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# Anatomical evidence for the projections from the basal nucleus of the amygdala to the primary visual cortex in the cat

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ABSTRACT

The amygdaloid complex receives information from all sensory systems, especially from vision. In the primate, the amygdala is reciprocally interconnected with some regions of high-order visual cortices such as TE and TEO and only projects to the primary visual cortex (V1, area 17) without direct projection from V1. However, in the cat little is known about the projection from the amygdala to the primary visual cortex. In this study, anatomical study is carried out in cats to determine whether the amygdala sends feedback projection to area 17. FlouroGold, a fluorescent dye was microinjected into area 17 in cats. In the basal nucleus in the amygdala, the retrograde labeled cells (about 30% of total number of the region of interest observed) are distributed widely in an irregular manner, neither in lamina nor in group. The results provide the first anatomical evidence of the amygdale projection to area 17 in the cat, which is a widely used animal model for vision research.



The amygdaloid is a complex of nuclei and specialized cortical areas located in the rostromedial part of the temporal lobe in high mammals. The amygdaloid complex has long been known to play an important role in the integration and control of emotional and autonomic behaviors, and mediation of certain aspects of learning and memory [3,4,21]. In the primate, the amygdala is reciprocally connected with most regions of the neocortex [16] and receives high-level sensory information through monosynaptic neocortical inputs [25]; furthermore, some studies show that the visual system provides the major sensory input and only the high-order visual cortex such as TE and TEO directly project to the amygdala [11,12]. Several lines of evidence indicate that the feedback projections are more extensive than the feedforward between the amygdala and the visual cortex [17,22,8,23].

Recently using anterograde and retrograde tracers, Amaral and Price [1,2] demonstrated that in the primate, the amygdala projects to visual cortex of "ventral stream" pathway including V4, V2, V1 in addition to TEO and TE, and these projections follow the rostrocaudal topography: rostral divisions of the amygdala preferentially project to more rostral parts of visual cortex whereas caudal divisions to more caudal [1,11]. Both superficial layers (border of I/II) and deep layers (V/VI) in area TE receive heavy projections from the magnocellular and intermediate divisions of the basal nucleus of the amygdala, but only superficial layers of area V1 receives projections primarily from the magnocellular division of the basal nucleus [1,5]. However, nothing is known about feedback projections from the amygdaloid complex to the primary visual cortex (area 17) in the cat, a largely used animal model, whose eyes are front located like human for vision research. The purpose of the study is to determine whether there is the projection from the basal nucleus of the amygdaloid complex to area 17 in the cat, using the FluoroGold retrograde tracing and evoked potentials (EPs) recording techniques.

Seven adult cats of either sex were used in the current study. All procedures were performed in strict accordance with the Guide for the Care and Use of Laboratory Animals described by the United States National Institutes of Health. All experiments were designed to minimize the number of animals used. The procedures were previously reported elsewhere [7,9,10] and are only briefly described here. Animals were initially anaesthetized with ketamine (25 mg/kg). During the experiments, anesthesia was maintained with i.v. infusion of sodium pentobarbital with a dose of 6 mg/(kg/h). The animals' physiological condition was monitored and kept in normal range throughout the experiment.

In histological study for three cats, the cortical area at Horsley–Clarke coordinates P0-6, L0-4 in area 17 was exposed. After removal of the dura, a micro-syringe filled with 2% fluorescent tracer FluoroGold (FG, Fluorochrome, Sigma–Aldrich Co., Switzerland) [27,18,24] was inserted perpendicular to the cortical surface and lowered to a depth of 500–1500 μm, the total amount of 4.0 μl FluoroGold (2%) was distributed in four sites in area 17 at

