Polarized Synaptic Interactions Between Intercalated Neurons of the Amygdala

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Royer, Sébastien, Marzia Martina, and Denis Paré. Polarized synaptic interactions between intercalated neurons of the amygdala. J Neurophysiol 83: 3509–3518, 2000. The intercalated (ITC) cell masses are small GABAergic cell clusters interposed between the basolateral (BL) complex and central (CE) nucleus of the amygdala. ITC cells receive excitatory afferents from the BL complex and generate feed-forward inhibition in the CE nucleus. Recently it was shown that ITC cells could gate impulse traffic between the BL complex and CE nucleus in a spatiotemporally differentiated manner. In addition, it was hypothesized that lateromedial inhibitory interactions between different ITC cell clusters played a critical role in this respect. Given the potential importance of such conditional computations, the present study aimed to characterize the connectivity existing among ITC cells. To this end, whole cell recordings of ITC neurons were obtained under visual guidance in coronal slices of the guinea pig amygdala. Electrical stimuli applied in the BL complex primarily elicited excitatory responses when they were applied at the same lateromedial level or more medially than the recorded ITC cells. As the stimulation site was moved laterally, the character of the response shifted toward inhibition. Both bicuculline and non-N-methyl-D-aspartate receptor antagonists abolished this BL-evoked inhibition, suggesting that it was not mediated by BL inhibitory cells projecting to ITC neurons. In keeping with this, local glutamate injections in and around the ITC clusters revealed that the most effective site to inhibit ITC cells were ITC clusters located laterally with respect to the recorded one. The activation of more medial ITC clusters evoked much smaller responses. Thus, connections between ITC clusters tend to run in a lateromedial direction. To identify the source of these directionally polarized synaptic interactions, the morphological features of ITC cells were analyzed by intracellular injection of Neurobiotin. This analysis revealed that the dendritic tree and axonal arbor of ITC cells are asymmetric in the lateromedial plane. In particular, their laterally directed dendrites were longer than the medial ones, whereas their laterally directed axon collaterals were shorter than the medial ones. It is concluded that the morphological asymmetry of ITC cells accounts for the directional polarization of inter-ITC connections. The significance of these findings for the gating of information transfer from the BL complex to the CE nucleus is discussed.

INTRODUCTION

Although much evidence points to the involvement of the amygdala in fear and anxiety (reviewed in Aggelton 1992), the computations carried out by this structure remain obscure. Fortunately, much data are available regarding its connectivity. For instance, most sensory inputs from the thalamus and cerebral cortex end in the basolateral (BL) amygdaloid complex (McDonald 1998; Turner and Herkenham 1991). On the output side, the central (CE) nucleus of the amygdala is the main source of brain stem projections mediating fear responses (Gentile et al. 1986; Hitchcock et al. 1989; Hopkins and Holstege 1978; Iwata et al. 1986; Kapp et al. 1979; Veening et al. 1984; Zhang et al. 1986). However, the type of cellular interactions that could allow the amygdala to adjust its outflow in accordance with the significance of sensory events remain unknown.

Recently, we have obtained evidence of cellular interactions that render the throughput of the amygdala sensitive to the timing and origin of sensory inputs in the BL complex (Royer et al. 1999). Indeed, we have shown that a group of inhibitory neurons called the intercalated (ITC) cell masses of the amygdala could control impulse traffic between the BL complex and the CE nucleus by subjecting CE cells to more or less feed-forward inhibition (Royer et al. 1999).

ITC cell masses are small clusters of GABAergic neurons (McDonald and Augustine 1993; Nitecka and Ben-Ari 1987; Paré and Smith 1993a) located between the BL complex and the CE nucleus (Millhouse 1986) (Figs. 1 and 2). They receive excitatory synaptic inputs from the BL complex (Royer et al. 1999) and project, among other targets, to the CE nucleus (Paré et al. 1993b).

