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Short Communication

Efferent connections of the parabigeminal nucleus to the amygdala and the superior colliculus in the rat: A double-labeling fluorescent retrograde tracing study

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ABSTRACT

The parabigeminal nucleus (Pbg) is a subcortical visual center that besides reciprocal connections with the superior colliculus (SC), also projects to the amygdala (Am). The Pbg–Am connection is part of a multineuronal pathway that conveys extrageniculate inputs of the retina to the Am, and it rapidly responds to the sources of threat before conscious detection. The present study demonstrates that Pbg projects bilaterally to Am and SC. The ipsilateral projections arise from separate cell populations, whilst the contralaterally projecting Pbg neurons emit branching axons that simultaneously innervate Am and SC.

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A recently revealed important function of the amygdala (Am) is that it acts as the brain's lighthouse, which constantly monitors the environment for stimuli which signal a threat to the organism (Davis and Whalen, 2001; Liddell et al., 2005). Observations of Adolphs et al. (1994) and of Young et al. (1995) that bilateral Am damage in humans compromises the recognition of fear in facial expressions while leaving recognition of face identity intact were immediately followed by a considerable number of investigations that confirmed and extended these significant data (Adolphs and Tranel, 2000; Breitner et al., 1996; Liddell et al., 2005; Morris et al., 2001; Whalen et al., 1998; Williams et al., 2004; reviewed in Usunoff et al., 2006). Importantly, the data from healthy volunteers when masking procedures were used, and in patients with extensive lesions of the striate cortex indicate that “unseen” fearful and fear-conditioned faces elicit in-

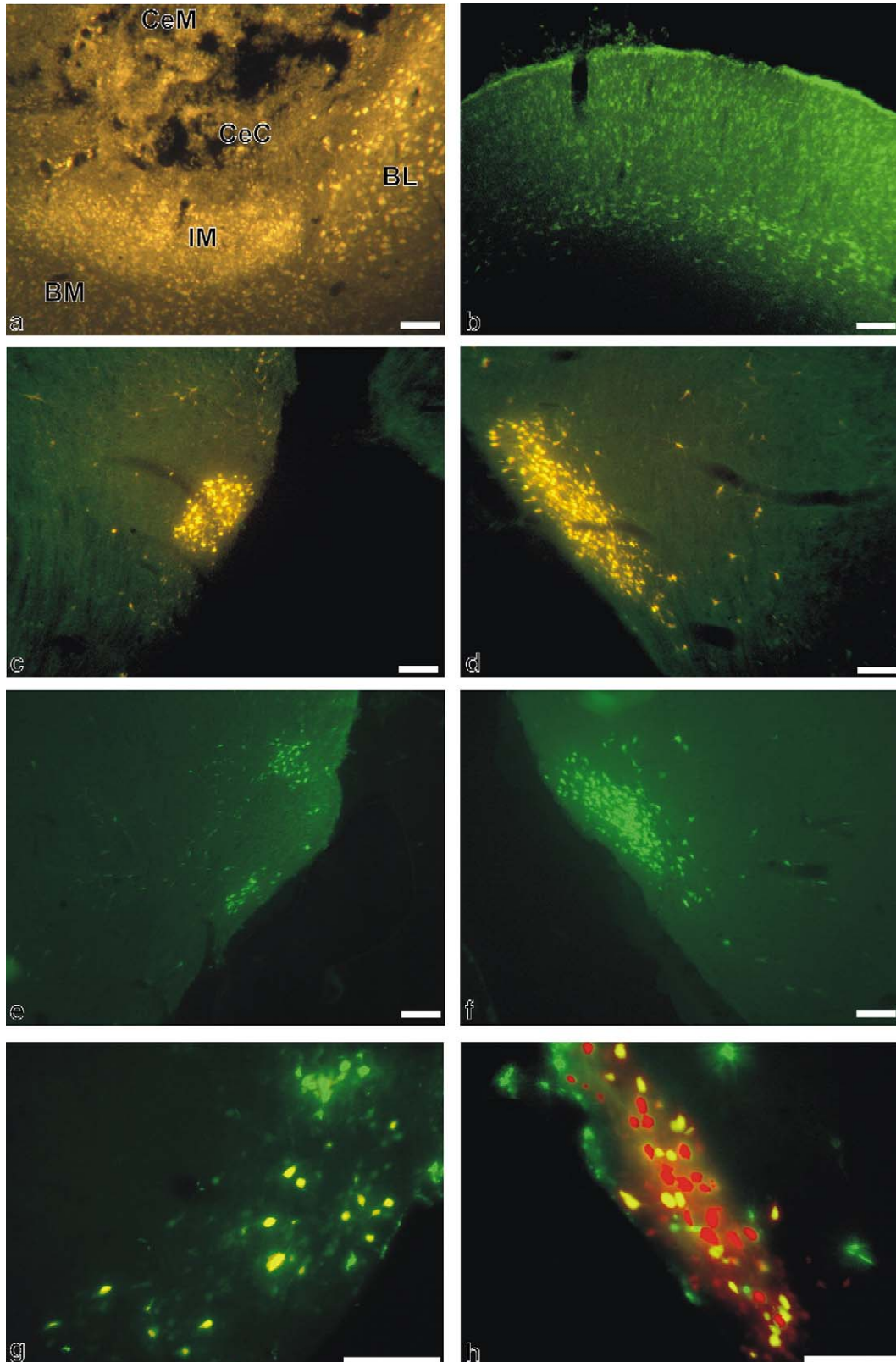
creased Am responses (Morris et al., 2001; Whalen et al., 1998). Apparently, extrageniculate pathways are involved. Morris et al. (2001) suggest that the retinal impulses reach the Am via a multisynaptic pathway: superior colliculus (SC) – pulvinar – Am. This multisynaptic chain was traced in previous studies (reviewed in Grieve et al., 2000; Morris et al., 2001). A second multineuronal chain was described by Linke et al. (1999). They traced to the Am axons from the supragenulate nucleus, the medial division of the medial geniculate nucleus, and from the posterior intralaminar nuclei. All these structures receive an afferent input from SC. According to Zald (2003), recent observations suggest that the Am may be the lynch-pin of the organism's ability to rapidly respond to sources of threat without explicit knowledge of the presence of the stimulus, i.e., before conscious detection.

