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RESEARCH ARTICLE

Evidence for direct projections from the basal nucleus of the amygdala to retrosplenial cortex in the Macaque monkey

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Abstract The role of the primate retrosplenial cortex (RSC) in memory processing and spatial navigation has been well established. Recently, processing emotionally salient information has been attributed to the RSC as well. Little anatomical data, however, exist linking the RSC with known emotional processing centers within the brain. The amygdala has been implicated as a substrate for modulating memory for emotionally salient events; yet no study to date has demonstrated that this area has a direct connection in the primate brain. With modern retrograde tracer injections into the RSC and adjacent cortical areas of the monkey (*Macaca fascicularis*), we demonstrate that there are efferent projections from the basal nucleus of the amygdala to the RSC and area 31. These projections offer anatomical data supporting the hypothesis that the RSC might receive emotionally salient input directly from the amygdala and suggest a role for the RSC as a node within a neural system potentially capable of integrating emotional information for use in memory or other cognitive processes.

Keywords Amygdaloid complex · Emotion · Memory

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Introduction

The functional and connectional organization of the retrosplenial cortex (RSC) is largely unexplored due to its relatively small cortical surface area and challenging location for surgery and other experimentation. The RSC is traditionally defined as cortical areas 29 and 30, which course along the dorsal bank of the callosal sulcus above the corpus callosum and extend onto the medial cortical surface immediately posterior to the splenium of the corpus callosum (Brodmann 1909; Vogt et al. 1995; Morris et al. 1999; Kobayashi and Amaral 2000; Morris et al. 2000; Vogt et al. 2001; Vogt and Laureys 2005; Vogt et al. 2005; Vogt et al. 2006). In both human and non-human primates, the RSC is located in roughly the same region (Fig. 1). However, in the non-human primate, the RSC is confined mostly to the upper bank of the callosal sulcus and does not extend significantly onto the medial surface. Area 31 is located on the posterior and superior extent of the caudal cingulate gyrus, juxtaposed between area 23 and area PGm (monkey) or 7 m (human). In some cases, area 31 is located within the caudal portions of the cingulate gyrus, but is most commonly found on the medial cortical surface. Adjacent cortices of the posterior cingulate, areas 23 and the precuneus, are often misrepresented as RSC or area 31, despite their distinct cytoarchitectural (Vogt et al. 1995) and immunohistochemical (Hof et al. 1995; Nimchinsky et al. 1997) differences. Recent studies have characterized this ensemble of posteromedial cortices en masse, focusing on the similar functional (Gusnard and Raichle 2001; Raichle et al. 2001; Vogt et al. 2006) and connectional (Parvizi et al. 2006) properties of this cortical region.

In rats (van Groen and Wyss 1990; van Groen and Wyss 1992; Wyss and Van Groen 1992; Van Groen and Wyss 2003; Shibata et al. 2004) and non-human primates

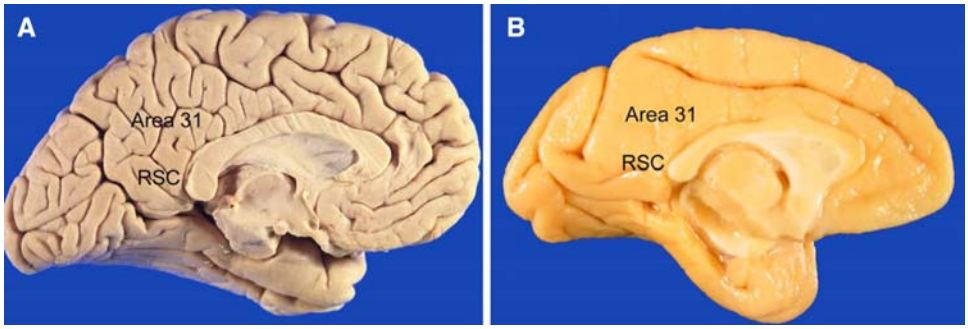

Fig. 1 Medial cortical surface of human and monkey. RSC and area 31 are found in roughly the same location on the medial cortical surface

(Kobayashi and Amaral 2003; Parvizi et al. 2006; Kobayashi and Amaral 2007), the RSC is connected with frontal, occipital, anterior cingulate and temporal lobes as well as the thalamus. Specifically, the RSC receives input from much of the temporal lobe, including the hippocampal formation, entorhinal cortex, subiculum, presubiculum, parasubiculum, parahippocampal, and perirhinal cortices (Kobayashi and Amaral 2003; Parvizi et al. 2006). One study reported projections from the anterior basolateral nucleus of the amygdala to the RSC in the rat (Dziewiatkowski et al. 1998).

Advances in neuroimaging and other technologies have allowed researchers to expose putative functional correlates for the RSC including memory (Valenstein et al. 1987; Shannon and Buckner 2004; Svoboda et al. 2006), spatial navigation (Wolbers and Buchel 2005) and consciousness (Fiset et al. 1999; Laureys et al. 1999; Raichle et al. 2001; Vogt and Laureys 2005). Of particular interest, some neuroimaging studies have indicated that the RSC is involved in emotional processing, due primarily to activation seen in the RSC with the presentation of emotionally salient stimuli (Maddock 1999; Maddock et al. 2003; Piefke et al. 2003; Cato et al. 2004).

