Neuroscience 141 (2006) 1599–1609

A SPATIALLY STRUCTURED NETWORK OF INHIBITORY AND EXCITATORY CONNECTIONS DIRECTS IMPULSE TRAFFIC WITHIN THE LATERAL AMYGDALA

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Abstract—The lateral nucleus of the amygdala is the entry point of most sensory inputs into the amygdala. However, the way information is processed and distributed within the lateral nucleus still eludes us. To gain some insight into this issue, we have examined the spatial organization of excitatory and inhibitory connections in the lateral nucleus. To this end, we performed whole-cell recordings of principal lateral amygdala neurons and studied their responses to local pressure applications of glutamate in coronal and horizontal slices of the guinea-pig amygdala. In coronal sections, glutamate puffs performed at a distance from the recorded cells usually evoked inhibitory responses, except when the recorded neuron was adjacent to the external capsule, in which case excitatory responses could be evoked from ejection sites along the external capsule. In contrast, glutamate puffs evoked a mixture of excitatory and inhibitory responses in horizontal slices. Excitatory responses were generally evoked from stimulation sites located lateral to the recorded cell whereas inhibitory responses were commonly elicited from medial stimulation sites, irrespective of their rostrocaudal position. These findings confirm and extend previous tract-tracing studies where it was found that intrinsic connections within the lateral amygdala prevalently run in the dorsoventral and lateromedial directions. However, our results also reveal a hitherto unsuspected level of spatial heterogeneity in the intrinsic circuit of the lateral amygdala. The prevalence of excitatory responses in horizontal slices coupled to the ubiquity of inhibitory responses in coronal slices suggest that the lateral amygdala network is designed to allow associative interactions within the rostrocaudal plane while preventing runaway excitation locally.

Key words: inhibition, glutamate, picrotoxin, microcircuit, fear, epilepsy.

Many data indicate that the amygdala plays a critical role in the acquisition, consolidation, and expression of emotional memories (Davis, 2000; LeDoux, 2000; McGaugh, 2000; Everitt et al., 2003). The basolateral complex of the amygdala (BLA), and particularly its lateral (LA) nucleus, has emerged as a critical component for these functions, in part because it represents the main input station of the amygdala for sensory inputs from the thalamus and cortex (Russchen, 1986; LeDoux et al., 1990; Turner and Herkenham, 1991; McDonald, 1998). However, the transformations performed by the LA on its sensory afferents remain unclear because our understanding of its intrinsic circuit is limited. The present study was undertaken to shed light on this issue.

There are two main types of neurons in the BLA (reviewed in McDonald, 1992a): (1) multipolar projection cells with spiny dendrites and highly collateralized axons and (2) GABAergic local-circuit neurons (McDonald, 1985; McDonald and Augustine, 1993; Paré and Smith, 1993) that are aspiny (or sparsely spiny) and express various combinations of peptides (Kemppainen and Pitkänen, 2000; Katona et al., 2001; McDonald and Betette, 2001; Mascagni and McDonald, 2003). Projection cells account for 80% of LA neurons (McDonald, 1992b), they use glutamate (Smith and Paré, 1994) as a transmitter, and they contribute most inter-nuclear projections of the amygdala (Stefanacci et al., 1992; Smith and Paré, 1994). Local-circuit cells constitute a heterogeneous group of neurons. According to the classification of McDonald (Mascagni and McDonald, 2003), there are at least four subtypes of BLA interneurons: parvalbumin (PV) expressing neurons, somatostatin positive interneurons, large cholecystokinin interneurons, and small bitufted cells that display various degrees of colocalization of cholecystokinin, calretinin and vasoactive intestinal polypeptide (McDonald and Pearson, 1989; Mascagni and McDonald, 2003).

