



Research article

Amygdalotrigeminal projection in the rat: An anterograde tracing study

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SUMMARY

Previous neurophysiological studies have demonstrated that the amygdala has a direct influence upon trigeminal motoneuron activity. The existence of a direct amygdalotrigeminal pathway in rats was proved by anterograde tracing with the neuroanatomical tracer, biotinylated dextran amine (BDA). After ipsilateral BDA application to the central nucleus of the amygdala (AmCe), widespread ipsilateral projections emerging from its medial subnucleus were traced to the trigeminal brainstem nuclear complex, including the principal sensory (Pr5) and mesencephalic trigeminal nucleus (Me5), and their premotoneurons and interneurons, located in the supratrigeminal, intertrigeminal and peritrigeminal nuclei. Sparse BDA-labeled axons and their terminals were also distributed in the contralateral Pr5, interpolar and caudal subnuclei of the spinal trigeminal nucleus. The central lateral amygdaloid nucleus gives rise to a light ipsilateral projection to the pontine part of the Me5. The present data indicate that AmCe sends massive efferents to the trigeminal nuclei in the brainstem, wherein its medial subnucleus sends the major input to them. The medial amygdaloid nucleus sparsely innervates Me5 neurons, specifically those located in its mesencephalic portion, while basomedial and basolateral efferents do not target the trigeminal nuclear complex. These results suggest that the amygdaloid input may modulate the activity of trigeminal sensory and motor neurons and, thus, the amygdala is possibly involved in the control of masticatory behavior.

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Abbreviations: ACo, anterior cortical amygdaloid nucleus; Am, amygdala; AmBl, basolateral nucleus of the amygdala; AmBm, basomedial nucleus of the amygdala; AmCe, central nucleus of the amygdala; AmLa, lateral nucleus of the amygdala; AmMe, medial nucleus of the amygdala; Astr, amygdalostriatal transition area; BDA, biotinylated dextran amine; CeC, central amygdaloid nucleus, capsular part; CeL, central amygdaloid nucleus, lateral division; CNS, central nervous system; DEN, dorsal endopiriform nucleus; I, intercalated nuclei of the amygdala; I5, intertrigeminal nucleus; LC, locus coeruleus; LPB, lateral parabrachial nucleus; LV, lateral ventricle; MGP, medial globus pallidus; Me5, mesencephalic trigeminal nucleus; MePV, medial amygdaloid nucleus anteroventral part; MPB, medial parabrachial nucleus; Mo5, motor trigeminal nucleus; opt, optic tract; PAG, periaqueductal gray; Pa5, paratrigeminal nucleus; Pir, piriform cortex; PLCo, posterolateral cortical amygdaloid nucleus; P5, peritrigeminal nucleus; Pr5, principal sensory trigeminal nucleus; Pr5DM, principal sensory trigeminal nucleus, dorsomedial part; scp, superior cerebellar peduncle; Sp5, spinal trigeminal nucleus; Sp5C, spinal trigeminal nucleus, caudal part; Sp5I, spinal trigeminal nucleus, interpolar part; Sp5O, spinal trigeminal nucleus, oral part; st, stria terminalis; Su5, supratrigeminal nucleus; 4V, 4th ventricle.

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1. Introduction

The human amygdala (Am) is an important brain structure, located deep within the ventromedial temporal lobe, ventral to the caudolateral striatum and the pallidum. The amygdaloid nuclear complex consists of several structurally and functionally distinct nuclei, generally divided on the basis of cytoarchitectonic, hodological, histochemical, and immunohistochemical studies, into a corticomедial complex and a basolateral complex. The latter is evolutionarily newer and comprises the lateral, basolateral and basomedial amygdaloid nuclei, while the former is phylogenetically older and encompasses the centromedial and cortical nuclei (reviewed in Amaral et al., 1992; McDonald, 1992; De Olmos et al., 2004). The Am is involved in the modulation of neuroendocrine functions, visceral efferent motor mechanisms, and in a vast range of normal behavioral functions and psychiatric con-

¹ Dr. Kamen Usunoff passed away unexpectedly on February 28, 2009 while on a study stay in Rostock, Germany during the preparation of the manuscript. He contributed equally to this paper and should be considered a first co-author.

ditions (for comprehensive reviews see Ben-Ari, 1981; Aggleton, 1992; Aggleton and Saunders, 2000), and has a wide variety of afferent and efferent connections throughout the central nervous system (CNS; Pitkänen, 2000). In particular, neuroanatomical studies in rats have demonstrated that Am sends efferents to a variety of brainstem regions including substantia nigra/ventral tegmental region, central gray, parabrachial nuclei, dorsal vagal complex and ventrolateral medulla (Hopkins, 1975; Price et al., 1987; Danielsen et al., 1989; Wallace et al., 1992; Tsumori et al., 2010).

The trigeminal brainstem nuclear complex in the rat, which receives somatosensory input from the orofacial region of the head, is located throughout the whole dorsolateral length of the brainstem, extending from the midbrain to the upper cervical spinal cord. The structure and connections of this complex in humans has been the subject of comprehensive reviews by Usunoff et al. (1997), and Waite and Ashwell (2004). It is an important sensorimotor center that is comprised sensory and motor nuclei. From rostrally to caudally, the trigeminal sensory nuclei include the mesencephalic trigeminal nucleus (Me5), the main or principal sensory nucleus (Pr5), and the spinal trigeminal nucleus (Sp5), which is further subdivided into three subnuclei, the oralis (Sp5O), the interpolaris (Sp5I) and the caudalis (Sp5C) (Olszewski, 1950; Olszewski and Baxter, 1954; Darian-Smith, 1973; Waite, 2004). The distinct trigeminal motor nucleus (Mo5) is located medially to the main sensory nucleus in the pontine tegmentum. Brainstem trigeminal nuclei also include the supratrigeminal nucleus (Su5) on the dorsomedial pole of Pr5, the intertrigeminal nucleus (I5), which lies between Pr5 and Mo5 (Waite, 2004), and the peritrigeminal nucleus (P5), located lateral and ventral to the trigeminal tract at the level of the Sp5I (Paxinos and Watson, 1998). The paratrigeminal nucleus (Pa5) has traditionally been thought of as a separate sensory component (Chan-Palay, 1978) but, at least in humans, it has been considered as another subdivision of the spinal nuclei (see Usunoff et al., 1997). Motor and premotor neurons, located in the Mo5, Su5 and I5, respectively, are associated with masticatory reflexes and the control of jaw movements (see Travers, 2004 for a recent review).

