Published in final edited form as:

Neuroscience. 2008 August 26; 155(3): 959–968. doi:10.1016/j.neuroscience.2008.06.028.

Unconditioned Stimulus Pathways to the Amygdala: Effects of Lesions of the Posterior Intralaminar Thalamus on Footshock-Induced c-Fos Expression in the Subdivisions of the Lateral Amygdala

Enrique Lanuza¹, Jose Moncho-Bogani², and Joseph E. LeDoux³

¹*Dept. de Biologia Cel·lular, Fac. CC. Biològiques, Universitat de València. Burjassot, València 46100. Spain.*

²*Departamento de Ciencias Médicas, Facultad de Medicina-CRIB, Universidad de Castilla-La Mancha. Albacete 02006. Spain*

³*Center for Neural Science, New York University. New York, NY 10003*

Abstract

The lateral nucleus of the amygdala (LA) is a site of convergence for auditory (conditioned stimulus) and footshock (unconditioned stimulus) inputs during fear conditioning. The auditory pathways to LA are well characterized, but less is known about the pathways through which footshock is transmitted. Anatomical tracing and physiological recording studies suggest that the posterior intralaminar thalamic nucleus, which projects to LA, receives both auditory and somatosensory inputs. In the present study we examined the expression of the immediate-early gene *c-fos* in the LA in rats in response to footshock stimulation. We then determined the effects of posterior intralaminar thalamic lesions on footshock-induced c-Fos expression in the LA. Footshock stimulation led to an increase in the density of c-Fos-positive cells in all LA subnuclei in comparison to controls exposed to the conditioning box but not shocked. However, some differences among the dorsolateral, ventrolateral and ventromedial subnuclei were observed. The ventrolateral subnucleus showed a homogeneous activation throughout its antero-posterior extension. In contrast, only the rostral aspect of the ventromedial subnucleus and the central aspect of the dorsolateral subnucleus showed a significant increment in c-Fos expression. The density of c-Fos-labeled cells in all LA subnuclei was also increased in animals placed in the box in comparison to untreated animals. Unilateral electrolytic lesions of the posterior intralaminar thalamic nucleus and the medial division of the medial geniculate body reduced footshock-induced c-Fos activation in the LA ipsilateral to the lesion. The number of c-Fos labeled cells on the lesioned side was reduced to the levels observed in the animals exposed only to the box. These results indicate that the LA is involved in processing information about the footshock unconditioned stimulus and receives this kind of somatosensory information from the posterior intralaminar thalamic nucleus and the medial division of the medial geniculate body.

Keywords

Emotional learning; fear conditioning; pain; freezing; electrolytic lesions; somatosensory thalamus

In recent years classical fear conditioning has become a leading model for studying the neural mechanisms of learning and memory in mammals (LeDoux, 2000; Maren, 2005). In this behavioral paradigm, a neutral (innocuous) conditioned stimulus (CS), such as a tone, is presented in association with an aversive unconditioned stimulus (US), such as a mild footshock. After a few such paired presentations, the animal begins to respond defensively to the neutral stimulus. Considerable evidence suggests the lateral nucleus of the amygdala (LA) is a site of auditory CS and somatosensory US convergence (Romanski et al., 1993; Bordi and LeDoux, 1994b) and is crucial for the formation of the association between the CS and US (Blair et al., 2001; Fanselow and LeDoux, 1999; LeDoux, 2000; Maren, 2001, 2005; but see Cahill et al., 1999). While the CS pathways to LA have been characterized in detail (LeDoux et al., 1990a; LeDoux et al., 1991; Bordi and LeDoux, 1994a), less is known about the origin of the US pathways to LA (see Shi and Davis, 1999; Lanuza et al., 2004).

Anatomical tracing and physiological recording studies suggest that the posterior intralaminar thalamic nucleus (PIN) and the medial division of the medial geniculate body (MGm) receive both auditory and somatosensory inputs (LeDoux et al., 1987; Bordi and LeDoux, 1994b) and project to LA (LeDoux et al., 1990b). While many cells in LA respond to both auditory CS-like and somatosensory US-like stimulation (Romanski et al., 1993), most cells in PIN process one or the other modality rather than both (Bordi and LeDoux, 1994a,b). Moreover, this thalamo-amygdaloid pathway is enriched in stathmin, a protein involved in the expression of learned and innate fear (Shumyatsky et al., 2005). The MGm/PIN complex is thus a likely candidate to provide the LA with information about a footshock US.

The expression of the immediate-early gene *c-fos* is widely used as a marker of cellular activity in the brain (Sagar et al., 1988). In the present study we therefore examined whether foot shock stimulation would increase *c-Fos* expression in different LA subnuclei and whether lesions of MGm/PIN would prevent the footshock-induced *c-Fos* expression. Previous studies on footshock-induced *c-Fos* expression in the amygdala gave rise to contradictory results (see Knapska et al., 2007). Some studies reported increased *c-Fos* expression in the LA after footshock (Schettino and Otto, 2001; Holahan and White, 2004; Knapska et al., 2006), whereas others found no footshock-induced increases in the LA (Pezzone et al., 1992; Smith et al., 1992; Rosen et al., 1998) or in the LA analyzed together with the basal nucleus (Milanovic et al., 1998; Radulovic et al., 1998). Recent anatomical data suggest that the LA is heterogeneous, and that the different subnuclei, and different anteroposterior divisions, may have different roles in fear conditioning (Pitkanen et al., 1997; Doron and LeDoux, 1999). This raises the possibility that footshock might induce differential *c-Fos* expression in subdivisions of LA. Due to the small size of these subdivisions, previous studies may have either been successful or failed to notice increases in *c-Fos* expression depending on the particular area of the LA chosen to count the *c-Fos*-labeled nuclei.

We therefore analyzed in detail footshock-induced *c-Fos* expression in each of the subdivisions of LA throughout the anteroposterior extension of the nucleus. To determine the role of the MGm/PIN complex in relaying US information to the LA, we examined the effects of unilateral electrolytic lesions of this thalamic complex on footshock-induced *c-Fos* activation in the LA ipsilateral to the lesioned thalamus, using the contralateral LA as a control.

EXPERIMENTAL PROCEDURES

Subjects

All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996) and were approved by the New York University Animal Care and Use Committee. Studies were performed using adult male Sprague-Dawley rats (Hilltop Laboratories, Scottsdale, PA)

which weighted 275–300 g on arrival at the laboratory. All rats were housed individually in clear plastic cages, with food and water available ad libitum, and kept on a 12 hours light-dark cycle with lights on at 7:00 am. All experiments were conducted during the light phase of the cycle. Rats were allowed to acclimate to the vivarium for at least five days before the start of the experiments.