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The parabigeminal nucleus (Pbg) is a small structure, located subpially along the lateral border of the mesencephalon, dorsocaudolateral to the substantia nigra. It contains densely arranged small neurons. Pbg is interconnected with several subcortical visual centers: SC, lateral geniculate body, striate-recipient zone of the pulvinar, and suprachiasmatic

nucleus (Baleydier and Magnin, 1979; Bina et al., 1993; Graybiel, 1978; Hall et al., 1989; Harting et al., 1991; Roldan et al., 1983; Watanabe and Kawana, 1979). Especially, the reciprocal connections between the Pbg and SC are so strong that Graybiel (1978) designated the Pbg as a satellite system of the SC.



We recently found (Usunoff et al., 2006) that the Pbg, an established subcortical visual structure, also projects to a key structure of the limbic system, the Am. The projection from the Pbg to Am might be an element of a third disynaptic connection from the SC to the Am, since this nucleus receives a significant input from the SC (Baleyrier and Magnin, 1979; Graybiel, 1978, reviewed in Usunoff et al., 2006). In order to understand whether the neurons of this small nucleus, with prominent efferent connections, are able to innervate more than one target by means of divergent axon collaterals, we performed a double labeling retrograde tracing study. Here we report that some neurons in Pbg emit branching axons that innervate SC and Am simultaneously, and additionally that these connections arise also from separate neuronal populations.