Previous physiological investigations have shown that Am has a direct influence upon trigeminal motoneuron activity because electrical stimulation of the central amygdaloid nucleus can produce rhythmic jaw movements in rats (Sasamoto and Ohta, 1982; Ohta, 1984). Besides, it has been demonstrated that long-lasting stimulation of the basal amygdaloid nucleus causes a delayed inhibition of the masseter reflex (Bobo and Bonvallet, 1975), mediated through the pars oralis of the Sp5 (Bonvallet and Bobo, 1975). This raises the intriguing possibility of a direct amygdalotrigeminal connection. However, none of these studies reveals the existence of monosynaptic projections from Am to trigeminal motoneurons. To date, evidence has only been provided for an amygdalofugal projection to the mesencephalic trigeminal nucleus in the rat (Krettek and Price, 1978; Post and Mai, 1980; Price and Amaral, 1981). In addition, an indirect descending pathway from the central amygdaloid nucleus via the pontine reticular formation to the contralateral Mo5 and ipsilateral supratrigeminal region has been traced in rats (Takeuchi et al., 1988a,b). Recently, a bilateral disynaptic pathway from the rat central amygdaloid nucleus to the Mo5 via the parvicellular reticular formation of the medulla oblongata, where many trigeminal premotor neurons are located, has been reported as well (Yasui et al., 2004; Tsumori et al., 2010). Nevertheless, the existence and organization of a direct amygdalotrigeminal pathway remains to be determined and, therefore, we decided to re-examine its real presence.

In the present study, we report efferent projections to the trigeminal nuclei in the brainstem from the amygdaloid nuclear complex, a key structure of the limbic system, studied by antero-

grade axonal tracing with the anterograde neuroanatomical tracer biotinylated dextran amine (BDA).

2. Materials and methods

The experiments in this study were carried out on 21 adult Wistar rats of both sexes, weighing 220–260 g. The surgical procedures involving animals and their care were conducted in conformity with the standards for animal experiments according to a protocol (Nr. 1414/2007) approved by the Animal Care and Use Committees at our universities and were consonant with the guidelines established by the NIH.

The animals were anesthetized with Thiopental (50 mg/kg, i.p.) and then placed on a stereotaxic apparatus (David Kopf, Tujunga, CA) in the flat skull position. Stereotaxic coordinates of the amygdaloid nuclei and the list of abbreviations used in the text and figures were obtained from the atlas of Paxinos and Watson (1998). Under aseptic conditions small craniotomies were performed. In four Am nuclei (central nucleus, AmCe – 10 rats; medial nucleus, AmMe – 6 rats; basomedial nucleus, AmBm – 3 rats; and basolateral nucleus, AmBl – 2 rats) 0.25–0.5 μ l 10% BDA (10,000 MW; Molecular Probes Europe BV, Leiden, The Netherlands) dissolved in 0.1 M phosphate buffer (PB; pH 7.4) was injected using a dorsal approach. Multiple unilateral injections were made by pressure over a period of 30–60 min using a pico-spritzer through a glass micropipette (20–50 μ m tip diameter) which was attached to a Hamilton microsyringe (Hamilton Co., Reno, NV). At the end of the injection, the pipette was held in place for 15 min to insure that the injected tracer had been absorbed into the tissue and to reduce the possibility of its spread. The site of microinjection was verified on coronal sections. After survival time of 7–14 days, the rats were deeply anesthetized and perfused transcardially with phosphate buffered saline (PBS), followed by 500 ml of 4% paraformaldehyde in PB. The brains were removed and postfixed overnight in the same fixative, sliced in the coronal plane and immersed in 0.5% paraformaldehyde in PBS containing 20% sucrose at 4 °C. Serial sections were cut at a thickness of 40 μ m on a Reichert Jung freezing microtome, collected in a free-floating state in PBS and then processed for tracer histochemistry. A commercial avidin–biotin–HRP complex (ABC) kit was used to visualize BDA (Vectastain ABC Kit, Vector Laboratories Inc., Burlingame, CA). Briefly, the sections were preincubated in PBS containing 0.1% bovine albumin (fraction V; Sigma Chemical Co., St. Louis, MO) for 20 min, and rinsed in PBS for 30 min. Subsequently they were incubated in the avidin–coupled biotinylated HRP solution for 45–60 min, and rinsed again in PBS for 30 min. The reaction product was developed with 0.06% 3,3'-diaminobenzidine (Sigma, St. Louis, MO) and 0.02% H₂O₂ in Tris buffer (0.05 M, pH 7.6) for 10–15 min in the dark. The sections were afterward rinsed in distilled water, mounted onto chrome alum-gelatin-coated slides and air dried overnight. Finally, the sections were counterstained with 0.025% cresyl violet, dehydrated, and coverslipped with Entellan (Merck, Darmstadt, Germany). The slides were then examined in a Zeiss Axioplan 2 research microscope and selected areas were photographed with an AxioCam MRC digital camera. Adobe Photoshop CS3 (Adobe Systems Inc., San Jose, CA) was used to adjust contrast and brightness of all photomicrographs.