In fact, there is a lateromedial correspondence between the position of ITC neurons, their projection site in the CE nucleus and the source of their excitatory afferents in the BL complex (Royer et al. 1999). In other words, the BL sites contributing the strongest excitatory inputs to ITC cells are generally located at the same lateromedial levels. When gradually more medial BL sites are stimulated, they elicit excitatory responses of progressively decreasing amplitude (Royer et al. 1999). In contrast, as more lateral BL sites are stimulated, the nature of the responses progressively shifts toward inhibition (Royer et al. 1999). Since previous work suggested that all internuclear amygdaloid projections of the BL complex appear to originate from glutamatergic cells (Paré et al. 1995; Smith and Paré 1994), the inhibition elicited by lateral stimulation sites was suggested to arise via the excitation of other ITC neurons located more laterally (Royer et al. 1999).

The present study was undertaken to test this hypothesis using electrical microstimulation of the BL complex and local glutamate injections in and around the ITC cell masses. Our results suggest that ITC cells inhibit each other and that this inhibition is directionally polarized, with laterally located ITC cells preferentially inhibiting more medial ones. Accordingly, the morphological characterization of ITC cells revealed an
asymmetry in their dendrites and axons that could account for this polarized connectivity.

**METHODS**

**Preparation of amygdala slices**

Coronal slices of the amygdala were obtained from Hartley guinea pigs (≈250 g). Prior to decapitation, the animals were anesthetized with pentobarbital (40 mg/kg ip) and ketamine (100 mg/kg ip) in agreement with the guidelines of the Canadian council on animal care. The brain was rapidly removed and placed in an oxygenated solution (4°C) containing (in mM) 126 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 1 MgCl₂, 2 CaCl₂, 26 NaHCO₃, and 10 glucose. Coronal slices (400 μm), prepared using a vibrating microtome, were stored for 1 h in an oxygenated chamber at 23°C. One slice was then transferred to a recording chamber perfused with an oxygenated physiological solution (2 ml/min). The temperature of the chamber was gradually increased to 32°C before the recordings began.

**Data recording and analysis**

Current-clamp 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 MgCl₂, 2 ATP-Mg, and 0.2 GTP-tris(hydroxy-methyl)aminomethane. In some experiments, Neurobiotin (0.5%) was added to the intracellular solution to visualize the recorded neurons. pH was maintained at 7.3–7.4 by adding 1N NaOH or HCl. The temperature of the chamber was gradually increased to 32°C before the recordings began.

Concentrations of drugs applied in the perfusate were (in μM) 10 bicuculline and 20 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). All drugs were obtained from RBI (Natick, MA). Local injections of glutamate were performed by applying air pressure pulses (0.01–0.05 s) to a patch pipette filled with a Ringer solution containing 0.5 mM glutamate.

An array of tungsten stimulating electrodes (80 μm diam; 80 kΩ) was positioned in the amygdala as shown in Figs. 1 and 2 (○). Electrical stimuli consisted of 50- to 300-μs current pulses (0.1–1 mA) passed through neighboring electrodes. Analyses were performed off-line with the software IGOR (WaveMetrics, Oregon) and home-made software running on Macintosh microcomputers. The input resistance (Rᵢ) of the cells was estimated in the linear portion of current-voltage plots. The membrane time constant was derived from single exponential fits to voltage responses in the linear portion of current-voltage relations.

**Morphological identification of recorded cells**

When recorded cells were dialyzed with Neurobiotin, the slices were removed from the chamber and fixed for 1–3 days in 0.1 M phosphate buffer saline (pH 7.4) containing 2% paraformaldehyde and 1% glutaraldehyde. Slices were then embedded in gelatin (10%) and sectioned on a vibrating microtome at a thickness of 60–100 μm. Neurobiotin-filled cells were visualized by incubating the sections in the avidin-biotin-horseradish peroxidase (HRP) solution (ABC Elite Kit, Vector Labs) and processed to reveal the HRP staining (Horikawa and Armstrong 1988).

**Nomenclature used to designate the different amygdala nuclei**

In the following, we will use the nomenclature defined by Krettek and Price (1978) to designate the different amygdala nuclei. It should be noted that these authors divided the CE nucleus of the rat and cat in two sectors, a lateral and a medial sector (CEL and CEM, respectively). In living guinea pig slices, it is impossible to distinguish further subdivisions in the CE nucleus.

