAT, lateral amygdaloid nucleus; OB, olfactory bulb; ON, optic nerve; OT, optic tract; PRH, perirhinal cortex; rs, rhinal sulcus.

ties, these depolarizing responses decreased in size and then became dominated by large hyperpolarizing potentials. The shape of the depolarizing potential also varied with stimulus intensity, having a broad shape at lower intensities (lasting for 100–200 ms), but narrowing to 5–10 ms or completely disappearing at higher ones. The IPSP shape also was found to vary with stimulus intensity, but in contrast to the EPSP, both its amplitude and duration grew monotonically with stimulus intensity.

A typical response, in this case evoked by PRH stimulation, is shown in Fig. 7A. Here the cell was depolarized to –62 mV with 0.21 mA DC current, and synaptic responses were evoked with intensities ranging from 0.23 to 1.5 mA (weaker shocks failed to elicit consistent responses). Depolarizing responses were evoked by the lower intensities (0.23, 0.25 mA), whereas the higher ones elicited hyperpolarizing responses; small depolarizing potentials embedded in the IPSP provided evidence that EPSPs continued to be elicited at the higher intensities (Fig. 7B).

In cases where the EPSP preceded the IPSP onset (65%, n = 11), evidence was obtained that the EPSPs would have continued to increase in size if not for the countering effects of the IPSP. For example, the PRH-evoked EPSPs in Fig. 8A increased in amplitude with stimulus intensity (compare EPSPs of 0.33, 0.50, and 0.83 mA traces), even though they are truncated more and more rapidly as the response becomes predominantly hyperpolarizing at higher intensities.

The duration of the IPSP always was found to vary directly with stimulus intensity (n = 17). Thus higher intensities produced IPSPs that could last several hundred milliseconds longer than IPSPs elicited at lower intensities. An example is shown in Fig. 7C, where IPSPs evoked by different intensities were scaled to have equal peak amplitudes. The responses evoked by the lower intensity stimuli, whereas peaking at a similar time as the responses evoked by the higher intensity shocks, had steeper decays leading to their more rapid termination (Fig. 7C). In addition, IPSP duration was measured from response onset to the time when the potential had decreased to 25% of the peak IPSP amplitude. A plot of duration as a function of stimulus intensity also showed the direct relation between IPSP duration and stimulus intensity (Fig. 7D).

Synaptic responses evoked from different PRH locations

Anatomic studies in cats have demonstrated that PRH projections to the LAT nucleus arise from the entire extent of the PRH cortex, although rostral levels contribute a denser projection (Russchen 1982a; Witter and Groenewegen 1986). To investigate the degree of PRH convergence onto individual LAT neurons, the PRH cortex was stimulated at different rostrocaudal sites (Fig. 9A). In all cells (n = 10), synaptic responses could be evoked from the entire extent of the PRH cortex with all sites showing similar intensity and synaptic response profiles, IPSP reversal potentials, and difficulty in evoking orthodromic spikes.

Nevertheless, some consistent differences emerged. First, stimulation of caudal PRH levels evoked smaller responses than were elicited from more rostral sites. For example, in the cell shown in Fig. 8, stimuli of identical strength (0.83 mA) evoked increasingly large EPSPs and IPSPs when applied at more rostral sites (compare S1, S2, and S3 in Fig. 8, A–C, respectively). Only very high stimulus intensities (1 mA) applied at the most caudal site (S4) produced IPSPs of comparable size. Further, the more rostral sites had lower thresholds for evoking EPSPs (compare minimal intensities for S1 and S2 versus S3 and S4 in Fig. 8).

Yet, the generation of an orthodromic spike depended less on the absolute size of the synaptic potentials than on the balance between the competing EPSPs and IPSPs evoked by the stimulus. Thus orthodromic spikes could be elicited from any level of the PRH cortex, not just from the site with the largest EPSPs. However, each PRH site elicited spikes only within a narrow range of stimulus intensities. For example, S3 at 0.23 mA readily evoked spikes whereas other intensities produced subthreshold synaptic potentials (Fig. 8C). In the cell of Fig. 8, only subthreshold responses to S1, S2, and S4 are displayed for clarity.

PSP onset and duration vary systematically with PRH stimulus site

Onset latency, defined as the first sustained deflection from baseline, whether positive or negative (as the evoked IPSP sometimes preceded the EPSP), was found to increase as the distance between the LAT nucleus and the stimulation site increased (Fig. 9, A and D). The average response laten-
cies to S1–S4 were 4.8 ± 0.5, 5.9 ± 0.7, 10.3 ± 1.9, 29 ± 3.5, respectively \((n = 8, P < 0.05)\). An example of this latency shift is shown in Fig. 9B.