There is an abundance of evidence from animal (LeDoux 2000; Davis et al. 2003; Bauman et al. 2004) and human (Adolphs et al. 1994; Adolphs et al. 1995; Whalen et al. 1998; Buchel and Dolan 2000; Whalen et al. 2004) studies to implicate the amygdala in emotional processing as well, particularly the detection of danger, vigilance, and the production of the conditioned fear response. The amygdala is located adjacent to the hippocampus in the rostral portion of the medial temporal lobe and has reciprocal connections with frontal, insular, anterior cingulate and temporal cortices (Amaral et al. 1992). The primate amygdaloid complex is comprised of 13 individual nuclei (Fig. 2) that are cytoarchitectonically, histochemically, and connectionally distinct (Amaral et al. 1992; Sorvari et al. 1995).

The RSC has recently been highlighted in a number of imaging studies suggesting that this region is involved in emotional processing; yet there is very little evidence directly linking the RSC to the amygdala, a known emotional processing output center. The current study was designed to investigate the potential direct connections between the amygdala and the RSC in light of studies that suggest RSC activation in emotionally related tasks.

An overview of the general cortical and subcortical connections of the experimental cases presented in this study has previously been published (Parvizi et al. 2006). Cases 1–4 from this study correspond to Cases M3-FB-30/23a, M1-FB-31, M2-FB-23a/b, and M5-FB-7m, respectively, in Parvizi et al. (2006).

Materials and methods

Two female (Cases 1 and 2) and two male (Cases 3 and 4) cynomolgus (*Macaca fascicularis*) weighing between 2.0 and 4.6 kg were utilized for the study (Table 1). The Institutional Animal Care and Use Committees at The University of Iowa approved all experimental and surgical protocols, which also conformed fully to AAALAC accreditation requirements and to the policies of Society for Neuroscience on the Use of Animals in Neuroscience Research and principles and complied with the policy of the U.S.P.H.S. on Humane Care and Use of Laboratory Animals.

Surgical procedures

Each monkey was immobilized with an intramuscular injection of Ketamine (10 mg/kg) before being anesthetized with an intravenous injection of Nembutal (30 mg/kg/h). The head was shaved, cleansed with Betadine and placed in a head holder device. A U-shaped skin flap was made and retracted occipitally. The temporalis muscles were then detached from their insertion points along the frontal and parietal bones and reflected bilaterally. A midline bone flap was removed and placed in a sterile saline solution. Approximately 25 ml of 25% Mannitol was administered intravenously in increments to reduce brain volume and counter cerebral edema. After a 30-minute stabilization period, a small dural incision lateral to the superior sagittal sinus was made and a dural flap was reflected medially exposing the underlying cortex. Cerebral veins bridging the cortex at the vertex and the superior sagittal sinus were

