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Acoustic stress activates tuberoinfundibular peptide of 39 residues neurons in the rat brain

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Abstract

Strong acoustic stimulation (105 dB SPL white noise) elicited *c-fos* expression in neurons in several acoustic system nuclei and in stress-sensitive hypothalamic nuclei and limbic areas in rats. In the present study, using this type of loud noise for 30 min, Fos-like immunoreactivity (Fos-ir) was investigated in neurons that synthesize tuberoinfundibular peptide of 39 residues (TIP39) in the rat brain: in the subparafascicular area of the thalamus, the posterior intralaminar complex of the thalamus and the medial paralemnisal nucleus in the lateral part of the pons. By double labeling, Fos-ir was shown in nearly 80% of TIP39-positive cells in the medial paralemnisal nucleus, 43% in the posterior intralaminar complex and 18.5% in the subparafascicular area 30 min after the end of a 30-min loud noise period. In control rats, only few neurons, including 0–4% of TIP39-positive neurons showed Fos-ir. While the majority of the Fos-ir neurons were TIP39-positive in the subparafascicular area and medial paralemnisal nucleus, a fairly high number of TIP39-immunonegative, chemically uncharacterized neurons expressed *c-fos* in the subparafascicular area and the posterior intralaminar complex of the thalamus. These observations clearly show that some TIP39 neurons in the so-called “acoustic thalamus” and the majority of TIP39 neurons in the medial paralemnisal nucleus are sensitive to loud noise and they may participate in the central organization of responses to acoustic stress. Furthermore, the present data suggest that non-TIP39-expressing neurons may play a prevalent role in the activity of the “acoustic thalamus”.

Keywords

Fos immunostaining; Paraventricular nucleus; Subparafascicular nucleus; Posterior intralaminar complex of the thalamus, medial paralemnisal nucleus; Acoustic stress

Introduction

Tuberoinfundibular peptide of 39 residues (TIP39) was purified from bovine hypothalamus based on selective activation of the parathyroid hormone 2 receptor (Usdin et al. 1999). Existing evidence suggests that it may modulate nociceptive information at the spinal cord level (Dobolyi et al. 2002), and that it may affect the hypothalamo-pituitary axis (Usdin et al. 2003). TIP39 increased the release of corticotropin-releasing hormone (CRH) from in vitro hypothalamic explants, while intracerebroventricular administration of the peptide resulted in elevated plasma ACTH level (Ward et al. 2001). In addition, intracerebroventricular administration of TIP39 had anxiolytic- and antidepressant-like effects (LaBuda et al. 2004). Furthermore, increased fear and stress-related anxiety-like behavior have been demonstrated in mice lacking TIP39 (Fegley et al. 2008).

TIP39 is synthesized in only three neuronal cell groups in the rat (Dobolyi et al. 2002, 2003a, b), mouse (Faber et al. 2007) and macaque brains (Bagó et al. 2009). One of the major TIP39 cell groups comprises of neurons in the subparafascicular area of the posterior thalamus including the rostral subparafascicular nucleus (rSPF) and the subparafascicular nucleus (SPF) itself (also referred to as the magnocellular subparafascicular nucleus). The second group of TIP39-immunoreactive (TIP39-ir) neurons are scattered throughout the posterior intralaminar complex of the thalamus (PIL) that includes the parvocellular subparafascicular nucleus (SPFp), the posterior intralaminar thalamic nucleus (PIN), and cells in the most caudal and lateral part of the zona incerta (ZI). The third TIP39 cell group is located within the medial paralemniscal nucleus (MPL), in the rostro-lateral pons, just medial to the lateral lemniscus (Dobolyi et al. 2002, 2003b). From these cell groups, TIP39-ir fibers project to several fore- and hind-brain areas including the parvocellular subdivisions of the hypothalamic paraventricular nucleus (pPVN) and several limbic cortical areas and nuclei (Dobolyi et al. 2003a, b).