The onset latency of PRH-evoked PSPs also was found to decrease with increasing stimulus intensity. The magnitude of this effect, however, depended on the stimulation site. Rostral sites had not only shorter onset latencies but also displayed less latency variations with changes in intensity. Thus in Fig. 9, the most caudal site evoked IPSPs at latencies of 17 and 6.2 ms with low and high intensities, compared with 6 and 2.5 ms with the rostral-most site.

The duration of PRH-evoked IPSPs (as defined above) also varied systematically with stimulation location, with more rostral sites evoking longer duration PSPs compared with caudal sites. In the example shown in Fig. 9C, the IPSP duration evoked by S4 was 409 ms compared with 528 ms at S1 and intermediate values with S2 and S3. As with onset latency, the duration varied with stimulation distance (Fig. 9D); however, the change in duration was 13.2 ms/mm, some 30-fold greater than the latency variations (0.4 ms/mm).

**Fig. 5.** Reversal potentials of simple and complex SP-related IPSPs. A: averaged bipolar recordings of ENT SPs and related intracellular events \((B)\) recorded at different \(V_m\)s \((-106 to -61 mV)\) as determined by DC current injection. Simple SP-related IPSPs reversed at \(-93.5 mV\) whereas complex SP-related IPSPs reversed at a similar value \((-91 mV)\). ENT-evoked IPSPs had a slightly more depolarized reversal potential \((-87.5 mV)\) in this cell. Rest \(-76 mV.\)

**Fig. 4.** IPSPs evoked by cortical and intra-amygdaloid stimuli have similar reversal potentials. A: IPSPs elicited at different \(V_m\)s by PRH \((A1)\) and BM \((A2)\) stimulation in same LAT neuron. Each trace in this and the following figures is an average of 4–9 individual sweeps, unless otherwise stated. Evoked responses consist of a short latency EPSP followed by a long lasting IPSP. Although PRH stimulation evoked larger synaptic potentials than did BM stimuli in this cell, evoked IPSPs had similar reversal potentials. Rest \(-71 mV.\) A3: plot of IPSP amplitude \((\Delta V)\) vs. \(V_m\) at IPSP peak vs. \(V_m\) as determined by DC current injection. Zero-crossing of fitted lines gives reversal potentials of IPSPs. B: plots of voltage change from resting potential \((\Delta V)\) vs. DC current \((I)\) before stimulation \((R_{IN})\) and at IPSP peak \((R_{PEAK})\) for PRH- \((B1)\) and BM- \((B2)\) evoked IPSPs. \(R_{IN}\) estimated from slopes of fitted lines (slope resistance). C: IPSPs evoked by direct LAT stimulation in a different neuron. C1: IPSPs evoked from different \(V_m\) as determined by DC current injection. C2: plot of IPSP amplitude \((\Delta V)\) at IPSP peak vs. \(V_m\). IPSP reversal potential of \(-79.6 mV\) was similar to those obtained for ENT- and BM-evoked IPSPs in this cell \((-77.5 and -80.5 mV, respectively)\). See text. Rest \(-65 mV.\) C3: plots of voltage change from resting potential \((\Delta V)\) vs. DC current \((I)\) before stimulation \((R_{IN})\) and at IPSP peak \((R_{PEAK})\). At its peak, IPSP reduced \(R_{IN}\) by \(-71\%.\)
related synaptic potentials; there is a large degree of convergence onto LAT neurons from widespread regions of the PRH cortex; and synaptic response profiles are highly dependent on stimulus intensity, implying a competitive interaction between excitatory and inhibitory inputs in the LAT nucleus.

Origin of inhibition within the LAT nucleus

Although glycinergic IPSPs of unknown origin have been observed in the LAT nucleus (Danobe and Pape 1995), GABA appears to be the main inhibitory transmitter in the BL complex. For instance, in vitro studies of LAT and BL neurons have shown that synaptically evoked IPSPs are sensitive to GABA_A and/or GABA_B-receptor antagonists (Danobe and Pape 1995; Rainnie et al. 1991b; Sugita et al. 1992, 1993; Washburn and Moises 1992). These IPSPs presumably resulted from the activation of local GABAergic interneurons as lesions deafferenting the amygdala produce little if in any decreases in GAD levels (Le Gal La Salle et al. 1978) and internuclear connections linking the different BL nuclei only consist of excitatory projections (Paré et al. 1995c; Smith and Paré 1994).