3. Results

3.1. AmCe injection site (10 rats)

In 10 cases studied the injection site involved the central nucleus of the amygdala (AmCe). In five animals in which a larger quantity of BDA was delivered (0.5 μ l), three of the cases had injections in the AmCe (Fig. 1A and C) without spread of the tracer into surround-

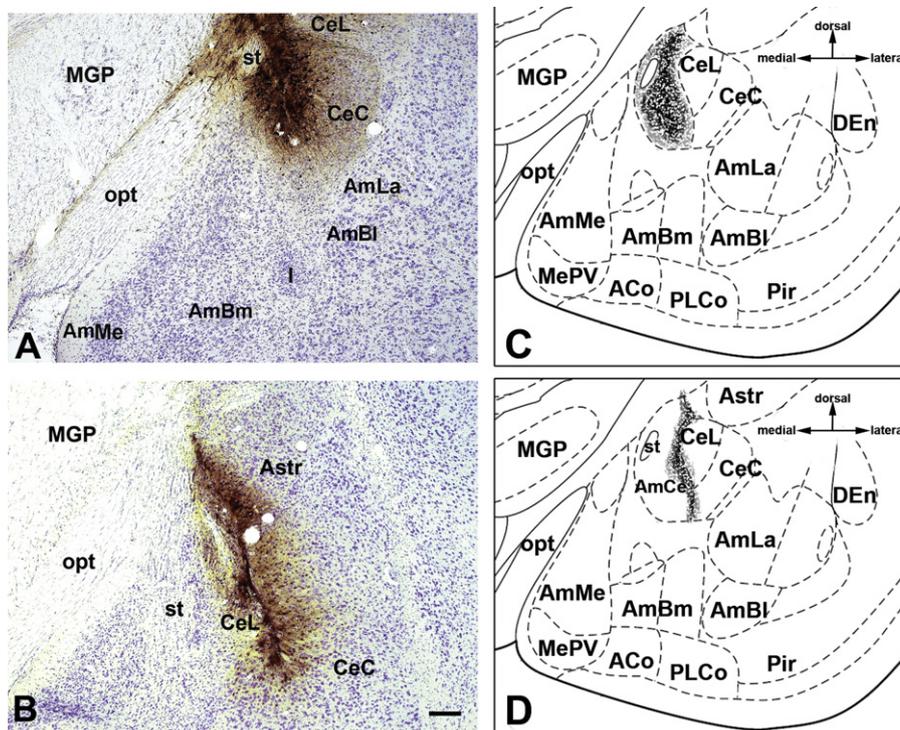


Fig. 1. (A and B) Photomicrographs showing the injection sites of BDA in the central amygdaloid nucleus (AmCe). (A) Injection site in the AmCe after application of 0.5 μ l BDA. The uptake of the tracer almost exclusively involves its medial subnucleus. (B) Injection site in the lateral subnucleus of AmCe (CeL) following application of 0.25 μ l BDA. Both sections were counterstained with cresyl violet after processing with the diaminobenzidine reaction. (C and D) Line drawings illustrating the sites of BDA injections into the AmCe (shaded area in C) and CeL (shaded area in D) at a coronal level of between Bregma -2.5 mm and -2.8 mm according to the rat brain atlas of Paxinos and Watson (1998). For further abbreviations see the list of abbreviations. Scale bars = 500 μ m (A and B); 1 mm (C and D).

ing structures, i.e., the intercalated cell masses of the Am. In the other two cases there was a small spread of tracer along the ventral part of the cannula track into the ventral portion of the amygdalo-striatal transition area and globus pallidus. These two cases were excluded from systematic examination. From five cases in which smaller injections (0.25 μ l BDA) were made, in four cases portions of AmCe were injected and in one of these cases there was a discrete focus in the lateral AmCe (Fig. 1B and D). In the fifth case the injection was primarily located in the intraamygdaloid portion of the bed nucleus of stria terminalis, ventral to the medial subnucleus of AmCe.

3.2. Projections of AmCe medial and lateral subnuclei

After unilateral injections of BDA in the AmCe, labeled efferent axons ascended from the injection site, with most of them coursing in the major efferent bundle of the Am, the stria terminalis. A smaller number of labeled axons coursed medially above the optic tract to join the ventral amygdalofugal pathway from which they descended to brainstem structures. Their first target was the lateral portion of the substantia nigra. Thereafter, the labeled axons ran dorsolaterally in the mesencephalic tegmentum towards the periaqueductal gray (PAG; Fig. 2A).

3.3. Labeling in the trigeminal nuclear complex

Entering the PAG, the labeled axons ran among the large neurons of the rostral portion of the Me5 (Fig. 2B and C) that are located at the lateral border of PAG and innervated also the small neurons belonging to Me5. Further caudally, labeled axons reached the pontine tegmentum, descending along the ventral border of Mo5 and then turning in a dorsal direction towards the dorsolateral pons. The fibers ran through Mo5 to P5, and laterally to it in I5 (Fig. 2D).

In the dorsolateral pons dense terminal fields of axonal arborizations were present in the medial and lateral parabrachial nuclei and in Me5, while no labeled axons entered the locus coeruleus (Fig. 2D–F). In Me5 dense pericellular baskets surrounded the large, typically pseudounipolar neurons, and the small neurons of this region were also contacted by amygdalofugal fibers. In Mo5 most, if not all, labeled axons represented passing fibers (Fig. 3A and B), whereas in Su5 along with numerous passing fibers discrete axonal arborizations were also present (Fig. 3C). Similar features were seen in the P5 and I5 as well (Fig. 3D–F).

In other nuclei of the trigeminal nuclear complex some labeling was present in the contralateral Pr5, as well as in Sp5I and Sp5C. The selective but relatively large injection of BDA in the medial subnucleus of AmCe gave rise to only a very sparse number of labeled axons in these latter nuclei (Fig. 4A–D). In Pr5 on the contralateral side robust labeled fibers were occasionally observed (Fig. 4A and B). However, in the contralateral Sp5I (Fig. 4C) and in the Sp5C (Fig. 4D) only scattered very fine terminal fibers were encountered.

The selective infiltration of the lateral part of AmCe was followed by a moderate number of labeled axons in the ipsilateral Me5 (Fig. 5A and B). The projection to the periaqueductal gray was very scanty and reliable data for a projection to the mesencephalic portion of Me5 could not be provided. However, in its pontine part unquestionable existence of labeled varicose axons was present (Fig. 5A and B).