Based on evidence obtained in the hippocampus (Freund and Buzsáki, 1996; Somogyi and Klausberger, 2005) and neocortex (Markram et al., 2004; Blatow et al., 2005), it is likely that these various types of interneurons structure the activity of principal cells in distinct ways because they target different postsynaptic domains and receive contrasting sets of inputs. For instance, PV interneurons of the BLA appear to be mainly involved in feedback inhibition: they receive strong excitatory inputs from BLA projection cells but very few from the cortex (Smith et al., 2000) and they form numerous inhibitory synapses on the somatic, axonal, and proximal dendritic domains of projection neurons (Pitkänen and Amaral, 1993; Sorvari et al., 1995; Smith et al., 1998; Kemppainen and Pitkänen, 2000; McDonald and Betette, 2001). Although their identity remains unclear, other types of interneurons likely generate feedforward inhibition in the BLA since cortical or thalamic stimulation evokes inhibitory postsynaptic potentials (IPSPs) in principal cells (Rainnie et al., 1991;

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Abbreviations: BLA, basolateral amygdaloid complex; EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; LA, lateral amygdala; PV, parvalbumin.

0306-4522/06$30.00+0.00
doi:10.1016/j.neuroscience.2006.04.077

Washburn and Moises, 1992b; Lang and Paré, 1997a,b, 1998; Danober and Pape, 1998; Bissière et al., 2003) and presumed interneurons can be orthodromically activated by such stimuli (Lang and Paré, 1998; Mahanty and Sah, 1998; Szinyei et al., 2000; Bauer and LeDoux, 2004).

Another outstanding question is whether the intrinsic inhibitory system of the BLA is spatially homogeneous or whether it constrains propagation of impulses in particular directions. In a previous study, we showed that feedback inhibition is spatially heterogeneous in the LA, with feedback interneurons inhibiting only projection cells located in the same coronal plane, leaving rostrocaudal interactions between principal cells relatively unrestricted (Samson et al., 2003). However, our previous study only examined the contribution of feedback interneurons. The present report extends this investigation to other types of BLA interneurons using local pressure applications of glutamate and whole-cell recordings of principal cells in coronal and horizontal slices of the guinea-pig amygdala.

EXPERIMENTAL PROCEDURES

Preparation of amygdala slices

Coronal and horizontal slices of the amygdala were obtained from Hartley guinea-pigs of either sex (250–300 g). Every effort was made to minimize the number of animals used and their suffering. Experiments were done with the approval of the Rutgers University Institutional Research Board and in accordance with the NIH guide for the care and use of laboratory animals. The animals were deeply anesthetized with pentobarbital (60 mg/kg, i.p.), ketamine (80 mg/kg, i.p.) and xylazine (12 mg/kg, i.p.) and then decapitated. The brain was extracted from the skull and placed in an oxygenated solution (4 °C) containing (in mM) 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgCL_2$, 2 $CaCL_2$, 26 $NaHCO_3$, and 10 glucose. Brain slices (400 μm) were prepared using a vibrating microtome and stored for one hour in an oxygenated chamber at 23 °C. One slice was then transferred to a recording chamber perfused with an oxygenated physiological solution (4 ml/min). The temperature of the chamber was gradually increased to 32 °C before the recordings began.

Data recording and analysis

Whole-cell recordings were obtained with borosilicate pipettes filled with a solution containing (in mM) 130 K-gluconate, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 10 KCl, 2 $MgCl_2$, 2 ATP-Mg, and 0.2 GTP-tris(hydroxy-methyl)aminomethane. pH was adjusted to 7.2 with KOH and osmolarity to ≈280 mOsm. The liquid junction potential was 10 mV with this solution and the membrane potential was corrected accordingly. The pipettes had a resistance of 4–8 MΩ when filled with the above solution. Recordings with series resistance higher than 15 MΩ were discarded. In some experiments, Neurobiotin (0.2%) was added to the intracellular solution to visualize the recorded neurons. Current clamp recordings were obtained with an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA, USA) under visual control using differential interference contrast and infrared video microscopy. Concentrations of drugs applied in the perfusate were (in μM) 10 bicuculline hydrochloride, 100 picrotoxin, 100 CGP-35348 (Martina et al., 2001).