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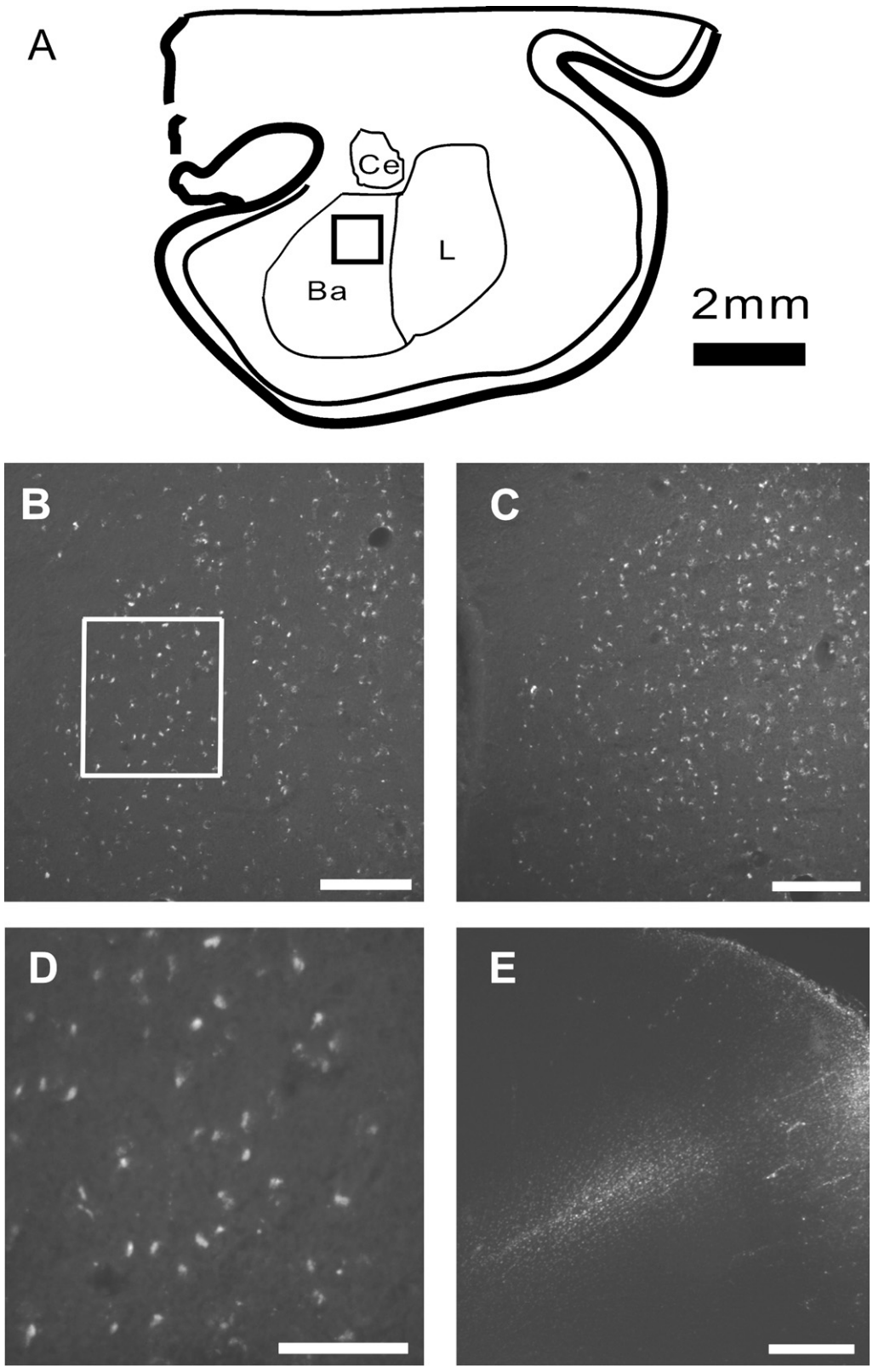


**Fig. 1.** Retrogradely labeled cells in the basal nucleus of the amygdala induced by injection of FluoroGold at area 17 of the cat. (A) A schematic view of a coronal section of the amygdaloid complex. The black square within the basal nucleus indicates the site where the photos were taken for (B) and (C). The left of the figure indicates the medial side, the right the lateral. Ba, basal nucleus; L, lateral nucleus; Ce, central nucleus; (B) and (C) two fluorescence photos of FluoroGold labeled cells in a coronal section of the basal nucleus at Horsley–Clarke coordinates P12 and P11, respectively; (D) a magnified photo taken from the square shown in (B); (E) a coronal section of area 17 showing the injection site and its diffusion area. Scale bars: 200 μm for B, C and D; 100 μm for E.