DISCUSSION

Previous work has shown that projection neurons of the LAT nucleus have very low firing rates (Gaudreau and Paré 1996; Paré and Gaudreau 1996), and that GABAergic inhibition may be an important factor in this respect. Thus the present investigation was undertaken to study how IPSPs affect the behavior of LAT projection neurons in vivo. Our results demonstrate that large amplitude, long-lasting monophasic IPSPs reversing around −85 mV dominate the activity of LAT cells; electrical stimuli applied in major input and output structures of the LAT nucleus evoke relatively invariant synaptic responses that are similar to spontaneous SP-related synaptic potentials.
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gested that they resulted from the activation of GABA\textsubscript{A} and GABA\textsubscript{B} receptors, respectively. The early and late IPSPs also could be distinguished by their reversal potentials (Rainnie et al. 1991b; Sugita et al. 1992, 1993; Washburn and Moises 1992). In LAT neurons for instance, the IPSP reversals were similar to those reported in other CNS neurons, i.e., around \(-70\, \text{mV}\) for GABA\textsubscript{A} and \(-110\, \text{mV}\) for GABA\textsubscript{B} mediated IPSPs (Sugita et al. 1993).

In addition, in vitro observations indicated that spontaneous and evoked GABA\textsubscript{A} and GABA\textsubscript{B} responses could occur independently in LAT neurons (Sugita et al. 1992), thus suggesting that the LAT nucleus contains two distinct GABAergic neuronal populations, each having access to different types of GABAergic receptors.

Although the present results confirmed some of the basic in vitro findings, such as the near coincident activation of a fast EPSP and IPSP and the presence of a long-lasting IPSP, significant differences were observed. First, spontaneous and evoked IPSPs were monophasic. That is, a clear separation of the IPSP into distinct early and late components was not observed in vivo. Second, these monophasic IPSPs (measured at their peak) reversed around \(-85\, \text{mV}\), a value in between those typically reported for GABA\textsubscript{A} and GABA\textsubscript{B} IPSPs, suggesting that Cl\textsuperscript{−} and K\textsuperscript{+} conductances contributed to the IPSP. Third, the electrophysiological features of evoked IPSPs were constant, irrespective of the stimulation site.

It could be argued that the use of barbiturates in our experiments has obscured the break between an early GABA\textsubscript{A} and late GABA\textsubscript{B} component because barbiturates prolonged the mean open time of GABA\textsubscript{A} channels (Barker and McBurney 1979), thus prolonging GABA\textsubscript{A}-mediated IPSPs (Thompson and Gähwiler 1992). However, biphasic GABAergic responses indistinguishable from those observed in vitro have been described in thalamocortical neurons intracellularly recorded in cats anesthetized with various drugs including urethane, sodium pentobarbital, and ketamine-xylazine (Contreras et al. 1996; Paré et al. 1991; Paré and Lang, unpublished results). Yet, pentobarbital perfusion has been shown to reduce GABA\textsubscript{B} IPSPs in neurons of the BL nucleus in vitro (Rainnie et al. 1991b).

IPSPs evoked by stimulating various input and output structures of the LAT nucleus were studied in an attempt to test the possibility that the LAT nucleus contains two populations of GABAergic interneurons having access to either GABA\textsubscript{A} or GABA\textsubscript{B} receptors and being involved in distinct intra-amygdaloid circuits (Sugita et al. 1992). Stimulation of the PRH and ENT cortices, basal forebrain (results not shown) as well as intra-amygdaloid stimuli (BM nucleus) all evoked similarly shaped monophasic IPSPs that reversed around \(-85\, \text{mV}\). Moreover, IPSPs evoked by direct LAT stimulation were monophasic, and had nearly identical reversal potentials to those of the BM- and cortically evoked IPSPs in the same cells.

In vivo IPSP profile differs from that found in vitro

In the in vitro studies of LAT and BL neurons (Rainnie et al. 1991a,b; Sugita et al. 1992, 1993; Washburn and Moises 1992), responses to afferent stimulation typically consisted of an early glutamatergic EPSP followed by a biphasic IPSP comprising an early Cl\textsuperscript{−}-mediated phase lasting \(-50\, \text{ms}\), and a late, longer-lasting, K\textsuperscript{+}-mediated component. The pharmacological sensitivity of these two IPSP phases sug-

**FIG. 8.** Synaptic responses evoked from different PRH sites. Synaptic responses were evoked in same neuron from 4 different stimulation sites along rostrocaudal extent of PRH cortex: S1 (A), S2 (B), S3 (C), and S4 (D). See scheme in Fig. 3A1 for relative positions of stimulating electrodes. Each panel depicts responses to a range of intensities (mA, values on right).