Experimental design

Four experimental groups were used. A control, **No-treatment group** of rats ($n = 6$) was carried out to determine the basal c-Fos expression in response to the conditions under which the animals were housed. These rats were sacrificed after living in the animal facility for 10 days without any specific experimental stimulation. Their brains were then processed for immunocytochemical detection of the c-Fos protein. To assess c-Fos expression induced by the surgery procedure and the conditioning apparatus, six rats (**Box group**) received sham surgery and four days later were placed in the conditioning box for 30 minutes. They were sacrificed 90 min after removal from the box. Immunocytochemical detection of c-Fos was then carried out. To characterize the footshock-induced c-Fos expression in the lateral amygdala, a group of nine animals (**Footshock group**) received sham surgery and, five days later, ten footshocks during a 30 minute stay in the conditioning box. Ninety minutes later they were sacrificed for c-Fos immunocytochemistry. Finally, to study the possible role of the posterior intralaminar thalamic complex in relaying footshock information to the amygdala, fourteen rats received unilateral electrolytic lesions of the MGm/PIN complex, and, after five days of recovery, received ten footshocks in the conditioning box. Ninety minutes later they were sacrificed for c-Fos immunocytochemistry. Eight of the fourteen rats had acceptable thalamic lesions (**Lesioned group**), and were included in the cell counting procedure.

Surgery

All surgical procedures were performed under anesthesia with sodium pentobarbital (45 mg/kg i.p.) complemented with atropine sulfate (0.4 mg/kg). Animals were placed in a stereotaxic frame and unilateral electrolytic lesions of the posterior intralaminar thalamic nuclei were performed using coordinates based on the atlas of Paxinos and Watson (1998) and a previous study (Shi and Davis, 1999). Electrolytic lesions were made passing constant (positive) current of 0.8 mA for 10 s through stainless steel electrodes (0.25 mm in diameter) insulated except for 500 μ m of the tip. Since the MGm/PIN complex is a long rostrocaudal structure, three lesions were made at anteroposterior (AP) coordinates -4.8 mm, -5.3 mm, -5.8 mm respectively. All lesions were placed at 2.6 mm from the midline and 6.5 below bregma. Sham electrolytic lesions were made by using the same procedure except that the dorsoventral coordinate was -4.0 mm, and no current was passed. Lesions and sham operations were performed counterbalanced in the left and right hemispheres.

Apparatus and stimulation procedure

Behavioral tests took place in a rodent chamber (Coulbourn Instruments, Lehigh Valley, PA, model E10-10) housed in a sound attenuating cubicle. Each animal on the Footshock and Lesioned groups received 10 footshocks (1 mA, 0.5 seconds each) during 30 minutes delivered through the grid floor of the chamber (shocker model E13-08, Coulbourn Instruments). The average intertrial interval was 160 sec. Freezing behavior, defined as immobility except for respiratory-related movements, was used as an index of fear. Freezing was timed for each animal during the 30 sec following the delivery of the first 5 shocks (post-shock freezing).

c-Fos immunocytochemistry

Ninety minutes after the end of stimulation, each animal was deeply anesthetized with an intraperitoneal injection (75 mg/kg) of sodium pentobarbital and perfused transcardially with

saline solution followed by 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). Following perfusion, the brains were removed and immersed in fixative for 4–6 hours at 4°C. Subsequently, each brain was immersed in a solution of 30% sucrose in phosphate buffer at 4°C until it sank. Serial, coronal 40 µm-thick sections were then cut from each brain with a freezing microtome and collected into six matching series. Alternate series were processed for c-Fos immunocytochemistry, for Nissl staining (with 1% acidic toluidin blue), or for acetylcholinesterase histochemistry (following the procedure given in Paxinos and Watson, 1998). The Nissl and acetylcholinesterase preparations facilitated the accurate localization of the cytoarchitectonic boundaries of the amygdaloid nuclei (especially of the subdivisions of the LA), as well as the extent of the lesion sites in the thalamus.

The tissue sections were processed for immunocytochemistry using the avidin-biotin complex method. Endogenous peroxidase activity in the tissue sections was suppressed by incubation in a 1% H₂O₂ solution for 30 min. The sections were then incubated for 24 hours at 4°C in anti-c-Fos IgG raised in rabbit (Cat. #: sc-52, Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:20000 in Tris buffered saline containing 0.3% Triton X-100 (Sigma, St. Louis, MO) and 2.5% normal goat serum (Vector Labs., Burlingame, CA). The sections were then incubated in biotinylated goat anti-rabbit IgG (Vector Labs.) diluted 1:200 in Tris buffered saline containing 0.3% Triton-X-100 for 2 hours at room temperature, followed by a further 2 hours in the Avidin-Biotin Elite complex (Vectastain Elite kit, Vector Labs.) diluted in similar buffer. The resulting peroxidase labeling was visualized using 0.025% diaminobenzidine (Sigma) as a chromogen in Tris buffer (pH 7.6), with 0.01% H₂O₂. Control sections were treated identically to the immunolabeled sections except for omission of the primary antibody or addition of an excess of blocking peptide (Santa Cruz Biotechnology) to the solution of primary antibody. In both controls we obtained no immunostaining.

Counting of Labeled Cells

Cells labeled with the c-Fos immunocytochemistry were counted using published guidelines (Saper, 1996). Labeled cells were counted in nine representative sections of the LA (one section every six), located approximately at the following anteroposterior coordinates from bregma, following the atlas of Paxinos and Watson (1998): -2.12, -2.30, -2.56, -2.80, -3.14, -3.30, -3.60, -3.80, -4.16. In these sections the subnuclei of the LA were depicted using a camera lucida and the labeled neurons drawn with the 10× objective. The criterion for a labeled neuron was a uniform brown or brown-to-black reaction product within the cell nucleus. The slides were coded so that the experimenter was blind to the experimental treatment of the animals. The results are expressed as mean ± SEM of the number of cells. To assess the density of labeling at each rostrocaudal level, the number of cells of each LA subnuclei was divided by the standard area of that level. Areas were calculated from the atlas of Paxinos and Watson (1998). Results of density are expressed as number of cells/mm².

Statistical analysis

The time that the animals spent freezing was analyzed with a two-way ANOVA, with shock number (shocks 1–5) as a within-subject factor, and group (Lesioned, Footshock) as a between subjects factor.

Regarding the analysis of the number of c-Fos-like immunoreactive (c-Fos-li) cells in LA, no differences were found in c-Fos-li cells in LA between the two hemispheres in every experimental group (one-way ANOVA: No-treatment group: $F_{1,10} = 0.227$; $p > 0.64$; Box group: $F_{1,10} = 0.045$; $p > 0.83$; Footshock group: $F_{1,16} = 0.003$; $p > 0.95$), except for in the group sustaining unilateral thalamic lesions (see Results). Therefore, data on the two hemispheres were pooled together in the No-treatment, Box and Footshock experimental groups. The total number of c-Fos-li cells in LA in the four experimental groups was compared

with a one-way ANOVA analysis. To analyze the density of c-Fos-labeled cells in the three LA subnuclei along the rostrocaudal extent of these structures, a two-way analysis of variance (ANOVA) with repeated measures was used, with the group (No-treatment, Box, Footshock, Lesioned) as between-subjects factor and the rostrocaudal level of the LA subnuclei as within-subjects factor. Finally, to compare the data obtained from the LA ipsilateral to the thalamic lesion with the data from the contralateral LA, an ANOVA with repeated measures was used, with the hemisphere (ipsilateral or contralateral to the lesion) and the rostrocaudal level of the LA subnuclei as within-subjects factors.