3.4. AmMe injection site (6 rats)

The six injections in the medial nucleus of the amygdala (AmMe) involved the nucleus to different extents. In three cases with more voluminous injections more than one of the four subnuclei (anterodorsal, anteroventral, posterodorsal and posteroventral) was involved, while concomitant spread to surrounding structures

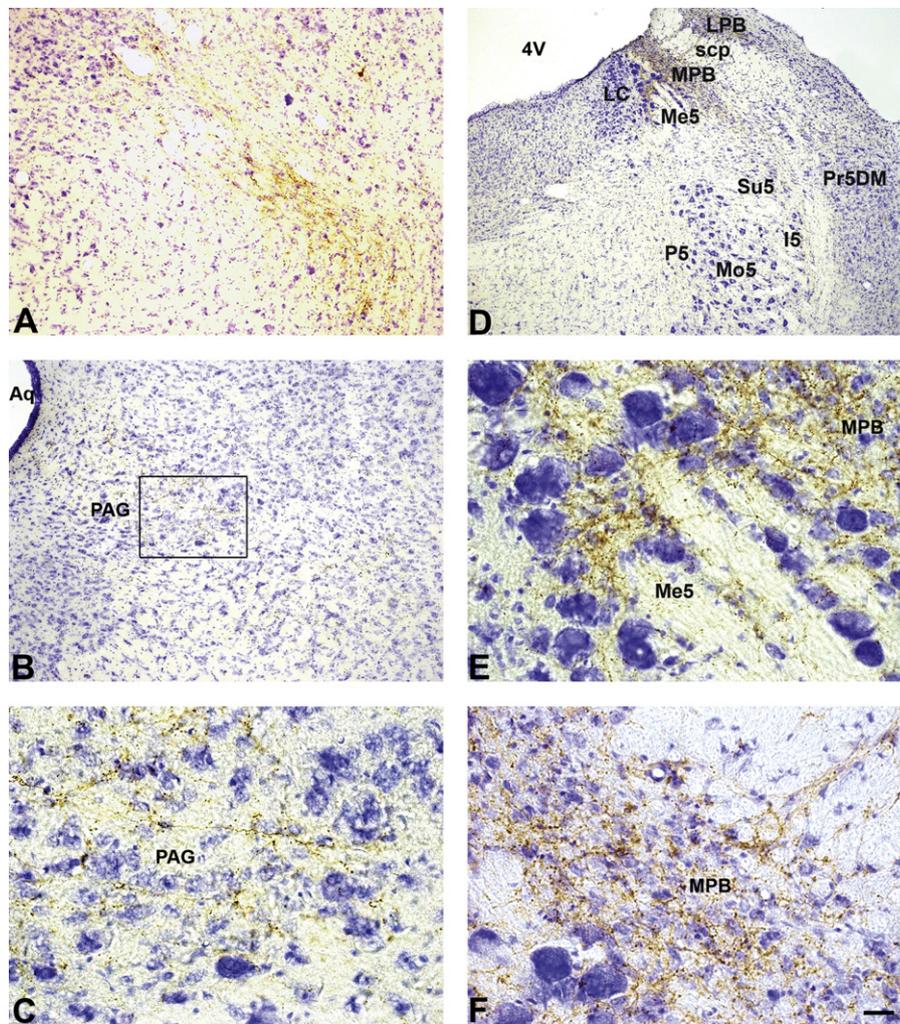


Fig. 2. Anterograde projections after unilateral injections in the central amygdaloid nucleus, medial subnucleus, to the ipsilateral rostral brainstem. (A) After unilateral injections of BDA in the AmCe, the labeled axons run through the tegmentum towards the PAG. (B) Photomicrograph of the midbrain at low magnification showing the discrete labeling of the PAG. (C) The inset in (B) at a higher magnification. Note that the labeled axons run among the mesencephalic trigeminal neurons lying at the lateral border of the PAG. (D) Low-power view of the dorsolateral pons at the level of the trigeminal brainstem nuclear complex. (E and F) are enlargements of parts of (D). (E) Dense field of BDA-labeled extensively ramified fibers and their terminals in the Me5. Note that the mesencephalic trigeminal neuronal perikarya are contacted by labeled fibers and terminals. A strong terminal labeling can also be seen in the medial parabrachial nucleus (MPB). (F) High magnification of labeling in the MPB. Note labeled varicose fibers passing perpendicular to the superior cerebellar peduncle and coursing between the MPB and LPB, lateral parabrachial nucleus. Scale bars = 100 μm (A); 200 μm (B and D); 50 μm (C, E and F).

was minimal. In the case of discrete injections, one fairly selective injection was located in the anterodorsal subnucleus, one in the posterodorsal subnucleus with some spread over the dorsal part of the posteroventral subnucleus, and one in the posteroventral subnucleus, with spread to the posteromedial cortical nucleus. The injection site of the most successful experiment is shown in Fig. 6A. The injection was placed in the posterior part of the anterodorsal subnucleus and the periphery of the injection encroached on the rostral poles of the posterodorsal and posteroventral subnuclei.

3.5. AmMe anterograde labeling

In the case with the large injection in the AmMe, there was a moderate projection to Me5. A fair number of labeled axons reached the PAG (Fig. 6B) and a small number of labeled axons arborized in the vicinity of neurons of the mesencephalic portion of Me5 (Fig. 6C). No labeled axons were observed in the pontine part of Me5 (Fig. 6D). Notably, in the case with discrete injection in the anterodorsal portion of AmMe a few axons were traced to the vicinity of the neurons of mesencephalic portion of Me5 but the discrete

injections in the posterior portions of AmMe were virtually negative.