**In vivo IPSP profile differs from that found in vitro**

Thus it is likely that in the present study, the hyperpolarizing potentials elicited by cortical and subcortical stimuli in LAT projection neurons were mediated mostly by GABAergic interneurons of the LAT nucleus. However, as shown in the companion paper (Lang and Paré 1997) and in a recent in vitro study (Danobe and Pape 1996), a synchronously activated intrinsic conductance also contributed to these hyperpolarizing potentials.

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ing the LAT nucleus (McDonald 1992; Millhouse and DeOlmos 1983), which presumably contact projection cells and interneurons (Paré et al. 1995c).

This attempt to demonstrate feedback inhibition was only partly successful in that the overlapping ranges of latencies of the BM- and PRH-evoked IPSP prevented us from ruling out the possibility that the BM-evoked IPSPs were mediated via an amygdalo-cortico-LAT loop rather than through antidromic activation of recurrent collaterals. Nevertheless, in at least some cells, the onset of the IPSP and/or EPSP was too fast (i.e., less than twice the shortest latency of a PRH-evoked IPSP) for the initial part of the response to be mediated via a cortical loop. Thus in these cases at least, the initial components of BM-evoked PSPs were mediated by feedback excitation and inhibition.

In light of these considerations, the finding that BM- and cortically evoked IPSPs were indistinguishable suggests that feedback IPSPs are mediated by the same interneurons responsible for the cortically evoked feed-forward inhibition. Again, this interpretation is subject to the caveat that a contribution from feed-forward inhibition due to cortical afferents cannot be excluded, particularly at later times during the IPSP. This indirect recruitment of cortical inputs may have obscured differences in shape or reversal potential.

Conversely, it is possible that feedback inhibition, resulting from antidromic activation of cortically projecting LAT neurons, contributed to cortically evoked IPSPs. However, although the LAT nucleus projects to the entire rostro-caudal extent of the PRH in cats (Room and Groenewegen 1986), the projection to the ENT is limited to ventrolateral ENT area (Smith and Paré 1994), whereas we stimulated the more caudally situated ventromedial ENT area. Because no consistent differences between ENT- and PRH-evoked IPSPs were found, either the contribution of feedback inhibition to PRH-evoked IPSPs is minimal relative to the feed-forward inhibition or both types of inhibition involve the same interneuronal population.

In sum, the relatively invariant nature of the evoked IPSPs, regardless of stimulation site and their similarity to SP-related IPSPs, lead us to conclude that independent activation of interneuronal subpopulations contacting only GABA_A or GABA_B receptors, respectively, appears not to occur in vivo. This difference from the in vitro situation may be ascribed to the intactness of the cortical and amygdaloid circuitry in vivo, which probably links the activation of the different GABAergic populations. Given the reported barbiturate-sensitivity of GABA_B IPSPs in some neurons (Rainnie et al. 1991b), this conclusion should be verified under other anesthetics. However, the results of the companion paper suggest that we were able to detect GABA_B IPSPs and that they never occurred independently of GABA_A IPSPs (Lang and Paré 1997).

**Convergence and divergence in corticoamygdaloid afferents**

The discrepancies between the in vivo and in vitro data appear to result from several factors, including the apparent

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**FIG. 9.** IPSP onset and duration vary with PRH stimulation site. A: scheme showing position of PRH stimulation electrodes on a ventral view of cat brain. B: initial part of responses are shown at fast sweep speed. Arrows indicate onset of responses. C: evoked response to identical stimuli applied at each site. Open circles, points where IPSP amplitudes have decreased to 25% of peak amplitudes. D: plot of IPSP duration and onset as a function of electrode location in terms of frontal plane level. Intracellular recordings were obtained at a frontal plane of ~12 mm. E: schematics showing postulated flow of activity after stimulation of rostral (E1) or caudal (E2) PRH sites.
down regulation of the $\text{GABA}_B$ IPSP and the presence of a synaptically activated $\text{Ca}^{2+}$-dependent $\text{K}^+$ conductance in vivo. These will be treated in detail in the companion paper (Lang and Paré 1997). Here we will discuss the role of the extensive intra-amygdaloid and intracortical connectivity, which is largely lost with in vitro preparations, in shaping the temporal characteristics of the evoked responses in vivo.