In the case of the one-way ANOVA, the differences in the means were further explored with Student-Newman-Keuls post-hoc analyses. In the case of the ANOVAs with repeated measures, post-hoc analyses were done when appropriate by means of multiple pair-wise comparisons with the Bonferroni adjustment.

RESULTS

Unilateral MGm/PIN lesions had no behavioral effect on foot-shock induced freezing

Unilateral lesions of the MGm/PIN complex were included in this study when they encompassed the MGm and the PIN. The largest and smallest lesions are shown in Figure 1. In the largest cases, damaged areas partially included the suprageniculate nucleus, the anterior pretectal nucleus and the posterior thalamic nucleus group. The unilateral thalamic lesions did not affect the freezing behavior elicited by the footshocks (Fig. 2), as revealed by a two-way ANOVA, with shock number (shocks 1–5) as a within-subject factor, and group (Lesioned, Footshock) as a between subjects factor. This analysis revealed no significant group $\times$ shock interaction ($F_{4,60} = 0.46782$, $p > 0.75$), and no significant main effect of the group ($F_{1,15} = 0.79788$, $p > 0.38$). There was a significant effect of the shock ($F_{4,60} = 12.0824$, $p < 0.0001$), due to the fact that freezing was relatively low after the first shock and increased with subsequent shocks.

Lesions of the MGm/PIN complex decreased c-Fos expression in the LA to the levels induced by the exposure to the conditioning box without foot-shock

The one-way ANOVA analysis of the level of c-Fos expression in the whole LA nucleus (Fig. 3A) revealed significant differences among the four groups ($F_{3,25} = 10.98$; $p < 0.0001$). The S-N-K post-hoc analysis showed that the c-Fos expression was significantly increased by the foot-shocks in the intact animals (Fig 4B) compared to the control animals exposed only to the conditioning box (Fig 4C) or non-treated (Fig 4D) (Table 1). Notably, the unilateral lesions of the MGm/PIT complex significantly attenuated the levels of footshock-induced c-Fos in the LA ipsilateral to the lesion (Fig 4A), so that the levels of c-Fos expression did not differ significantly from those observed in the animals exposed to the conditioning box or non-treated (Fig. 3A and Fig. 4). The levels of c-Fos expression in the Box group were significantly higher than those observed in the No-treatment group (Table 1), and therefore the manipulation and the exposure to the test cage importantly induced c-Fos expression in the LA.

The significant decrease of c-Fos expressing cells that results from the thalamic lesion is also revealed by the one-way ANOVA analysis comparing the level of c-Fos induction in the LA ipsilateral to the lesioned thalamus with the contralateral LA ($F_{1,14} = 6.2613$; $p < 0.05$; Fig. 3B).

Differential effects of lesions of the MGm/PIN complex in footshock-induced c-Fos expression in LA subnuclei

Dorsolateral (DL) subnucleus—The ANOVA analysis of the levels of c-Fos expression in the dorsolateral subnucleus of the LA in the four experimental groups revealed significant

main effects of the factors “group” ($F_{3,25} = 11.545$; $p < 0.001$) and “anteroposterior level” ($F_{8,18} = 5.82$; $p < 0.001$), as well as a significant interaction between these two factors ($F_{24,60} = 2.04$; $p < 0.05$). Therefore, the different experimental treatments induced changes in the level of c-Fos expression in this subnucleus and this induction was heterogeneous throughout the anteroposterior extent of the structure (Fig. 5). The corresponding post-hoc analysis of the significant effects revealed that the expression of the c-Fos protein in the DL subnucleus in the animals sacrificed ten days after arrival to the laboratory, without any stimulation (No-treatment group), was very low and homogeneous throughout its nine anteroposterior levels (non-significant simple effect of anteroposterior level in this group, $p > 0.99$). In contrast, rats placed in the conditioning box for 30 minutes, without receiving footshocks, showed a noticeable level of c-Fos expression in the DL (and also in many other forebrain areas). This expression was heterogeneous throughout the antero-posterior axis (significant simple effect of anteroposterior level in the Box group, $p < 0.05$). However, the density of c-Fos-li cells was only significantly higher than that of the No-treatment group in the two caudalmost sampled levels (coordinates -3.8 and -4.16 mm from bregma, $p < 0.05$ in both comparisons) (Fig. 5).

Footshock stimulation induced a strong c-Fos expression in DL that was low at the rostral levels, increased steadily caudally, and was highest in the caudal levels (significant simple effect of anteroposterior level in the Footshock group, $p < 0.001$). The increase in the expression of c-Fos was particularly relevant, as compared with the box group, in the central aspect of the dorsolateral subnuclei, at the levels -2.56 and -2.80 mm from bregma ($p < 0.05$ in both cases).

The electrolytic thalamic lesions strongly diminished the expression of c-Fos in the DL, eliminating the anteroposterior heterogeneity (non-significant simple effect of anteroposterior level in the Lesioned group, $p > 0.42$) and attenuating the c-Fos expression to the levels observed in the Box group, except for the caudalmost level (coordinate -4.16 mm from bregma) in which the c-Fos expression was even significantly lower than that observed at the corresponding level of the Box group (Fig. 5). In fact, the level of c-Fos expression in the DL of the Lesion group was significantly lower than that obtained in the Footshock group in all of the anteroposterior levels analyzed ($p < 0.05$ in every case). Finally, the levels of c-Fos expression of the Lesion and the No-treatment groups did not differ significantly in any of the comparisons of the corresponding anteroposterior levels ($p > 0.05$ for all cases).

The analysis of the level of c-Fos expression in the DL ipsilateral to the lesioned thalamus compared to the contralateral DL revealed significant effects of the factors “Hemisphere” ($F_{1,7} = 6.59$; $p < 0.05$) and “anteroposterior level” ($F_{2,14.4} = 6.39$; $p < 0.05$), as well as a significant interaction ($F_{2.39,16.78} = 4.3$; $p < 0.05$) between these two factors (in the latter two cases assuming sphericity, with the Greenhouse-Geisser correction of the degrees of freedom). Therefore, the thalamic lesion induced a significant reduction in the level of footshock-induced c-Fos expression in the DL ipsilateral to the lesion compared to the contralateral side. The post-hoc analysis of the comparison between hemispheres showed that the differences were significant in the anteroposterior levels -2.3 and -4.16 mm from bregma ($p < 0.05$ in both cases), and approached significance at the levels -2.8 mm ($p = 0.064$), -3.14 mm ($p = 0.052$) and -3.8 mm ($p = 0.052$) from bregma.