**Quantification of cellular densities**

Golgi observations (Millhouse 1986) and GABA immunohistochemical studies (Paré and Smith 1993b) have revealed that ITC cells occur in clusters as well as in thin strands of cells present in between these clusters. As a result, ITC neurons form a more or less continuous reticulated sheet of cells (Millhouse 1986). To ensure that our recordings were obtained from ITC cells, as opposed to interneurons located at the periphery of neighboring nuclei, we only studied cells occurring in clusters, the ITC cell masses proper. ITC cell masses could be easily identified because the diameter of ITC cells was smaller and the ITC cell masses contained a much higher number of neurons per surface area than neighboring nuclei (see RESULTS). This was quantified by measuring the number of cells observed at all focal planes with IR-DIC in a 400-μm-thick slice and dividing it by the surface area of the cluster. It should be understood that these “cellular densities” are not accurate stereological estimates, but represent a simple index contrasting the cellular distribution seen in the different amygdala nuclei with IR-DIC in living slices. An ocular micrometer was used to measure surface areas and neuronal diameters.

**RESULTS**

In trans-illuminated slices, variations in the opacity of the tissue allowed identification of the fiber bundles delimiting the different nuclear groups of the amygdala. For instance, the intermediate capsule, which separates the BL complex from...
the CE nucleus and in which ITC cell masses are embedded, could be easily identified. Under high magnification and with IR-DIC, ITC cell masses (Fig. 2, arrows) could be discriminated from neighboring nuclei because they contained smaller-sized neurons (soma diameter: ITC, 10.8 ± 0.36 μm, mean ± SE; BL complex, 18.2 ± 0.8 μm; CE, 22.9 ± 1.35 μm), and they had a much higher cellular density (ITC masses: 70 ± 4.5; BL complex: 22 ± 3.7; CE: 27 ± 1.9 neurons/10^4 mm^2) (for details, see Royer et al. 1999).

Using these anatomical criteria to aim our recording electrodes, a total of 105 ITC neurons were studied at the sites indicated in Fig. 2, arrows. These cells had a resting potential of −75.9 ± 0.77 mV, a R_{in} of 645.5 ± 34.10 MΩ, and a membrane time constant of 29.8 ± 1.64 ms. On depolarization just above threshold, these neurons generated action potentials averaging 76.9 ± 0.86 mV in amplitude and 1.07 ± 0.043 ms in duration (at half-amplitude).

**Synaptic responses of ITC cells to electrical stimulation of the BL complex**

If, as suggested in the introduction, the inhibition displayed by ITC neurons in response to BL inputs is generated by other ITC cells located more laterally, a systematic relation should be found between the position of the BL stimulation sites exciting laterally located ITC cells and inhibiting more medial ones. To test this prediction, cells were recorded sequentially in two or more ITC cell masses of the same slices, and their responses to electrical stimuli applied in the BL complex were compared. The stimulating electrodes consisted of an array of tungsten electrodes (inter-tip spacing ≈ 130 μm) represented by black dots in the scheme of Figs. 1 and 2. The stimuli were brief current pulses applied through neighboring electrodes. During these tests, the cells were depolarized to around −65 mV by intracellular current injection.