In our experiments, the extensive convergence of cortical inputs on LAT neurons was demonstrated by the possibility of evoking synaptic responses in LAT neurons from all levels of the PRH cortex as well as from the ENT cortex. A previous in vivo study performed in rats also demonstrated that LAT neurons receive convergent inputs from multiple sources (Mello et al. 1992). The variations in amplitudes and onset latencies of PRH-evoked IPSPs as a function of the stimulation site were consistent with the anatomic data on PRH projections to the LAT nucleus. Thus the shorter latency of responses to rostral PRH stimuli is consistent with the shorter distance separating the LAT nucleus from rostral PRH levels as compared with caudal PRH sites (Fig. 9E).

In addition, the larger size of the responses evoked from rostral sites fits with the denser projection of rostral PRH levels to the LAT nucleus (Russchen 1982a; Witter and Groenewegen 1986; Witter et al. 1986).

Given the extensive corticocortical connections within the PRH cortex and considering the fact that the entire PRH cortex projects to the LAT nucleus (Russchen 1982a; Witter and Groenewegen 1986; Witter et al. 1986), the PRH-evoked IPSPs must have resulted from the direct activation of corticoamygdaloid neurons at the stimulation site and from the activation, via corticocortical circuits, of such neurons throughout a great extent of the PRH cortex (Fig. 9E).

Consequently, PRH inputs arising from different rostrocaudal levels reach the LAT nucleus asynchronously, producing a temporally distributed activation of LAT inhibitory interneurons that might cause the “early” and “late” inhibitory responses seen in vitro to overlap, thus explaining the lack of a clear break between the $\text{GABA}_A$ and $\text{GABA}_B$ responses in vivo. In parallel with the spread of activity at the cortical level, intrinsic connections within the LAT nucleus (Krettek and Price 1978; Pitkänen et al. 1995; Russchen 1982b; Smith and Paré 1994) also must contribute to distribute PRH influences in time and space.

In addition, it is likely that this spread of activity across the cortex is partly responsible for the longer duration of IPSPs evoked from rostral PRH sites as compared with caudal ones, since stimuli applied at rostral levels would recruit the shortest corticoamygdaloid pathways first and the longest pathways last (Fig. 9E1), resulting in a relatively longer activation of LAT interneurons. In contrast, IPSPs evoked by stimulation of caudal PRH sites would be relatively compressed in time because the longer corticoamygdaloid fibers would be activated before the shorter ones (Fig. 9E2). However, given the differences in IPSP latencies from these sites, which reflect the differing conduction times, only 10–20% of the duration difference can be accounted for in this manner. The remaining difference must be due to other mechanisms that lead to a more sustained activation of LAT interneurons and/or to greater activation of the inhibitory intrinsic membrane conductances present in LAT projection neurons.

A balance of excitation and inhibition

The large IPSPs observed here in vivo, and previously in vitro (Sugita et al. 1992, 1993; Takagi and Yamamoto 1981), must play an important role in shaping the responses of LAT neurons. Consistent with these intracellular observations, the large majority of LAT neurons have transient (i.e., 1–2 spikes) responses to sustained auditory stimuli (Bordi and LeDoux 1992). Moreover, in a double shock paradigm of medial geniculate inputs, LAT neurons were found to have a reduced response to stimuli for delays ≤150 ms (Clugnet et al. 1990).

The present results suggest that this inhibition is due to activation of much the same intra-LAT circuitry and intrinsic conductances, regardless of the afferent source activated. Instead, the balance of inhibition and excitation evoked from a particular site, appears to depend most strongly on the stimulus intensity, with low intensities producing depolarizing responses and high intensities hyperpolarizing ones. Given the large degree of divergence in the PRH-LAT projection, this pattern may result from the more extensive dendritic trees of the projection cells as compared with those of interneurons (Hall 1972). Thus projection neurons would be more likely to receive synaptic inputs from a particular source than inhibitory interneurons. Therefore low intensity stimuli could produce excitation of projection neurons while still subthreshold for evoking firing of interneurons. With higher stimulus intensities, interneurons would fire, and because of their strategically located synapses onto the soma and proximal dendrites (Carlsten 1988), inhibit the projection neurons, despite their receiving increasingly strong excitatory input.

Thus we propose that information processing in the LAT nucleus may be linked to quantitative, rather than qualitative differences in activation of intra-LAT circuitry, with the balance of excitation and inhibition being more relevant than the absolute magnitude of the evoked responses, or the particular afferent stimulated, for determining the response of a LAT neuron. Thus we predict that large-scale generalized activity in LAT afferents should not be as effective in activating the LAT nucleus as would the activity of relatively small cortical neuronal ensembles. Simultaneous recordings from the PRH cortex and LAT nucleus should be performed to address these issues.

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REFERENCES