Based on their topography and neuronal connections, the SPFp, PIN and ZI have been referred to as the “acoustic thalamus” (LeDoux et al. 1984, 1990; Campeau and Watson 1997, 2000; Campeau et al. 1997). Neurons in the medial paralemniscal nucleus, which was recently characterized cytoarchitectonically (Varga et al. 2008), are connected to ascending acoustic pathways by projections to the inferior colliculus and the medial geniculate body (Dobolyi et al. 2003a, b) and receive major auditory input from the periolivary area, the inferior colliculus, and the auditory cortex (Varga et al. 2008). These connectional data suggest that TIP39 may be involved in some kind of auditory-related mechanisms, including responses to acoustic stress. Loud noise has been recognized as a strong stressful stimulus that stimulates the HPA axis by activating neurons in the pPVN (Henkin and Knigge 1963; Borrell et al. 1980; Campeau and Watson 1997; Campeau et al. 1997; Burow et al. 2005), especially neurons that produce CRH (Helfferich and Palkovits 2003). With tract-tracing techniques, direct and indirect (multisynaptic) neuronal projections from the “acoustic thalamus” to the PVN have been demonstrated in rats (Campeau and Watson 2000; Palkovits et al. 2004). Lesions of the “acoustic thalamus” block noise-induced corticosterone release, and acoustic stress induced *c-fos* mRNA expression in the PVN (Campeau et al. 1997; Burow et al. 2005). The PVN receives a relatively dense TIP39-ir innervation (Dobolyi et al. 2003b) that disappeared almost completely after lesion of the “acoustic thalamic” nuclei or transections of their projections to the hypothalamus (Dobolyi et al. 2003a).

In the present study, double immunolabeling for TIP39 and Fos peptide [an indicator of acute activation of neuronal cells (Morgan and Curran 1989; Bullitt 1990; Herdegen and Leah 1998)] was performed following acoustic stimuli to investigate the possible involvement of TIP39-expressing neurons in acoustic stress. Rats were exposed to loud noise at 105 dB SPL for 30 min. Auditory stimulation of this intensity and duration strongly elevates plasma

corticosterone (Campeau and Watson 1997), depletes the level of CRH in the median eminence (Feldman et al. 1972; Weidenfeld et al. 1989), and elicits strong *c-fos* activation in the auditory cortex and subcortical auditory areas and nuclei (Campeau and Watson 1997; Helfferich and Palkovits 2003; Burow et al. 2005).

Materials and methods

Animals

Adult, male Wistar rats (250–330 g body weight; Gödöllő, Hungary) were used in this study ($n = 16$). All experiments were carried out in accordance with the Guide for Care and Use of Laboratory Animals of the Ethics Committee of Semmelweis University, Budapest (based on the European Communities Council Directive of 24 November 1986).

Rats were kept three per cage at a temperature of $22 \pm 1^\circ\text{C}$ with 12–12 h light/dark periods (lights on at 6.00 a.m.), and supplied with dry rat food and drinking water ad libitum.

Sound stimulation

Prior to the experiment, the tympanic membranes of the rats were checked to exclude incidental middle ear infections, which might disturb the test. The rats were moved from their usual environment, in their own cages, to the test-room 2 days before the experiment where they were provided with food and drink ad libitum, and left undisturbed. For stimulation, animals ($n = 6$) were exposed to “white noise” (all frequency components equally represented), at 105 dB SPL (dRre: 20 μPa) with linear intensity, for 30 min, as this protocol has been shown previously to act as an acoustic stressor (Campeau and Watson 1997; Helfferich and Palkovits 2003). To produce homogenous noise, two GSI 16 audiometer external loudspeakers (Model No. 1716, manufacturer: Grason Stadler, Littleton, MA, USA) were set up 2 feet from the cages. The smooth distribution of sound was tested at several locations in the cages with a Brüel-Kjaer sound intensity meter. (The average of the different reading was 103–106 dB SPL.) Six rats served as controls and were kept in the same environment but without any acoustic stimulation. The background noise was 55 dB. After the stimulation, rats were undisturbed for 30 min then sacrificed under anesthesia with Ketamine (100 mg/kg b.w.) and Rompun (15 mg/kg b.w.) and perfused cardially with a Zamboni's fixative. Control rats ($n = 6$) were kept under the same surroundings for 60 min but without acoustic stimulation. Naive rats ($n = 3$) that did not receive any experimental interventions were also killed. They were anesthetized immediately after their removal from their cages and perfused transcardially.

Immunohistochemistry

The brains were removed and post-fixed in the fixative solution for 4 h, then cryoprotected in 0.1 M phosphate buffer (pH 7.4; PB) containing 30% sucrose for at least 24 h. Brains were cut in coronal sections of 40 μm thickness on a freezing microtome (Frigomobil, Reichert-Jung) and collected for floating immunostaining using the avidin–biotin peroxidase (ABC, Vectastain) method.