Whereas the synaptic response profiles of neurons recorded

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**FIG. 2.** Synaptic responses of ITC cells to electrical stimulation of the BL complex. Top: schematic view of the amygdala slice with ITC cells (black ovals) and stimulating electrodes (black dots) in the BL complex. Arrows point to ITC cell masses. Stimuli consisted of electrical pulses (50 μs, 0.2 mA) applied through neighboring electrodes. Numbers indicate stimulation sites. Responses of 2 ITC cells (A, ITC1; B, ITC2) were recorded in the same slice (see position in scheme). Their responses and the corresponding stimulation sites are aligned. In B (ITC2), synaptic responses were first evoked in Ringer (control), then in Ringer containing 10 μM bicuculline (bicuculline), 20 min after return to control Ringer (recovery), and finally in Ringer containing 20 μM 6-cyano-7-nitroquinoxalene-2,3-dione (CNQX). *Insets:* response of both cells to stimulation of site 9 with an expanded time base (identical x-axis for both insets).
sequentially in the same ITC cell masses were nearly identical (13 comparisons carried out in 7 slices; data not shown), systematic differences were observed when ITC neurons located at different lateromedial levels were compared (11 pairs of ITC cells recorded in 8 slices). The result of such an experiment is shown in Fig. 2. Note that the position of recorded cells is indicated in the scheme (Fig. 2, ITC1 and ITC2) and that their responses to BL stimuli are shown (Fig. 2, A and B, respectively) in register with the corresponding stimulating sites (Fig. 2, • and numbers).

In agreement with previous findings (Royer et al. 1999), BL sites located at the same lateromedial levels or slightly more medially with respect to the recorded cells, elicited apparently pure excitatory responses (ITC1, sites 8–12 in Fig. 2A; ITC2, sites 13–17 in Fig. 2B, control), whereas the responses shifted toward inhibition when gradually more lateral sites were stimulated. Although this relationship held irrespective of the lateromedial position of ITC cells, the spatial extent of the BL stimulation sites affecting ITC cells was more restricted for laterally located than medially located ITC cells. For instance, the number of BL stimulating sites eliciting synaptic responses in ITC cells ventral to the CE_M was 11.6 ± 0.51 compared with 16.6 ± 1.40 sites for ITC cells ventral to the CE_M (t-test, P < 0.05).

In support of our hypothesis, a close correspondence was found between the BL stimulation sites inhibiting medially located ITC cells and exciting laterally located ones, provided that the recordings were obtained from neighboring ITC cell clusters. A striking example of this is shown in Fig. 2. Note the nearly perfect correspondence between the BL sites eliciting orthodromic spiking in the laterally located ITC cell (ITC1 in Fig. 2) and those eliciting inhibitory postsynaptic potentials (IPSPs) in the more medial one (ITC2 in Fig. 2). Although such perfect matches occurred infrequently, the concordance was generally very good when the response profiles of cells located in neighboring ITC cells masses were compared. Partial or no overlap between the sites exciting laterally located ITC cells and inhibiting more medial ones was seen when more distant ITC cell masses were compared.

To address this issue quantitatively, we compared the response profile of ITC cells located medial to the CE_M (n = 5), under the CE_M (n = 8), and below the CE_M (n = 5). The position of the stimulation sites eliciting the largest IPSPs and excitatory postsynaptic potentials (EPSPs), at sub-threshold stimulation intensities, were averaged for each group of ITC cells. See Fig. 2 to appreciate the relation between the stimulation site numbers and their position. Consistent with the impression gained from the visual inspection of the data, the position of the stimulation site eliciting the maximal EPSP in ITC cells ventral to the CE_L (7.2 ± 0.37) was not significantly different (t-test, P > 0.05) from that evoking the largest inhibitory response in ITC cells ventral to the CE_M (8.2 ± 0.31). Similarly the position of the stimulation site eliciting the maximal EPSP in ITC cells ventral to the CE_M (13.5 ± 0.86) was not significantly different (t-test, P > 0.05) from that evoking the largest inhibitory response in ITC cells medial to the CE_M (13.2 ± 1.94). In contrast, a statistically significant difference (t-test, P < 0.05) was found between the position of the sites eliciting the maximal EPSP in ITC cells ventral to the CE_L (7.2 ± 0.37) and those evoking the largest inhibitory response in ITC cells medial to the CE_M (13.2 ± 1.94).

**Pharmacological sensitivity of the BL-evoked ITC inhibition**

If the inhibition elicited in ITC neurons by BL stimuli is generated by the glutamatergic activation of ITC cells located more laterally, by opposition to the direct recruitment of GABAergic axons in the BL complex, then the inhibition should be abolished by addition of bicuculline or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) to the perfusate. Such an experiment is shown in Fig. 2B where BL-evoked responses of the same ITC cells were studied in control Ringer (Fig. 2B, control), in the presence of bicuculline (Fig. 2B, bicuculline), and after recovery, in the presence of CNQX (Fig. 2B, CNQX). See figure legend for methodological details.