Horsley–Clarke coordinates P1-6, L1-3. The micro-syringe was routinely kept in the brain for 10 min after the injection. The incision on the animal's head was closed in layers after the tracer was distributed in the intended location, and the animal was returned to its home cage when it became alert from the anesthesia.

After a 15-day survival period, the animal was deeply anesthetized and perfused conventionally using a saline rinse and aldehyde fixative. The part of brain including area 17 and the amygdala were removed for frozen sectioning. They were cut in 40 μm coronal sections and observed under a fluorescent microscope. Conventional Nissl staining was employed for identifying the structure of the amygdala.

In the evoked potential experiments of four animals, gallamine triethiodide (8–10 mg/(kg/h)) was used for immobilization and they were artificially respired. Contact lenses were used to protect the cornea. The end-tidal was kept at about 4%. Area 17 and the cortical area covering the amygdala at Horsley–Clarke coordinates A9-15, L6-12 were exposed. The dura covering the latter cortex was removed. The double electrodes (tip distance: 0.5–0.8 mm) were inserted perpendicularly, via the latter cortex, to the basal nucleus of the amygdala for electrical stimulation. The duration of electrical pulse was 0.2 ms and intensity ranged from 0.3 mA to 1.0 mA at 4 Hz. The evoked potentials were recorded from different sites in area 17 with Ag–AgCl electrode with a diameter of 0.5 mm. The EP signals were amplified and stored in an electrophysiological system (model: U-ML, MacLab, Powerlab, Australia) for analysis. The latency of EPs was defined as a peak latency of the EPs trace, which is the time from the onset of the electrical stimulus to the peak of each EP component. At the end of experiments, the stimulus sites in the amygdala were marked with electrolytic lesions (0.5 mA for 5 s) and