Sections were rinsed in PB, treated with 0.5% Triton X-100 for 1 h, then the endogenous peroxidase activity was blocked by 15 min incubation in 3% H_2O_2 . After washing in 0.1 M PB, sections were treated with 10% normal goat serum for 1 h, rinsed in 0.1 M PB and incubated in *c-fos* polyclonal antiserum raised in rabbit (Ab-5, Calbiochem-Novabiochem, San Diego, CA, USA; 1:6,000) overnight at 4°C . Next, biotin-labeled secondary antibody, raised in sheep was added (biotinylated anti-rabbit IgG, Vector Laboratories, Burlingame, CA, USA), and the sections processed using the ABC technique with a rabbit Vectastain Elite ABC-peroxidase Kit (Vector Labs.). The tissue bound peroxidase was visualized by nickel enhanced diaminobenzidine (NiDAB, Sigma Chemical Co., St. Louis, MO, USA) chromogen reaction.

For double labeling, sections were placed in anti-TIP39 primary antibody (1:3,000) overnight followed by biotinylated anti-rabbit IgG. This antibody was detected using DAB as the chromogen without nickel intensification. Following the immunohistochemical procedures, sections were mounted on gelatinized slides, dried overnight, dehydrated in ethanol followed by xylene and coverslipped. The brown perikaryonal reactions for TIP39 were well distinguished from dark blue cell nuclei stained for Fos. In a control experiment, following the development of the c-fos primary antibody, another biotinylated anti-rabbit secondary antibody was applied and the DAB reaction was run. Sections were mounted onto gelatin-coated slides, dried and coverslipped.

Quantitative analysis

TIP39 immunostained neurons were counted in serial sections through the entire rostro-caudal length of the investigated six brain nuclei (Table 1) from three naive rats. The numbers of TIP39-positive/Fos-positive and TIP39-positive/Fos-negative were counted on 4–10 representative sections from 4 to 6 stressed and 6 control rats. Data are presented as mean $\pm$ SEM (Table 1). In 2–4 sections from three stressed animals, the total number of Fos-ir cells was counted in each investigated cell group. The ratio of the Fos-positive/TIP39-positive and the Fos-positive/TIP39-negative cells were calculated and are shown in Fig. 1. For comparison of the number of TIP39-positive neurons from stressed and control rats, the Student's *t* test was performed.

Results

Exposure of rats to a 30-min intense noise produced strong Fos activation 30 min after the end of stimulus in almost all auditory nuclei (cochlear, superior olivary, trapezoid, lateral lemniscal, medial geniculate nuclei) and temporal (auditory) cortical neurons. These findings were consistent with those reported in our previous experiments with application of loud noise in similar conditions (Helfferich and Palkovits 2003; Palkovits et al. 2004). In addition, neurons in several brain areas sensitive to other types of stressful stimuli (piriform, infralimbic and cingulate cortex, midline thalamic nuclei, septohippocampal and lateral septal nuclei, some of the hypothalamic (preoptic, paraventricular, anterior hypothalamic, dorsomedial, supramamillary), dorsal raphe and parabrachial nuclei also contained strong Fos immunostaining. The strong Fos immunoreactivity seen in the parvocellular paraventricular neurons is illustrated (Fig. 2b). This part of the nucleus is fairly well innervated by very fine varicose TIP39-ir fibers, in contrast to the magnocellular portion of the nucleus, which is devoid of TIP39 immunoreactivity (Fig. 2a).

A fairly high number of TIP39-ir neurons are located at the diencephalic-mesencephalic junction, in the subparafascicular area (Table 1). Relatively few TIP39-ir neurons were activated here by loud noise as indicated by Fos expression (Fig. 3). Less than 9% of TIP39-ir neurons were Fos-ir in the *rostral subparafascicular nucleus*, and 23.9% in the *subparafascicular nucleus* (Table 1). Even these small percentages represent a significant elevation in the number of TIP39-ir neurons activated in response to loud noise ($p < 0.01$), since the numbers of Fos-ir/TIP39-ir cells were very low in these nuclei of the control animals (Table 1). The majority of the double-labeled cells were located in the caudal portion of the nucleus, between the fasciculus retroflexus and the beginning of the cerebral aqueduct (Fig. 3). In addition to Fos-positive/TIP39-positive neurons, 18% of Fos-ir neurons in the subparafascicular area were TIP39-negative (Fig. 1; Table 1).