Bath application of bicuculline greatly reduced (average of 92 ± 8.3%; n = 5) the amplitude of BL-evoked IPSPs and usually enhanced the amplitude (average increase of 50 ± 4.9%) and duration (average increase of 77 ± 27.1%) of BL-evoked EPSPs. In agreement with our hypothesis, CNQX application abolished both the BL-evoked EPSPs and IPSPs (n = 5), thus suggesting that the inhibition seen in ITC cells was not generated by GABAergic elements of the BL complex excited directly by the electrical stimuli, but by the glutamatergic activation of GABAergic cells projecting to the recorded neuron. This point was tested in five cells and the same result was obtained in every cases.

**Effect of local pressure application of glutamate**

To identify the GABAergic neurons inhibiting ITC cells, local pressure glutamate injections were performed in and around the ITC cell masses while recording from ITC neurons (Figs. 3 and 4). In these experiments, the orientation of the slices in the recording chamber was adjusted so that the Ringer flow would facilitate the diffusion of glutamate away from the recorded cell (Fig. 4, arrows).

For these tests, a total of 12, 22, and 11 neurons were recorded in ITC cell masses located medial to, below, or lateral to the CE_M, respectively. One or more local glutamate injection sites were studied in each cell, and response reliability was ascertained by applying the same stimuli several times at each site. Examples of such experiments are shown in Fig. 3 depicting the response of two ITC neurons (Fig. 3, B and C), both recorded in an ITC cell mass located below the CE_M (Fig. 3A, recording pipette). Note that the glutamate injection sites are indicated by numbers in the scheme (Fig. 3A).

In disagreement with the possibility that the indirect activation of GABAergic CE neurons was responsible for BL-evoked IPSPs in ITC cells, glutamate ejection in the CE_L (n = 6; Fig. 3B5) or CE_M (n = 2; not shown) elicited no responses in ITC cells (irrespective of their lateromedial position). To verify whether this lack of response reflected the compromised connectivity of the slice, the CE ejection site was gradually moved closer to the recording pipettes. No response was seen until glutamate directly excited the recorded cells (distance ≤150 μm, n = 3; not shown).

Consistent with the results obtained with electrical stimuli, the synaptic responses evoked by glutamate injections in the BL complex varied with the lateromedial position of the injection site with respect to the recorded cell. When the injection and recording sites were at the same levels, EPSPs were elicited (Fig. 3C2; n = 5). When the injection site was located...
lateral with respect to the recorded cell, small inhibitions (Fig. 3B4; n = 2) or no responses (n = 11) were observed.

In contrast, glutamate injection in ITC clusters located laterally to the recorded cells (n = 25) produced multiphasic hyperpolarizing responses (Fig. 3, B3 and C3) with an average peak amplitude of 6.4 ± 0.92 mV. The duration of this hyperpolarization (range ∼0.5–3.5 s; average of 2.2 ± 0.41 s) was longer than the excitation caused by glutamate injections performed in the immediate vicinity of the recorded ITC cells (Fig. 3C1, average of 1.2 ± 0.2 s; range 0.5–2 s; n = 8; t-test, P < 0.05). This difference probably reflects the recruitment of many ITC cells at different latencies as glutamate diffused from the injection site. The hyperpolarization evoked in ITC neurons by glutamate injection in more laterally located ITC cell clusters could be reversed in polarity by membrane hyperpolarization (compare Fig. 3C, 3 and 3′) and was virtually abolished by bath application of bicuculline (n = 4; data not shown).

It should be emphasized that the same results were obtained when the effect of several glutamate injection sites was compared in the same ITC cell (n = 5). As shown in Fig. 3B, the amplitude of the evoked inhibition was maximal with glutamate injections in laterally located cell clusters (Fig. 3B3), it decreased when the ejection pipette was moved ventrally (Fig. 3B4), and it disappeared completely when the pipette was positioned dorsally (Fig. 3B5).