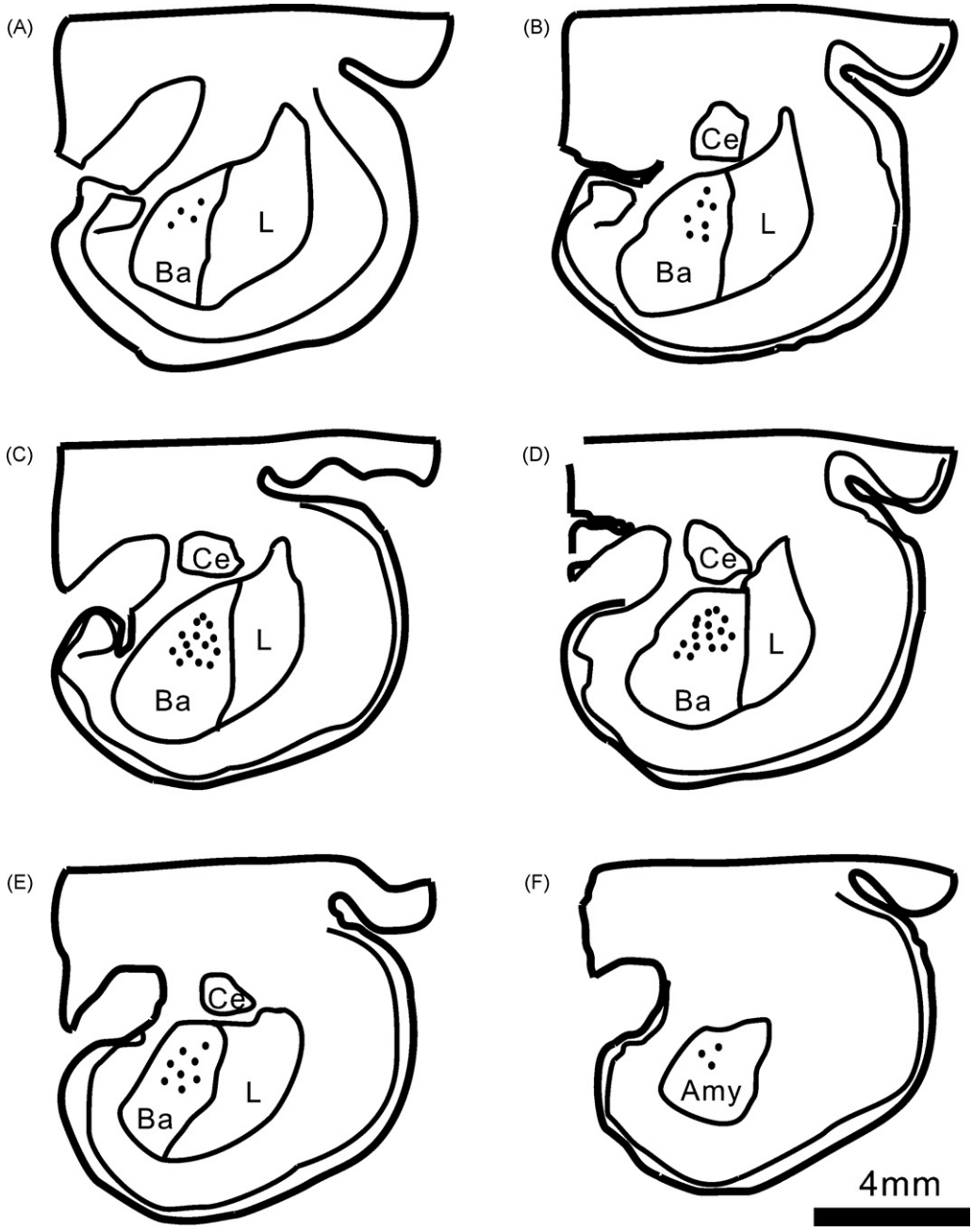


**Fig. 2.** Distribution of labeled cells in the basal nucleus in the amygdala of a cat shown in different coronal sections of Horsley–Clarke coordinates at 10.2 (A), 10.5 (B), 11.0 (C), 12.0 (D), 12.2 (E) and 12.5 (F), respectively. Ba, basal nucleus; Ce, central nucleus; L, lateral nucleus; Amy, the amygdala.

the animals were perfused and the part of the brain was removed for frozen sectioning. The stimulus site was identified using Nissl staining.

Fig. 1 shows a few coronal sections of retrograde labeled cells in the basal nucleus of the amygdala induced by injection of FluoroGold on the ipsilateral side in area 17 of a typical cat. These labeled pyramidal cells were located in a superior area of about 1 $mm^2$ and widely distributed throughout sections from A10.2 to A12.5, where the basal nucleus could be clearly identified in the Nissl stained sections, along the rostrocaudal axis in an irregular manner neither in lamina nor in grouping. The mean cell density of the labeled cells (about 360 cells/$mm^2$) was about 30% of the total cell density (about 1200 cells/$mm^2$, which was counted from the Nissl stained neighboring section) in the region of interest observed in the amygdala. The similar retrograde labeled neurons were observed in the basal nucleus for all three cats of either sex studied without exception though there was significant variation caused by difference in individuals and experimental process. This indicates that in the cat there is a direct feedback projection from the basal nucleus of the amygdala to area 17.

The serial coronal sections containing FluoroGold labeled cells are shown in the amygdala at Horsley–Clarke coordinates from A10.2 to A12.5 in Fig. 2. The labeled cells were continuously located along a rostrocaudal axis, with maximal distribution at A11–A12, in the superior part of the basal nucleus although the injection sites of retrograde tracer are separately in area 17. The volume the labeled cells occupied was about 1–2 $mm^3$ that is approximately 30–60 times larger than the total volume of diffusing regions (about 0.03 $mm^3$) in area 17 based on the estimation of labeled cells around the four injected sites. This may suggest a convergent projection from the amygdala to area 17, in addition to its extended diffusing projection.

The typical evoked potentials recorded in area 17 by electrical stimulation in the basal nucleus are shown in Fig. 3A and B. The fast positive wave appeared initially at 4.3–4.5 ms and peaked at 10.8 ms after the onset of stimuli. The distance between the amygdala and area 17 is estimated to be ranged from about 30 mm to 37 mm according to Horsley–Clarke coordinates. We suppose that if the fastest signal from the amygdala to area 17 was via monosynaptic connections, then the fastest speed of direct feedback signals from amygdala to area 17 would be estimated to be around 3–8 m/s, suggesting that they may be presumably conducted through the finest myelinated nerve fibers similar to Gasser's type B periphery fibers with conduction speed 3–15 m/s [6,15]. The late slow negative wave peaked at 57 ms after the stimulus onset may reflect cortical activities induced by signals through various indirect multi-synaptic pathways from the amygdala to area 17. It is worthy to note that when the stimulating current in the amygdala decreased from 1.0 mA to 0.4 mA, the fastest positive response declined only to 73%, while the late negative response declined to as low as 47%, speculating that the later may not be via monosynaptic pathways. The amplitude distributional maps demonstrate that the recorded evoked potentials were located extensively with the maximal response sites concentrated in an area of P2-5 and L2-3 in Horsley–Clarke coordinates, suggesting that this feedback signals from the amygdala may influence area 17 in a diffuse manner.