Ventrolateral (VL) subnucleus—The ANOVA analysis of the levels of c-Fos expression in the ventrolateral subnucleus of the LA in the four experimental groups revealed a significant main effect of the factor “group” ($F_{3,25} = 9.16$; $p < 0.001$) but no effect of the factor “anteroposterior level” ($F_{4,22} = 0.58$; $p > 0.6$), and no significant interaction between these two factors ($F_{12,72} = 0.84$; $p > 0.6$). Therefore, the level of c-Fos expression in this subnucleus was affected by the different treatments but no heterogeneity in the anteroposterior extent of the structure was present (Fig. 6). Since the interaction between the “group” and the

“anteroposterior level” resulted non-significant, the post-hoc comparisons performed were the among the four groups without taking into account the values at the five anteroposterior levels. This post-hoc analysis revealed that no significant differences were present in the expression of the c-Fos protein in the VL subnuclei in the No-treatment, Box and Lesioned groups ($p > 0.49$ in the different comparisons). Therefore, in contrast with the DL, the VL subnucleus was not activated by the exposure to the conditioning box and, similar to the result obtained in the DL, the thalamic lesions attenuated the expression of c-Fos to the levels observed in the Box and No-treatment groups. Footshock stimulation induced a strong c-Fos expression in the VL, making it significantly different to the Box ($p < 0.05$), to the Lesioned ($p < 0.01$) and to the No-treatment ($p < 0.001$) groups (Fig. 6).

The comparison of the footshock-induced c-Fos expression in the VL ipsilateral to the lesioned thalamus compared to the contralateral VL revealed a significant main effect of the factor “Hemisphere” ($F_{1,7} = 7.16$; $p < 0.05$), and no significant effects of the “anteroposterior level” ($F_{4,4} = 0.76$; $p > 0.6$) neither of the “Hemisphere $\times$ anteroposterior level” interaction ($F_{4,4} = 2.59$; $p > 0.1$). Therefore, the lesion of the MGm/PIN thalamic complex induced a significant reduction in the level of footshock-induced c-Fos expression in the VL ipsilateral to the lesion compared to the contralateral side but, consistent with the comparison between groups, there was no effect of the anteroposterior level.

Ventromedial (VM) subnucleus—The ANOVA analysis of the levels of c-Fos expression in the ventromedial subnucleus of the LA in the four experimental groups revealed significant main effects of the factors “group” ($F_{3,25} = 11.06$; $p < 0.001$) and “anteroposterior level” ($F_{6,20} = 3.29$; $p < 0.05$), as well as a marginally significant interaction between these two factors ($F_{18,66} = 1.76$; $p = 0.05$). The interaction between these two factors was positively significant assuming sphericity, which could be done with the Greenhouse-Geisser correction of the degrees of freedom ($F_{8,6,71,9} = 3,492$; $p < 0.01$). Therefore, the experimental treatments induced significant changes in the level of c-Fos expression in this subnucleus and this changes showed heterogeneity throughout the anteroposterior extent of the structure (Fig. 7). The analyses of the simple effects of the anteroposterior level revealed that this factor had a significant effect only the Footshock group ($p > 0.1$ for the No-treatment, Box and Lesioned groups; $p < 0.001$ for the Footshock group). The pos-hoc pair-wise comparisons among groups revealed that the expression of the c-Fos protein in the VM subnucleus in the animals sacrificed ten days after arrival to the laboratory, without any stimulation (No-treatment group), was similar to that observed in the VM of the animals with thalamic lesions (Lesioned group) throughout its seven anteroposterior levels (Fig. 7).

Rats placed in the conditioning box for 30 minutes, without receiving footshocks, showed a conspicuous level of c-Fos expression in the VM. In fact, the density of c-Fos-li cells was significantly higher than that observed at the corresponding levels in the No-treatment group at the caudal aspect of the subnucleus (coordinates -3.3 , -3.6 and -3.8 mm from bregma). Therefore, in contrast to the VL and similar to the DL, the VM is activated by the exposure to the conditioning box in its caudal levels (Fig. 7).

Footshock stimulation induced a noticeable c-Fos expression in the rostral aspect of the VM as compared to the group exposed to the conditioning box. The comparison of the density of c-Fos-labeled cells revealed a significantly higher expression of footshock-induced c-Fos in the anteroposterior coordinate -2.56 ($p < 0.05$) and a marginally significant difference at -2.8 mm ($p = 0.063$) (Fig. 7). Noteworthy, these are the same levels at which the footshock induced a significant effect in the DL subnucleus. In the animals with thalamic lesions the expression of c-Fos was attenuated to the levels observed in the Box and the No-treatment groups (no differences of the Lesioned groups with these two groups were significant, $p > 0.1$ in all cases).

Therefore, the MGM/PIN lesions abolished the differential activation observed in the rostral aspect of this subnucleus.

The analysis of the footshock-induced c-Fos expression in the VM ipsilateral to the lesioned thalamus compared to the contralateral VM revealed a significant effect of the factor "Hemisphere" ($F_{1,7} = 13.45$; $p < 0.01$), a marginally significant effect of the "anteroposterior level" ($F_{6,2} = 13.47$; $p = 0.71$) and no significant "Hemisphere $\times$ anteroposterior level" interaction ($F_{6,2} = 1.47$; $p > 0.4$). Therefore, consistent with the results obtained in the comparison between groups, in the VM subnucleus the thalamic lesion abolished the footshock-induced c-Fos expression compared to the contralateral side.

DISCUSSION

The present study provides, as far as we know, the first evidence showing that footshock (the most common aversive stimulus in fear conditioning) induces a heterogeneous expression of c-Fos in the LA both in terms of the LA subnuclei and also regarding the anteroposterior extent of this structure. Previous studies of the expression of c-Fos after footshock produced contradictory findings. Campeau et al. (1991) found that footshock induced a significant elevation of *c-fos* mRNA in the amygdala as a whole (without considering different nuclei), whereas Smith et al. (1992), using in situ hybridization of *c-fos* mRNA, found no effect in the amygdala after footshock. Other studies reported that footshock induced c-Fos expression in some amygdaloid nuclei such as the medial (Pezzone et al., 1992; Rosen et al., 1998) or the central nucleus (Milanovic et al., 1998; Radulovic et al., 1998). Recently, several studies (Schettino and Otto, 2001; Holahan and White, 2004; Knapska et al., 2006) have reported a footshock-induced c-Fos expression in the LA, but the possible differences among the LA subdivisions were not studied. The fact that the rest of previous studies found no footshock-induced c-Fos expression in LA is unlikely to be due to the footshock parameters used. Our shock parameters (intensity: 1 mA; duration: 0.5 sec; number of shocks: 10) are well within the range of intensity (0.7–2 mA), duration (0.5–2 sec) and number of shocks (1–30) used in the other studies (Pezzone et al., 1992; Smith et al., 1992; Milanovic et al., 1998; Radulovic et al., 1998; Rosen et al., 1998). More likely, the difference with past studies is due to anatomical sampling issues. The use of small sample areas, which may in some cases accompany an inconsistent sampling from different levels of the amygdala, probably contributes to the discrepancies obtained, since, as shown in this study, the footshock-induced c-Fos expression is heterogeneous within the LA. In fact, one of the studies that reported a significant footshock-induced c-Fos expression in the LA (Holahan and White, 2004) chose the coordinate 2.8 mm posterior to bregma to count the c-Fos-positive nuclei. As can be observed in Fig. 5, Fig. 6 and Fig. 7, it is this anteroposterior level the one showing a significant footshock-induced c-Fos expression in the three LA subnuclei. Anatomical data on the thalamic projections to the LA has also revealed the organization of LA into anterior and posterior segments (Doron and LeDoux, 1999), and the present results confirmed the functional relevance of this regional heterogeneity.