The projection of Pbg to the Am is demonstrated in Figs. 1a, c, d. Fluoro-Gold (FG), one of the most effective fluorescent retrograde tracers, was stereotaxically injected in the central nucleus of the Am (Fig. 1a). After 4 days, the tracer was retrogradely transported to the cells of origin of the afferent pathways of the central Am nucleus in numerous regions (Usunoff et al., 2006), among those also the Pbg. The Pbg–Am connection is bilateral (Figs. 1c, d). On the ipsilateral side, the Pbg–Am neurons are concentrated in the central portion of Pbg, leaving the dorsal and ventral parts of the nucleus unlabeled (Fig. 1c). The crossed connection is more prominent. On the contralateral side the labeled neurons are located throughout the Pbg (Fig. 1d).

The projection of Pbg to the SC is shown in Figs. 1b, e, f. The superficial layers of SC were evenly infiltrated with fluoro-emerald (FE, D1820, Molecular Probes) (Fig. 1b). The Pbg–SC connection is also bilateral. On the ipsilateral side, two sharply delineated neuronal groups in Pbg are present – dorsal and ventral (Fig. 1e). Contralaterally projecting Pbg cells are distributed throughout the dorsoventral extent of the nucleus but are mainly concentrated in the central portion (Fig. 1f).

The simultaneous tracing of Pbg–Am and Pbg–SC connections is demonstrated in Fig. 1g, h. In double tracing experiments, we injected FG in the central nucleus of the Am, and SC was infiltrated with FE. These two tracers are fluorescing at different wavelengths, so that every field was observed and photographed with two filters. Upon excitation, FG emits strong yellow fluorescence, whilst the fluoro-emerald-labeled neurons exhibit a green fluorescence. It is evident that on the ipsilateral side (Fig. 1g) Pbg–Am and Pbg–SC tracts arise largely – if not exclusively – from different cell

populations, as already indicated in Figs. 1c, e. On the contralateral side (Fig. 1h), the cells of origin of these two pathways are mixed. There are apparently three populations: neurons that project only to the Am, cells that project only to the SC (a smaller number), and double-labeled neurons that simultaneously innervate Am and SC by means of divergent axon collaterals. The fluoro-emerald is not so effective as retrograde tracer as the FG, yet it has a significant advantage: fluoro-emerald is transported also anterogradely. Axons arise from the injected SC that could be followed to the ipsilateral Pbg, where they form a very dense terminal field, in the central region, where the Pbg–Am cells are located.

Pbg emits bilateral projections to SC and Am, and also to the lateral geniculate body, which is bilateral in some species (Harting et al., 1991). The ipsilateral connection to the SC arises in the dorsal and ventral subgroups of Watanabe and Kawana (1979) and the middle subgroup project to the Am. Exactly this group receives a dense terminal input from the superficial layers of the SC. Thus, there is a point-to-point multineuronal chain: SC – Pbg – Am. This characteristic labeling in Pbg was first demonstrated by Graybiel (1978) in the cat (see her “band” in Fig. 6B, Graybiel, 1978). Otherwise, the ipsilaterally projecting Pbg neurons in the cat have a broader distribution (Graybiel, 1978; Roldan et al., 1983). The crossed connections are supplied by two neuronal types. Some cells project either to the Am or to the SC, and there are also neurons that simultaneously reciprocate the input from the SC, and project to the central nucleus of Am. Thus, a one and the same cell can project to visual and limbic structures.

The present results extend the previous observation (Usunoff et al., 2006) that Pbg might be included in the multineuronal subcortical pathways that transfer the retinal inputs to the Am, thus rapidly informing the brain’s lighthouse about sources of threat before conscious detection (Adolphs and Tranel, 2000; Davis and Whalen, 2001; Liddell et al., 2005; Morris et al., 2001; Whalen et al., 1998; Williams et al., 2004; Zald, 2003). However, the present findings in the rodent brain should be interpreted for the human neuronal circuitry with a caution as the projections of Pbg upon the lateral geniculate body display very significant species differences (Harting et al., 1991).