About 10% of the TIP39-ir cells in the brain were present in the posterior intralaminar complex of the thalamus (Table 1), one-third of them were located in the *parvocellular subparafascicular nucleus*. One half of these TIP39-ir cells were immunostained with Fos after loud noise (Fig. 4). In control rats, very few TIP39-ir cells showed Fos immunoreactivity (Table

1). In this nucleus, like in other compartments of the PIL, the majority of Fos-ir cells were TIP39-negative (Fig. 1).

The total number of TIP39-positive cells was relatively low in the *posterior intralaminar thalamic nucleus*: less than 4% of the total number of TIP39-ir cells in the brain were located here. About the half of this small number of cells showed Fos-ir in response to loud noise, which was significantly higher ($p < 0.01$) than those in control rats (Table 1). More than 90% of the neurons in the PIL activated by loud noise were TIP39-negative (Fig. 1).

Only 2.4% of TIP39-ir cells are scattered in the caudo-lateral part of the *zona incerta*, and only one-third of them showed Fos-ir in response to loud noise (Table 1). In control rats, almost all of the TIP39-ir cells were Fos-negative ($p < 0.001$). The majority of the Fos-ir cells here were TIP39-negative (Fig. 1).

A large collection of TIP39-ir neurons (more than one-third of the TIP39-ir cells in the brain) is present in the lateral part of the rostral pons, in the *medial paralemniscal nucleus* (Fig. 5). Here, approximately four out of five TIP39-ir neurons showed Fos immunostaining in response to loud noise (Table 1). The numbers of double-labeled Fos- and TIP39-positive neurons in the control rats were very low (Table 1), significantly ($p < 0.001$) less than in the treated group. The ratio of Fos-positive/TIP39-negative neurons was relatively low in this nucleus (Fig. 1).

Discussion

Loud noise is recognized as a strong stressful stimulus that activates the hypothalamo-pituitary-adrenal axis. Plasma corticosterone and ACTH levels are elevated minutes after stimuli (Henkin and Knigge 1963; Borrell et al. 1980; Campeau and Watson 1997; Campeau et al. 1997; Campeau and Watson 2000; Burow et al. 2005), and depletion of CRH immunoreactivity in the median eminence is seen (Feldman et al. 1972; Weidenfeld et al. 1989). Remarkable elevations in plasma corticosterone levels were elicited by 105 dB SPL, the acoustic stimulus used in this study.

As reported previously (Borrell et al. 1980; Friauf 1995; Adams 1995; Campeau and Watson 1997; Campeau et al. 1997; Helfferich and Palkovits 2003; Burow et al. 2005), loud noise elicited *c-fos* activation in almost all of the nuclei of the auditory system and in auditory cortical neurons, as well as in several other neurons in regions that are generally known as “stress sensitive” brain areas (for a review see Pacak and Palkovits 2001). Strongly Fos-ir neurons were seen in the pPVN after acoustic stress (Campeau and Watson 1997; Campeau et al. 1997; Helfferich and Palkovits 2003), particularly in CRH-containing PVN neurons. Campeau and Watson (2000) reported that neurons in the medial aspect of the PIN adjacent to the SPFP nucleus project to the pPVN. This region, also called “acoustic thalamus” receives strong acoustic input from the inferior colliculus (see Campeau and Watson 2000).

In the present study, loud noise represents a strong stressful stimulus indicated by the elicited strong Fos activity in the pPVN. The Fos-ir cells overlapped the area where CRH cells are located (Helfferich and Palkovits 2003) and TIP39-ir fibers establish a dense network of fibers (Fig. 2a). This dense network of TIP39 fibers in the pPVN was eliminated by lesioning of neurons in the PIL and the SPF, or by transecting their fibers that project to the hypothalamus (Dobolyi et al. 2003a). Thus, TIP39-ir neurons in the PIN, as well as in the subparafascicular area project to the pPVN (Dobolyi et al. 2003a). However, a relatively low percentage of TIP39-positive neurons in these areas expressed Fos protein in response to stressful acoustic stimulation (Table 1). Whether the TIP39-Fos double-labeled cells are those TIP39-ir neurons in the PIN and the subparafascicular area that may interconnect the hypothalamus, especially the PVN and the acoustic system, could be a subject for further studies. It should be mentioned, that a high percentage of Fos-positive neurons in the SPFP, PIL and the ZI activated by loud

noise were TIP39 negative (see Table 1). These neurons may also project to the hypothalamus and contribute to the acute stress response elicited by a loud noise and may express neuropeptides other than TIP39. The calcitonin gene-related peptide (CGRP) could be one of the candidates that is expressed in several neurons in the PIL based on its presence in the area (Shimada et al. 1985; Inagaki et al. 1990; Yasui et al. 1991; Coolen et al. 2003; Dobolyi et al. 2005).