To study the interactions between ITC neurons belonging to the same or different ITC cell masses, glutamate was injected successively in three positions (Fig. 4A): 50–100 μm from the recorded ITC cell as well as in ITC clusters located medially or laterally with respect to the recorded cell. Responses to these three glutamate ejection sites were compared in the same cells (n = 5; Fig. 4, B and C). Two such experiments are shown in Fig. 4, B and C.

Consistent with the hypothesis that the connectivity existing between ITC′ neurons is polarized lateromedially, the hyperpolarization evoked by glutamate ejection was larger when it was performed in ITC clusters located laterally (Fig. 4, B1 and C1) versus medially (Fig. 4, B3 and C3) to the recorded one (area of hyperpolarization in mV/s: 11.33 ± 1.84 vs. 2.75 ± 1.09, respectively; t-test P < 0.05). The initial response to glutamate injections close to the recorded cells (Fig. 4, B2 and C2) was a hyperpolarization (average peak amplitude 4.8 ± 0.77 mV) followed by a depolarization, suggesting that ITC cells located in the same clusters are interconnected. The secondary depolarization was probably due to the direct action of glutamate on the recorded cells and/or to the activation of excitatory neurons in the vicinity of the ITC cluster.

To quantify the polarization of the connections existing between two neighboring ITC masses, we performed local glutamate injections in one of the two clusters while recording in the other, and vice versa (n = 11; Fig. 4D, 1 and 2). Again, the hyperpolarization evoked by glutamate injections in laterally versus medially located ITC clusters was consistently larger.
Morphology of ITC cells

To determine whether the morphological features of ITC cells accounted for their directional interconnections, as evidenced in the preceding text, a total of 46 ITC cells, recorded at different lateromedial levels of the amygdala, were filled with Neurobiotin (Figs. 5 and 6). Of these cells, 30 were recovered.

Most ITC neurons had ovoid cell bodies (Figs. 5, A–C, and 6B1), whose longest axis averaged 15.95 ± 1.27 μm. Note that this is more than what was measured prior to the recordings, with IR-DIC (10.8 ± 0.36 μm). The difference probably results from the deformation and/or swelling caused when the patch pipette was retracted from the cells at the end of the experiments.

ITC cells had spiny dendritic trees that were elongated in the lateromedial axis, paralleling the inclination of the intermediate capsule at the recording site (Fig. 5, A and B). The dendritic arbor of ITC cells were distributed asymmetrically about their soma (Fig. 5, A and C). Indeed, laterally directed dendrites tended to be longer (≥450 μm) than those extending medially (rarely >150 μm). On average, the longest laterally and medially directed dendritic branches measured 285 ± 46.7 versus 95 ± 18.0 μm (t-test, P < 0.05). Two representative examples of this are shown in Fig. 5, A and C. Our unique example of an ITC cell with a symmetric dendritic tree is shown in Fig. 5B. A few ventrally or dorsally directed dendrites were observed, but they did not exceed 150 μm in length.

The axonal arborization of ITC cells was also asymmetric in the lateromedial axis but in a direction opposite to that of the dendrites. Typically the axon emerged from the medial side of the soma (Fig. 5A), coursed for 30–150 μm, and then gave rise to several collaterals (Figs. 5C and 6, A and B). Laterally and ventrally directed collaterals were short and infrequent. Medially directed axonal branches were much longer and generally gave rise to one or two extensive dorsal axon collaterals in the CE nucleus (Fig. 6, A and B). The difference in length between the longest medially (306.7 ± 67.7 μm) and laterally (149.7 ± 36 μm) directed axon collaterals was statistically significant (t-test, P < 0.05). The axon collaterals of ITC cells bore small varicosities occurring at intervals ranging between 2 and 11 μm (Figs. 5A and 6, A and B). In the CE nucleus, they sometimes formed dense clusters of varicosities (≥3 μm in diameter) where the intervaricose segments was barely visible (Fig. 6A).