Ten male Wistar rats, weighing 260–320 g, were used. In 8 animals 0.25 μ l 2% FG was stereotaxically injected in the right central nucleus of the Am (coordinates according to bregma:

Fig. 1 – (a) Injection focus of Fluoro-Gold (FG) in the amygdala (Am). The central necrotic zone involves the medial (CeM) and the capsular (CeC) subnuclei of the central amygdaloid nucleus. BL – basolateral amygdaloid nucleus, BM – basomedial amygdaloid nucleus, IM – intercalated cell masses. Calibration bar = 100 μ m. (b) By means of several small injections of fluoro-emerald (FE), the superficial layers of the superior colliculus (SC) are regularly infiltrated. Calibration bar = 100 μ m. (c) Retrogradely labeled neurons in the central portion of the Pbg, ipsilateral to the injection of FG in the Am. Calibration bar = 100 μ m. (d) Retrogradely labeled neurons in the Pbg, contralateral to the injection of FG in Am. Calibration bar = 100 μ m. (e) Two groups (dorsal and ventral) of labeled neurons in Pbg following injection of FE in the ipsilateral SC. Calibration bar = 100 μ m. (f) Retrogradely labeled neurons in Pbg contralateral to the injection of FE in SC. Calibration bar = 100 μ m. (g–h) Double labeling experiment with injections of FG in Am and FE in SC. (g) The dorsal neuronal group of the ipsilateral Pbg exhibits green fluorescence after SC injection. The yellow fluorescence of FG marks the central Pbg neurons projecting to AM. There are no double-labeled neurons in the Pbg ipsilateral to the injection. Calibration bar = 100 μ m. (h) In the contralateral Pbg there are neurons projecting only to Am (yellow), quite a few neurons project only to SC (green), and there is a substantial number of double-labeled neurons (red). Calibration bar = 100 μ m.

AP -2.5, L -4.2, DV -8.2; Paxinos and Watson, 1998) and four small injection foci of fluoro-emerald (1.5 μ l each, 10% fluoro-emerald) were placed in the superficial layers of the SC on the same side (coordinates according to bregma: 1: AP -6.0, L -0.8, DV -4.2; 2: AP -6.0, L -2.0, DV -4.5; 3: AP -7.0, L -0.8, DV -4.0; 4: AP -7.0, L -2.0, DV -4.2; Paxinos and Watson, 1998) (Fig. 2). Since FG is considerably more effective retrograde tracer than

fluoro-emerald, in order to compare the Pbg-SC and Pbg-Am connections, in one rat only the Am received a FG injection, and in another rat the SC was infiltrated with $2 \times 0.25 \mu$ l FG. Following survival time of 4 days, the animals were transcardially perfused with 100 ml phosphate-buffered saline, followed by 500 ml of 4% paraformaldehyde in phosphate buffer (pH 7.2), and finally with 100 ml of the same fixative to