Loud noise did not produce Fos activation in all of the TIP39-positive neurons in the PIL (Table 1). The presence of a high number of Fos-positive but TIP39-negative cells in the PIL of acoustically stimulated animals supports the hypothesis that brain TIP39-expressing neurons could be involved in several other types of neuronal functions, such as nociceptive, neuroendocrine, or behavioral responses (Ward et al. 2001; Usdin et al. 2003; Wang et al. 2006; Fegley et al. 2008).

In response to loud noise, the vast majority (79.4%) of TIP39-ir neurons in the medial paralemniscal nucleus were also labeled with Fos. The TIP39-positive MPL neurons are bilaterally connected to various compartments of the auditory system: they project to the inferior colliculus and the medial geniculate (Dobolyi et al. 2003a) and receive afferents from the periolivary area, the inferior colliculus, the medial geniculate body, and the auditory cortex (Varga et al. 2008). The MPL is also connected with the other TIP39-containing areas, the PIL, the SPF and the ZI (Varga et al. 2008).

In addition to the MPL, there are different cell groups in the lateral ponto-mesencephalic tegmentum, which are anatomically and functionally also connected to the auditory system. The *ventrolateral tegmental area*, an area involved in audiomotor behavior (Herbert et al. 1997) is located ventral to the MPL. (The rubrospinal tract separates these two cell groups, the MPL is located dorsal to it.). This area may correspond to the ventrolateral portion of the posterior half of the pontine reticular nucleus just above the superior olivary complex, which is the vocalization center in monkeys (Hage and Jürgens 2006; Hannig and Jürgens 2006). Another area has been localized in the lateral mid-brain tegmentum under the name of “paralemniscal zone” (Henkel 1981). The possible role of neurons in this zone has been recognized in pinna movements based on their projections to neurons of the facial motor nucleus. The above-mentioned two areas are anatomically distinct entities. They have their own specific patterns of afferent and efferent projections. The MPL can be topographically and cytoarchitectonically distinguished from these cell groups. One of the major differences: these areas do not contain TIP39 expressing neurons. The second, loud noise applied in this study elicited minor if any Fos activation in neurons in the paralemniscal zone or in the ventrolateral tegmental area.

Theoretically, the acoustic stress (loud noise) may activate the HPA axis through different neuronal circuits: (1) a direct cochlear nuclei–“acoustic thalamus” or (PIL)–PVN pathway; (2) Campeau and Watson (2000) suggested the existence of routes from the “acoustic thalamus” through the limbic system, which might influence the HPA axis; (3) a direct (monosynaptic) projections from the MPL to the hypothalamus, or the existence of a MPL–PIL–PVN pathway also cannot be excluded. The present findings suggest that TIP39-immunoreactive neurons may participate somehow in the auditory system–hypothalamic connections, and are involved in the central organization of the acoustic stress.

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Abbreviations

ABC	Avidin–biotin complex
ACTH	Adrenocorticotropin
AH	Anterior hypothalamic nucleus
aq	Cerebral aqueduct
cc	Crus cerebri
CGRP	Calcitonin gene-related peptide
CRH	Corticotropin-releasing hormone
DAB	Diaminobenzidine
dB SPL	Decibel (sound pressure level, A scale)
DL	Dorsal nucleus of the lateral lemniscus
DLG	Lateral geniculate body, dorsal part
fr	Fasciculus retroflexus
HPA	Hypothalamo-pituitary-adrenal
IC	Inferior colliculus
ll	Lateral lemniscus
MPL	Medial paralemniscal nucleus
mPVN	Magnocellular part of the paraventricular nucleus
mt	Mamillothalamic tract
p	Pyramidal tract
PB	Phosphate buffer
pc	Posterior commissure
PIL	Posterior intralaminar complex of the thalamus
PIN	Posterior intralaminar thalamic nucleus
pPVN	Parvocellular part of the paraventricular nucleus
PVN	Paraventricular nucleus of the hypothalamus
RP	Pontine reticular nucleus, pars oralis
rSPF	Rostral subparafascicular nucleus
SM	Supramamillary nucleus
sp	Superior cerebellar peduncle
SPF	Subparafascicular nucleus
SPFp	Parvocellular subparafascicular nucleus
SUT	Subthalamic nucleus
TIP39	Tuberoinfundibular peptide of 39 residues
VL	Ventral nucleus of the lateral lemniscus
Vth	Root of the trigeminal nerve
ZI	Zona incerta