3.6. AmBm injections (3 rats)

One large injection that was located in the basomedial nucleus of the amygdala (AmBm) slightly encroached on the cortical nuclei ventrally, the intraamygdaloid portion of bed nucleus of stria terminalis and the intercalated cell masses (dorsally). Two additional discrete injections were fairly restricted to the AmBm. Only in the case with a large BDA injection there, an unconvincing projection towards the mesencephalic portion of Me5 was encountered whereas in the cases with the discrete injections, tracing findings were absolutely negative.

3.7. AmBl injections (2 rats)

The two experiments with basolateral nucleus of the amygdala (AmBl) injections also provided negative results.

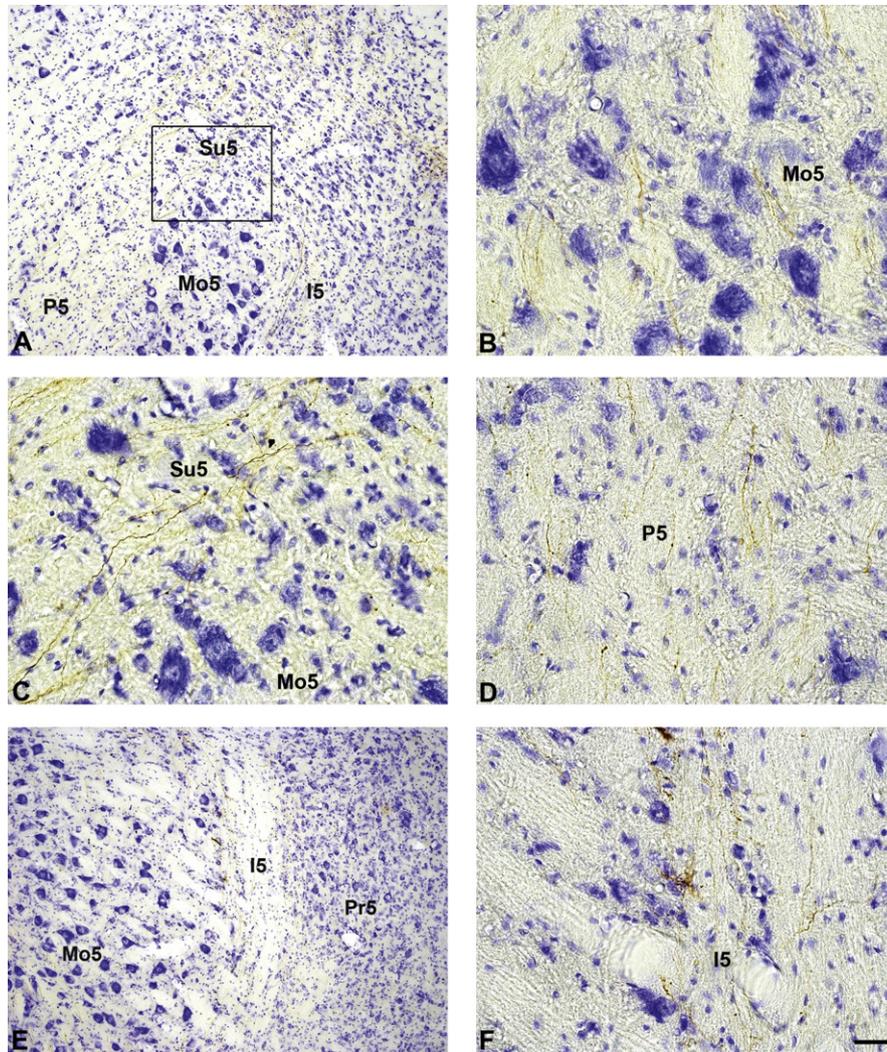


Fig. 3. Anterograde amygdaloid projections to the ipsilateral pontine trigeminal nuclei. (A) Anterograde labeling of central nucleus of the amygdala projections to the Mo5 and associated trigeminal nuclei. (B) Note the labeled axons with boutons en passant and boutons terminaux around, abutting on the large trigeminal motoneurons. (C) High-resolution view of the boxed area in (A) reveals multiple BDA-labeled varicose fibers crossing the Su5. Distinct labeling is also visible in the P5 (D) and I5 (E and F). (F) Higher magnification of the central part of (E) containing the I5. Scale bars = 100 μm (A, D and F); 50 μm (B and C).

4. Discussion

The present study describes the existence of a direct amygdalofugal pathway to the trigeminal nuclear complex in the rat. Particularly, the medial subnucleus of the AmCe sends extensive unilateral projections to all the trigeminal sensory nuclei, including Su5, I5 and P5, as well as relatively light projections to the contralateral Pr5, Sp5I and Sp5C. On the other hand, the lateral part of AmCe projects mainly to the ipsilateral pontine portion of the Me5. At the same time, AmMe sparsely innervates mesencephalic trigeminal neurons only, specifically those located in its mesencephalic portion, while AmBm and AmBl efferents do not target the trigeminal nuclear complex.

4.1. Amygdalotrigeminal projections

Classical hodological studies, carried out by silver impregnation of degenerating axons, describe amygdalofugal projections to certain basal telencephalic and hypothalamic structures but not to the brainstem (Nauta, 1961; Cowan et al., 1965). The introduction of modern, more sensitive techniques for tracing axonal connections has led to the description of a much more extensive subcortical distribution of amygdaloid fibers (reviewed by Pitkänen, 2000).