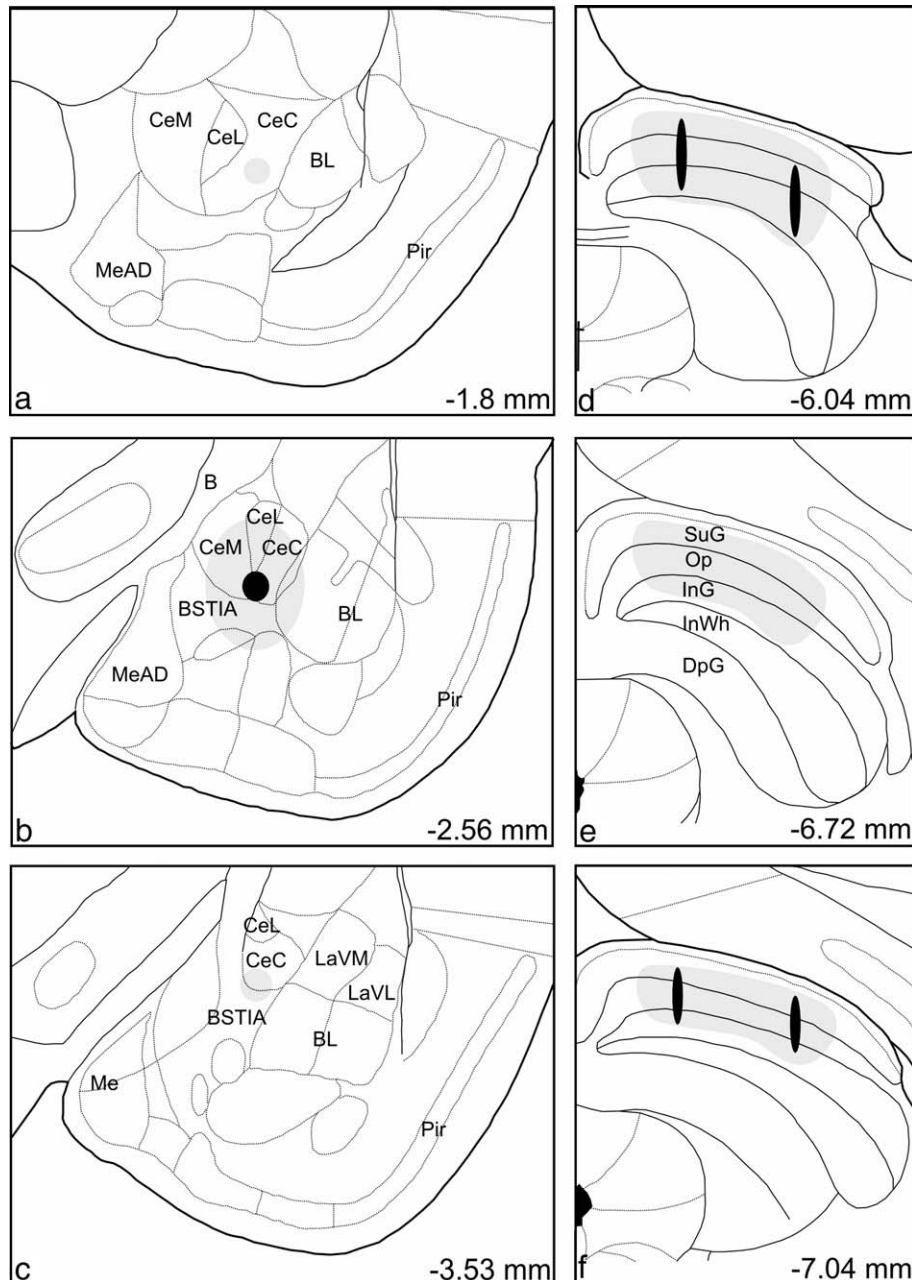


Fig. 2 – Schematic diagrams showing cores (black) and halos (gray) of injections sights of Fluoro-Gold in the amygdala (a–c) and fluoro-emerald in the superior colliculus (d–f) (schematic frontal planes posterior to bregma, modified after Paxinos and Watson, 1998). Abbreviations: B – basal nucleus of Meynert, BL – basolateral amygdaloid nucleus, BSTIA – bed nucleus of stria terminalis, intraamygdaloid division, CeC – central amygdaloid nucleus, central part, CeL – central amygdaloid nucleus, lateral part, CeM – central amygdaloid nucleus, medial part, DpG – deep gray layer, InG – intermediate gray layer, InWh – intermediate white layer, LaVL – lateral amygdaloid nucleus, ventrolateral part, LaVM – lateral amygdaloid nucleus, ventromedial part, Me – medial amygdaloid nucleus, MeAD – medial amygdaloid nucleus, anterodorsal part, Op – optic nerve layer, Pir – piriform cortex, SuG – superficial gray layer.

which 5 g sucrose were added. The brains were stored in 20% sucrose in the same fixative for 2–5 days at 4 °C. Serial sections, 30 µm thick, were cut on a cryostat and stored in phosphate buffer. The sections were mounted, air-dried and coverslipped with Gel Mount Aqueous Mounting Medium (Sigma) and sealed with nail-varnish. The sections were observed in Nikon and Leitz Aristoplan fluorescent microscopes with filter sets detecting the respective maxima of emission: 536 nm (FG) and 518 nm (fluoro-emerald). Photomicrographs of selected fields were taken with a digital camera (7.3 three Shot Colour, Visitron Systems, Diagnostic Instruments).

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