The results of the present work, together with those of previous studies (Schettino and Otto, 2001; Holahan and White, 2004; Knapska et al., 2006), suggest that the c-Fos expressing neurons in the LA mediate a representation of the footshock unconditioned stimulus, in addition to the associative role that they perform in different fear conditioning tasks. Other lines of evidence give support to this view. First, electrophysiological data have shown the presence of cells in the LA (especially in the VL) that respond exclusively to somatosensory (foot-shock) stimulation (Romanski et al., 1993). Secondly, some of thalamic cells in the MGM/PIN complex that project to the amygdala respond solely to somatosensory stimuli (Bordi and LeDoux, 1994b). Finally, recent behavioral data have revealed that bilateral lesions (or inactivation) of the LA impaired the unconditioned responses induced by a brief train of eyelid

shocks (Blair et al., 2005). Altogether, these evidences strongly support the hypothesis that the LA processes at least some aspects (maybe the emotional properties) of aversive unconditioned stimuli.

Significant c-Fos expression occurred in LA not only in response to footshock but also as a consequence of placing the animals in a novel environment such as the conditioning box. However, the level of c-Fos expression induced by the conditioning box was significantly less than that induced by the shocks. Previous studies found that novelty induces increases in c-Fos expression in a variety of brain regions, including the amygdala (Handa et al., 1993; Papa et al., 1993; Radulovic et al., 1998), although none of the previous studies described this phenomenon for the LA. Nevertheless, unit recording studies show that LA cells respond briskly to novel stimuli and that they rapidly habituate (Bordi and LeDoux, 1992). FMRI studies in humans also show amygdala responses to novel stimuli (Breiter and Rauch, 1996). Our results further refined the description of this activation showing that novelty-induced c-Fos expression takes place exclusively in the caudal aspect of the DL and VM subnuclei. This finding is consistent with the fact that the caudal LA receives the strongest projections to this nucleus originated by the hippocampal formation (McDonald, 1998). This amygdalo-hippocampal projection has been previously implicated in processing novel stimuli (O'Keefe and Nadel, 1978). Interestingly, contextual fear conditioning occurs more easily in new environments, and extensive context pre-exposure diminishes the strength of conditioning (Killcross et al., 1998), a process referred to as latent inhibition (Lubow and Moore, 1959). In contrast, a short pre-exposure to the conditioning context is necessary for the normal acquisition of contextual fear conditioning. Thus, delivering the footshock immediately after placing the animal in the conditioning box results a deficit in context-induced freezing in later tests (Fanselow, 1986). The immediate shock deficit is probably due to an inability to form a representation of the contextual stimuli prior to receiving the shock (Matus-Amat et al., 2004; Landeira-Fernandez et al., 2006). The present results show that the acquisition of contextual information that takes place during context pre-exposure activates c-Fos expression in the LA. This c-Fos activation may reflect plasticity in the LA related to the novel context needed for the acquisition of contextual fear conditioning if footshock or other aversive stimulation occurs, or, on the contrary, needed for the animal to learn that the new context is safe if no aversive stimulus occur, as it is the case in our Box group.

The footshocks induced high levels of c-Fos expression in the whole VL, in the anterior subdivision of the VM and in the middle DL subnuclei of the LA (Fig. 5, Fig. 6 and Fig. 7), whereas in the posterior part of the DL the footshock-induced c-Fos expression is approximately similar to that induced by the new environment. Note that the VL subnuclei is present only at the rostral and middle levels of the LA, matching approximately the anteroposterior location of the parts of the DL and VM activated by the footshocks.

One of the main candidates to send somatosensory information to the anterior part of the LA is the posterior intralaminar thalamic nucleus (LeDoux et al., 1987; Doron and LeDoux, 1999; Shi and Davis, 1999). The anterior division of the LA receives an important projection from the PIN, which originates in the whole nucleus, but especially in its dorsal tip (Doron and LeDoux, 1999). This part of the PIN has been shown to receive convergent projections from the spinal cord and the inferior colliculus (LeDoux et al., 1987). In contrast, the posterior division of the LA receives a lighter projection from the PIN, which arises mainly from its ventrolateral wing (Doron and LeDoux, 1999), which does not receive spinal cord input (LeDoux et al., 1987). On the other hand, the disgranular part of the parietal insular cortex has been suggested to play an important role in relaying nociceptive information to the amygdala (Shi and Cassell, 1998; Shi and Davis, 1999). This structure projects to the entire rostrocaudal extent of the LA, but also with a preference for the anterior part (Shi and Cassell, 1998), and therefore may contribute to the c-Fos activation found in the LA after footshock.

Our results from the central DL and anterior VM subnuclei and the VL subnucleus are in agreement with the available anatomical data on the somatosensory projections to the LA. The data suggest that the anterior part of the LA receives somatosensory (or nociceptive) information derived from the footshock, whereas the caudal part of the LA receives novelty (possibly context-related) information.

To further clarify the possible role of the MGm/PIN complex in relaying pain information to the LA, we performed unilateral electrolytic lesions of this structure and characterized c-Fos expression in the LA ipsilateral to the lesion, using as controls both the contralateral side and the rest of the experimental groups. This approach is possible because the thalamo-amygdaloid projections are strictly ipsilateral (Ottersen and Ben-Ari, 1979; LeDoux et al., 1985, 1990b, 1991; Turner and Henkerham, 1991) and the LA does not have commissural connections (Pitkanen et al., 1995).

Both the comparison with the the contralateral hemisphere and the comparison with the rest of the experimental groups revealed that the MGm/PIN lesions decreased the number of c-Fos-positive cells in the LA ipsilateral to the lesioned thalamus, reducing it to the level seen in LA following exposure to the conditioning box. These results suggest that, while the information about the new environment (the conditioning box) is normally reaching the LA from unimodal or multimodal (including somatosensory) cortical areas and from the hippocampus (McDonald, 1998), the thalamic lesion affected the direct transmission of nociceptive information from the thalamus to the LA. This supports previous ideas of the MGm/PIN complex being an important relay station on the transfer of somatosensory and nociceptive information to the LA (LeDoux et al., 1987; Romanski et al., 1993; Bordi and LeDoux, 1994b; Shi and Davis, 1999). However, it should be kept in mind that electrolytic lesions affect both cells and fibers of passage and therefore clarification of the roles of these two components needs further study. In this regard, it is relevant to point out that the lesions used in this work were substantially smaller and more centered on the MGm/PIN than those used in a previous study (Lanuza et al., 2004), and therefore did not affect in a large extent the spinothalamic fibers running medial to the MGm/PIN complex.