Hopkins and Holstege (1978) followed amygdaloid tracts to the caudal brainstem in cats but did not describe a projection to the trigeminal nuclear complex. In fact, this may be related to possible species differences. Besides, Holstege et al. (1985) showed that some fibers arising from the bed nucleus of the stria terminalis could be traced to the rostral part of the Sp5C and stressed that these projections were virtually identical to ones derived from the medial subnucleus of AmCe (Holstege et al., 1985). In this regard, these authors offered the possibility that the bed nucleus of the stria terminalis and the AmMe and AmCe should be considered as one anatomical entity. A projection to the caudal (pontine) portion of Me5 was noticed in the autoradiographic experiments of Post and Mai (1980) and of Price and Amaral (1981). The application of the BDA technique enabled us to describe an unexpectedly strong projection from the AmCe to the entire rostrocaudal extent of the Me5. The projection is so massive that the density of BDA labeled terminals in the Me5 rivals the density in the generally appreciated strong projection of the amygdala to the parabrachial nuclear complex (see Fig. 2E). In addition to the findings of Canteras et al. (1995), we were able to demonstrate for the first time that the AmMe sends a scant descending projection to the Me5, indeed only to its rostral part. Our results suggest that both the neuronal types in Me5 receive an amygdaloid input. The projection to the

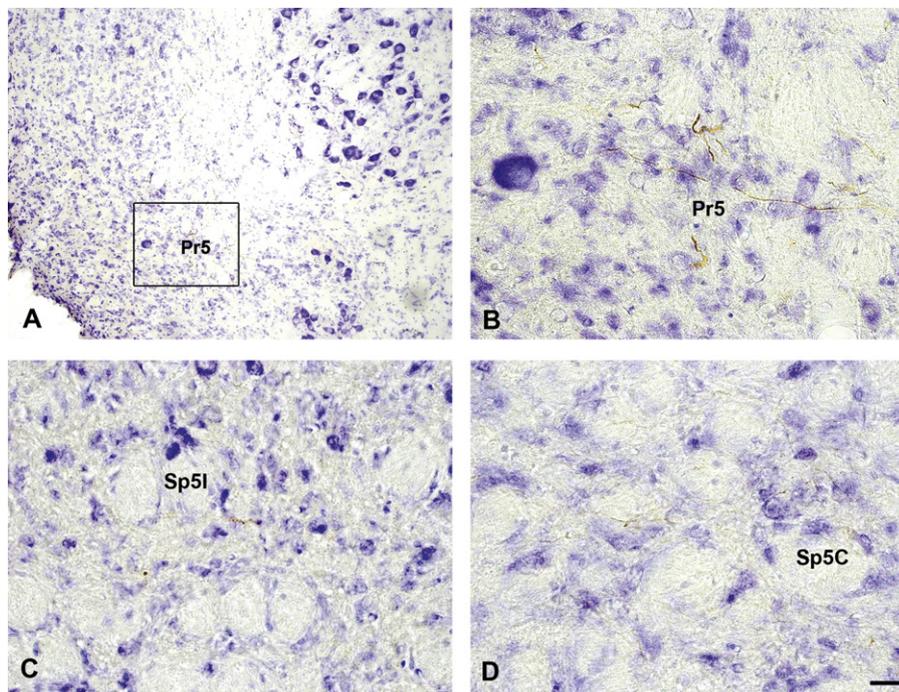


Fig. 4. Distribution of labeled axons and terminals within the contralateral Pr5 and Sp5 after a large injection of BDA in the medial subnucleus of AmCe. (A) Anterograde labeling in the Pr5. (B) Higher magnification of the area inside the rectangle in (A) demonstrating robust labeled varicose fibers and putative terminals in this nucleus. Examples of faint terminal labeling in the neuropil of the contralateral Sp5I (C) and Sp5C (D). Scale bars = 100 μm (A, C and D); 50 μm (B).

pseudounipolar neurons is especially evident in the pontine part of the Me5, where the densely arranged mesencephalic trigeminal perikarya are surrounded by numerous labeled endings. The amygdaloid axons are also in contact with the small multipolar neurons located in close vicinity. The affiliation of the latter to Me5 had been a matter of debate, but their constant presence in the cat (Walberg, 1984; Nomura et al., 1985) and rat (Liem et al., 1991; Luo et al., 1991) MTN has now been verified by means of retrograde tracer studies. In addition, the papers of Lazarov (2000, 2007) provided firm evidence that they are really MTN neurons and represent GABAergic interneurons.

On the other hand, we found no labeled axons in the locus coeruleus. This is in agreement with previous findings in rats and cats (Krettek and Price, 1978). In the monkey, Price and Amaral (1981) have reported that only the ventral aspect of the locus coeruleus receives a substantial input from the central amygdaloid nucleus and that the area of the nucleus subcoeruleus is also slightly labeled. It should be noted, however, that a distinct amygdalo-coeruleal projection, arising from the central nucleus was recently suggested by Reyes et al. (2008).

Our finding on a very scant projection of the AmBm to the periaqueductal gray confirms the detailed study of Petrovich et al. (1996) on the AmBm efferents that establish only a minute projection to the periaqueductal gray from the anterior basomedial nucleus and the anterior cortical nucleus.

4.2. Functional implications

The brainstem neuronal circuitry, responsible for the production of jaw movements is made up of trigeminal sensory and motor neurons, interneurons and premotoneurons. Intrinsic connections also exist between neurons in different components of the trigeminal nuclear complex which underlie modulatory influences between rostral and caudal trigeminal brainstem neurons.

The trigeminal sensory nuclear complex has traditionally been considered as an essential brainstem relay site of nociceptive infor-

mation. Indeed, it is well known that its rostral nuclei as well as the Sp5C are involved in integration of nociception from oral and perioral regions. On the other hand, there is growing evidence that the amygdala is an unexpectedly important subcortical nociceptive center (Usunoff et al., 2006a,b). The input of pain sensation is conducted by the spino (trigemino)–parabrachial–amygdaloid pathway to the AmCe, and we recently demonstrated that the amygdala receives a monosynaptic input from the dorsal horn of the spinal cord and from the Sp5 (Usunoff et al., 2006a,b). The present data suggest a further sensory involvement of the amygdala, e.g., a very strong monosynaptic influence over both the primary proprioceptive neurons of the Me5 and their interneurons, located in Su5, I5 and P5. Although the Pa5 is not a specific site for visceronociceptive function, most physiological and anatomical studies suggest that the Pa5 might be a good candidate contributing to the integration of peripheral nociceptive information (for references see Ma et al., 2007).