DISCUSSION

This study was prompted by recent findings suggesting that the throughput of the amygdala can be modified depending on the origin and timing of sensory inputs (Royer et al. 1999). Key to this phenomenon are the ITC cells, which receive glutamatergic inputs from the BL complex and generate feed-forward inhibition mediated by GABA in the CE nucleus (Paré et al. 1993a,b; Royer et al. 1999). It was found that ITC neurons exhibit an asymmetric response profile to BL stimulation such that ITC cells are excited by BL inputs originating at the same lateromedial level or more medially, whereas inputs originating laterally tend to elicit more inhibition (Royer et al. 1999). Consequently the amount of feed-forward inhibition generated by ITC cells in the CE nucleus can be enhanced or reduced...
depending on the particular combination of BL sites that are excited. Considering the potential importance of such conditional operations, the present study was undertaken to determine the origin of the inhibition generated in ITC cells by the activation of some BL sites. Our results suggest that it is generated by ITC cells inhibiting each other. However, the connections existing between ITC cells are directionally polarized with laterally located ITC cells prevalently inhibiting more medial ones. Our results also indicate that the origin of this directionality lies in the morphological properties of ITC neurons, their dendrites extending over longer distances in the lateral than the medial direction whereas their axons show the opposite trend.

In the following discussion, we will consider the evidence supporting these conclusions and speculate on their significance for the computations carried out by the amygdala.

**BL-evoked inhibition of ITC cells involves the glutamatergic activation of GABAergic ITC neurons inhibiting other ITC cells**

Two lines of evidence argue against the possibility that the BL-evoked inhibition arises from GABAergic neurons of the BL complex with projections to the ITC cells masses. First, blocking non-NMDA glutamate receptors by addition of CNQX to the perfusate abolished the inhibition evoked by electrical stimulation of the BL complex. Second, when local applications of glutamate were used, excitation of ITC cells located laterally to the recorded ITC neurons produced the largest IPSPs. When the ejection site was moved ventrally or dorsally, the inhibitory response rapidly decreased in amplitude or disappeared.

This interpretation is consistent with the result of Golgi and immunohistochemical studies (Carlson 1988; Hall 1972; reviewed in McDonald 1992) indicating that GABAergic cells of the BL complex have axons that remain confined within the nucleus where their soma is located. It is also supported by tract-tracing studies where the articulation of axons arising in the BL complex and ending in other amygdala nuclei was examined in the electron microscope (Paré et al. 1995; Smith and Paré 1994). Indeed, these studies have revealed that BL axons projecting to other nuclei of the amygdala only form asymmetric synapses with their targets, a type of synaptic specialization typically associated to excitatory synapses (Colonnier 1981). Moreover, these axon terminals were enriched in glutamate but not GABA (Smith et al. 1994).

**Directionally polarized connections between different ITC cell masses**

Unfortunately, little anatomical evidence exists concerning intra-ITC connections. In a Golgi study, Millhouse (1986) noted that ITC cells had axon collaterals coursing in the intermediate capsule. In addition, Paré et al. (1993b) reported the presence of a few retrogradely labeled ITC cells following an extremely small cholera toxin injection in the intermediate capsule.

In the present study, we obtained evidence suggesting that inter-ITC connections preferentially run in a lateromedial direction. First, a close correspondence was found between the sites inhibiting ITC cells and those exciting laterally located ITC neurons. In contrast, more medial stimulation sites elicited prevalently excitatory responses. Second, local intra-ITC injection of glutamate consistently evoked larger inhibitory responses when they were performed in ITC clusters located laterally compared to medially with respect to the cluster.
containing the recorded ITC cell. This result was obtained when lateral and medial injection sites were compared in the same or different ITC cells recorded in the same slice. The possibility that the progenitors of this inhibition were located in neighboring nuclei appears unlikely as control injections performed dorsally or ventrally evoked no or smaller responses.

Morphology of ITC cells accounts for the directional polarization of inter-ITC connections

A striking feature of ITC cells is that their dendritic tree and axonal arbor are asymmetric in the lateromedial plane. In particular, laterally directed dendrites were three times longer than medial ones whereas laterally directed axon collaterals were twice shorter than medial ones. As a result of these asymmetries, the interactions between ITC cells are biased to favor interactions in the lateromedial direction.