In the animals sustaining MGm/PIN thalamic lesions, the comparison of c-Fos expression in the different LA subnuclei of the lesioned hemisphere with the corresponding structure in the contralateral LA revealed a somewhat minor effect of the lesions than that observed in the comparison with the intact animals in the other experimental conditions. Specifically, the effect of the thalamic lesion on the heterogeneous c-Fos expression in the different anteroposterior levels of the VM, that was significant in the comparison with the rest of the experimental groups, was not significant when compared with the contralateral VM. These discrepancies can be attributed to the existence of important reciprocal connections between different amygdaloid nuclei, such as the basal and accessory basal nuclei (Savander et al., 1997) that are in turn strongly interconnected with the LA (Pitkanen et al., 1997). Therefore, the diminished activation of the amygdala ipsilateral to the lesion may affect the neural activity in the contralateral amygdala.

The thalamic lesions affected c-Fos expression in the whole rostrocaudal extension of the LA, and in some instances the level of c-Fos was even decreased in comparison with the Box group (Figure 5, Figure 6, and Figure 7). Since the somatosensory part of the PIN projects to the anterior LA division, the lesion may be expected to cause a stronger decrease in that territory. However, it has to be remembered that the MGm/PIN is a multimodal complex, receiving visual (Yamasaki et al., 1986; Linke et al., 1999) and viscerosensitive (Saper and Loewy, 1980; Yasui et al., 1989) inputs in addition to auditory and somatosensory information (LeDoux et al., 1987). Therefore the MGm/PIN lesion apparently caused a general deficit of sensory

inputs to the LA, which may be the reason for the reduction of c-Fos expression in the whole rostrocaudal extent of the nucleus.

The unilateral thalamic lesions did not affect post-shock freezing (Fig. 2). Thus, this behavioral measure did not correlate with the decreased expression of c-Fos caused by the lesion. Although unilateral amygdala lesions have been shown to partially disrupt auditory fear conditioning (LaBar and LeDoux, 1996), unilateral lesions of MGm/PIN do not appear to have an effect on this task (Romanski and LeDoux, 1992). However, post-shock freezing was not assessed in that previous study. Bilateral electrolytic thalamic lesions impair post-shock freezing when they are large enough as to include the MGm/PIN and the medial subparafascicular and the posterior triangular nuclei (Lanuza et al., 2004), thus suggesting that other structures along with the MGm/PIN are involved in the transmission of pain information to the amygdala. It has been suggested that nociceptive information may also travel to the LA through an indirect thalamo-cortico-amygdala route (Shi and Cassell, 1998; Shi and Davis, 1999), originating in the ventral posterolateral thalamic nucleus (which is unaffected by the lesions) or other alternative pathways (Lanuza et al., 2004). Therefore the lesions used in the current study might not totally deprive the LA from its somatosensory input, allowing the behavioral response (freezing) to be unaffected.

Finally, footshock-induced c-Fos expression in the LA may also be related to the acquisition of contextual fear conditioning. The behavioral procedure used in the present study induced strong freezing behavior during the time spent in the conditioning box, and therefore a strong fear to the context. A previous study has dissociated these two experimental situations (footshock and contextual fear conditioning) using the immediate shock deficit paradigm. In this paradigm, foot-shock given immediately after the rat is placed in the chamber fails to produce context conditioning and is thus a way to assess the effects of the foot-shock itself. The results show that the expression of the immediate-early genes *egr-1* and *egr-3* increases in the dorsolateral aspect of the LA after both footshock and contextual conditioning, with a larger increase in the expression of *egr-1* in the case of contextual conditioning (Malkani and Rosen, 2000). The effects of footshock and those of the acquisition of contextual fear conditioning on c-Fos expression are indistinguishable at the present level of analysis.

In summary, the unconditioned stimulus (footshock) induces neuronal activation (c-Fos expression) in the three subnuclei of the LA. This induction shows a regionalization in the antero-posterior axis, being significant in the intermediate levels of the DL and in the anterior levels of the VM. In contrast, the VL subnucleus shows homogeneous c-Fos induction throughout. Lesions of the MGm/PIN thalamic complex suggest that its projections to the LA are involved in the footshock-induced c-Fos expression.

Acknowledgments

This research was supported in part by National Institute of Health grants R01 MH46516, R37 MH38774, P50 MH58911, and K05 MH067048, by a grant of the W.M. Keck Foundation to New York University. EL received a Fulbright-Spanish Ministry of Education and Science postdoctoral fellowship. The authors are grateful to Dr. Fernando Martínez-García for reading and commenting on the manuscript, and to Guillermo Ayala (Dept. of Statistics, Univ. of Valencia) for his help with statistics.

REFERENCES

- Blair HT, Schafe GE, Bauer EP, Rodrigues SM, LeDoux JE. Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. *Learn Mem* 2001;8:229–242. [PubMed: 11584069]
- Blair HT, Sotres-Bayon F, Moita MA, Ledoux JE. The lateral amygdala processes the value of conditioned and unconditioned aversive stimuli. *Neuroscience* 2005;133:561–569. [PubMed: 15878802]