We studied the responses of principal LA neurons to local pressure applications (12 p.s.i., 60 ms) of glutamate performed at various positions with respect to the recorded cells. These tests were performed in coronal (Fig. 1A) and horizontal slices (Fig. 1B).

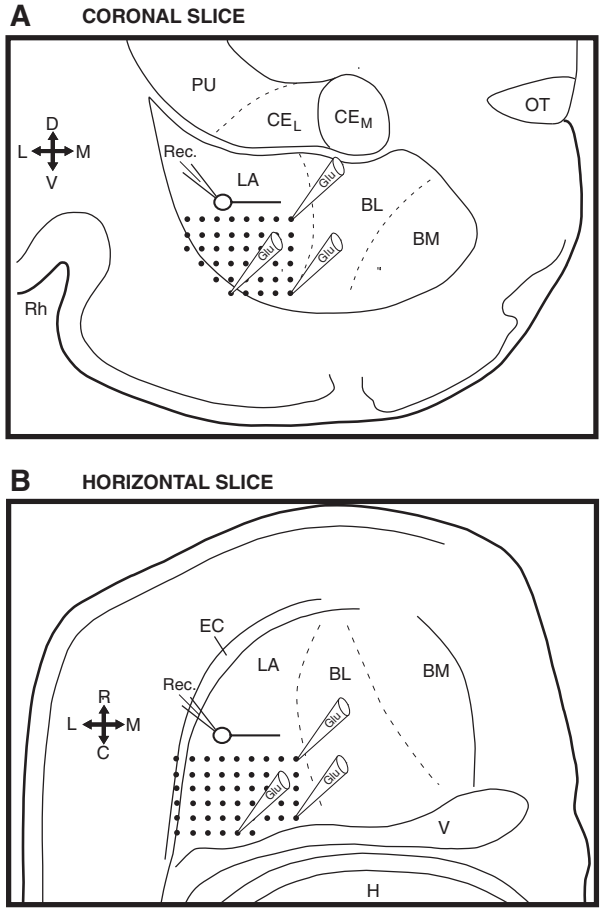

Fig. 1. Scheme illustrating the approach used in this study. Coronal (A) or horizontal (B) slices of the guinea-pig amygdala were prepared and whole-cell patch recordings of principal LA neurons (empty circle) were obtained under visual guidance. Local pressure applications of glutamate were performed at various sites (filled circles) around the recorded cells. Glutamate was applied via a patch pipette (Glu) filled with artificial cerebrospinal fluid containing 0.5 mM glutamate. The orientation of the slices is indicated by the crosses (D, dorsal; V, ventral; L, lateral; M, medial; R, rostral; C, caudal).

The glutamate puffs were performed via a patch pipette filled with artificial cerebrospinal fluid containing 0.5 mM glutamate. The glutamate concentration and stimulation parameters were chosen on the basis of previous experiments with this technique (Royer et al., 2000). Tests conducted with these stimulation parameters revealed that the effect of glutamate is extremely circumscribed in space: direct responses vanish when the ejection pipette is moved >25–50 μm from the somata of recorded cells. Accordingly, the glutamate ejection pipette was initially positioned 100 μm from the recorded cells and then moved in steps of 100 μm toward the periphery of the LA nucleus.

When studying the synaptic responses elicited by glutamate, the membrane potential of the recorded cells was set to −65 mV by intracellular current injection and at two more negative membrane potentials to determine the reversal potential of evoked responses. Three stimuli were applied at each membrane potential. A glutamate ejection site was considered effective only if three consecutive stimuli elicited responses of relatively constant latencies. Synaptic events with amplitudes <0.5 mV were ignored. Values are expressed as means±S.E. To determine whether response amplitudes between cell groups differed significantly, *t*-tests were computed using a fixed level of significance ($P<0.05$). Analyses were