The results of this study provide the first anatomical evidence for the direct feedback projection from the basal nucleus of the amygdala to the primary visual cortex in the cat. The labeled amygdala cells of about 30% in the total in the region of interest, which were located in the superior part of the basal nucleus, demonstrate a similar topographic organization of feedback projection to area 17 observed in monkeys [1]. Cats have been widely used in vision research for more than half a century, during which a huge amount of data on functions of visual cortical areas was largely accumulated to gain better understanding of the cerebral cortex. Thus, in cats, the functions of the substantial feedback projection from the amygdala to lower visual cortex remain elusive further.

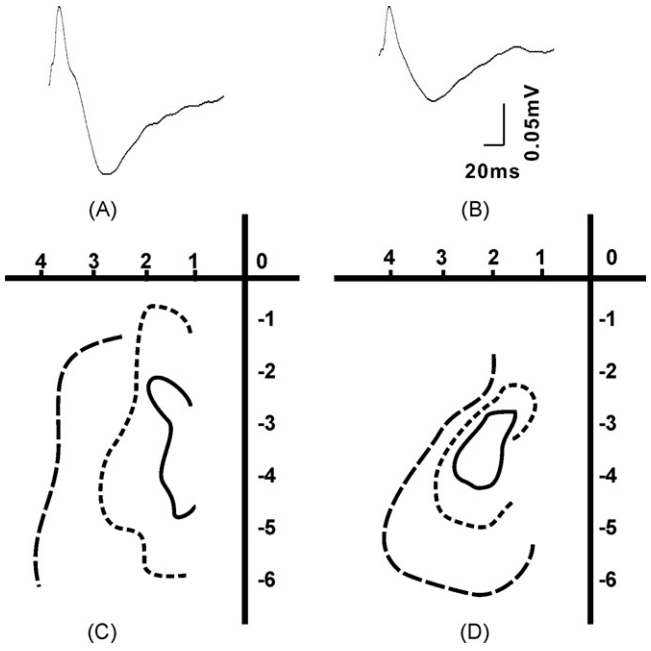


**Fig. 3.** Cortical evoked potentials at area 17 elicited by electrical stimulation of the basal nucleus in the amygdala of a cat. (A) and (B) wave forms of evoked potentials elicited by different intensity of impulse current 1.0 mA and 0.4 mA, respectively. Note that as stimulus current decreased the amplitude of late negative wave appeared to decline more than the positive one. (C) and (D) the iso-amplitude distributional maps of the positive peak response in evoked potentials recorded from area 17 of two cats in the Horsley–Clarke coordinates. Iso-amplitude lines in C indicate: long dashed line: 5 μV; short dashed line: 10 μV; solid line: 15 μV, and lines in D: long dashed line: 10 μV; short dashed line: 25 μV; solid line: 30 μV.

Recently, a functional magnetic resonance imaging (fMRI) study combined with behavioral tests has shown that the functional connection between the amygdala and area 17 is negative during conscious fear perception and there is no such connection between them in nonconscious fear, suggesting that reentrant feedback necessary to afford such awareness [26,14]. In the cat, the modulatory feedback effect of higher level cortical areas, such as area 21a, PMLS have been demonstrated to significantly improve spatial frequency sensitivity, orientation selectivity, direction selectivity and even neural oblique effect of neurons in area 17 [9,10,13,19,20]. Whether the amygdala has substantial control over visual information processing is worthy to study further. The study on the physiological roles of amygdala projections to striate and extrastriate cortical areas are under investigation in the laboratory.

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