- Bordi F, LeDoux JE. Sensory tuning beyond the sensory system: an initial analysis of auditory response properties of neurons in the lateral amygdaloid nucleus and overlying areas of the striatum. *J Neurosci* 1992;12:2493–2503. [PubMed: 1613543]
- Bordi F, LeDoux JE. Response properties of single units in areas of rat auditory thalamus that project to the amygdala. I. Acoustic discharge patterns and frequency receptive fields. *Exp Brain Res* 1994a; 98:261–274. [PubMed: 8050512]
- Bordi F, LeDoux JE. Response properties of single units in areas of rat auditory thalamus that project to the amygdala. II. Cells receiving convergent auditory and somatosensory inputs and cells antidromically activated by amygdala stimulation. *Exp Brain Res* 1994b;98:275–286. [PubMed: 8050513]
- Breiter HC, Rauch SL. Functional MRI and the study of OCD: from symptom provocation to cognitive-behavioral probes of cortico-striatal systems and the amygdala. *Neuroimage* 1996;4:127–138.
- Cahill L, Weinberger NM, Roozendaal B, McGaugh JL. Is the amygdala a locus of 'conditioned fear'? Some questions and caveats. *Neuron* 1999;23:227–228. [PubMed: 10399929]
- Campeau S, Hayward MD, Hope BT, Rosen JB, Nestler EJ, Davis M. Induction of the c-fos proto-oncogene in rat amygdala during unconditioned and conditioned fear. *Brain Res* 1991;565:349–352. [PubMed: 1842702]
- Doron NN, LeDoux JE. Organization of projections to the lateral amygdala from auditory and visual areas of the thalamus in the rat. *J Comp Neurol* 1999;412:383–409. [PubMed: 10441229]
- Fanselow MS. Associative vs topographical accounts of the immediate shock-freezing deficit in rats: implications for the response selection rules governing species-specific defensive reactions. *Learn Motiv* 1986;17:16–39.
- Fanselow MS, LeDoux JE. Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron* 1999;23:229–232. [PubMed: 10399930]
- Handa RJ, Nunley KM, Bollnow RM. Induction of c-fos mRNA in the brain and anterior pituitary gland by a novel environment. *NeuroReport* 1993;4:1079–1082. [PubMed: 8219031]
- Holahan, White NW. Amygdala c-Fos induction corresponds to unconditioned and conditioned aversive stimuli but not to freezing. *Behav Brain Res* 2004;152:109–120. [PubMed: 15135974]
- Killcross AS, Kiernan MJ, Dwyer D, Westbrook RF. Loss of latent inhibition of contextual conditioning following non-reinforced context exposure in rats. *Q J Exp Psychol* 1998;51B:75–90.
- Knapka E, Nikolaev E, Boguszewski P, Walasek G, Blaszczyk J, Kaczmarek L, Werka T. Between-subject transfer of emotional information evokes specific pattern of amygdala activation. *Proc Natl Acad Sci* 2006;103:3858–3862. [PubMed: 16497832]
- Knapka E, Radwanska K, Werka T, Kaczmarek L. Functional internal complexity of amygdala: focus on gene activity mapping after behavioral training and drugs of abuse. *Physiol Rev* 2007;87:1113–1173. [PubMed: 17928582]
- LaBar KS, LeDoux JE. Partial disruption of fear conditioning in rats with unilateral amygdala damage: correspondence with unilateral temporal lobectomy in humans. *Behav Neurosci* 1996;110:991–997. [PubMed: 8919001]
- Landeira-Fernandez J, DeCola JP, Kim JJ, Fanselow MS. Immediate shock deficit in fear conditioning: effects of shock manipulations. *Behav Neurosci* 2006;120:873–879. [PubMed: 16893293]
- Lanuza E, Nader K, LeDoux JE. Unconditioned stimulus pathways to the amygdala: effects of posterior thalamic and cortical lesions on fear conditioning. *Neuroscience* 2004;125:305–315. [PubMed: 15062974]
- LeDoux JE. Emotion circuits in the brain. *Annu Rev Neurosci* 2000;23:155–184. [PubMed: 10845062]
- LeDoux JE, Ruggiero DA, Reis DJ. Projections to the subcortical forebrain from anatomically defined regions of the medial geniculate body in the rat. *J Comp Neurol* 1985;242:182–213. [PubMed: 4086664]
- LeDoux JE, Ruggiero DA, Forest R, Stornetta R, Reis DJ. Topographic organization of convergent projections to the thalamus from the inferior colliculus and spinal cord in the rat. *J Comp Neurol* 1987;264:123–146. [PubMed: 2445791]
- LeDoux JE, Cicchetti P, Xagoraris A, Romanski LM. The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. *J Neurosci* 1990a;10:1062–1069. [PubMed: 2329367]

- LeDoux JE, Farb C, Ruggiero DA. Topographic organization of neurons in the acoustic thalamus that project to the amygdala. *J Neurosci* 1990b;10:1043–1054. [PubMed: 2158523]
- LeDoux JE, Farb CR, Romanski LM. Overlapping projections to the amygdala and striatum from auditory processing areas of the thalamus and cortex. *Neurosci Lett* 1991;134:139–144. [PubMed: 1815147]
- Linke R, De Lima AD, Schwegler H, Pape H-C. Direct synaptic connections of axons from superior colliculus with identified thalamo-amygdaloid projection neurons in the rat: possible substrates of a subcortical visual pathway to the amygdala. *J Comp Neurol* 1999;403:158–170. [PubMed: 9886041]
- Lubow RE, Moore AU. Latent inhibition: the effect of nonreinforced pre-exposure to the conditional stimulus. *J Comp Physiol Psychol* 1959;52:415–419. [PubMed: 14418647]
- Malkani S, Rosen JR. Specific induction of early growth response gene 1 in the lateral nucleus of the amygdala following contextual fear conditioning in rats. *Neuroscience* 2000;97:693–702. [PubMed: 10842014]
- Maren S. Neurobiology of Pavlovian fear conditioning. *Annu Rev Neurosci* 2001;24:897–931. [PubMed: 11520922]
- Maren S. Synaptic mechanisms of associative memory in the amygdala. *Neuron* 2005;47:783–786. [PubMed: 16157273]
- Matus-Amat P, Higgins EA, Barrientos RM, Rudy JW. The role of the dorsal hippocampus in the acquisition and retrieval of context memory representations. *J Neurosci* 2004;24:2431–2439. [PubMed: 15014118]
- McDonald AJ. Cortical pathways to the mammalian amygdala. *Prog Neurobiol* 1998;55:257–332. [PubMed: 9643556]
- Milanovic S, Radulovic J, Laban O, Stiedl O, Henn F, Spiess J. Production of the Fos protein after contextual fear conditioning of C57BL/6N mice. *Brain Res* 1998;784:37–47. [PubMed: 9518543]
- O'Keefe, J.; Nadel, L. *The Hippocampus as a Cognitive Map*. Oxford, UK: Clarendon Press; 1978.
- Ottersen OP, Ben-Ari Y. Afferent connections to the amygdaloid cortex of the rat and cat: I. Projections from the thalamus. *J Comp Neurol* 1979;187:401–424. [PubMed: 489786]
- Papa M, Pellicano MP, Welzl H, Sadile AG. Distributed changes in c-fos and c-jun immunoreactivity in the rat brain associated with arousal and habituation to novelty. *Brain Res Bull* 1993;32:509–515. [PubMed: 8221144]
- Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates*. 4th ed. San Diego, CA: Academic Press; 1998.
- Pezzone MA, Lee WS, Hoffman GE, Rabin BS. Induction of c-fos immunoreactivity in the rat forebrain by conditioned and unconditioned aversive stimuli. *Brain Res* 1992;597:41–50. [PubMed: 1477734]
- Pitkanen A, Stefanacci L, Farb CR, Go GG, LeDoux JE, Amaral DG. Intrinsic connections of the rat amygdaloid complex: projections originating in the lateral nucleus. *J Comp Neurol* 1995;356:288–310. [PubMed: 7629320]
- Pitkänen A, Savander V, LeDoux JE. Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. *Trends Neurosci* 1997;20:517–523. [PubMed: 9364666]
- Radulovic J, Kammermeier J, Spiess J. Relationship between Fos production and classical fear conditioning: effects of novelty, latent inhibition, and unconditioned stimulus preexposure. *J Neurosci* 1998;18:7452–7461. [PubMed: 9736664]
- Romanski LM, LeDoux JE. Equipotentiality of thalamo-amygdala and thalamo-cortico-amygdala circuits in auditory fear conditioning. *J Neurosci* 1992;12:4501–4509. [PubMed: 1331362]
- Romanski LM, Clugnet MC, Bordi F, LeDoux JE. Somatosensory and auditory convergence in the lateral nucleus of the amygdala. *Behav Neurosci* 1993;107:444–450. [PubMed: 8329134]
- Rosen JB, Fanselow MS, Young SL, Sitcoske M, Maren S. Immediate-early gene expression in the amygdala following footshock stress and contextual fear conditioning. *Brain Res* 1998;796:132–142. [PubMed: 9689463]
- Sagar SM, Sharp SR, Curran T. Expression of c-fos protein in the brain: metabolic mapping at the cellular level. *Science* 1988;240:1328–1331. [PubMed: 3131879]
- Saper CB. Any Way You Cut It: A New Journal Policy for the Use of Unbiased Counting Methods. *J. Comp. Neurol* 1996;364:5. [PubMed: 8789271]