3V

Third ventricle

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Fig. 1. Percentage of Fos-positive/TIP39-positive and Fos-positive/TIP39-negative neurons in the subparafascicular area, the posterior intralaminar thalamic complex and the medial paralemniscal nucleus 30 min after the end of a 30-min loud noise period. Mean $\pm$ SEM of Fos-positive cells in selected sections from 3 rats (number of sections/rat): rSPF: (4), SPF: (4), SPFP: (10), PIN: (12), ZI: (10), MPL: (4). For abbreviations see full list

Fig. 2. Fos-ir cells in the hypothalamic paraventricular nucleus in control rats (**a**) and 60 min after the beginning of a 30-min application of loud noise (**b**). TIP39-ir fibers can be distinguished in panel **a**, in which there is little Fos labeling. For abbreviations see full list. *Scale bar* 50 μ m

Fig. 3. Fos-positive TIP39-ir neurons in the subparafascicular nucleus after a loud noise stress. Localization of the subparafascicular nucleus at the level of the diencephalic-mesencephalic junction in coronal sections (**a**, **b**). The area outlined by the rectangle in **a** is shown in **c**. **c** The area outlined by the rectangle in **c** is shown in **d**. **d** Fos immunoreactivity (black dots) is present in cell nuclei while TIP39 immunoreactivity (brown) is located in perikarya. Examples of double-labeled cells (arrow), cells containing only Fos (arrowhead) and only TIP39 (two small arrows) are indicated. For abbreviations see full list. Scale bars 500 μ m (**a**), 200 μ m (**b**), 100 μ m (**c**), 20 μ m (**d**)

Fig. 4.

Fos-positive TIP39-ir neurons in the parvocellular subparafascicular nucleus (SPFp) after a loud noise stress. **a** Coronal section 4.5 mm caudal to the level of the bregma. The area outlined by the rectangle is shown in **b**. Cells have very dense Fos immunoreactivity in the intergeniculate leaflet of the lateral geniculate body (*arrow*). **b** Double-labeled cells in the SPFp are evident, as well as a high number of cells with Fos-positive cell nuclei without TIP39 immunolabeling. For abbreviations see full list. *Scale bars* 500 μm (**a**) and 50 μm (**b**)

Fig. 5. Fos-positive TIP39-ir neurons in the medial paralemniscal nucleus (MPL) 30 min after the end of a 30-min loud noise period. Topographical localization of the MPL in the lateral portion of the rostral pons is shown in TIP39/Fos double labeled (**a**), and TIP39 immunolabeled (**b**) coronal sections. **c** A fairly large number of double-labeled cells can be seen in the MPL at higher magnification. For abbreviations see full list. *Scale bars* 500 μ m (**b**), 400 μ m (**a**) and 50 μ m (**c**)

Table 1

Number and percent differences in Fos immunoreactivity in TIP39-positive and TIP39-negative neurons in the subparafascicular area, the posterior intralaminar complex of the thalamus and the medial paralemniscus 30 min after the end of an audiogenic stress (30-min loud noise, 105 dB SPL)

Nuclei	Number of TIP39-ir neurons	Rostro-caudal extension of TIP39-ir cells	Controls	Audiogenic stress		
				Total no. of Fos +/TIP39 + cells	% of the total TIP39-ir cells	% of the total Fos-ir cells
SPF area	1,031 ± 29	B-3.8-4.6	14 ± 3	201 ± 21	19.5	18.0
RSPF	303 ± 71	B-3.8-4.1	6 ± 2	27 ± 9	8.9	9.9
SPF	728 ± 57	B-4.1-4.6	8 ± 1	174 ± 28	23.9	21.0
PIL complex	204 ± 33	B-4.2-5.7	9 ± 1	89 ± 6	43.6	11.7
SPFp	74 ± 10	B-4.2-4.8	2 ± 0	36 ± 6	48.6	14.1
PIN	82 ± 17	B-4.6-5.5	1 ± 1	36 ± 7	43.9	8.6
ZI	48 ± 14	B-4.9-5.7	6 ± 1	17 ± 8	17.8	14.9
MPL	746 ± 60	B-8.0-8.5	4 ± 1	592 ± 55	79.4	85.9
	<i>n</i> = 3	<i>n</i> = 3	<i>n</i> = 4	<i>n</i> = 4-6		<i>n</i> = 3

The names and quantitative data of the 3 major TIP39 area/complex/cell group are written in bold

B bregma