The Sp5 is an important relay station in the transmission of orofacial sensory information (Tracey, 1985). Its oral subdivision, the Sp5O conveys information for nociceptive reflexes (e.g., the jaw-opening reflex) from the orofacial region, including the tooth pulp (Jacquin and Rhoades, 1990). Local-circuit neurons located in the Sp5O and Sp5I send their axons to the Mo5 as well.

Previous intracellular tracing studies have demonstrated that in the cat and rat the Pr5 and the dorsal parts of Sp5O contain a high density of medium-sized and small premotoneurons projecting to the jaw-closing motoneurons (Yoshida et al., 1998) and either to the jaw-opening or to the jaw-closing motoneurons (Shigenaga et al., 2000), respectively.

The Pr5 can be subdivided into dorsal and ventral parts which have been proposed to subserve the different functions of relaying and processing of sensory signals from the head, such as tactile sensations and trigeminal proprioception. The dorsal part contains a high density of medium-sized and small neurons, many (45%) of which are glutamatergic (Jacobs and Miller, 1999), constituting most of the projection neurons, while the rest are GABAergic

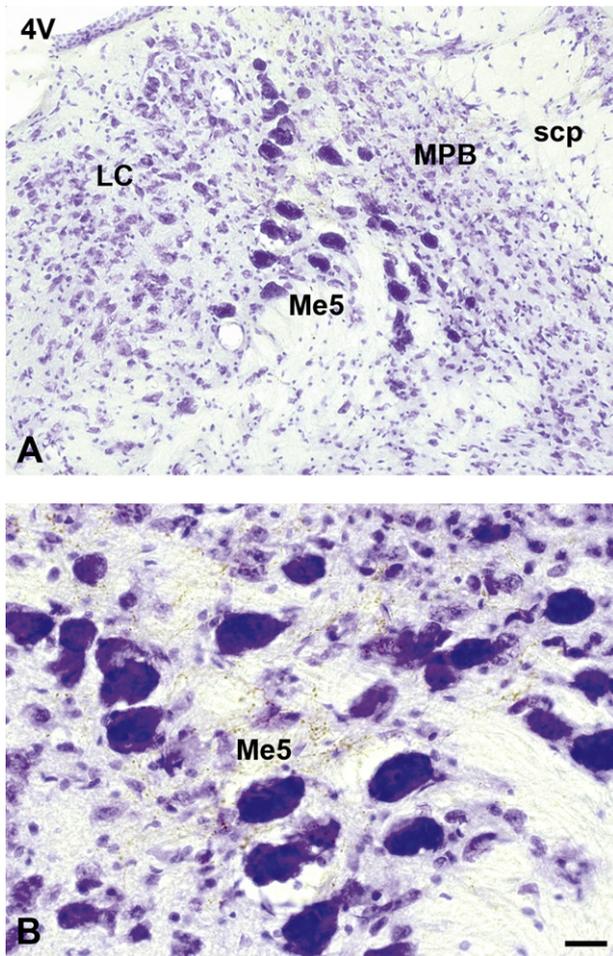


Fig. 5. Anterograde projections from the lateral subnucleus of the central amygdaloid nucleus to the pontine part of the ipsilateral Me5. (A) Low-power microphotograph of the dorsolateral pons at the level of Me5. Note that the adjacent locus coeruleus (LC) is devoid of BDA-labeling while some labeling is evident in the MPB. (B) Higher magnification of the middle part of the section in (A) rotated about 45° counter-clockwise. Light but distinct terminal labeling is found in the neuropil of the rostral portion of the Me5 and around the perikarya of both large pseudounipolar and smaller multipolar mesencephalic trigeminal neurons. Scale bars = 100 μm (A); 50 μm (B).

(Haring et al., 1990) or glycinergic (Rampon et al., 1996). This nucleus is believed to have little impact on orofacial proprioception but is involved in processing of orofacial tactile sensation (Sessle, 1987). The Pr5 has recently been proposed to be a central pattern generator of fictive masticatory movements as neurons in the nucleus were found to fire in phase with the fictive motor program (Tsuboi et al., 2003).

There is evidence that neurons in Me5 subserve not only masticatory muscle reflex mechanisms but also exert direct influence upon the brainstem circuitry responsible for oral-motor behaviors. In fact, cranial proprioceptive signals reach Me5 neurons and are then transmitted, either directly or via brainstem premotor neurons, to the Mo5 for the control of jaw movements (Holstege et al., 1995). Me5 neurons, indicated to be glutamatergic, play a significant role in oral-motor circuits relaying critical proprioceptive information to the CNS (reviewed in Lazarov, 2000, 2007). Besides, it has recently been shown that their proprioceptive properties could be affected by nociceptive signals from the masticatory muscles via central neural mechanisms (see Lazarov, 2007). Mesencephalic trigeminal neurons give off axon collaterals that terminate not only in the Mo5 but also in other brainstem nuclei including the Su5, I5, Pr5, Sp50, the lateral reticular formation of the medulla

(Raappana and Arvidsson, 1993), and thus a variety of neural circuits participate in the control of mastication. Furthermore, some of the Me5-targeted nuclei may have connections through polysynaptic pathways with the tuberomammillary nucleus and small Me5 neurons are reciprocally connected with the hypothalamus (Ericson et al., 1989). Thus, Me5 neurons receive divergent inputs from various brainstem structures on their somata and on their terminals within the Mo5 that can presynaptically modulate their output through different mechanisms (Luo and Li, 1991).