- Saper CB, Loewy AD. Efferent connections of the parabrachial nucleus in the rat. *Brain Res* 1980;197:291–317. [PubMed: 7407557]
- Savander V, LeDoux JE, Pitkanen A. Interamygdaloid projections of the basal and accessory basal nuclei of the rat amygdaloid complex. *Neuroscience* 1997;76:725–735. [PubMed: 9135046]
- Schettino LF, Otto T. Patterns of Fos expression in the amygdala and ventral perirhinal cortex induced by training in an olfactory fear conditioning paradigm. *Behav Neurosci* 2001;115:1257–1272. [PubMed: 11770057]
- Shi C-J, Cassell MD. Cascade projections from somatosensory cortex to the rat basolateral amygdala via the parietal insular cortex. *J Comp Neurol* 1998;399:469–491. [PubMed: 9741478]
- Shi C-J, Davis M. Pain pathways involved in fear conditioning measured with fear-potentiated startle: lesion studies. *J Neurosci* 1999;19:420–430. [PubMed: 9870970]
- Shumyatsky GP, Malleret G, Shin RM, Takizawa S, Tully K, Tsvetkov E, Zakharenko SS, Joseph J, Vronskaya S, Yin D, Schubart UK, Kandel ER, Bolshakov VY. Stathmin, a gene enriched in the amygdala, controls both learned and innate fear. *Cell* 2005;123:697–709. [PubMed: 16286011]
- Smith MA, Banerjee S, Gold PW, Glowa J. Induction of c-fos mRNA in rat brain by conditioned and unconditioned stressors. *Brain Res* 1992;578:135–141. [PubMed: 1511271]
- Turner BH, Henkerham M. Thalamoamygdaloid projections in the rat: A test of the amygdala's sensory processing. *J Comp Neurol* 1991;313:295–325. [PubMed: 1765584]
- Yamasaki DG, Krauthamer GM, Rhoades RW. Superior collicular projection to intralaminar thalamus in rat. *Brain Res* 1986;378:223–233. [PubMed: 3730874]
- Yasui Y, Saper CB, Cechetto DF. Calcitonin gene-related peptide immunoreactivity in the visceral sensory cortex, thalamus and related pathways in the rat. *J Comp Neurol* 1989;290:487–501. [PubMed: 2613940]

ABBREVIATIONS

CS, Conditioned stimulus; DL, Dorsolateral subnucleus of the LA; LA, Lateral nucleus of the amygdala; MGm, Medial division of the medial geniculate body; PIN, Posterior intralaminar thalamic nucleus; US, Unconditioned stimulus; VL, Ventrolateral subnucleus of the LA; VM, Ventromedial subnucleus of the LA.

Figure 1.

Photographs of the largest (A) and smallest (B) lesions of the thalamic MGm/PIN complex included in this study. Abbreviations: APT: anterior pretectal nucleus; csc: commissure of the superior colliculus; DpMe: deep mesencephalic nucleus; Hipp: hippocampus; MGd: medial geniculate nucleus, dorsal part; MGv: medial geniculate nucleus, ventral part; ml: medial lemniscus; PAG: periaqueductal gray; PoT: posterior thalamic nuclear group, triangular part; SC: superior colliculus; SG: supragenicular thalamic nucleus; SNR: substantia nigra, reticular part; for the rest of abbreviations, see list. Scale bar: 1 mm.

Figure 2.

Post-shock freezing in the first 30 seconds following the first five footshocks display by the animals of the Footshock and the Lesion groups. The unilateral lesions of the thalamic MGm/PIN complex had no effect on the post-shock freezing behavior. Error bars are SEM.

Figure 3.

Total number of c-Fos-li nuclei in the LA. A. Expression of c-Fos induced by the different experimental treatments (No-treatment, Box, Lesion and Footshock groups). The data of the Lesion group correspond only to the LA ipsilateral to the lesioned MGm/PIN complex B. Comparison of footshock-induced c-Fos expression in the LA ipsilateral versus the LA contralateral to the lesioned thalamus. Data represent mean $\pm$ SEM.

Figure 4.

Photomicrographs of c-Fos-like immunopositive nuclei in transverse sections through the LA in rats of the different experimental groups (Lesioned, Footshock, Box, and No-treatment groups). In A, the LA ipsilateral to the MGM/PIN thalamic lesion is shown. Abbreviations: AStr: amygdalostriatal transition area; BLA: basolateral amygdaloid nucleus; CeA: central amygdaloid nucleus; CPu: caudate putamen; DEn: dorsal endopiriform nucleus; for the rest of abbreviations, see list. Scale bar: 200 μ m.

La DL

Figure 5.

Distribution of the density of c-Fos-li nuclei in the nine rostrocaudal levels of the dorsolateral LA subnucleus in the No-treatment, Box, Lesion and Footshock groups. The small LA representations at the bottom of the graph show the area of the DL subnucleus at each rostrocaudal level (in grey). Data represent mean $\pm$ SEM.

Figure 6.

Distribution of the density of c-Fos-li nuclei in the five rostrocaudal levels of the ventrolateral LA subnucleus in the No-treatment, Box, Lesion and Footshock groups. The area of the VL subnucleus at each rostrocaudal level is depicted in grey in the small LA representations at the bottom of the graph. Data represent mean $\pm$ SEM.

La VM

Figure 7. Distribution of the density of c-Fos-li nuclei in the seven rostrocaudal levels of the ventromedial LA subnucleus in the No-treatment, Box, Lesion and Footshock groups. The small LA representations at the bottom of the graph display in grey the area of the VM subnucleus at each rostrocaudal level. Data represent mean $\pm$ SEM.

Table 1

Results of the post-hoc Student-Newman-Keuls test comparing the total number of Fos-positive cells in the LA in the four experimental groups. The significance refers to the comparison between the two most distant means within the subgroup. As it can be observed, the Fos values for the No-treatment, Box and Shocked animals are significantly different, and the Lesion animals may be grouped with the No-treatment or with the Box groups.

Group	<i>n</i>	Subgroup		
		1	2	3
No treatment	6	27,42		
Lesion	8	163,37	163,37	
Box	6		252,33	
Shocked	9			452,94
<i>Significance</i>		,099	,272	1,000