Figure 7 provides a schematic illustration of this point. The two ITC cells of Fig. 7A were represented in accordance with the average morphological data obtained in this study. However, note that dorsally and ventrally directed processes were not included for simplicity. From this scheme, it is obvious that the particular features of ITC dendrites and axons conspire to polarize the propagation of inhibition lateromedially, in agreement with our physiological observations. Indeed, the longer medial axonal collaterals will predispose ITC cells to influence more ITC cells in the medial than in the lateral direction, particularly since the laterally directed dendrites of recipient cells are longer than medial ones.

In addition, our data suggest that the asymmetric morpho-

FIG. 6. Axon collaterals of ITC neurons. Same orientation as in Fig. 5 (lateral to the left). A: drawing of an ITC axon. The horizontal segment of this axon remained confined in the intermediate capsule but gave rise to 2 dorsally directed collaterals: the most lateral (left) ascended in the CE₉₉ nucleus, whereas the other one (right) coursed medially to the CE₉₉. Microphotographs show selected portions of the axon. Note the presence of numerous varicosities. B1: photomontage illustrating an ITC cell. Arrowheads in B1 indicate the portion of the axon shown in the drawing to the right (B2). This cell did not have a medially directed axon collateral.

FIG. 7. Postulated interactions between ITC cells. A: schematic reconstruction of 2 ITC cells using the average morphological data obtained in this study. For clarity, dorsally and ventrally oriented processes are not shown. B: view of inter-ITC interactions at a macroscopic scale. ITC cells clusters are depicted at their typical position in coronal sections.
logical features of ITC cells might allow interactions between neurons as distant as 600 μm, more than the typical distance between neighboring ITC clusters. This point is illustrated in Fig. 7B, which provides a macroscopic view of potential interactions between neighboring ITC cells masses. In this scheme, the various ITC cell clusters are represented in their typical location. Note that the only zone of potential synaptic interactions between ITC cells belonging to different clusters is that between medial axons and lateral dendrites. In this context, it seems that the role of the shorter lateral axons and medial dendrites might be limited to the formation of synaptic contacts between ITC cells belonging to the same cluster.

One important issue, not addressed in the present study, is the nature of inter-ITC interactions in the rostrocaudal plane and their consequences for impulse traffic between the BL complex and CE nucleus. Future investigations should aim to address this point.

Implications of inter-ITC connections for intra-amygdaloid computations

Figure 8 integrates the findings of the present study with those of Royer et al. (1999) to consider how ITC cells might affect impulse traffic from the BL complex to the CE nucleus. Let us consider an hypothetical case where sensory inputs activate a group of projection cells of the BL nucleus. This BL input will excite both CE<sub>M</sub> cells and the interposed ITC cells. In turn, these ITC cells will generate feed-forward inhibition in CE<sub>M</sub> neurons and, in so doing, attenuate the excitatory effect of the BL inputs. However, if the excitation of BL neurons is preceded by the activation of LA cells, say by thalamic auditory inputs, this attenuation will be reduced or abolished via the activation of laterally located ITC neurons, which, as shown in the present study, can inhibit those located more medially.

Two factors suggest that the cellular interactions involved in the above scenario can be transposed lateromedially (dorsally in rats). First, the position of ITC cells, their projection site in the CE nucleus and the origin of their excitatory afferents in the BL complex are in register (Royer et al. 1999). Second, the connections between ITC cells preferentially run in a lateromedial direction, irrespective of their location in the intermediate capsule (present study).

We speculate that such cellular interactions can implement conditional computations in the amygdala, rendering its throughput sensitive to the timing and origin of sensory inputs. The fact that amygdala lesions can abolish the stereotyped fear reactions of laboratory animals to sensory stimuli which they have never experienced suggest that, hard-wired in the amygdala, are circuits which can carry out such computations. Identifying these circuits and analyzing how (or whether) they are affected by experience are major challenges for future investigations.

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