The Mo5 is divided cytoarchitecturally into a dorsolateral subdivision of jaw-closing motoneurons and a ventromedial subdivision of jaw-opening motoneurons (Mizuno et al., 1975), and is associated with masticatory reflexes and the control of jaw movements (for functional somatotopic organization of Mo5, see Weijis, 1996). It has been established by studies using the intraaxonal HRP tracing method that the dorsolateral subdivision of the Mo5 receives projections from jaw muscle spindle and periodontal afferents, whose cell bodies are located in the Me5 (Mizuno et al., 1975; see also Bae et al., 1999 and references therein). Trigeminal motor neurons are the final output neurons of the brainstem responsible for a variety of orofacial motor functions. Until recently, it was believed that although mastication is controlled by motoneurons innervating antagonistic muscles, the trigeminal motor system is devoid of reciprocal inhibition. However, it has been reported that some of premotoneurons contain the main excitatory or main inhibitory transmitters in the CNS, such as glutamate, GABA and glycine, respectively (Turman and Chandler, 1994a,b) and that jaw-closing motoneurons receive excitatory and inhibitory inputs from premotoneurons (Bae et al., 1999; Pang et al., 2009). Further, it is well known that not only motoneurons, but a variety of interneurons and projection neurons containing glycine and/or GABA (Bae et al., 2002) are located within the anatomical confines of the rat Mo5 (see also Luo and Dessem, 1999 and references therein). For instance, Su5 and I5 commissural interneurons receive a variety of convergent inputs, provide input to trigeminal motoneurons and, thus, function in the rhythmical production of jaw movements. Intertrigeminal neurons receive convergent input from perioral afferents and from oral regions of the cerebral cortex, and are hypothesized to be involved in the sensorimotor regulation of orofacial behaviors engaged with explorative or manipulative functions (Olsson and Westberg, 1989). Supratrigeminal neurons are also rhythmically active during cortically induced fictive mastication in the rat (Inoue et al., 1992), suggesting that they have a role in central rhythm patterning. Besides, the Su5 is the most prominent termination area for Me5 neurons. It is likely that mesencephalic trigeminal neurons activate Su5 neurons which in their turn will inhibit trigeminal motoneurons innervating jaw-closing muscles. Indeed, interneurons in the Su5 mediate neuronal activity to ipsilateral and contralateral trigeminal motoneurons, 90% of them are GABAergic and are obviously inhibitory to jaw-closing motoneurons (Jerge, 1963; Luo and Li, 1990; Ginestal and Matute, 1993; Enomoto et al., 2002).

For many years it has been well known that the amygdala is involved in emotional behavior, especially in fear conditioning and fear-motivated learning, but is also involved in conditioning using appetitive stimuli such as food, sex, and drugs (for review, see Davis, 1992). Moreover, many of its target areas are known to be involved in the expression of fear and defensive responses. In particular, it is believed that neurons in AmCe projecting to the Mo5 play a key role in the recognition of fear in facial expressions (Davis, 1992). Here we demonstrate that AmCe-Me5 pathway may be another possible pathway responsible for the control of jaw movements closely related to emotional behavior since Me5 provides sensory feedback from orofacial tissues to trigeminal motor neurons (Lazarov, 2000, 2007). Though the majority of neurons in AmCe are considered GABAergic and inhibitory, the neurochemical nature of the amygdala

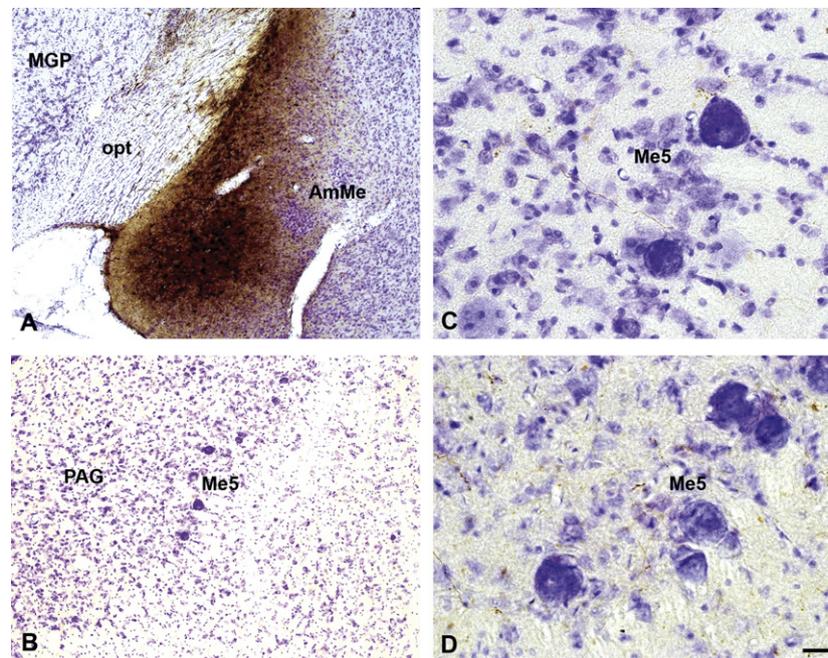


Fig. 6. A section through the rostral part of the Me5 showing the distribution of terminal labeling following a large injection of BDA into medial amygdaloid nucleus (AmMe). (A) Injection site in the posterior part of its anterodorsal subnucleus without concomitant labeling spread in the surrounding nuclei of the amygdala. (B) Low magnification of the midbrain at the level of the rostral Me5. A fair number of labeled axons reach the PAG. (C and D) A small number of smoother labeled axons arborize in the vicinity of large mesencephalic trigeminal neurons. Throughout the neuropil of the Me5 a meshwork of fine labeled fibers with varicosities is also present. (C) Higher magnification of the nucleus shown in (B). Scale bars = 200 μm (A); 100 μm (B); 40 μm (C and D).

dalotrigeminal projection and its functional relevance still remain to be elucidated in details.

On the other hand, we were not able to show that output signals from the basolateral amygdaloid complex influence trigeminal neurons. Neurons in this subdivision, which are thought to be glutamatergic, receive direct or highly processed input from the sensory systems. The lack of data on a direct trigemino-Am tract, however, does not allow us to conclude whether the connection between the amygdaloid and trigeminal nuclear complexes is reciprocal or the Am receives very few collaterals from the trigeminal axons that profusely innervates other target structures as we have already described (Usunoff et al., 2006a).

In conclusion, it can be inferred that the amygdala provides modulatory inputs that affect the orofacial sensory-motor reception processing. Nonetheless, the present findings in the rodent brain should be interpreted for the human neuronal circuitry with caution.

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