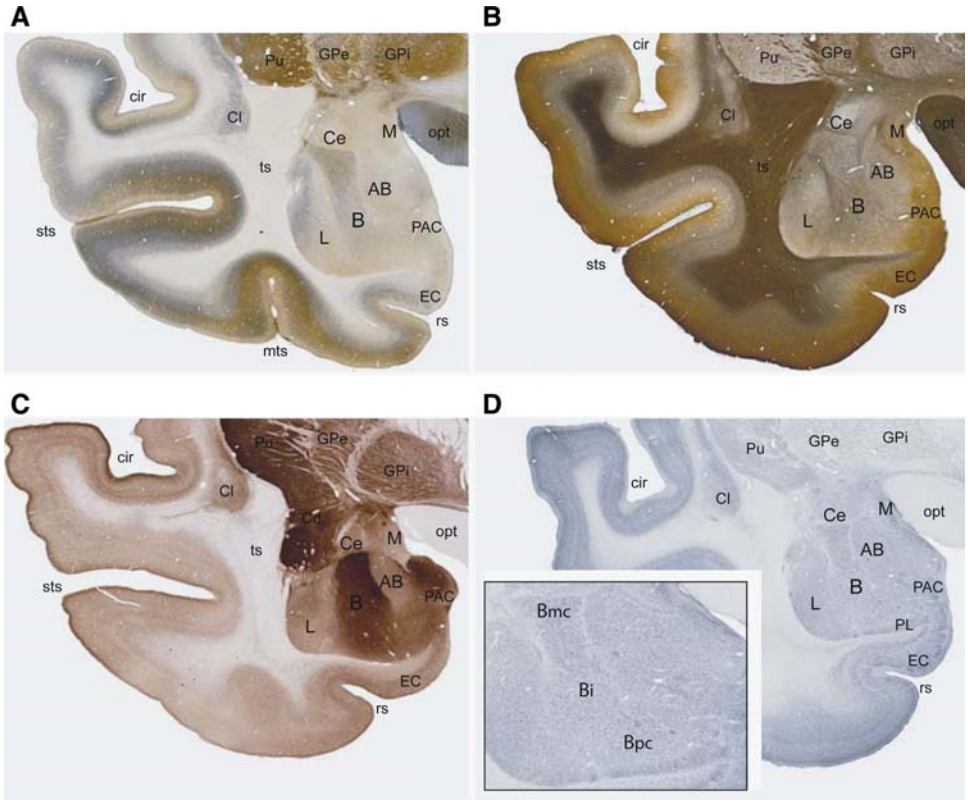

Fig. 2 Divisions of the monkey amygdala identified using four different histological techniques. **a** Calbindin and parvalbumin double stain. **b** Myelin. **c** Acetylcholinesterase. **d** Neu-N. Inset in (**d**) highlights the three divisions of the basal nucleus: parvicellular, intermediate and magnocellular divisions. *AB* accessory basal; *B* basal; *Bi* basal nucleus, intermediate division; *Bmc* basal nucleus, magnocellular division; *Bpc* basal nucleus, parvicellular division; *Ce* central nucleus; *cir* circular suclus; *Cl* claustrum; *EC* entorhinal cortex; *Gpe* globus pallidus, external segment; *Gpi* globus pallidus internal segment; *L* lateral nucleus; *M* medial nucleus; *opt* optic tract; *PAC* periamgydalar cortex, *PL* paralaminar nucleus; *Pu* putamen; *rs* rhinal sulcus; *sts* superior temporal sulcus; *ts* temporal stem

Table 1 Case summary table

Case No.	Sex	Weight (kg)	Injection site	Hemisphere	Tracer	Amount (μl)
Case 1	F	2.0	RSC	Right	FB	0.5
Case 2	F	4.5	Area 31	Left	DY	0.8
Case 3	M	2.0	Area 23	Right	FB	0.5
Case 4	M	4.6	PGm	Right	FB	0.6

Summarizes sex, weight, injection site, amount and type of retrograde tracer used in each case. *DY* Diamidino Yellow; *FB* Fast Blue; *kg* kilogram; *μ* microliter

ligated and separated to allow access to the medial surface. The interhemispheric fissure was then packed with saline-soaked cotton pads and gently retracted to expose the medial cortex. The target injection sites were identified by proximity to the corpus callosum and position relative to the visible hemispheric sulcal patterns. All injections were viewed with a surgical microscope to ensure pial penetration between cortical arterioles and veins.

Injections were made with a 31-gauge stainless steel needle attached to a 1-μl Hamilton microsyringe directly into four areas of the posteromedial cortices: RSC, area 23, area 31, and PGm. A retrograde tracer, either Fast Blue (FB) or Diamidino Yellow (DY), was injected at a cortical depth of approximately 2 mm by pressure injection. Each injection consisted of 0.4–0.6 μl of a 4% in phosphate-buffered saline PBS solution of the chosen tracer. Digital pictures were taken as a first step to determine the cerebral topography of the injection site.

After the injections were completed, the dura was repositioned and closed using 5-0 silk. The bone flap was replaced and anchored and the temporalis muscles were sutured over the bone flap to ensure stability. Finally, the skin flap was closed using sterile stainless steel staples.

After a survival period of 24–27 days, the monkey was anesthetized with Nembutal (5 mg/kg), and perfused transcardially with 1 l 0.9% saline, followed by 2 l of chilled 4% paraformaldehyde in 0.1 M (pH 7.4) phosphate buffer (PB). The brain was flushed with 1 l of 10% sucrose in 0.1 M PB, followed by 1 l of 30% sucrose in 0.1 M PB. After removal, the brain was placed in 30% sucrose in 0.1 M PB and allowed to equilibrate for 2–4 days for cryoprotection. The remaining meninges were then removed and the brain was photographed from all angles to include the cerebrum and its sulcal and gyral patterns, the brainstem, cerebellum, and cervical spinal cord.

Data analysis

The cerebellum and cervical spinal cord were removed from the rest of the brain. The remaining hemispheres and brainstem were frozen with dry ice and cut in the coronal plane at a thickness of 50 μm using a sliding microtome. The sections were divided into ten series of which several were used for fluorescent visualization of the efferent

connections of each injected cortical area. These sections were mounted on gelatin-subbed slides, dried overnight, and cover-slipped with DPX neutral mounting medium (Aldrich Chemicals, USA), and stored in a dark cold room (4°C) in light-tight boxes until they were used for data analysis. The remaining series were used for the cytoarchitectural analysis and immunohistochemistry.

Retrograde fluorescence material was studied using epi-fluorescent illumination and viewed under a Nikon Optiphot-2 Microscope. The cell bodies of FB-labeled cells appear bright blue and the nuclei of DY-labeled cells appear bright yellow when viewed under fluorescent illumination. Data from all tissue sections were collected with the use of the 2003 updated Neurolucida System, version 5.1 (MBF Bioscience Inc., Williston, VT). This software was loaded onto a Micron Millenia LXA computer connected to a Nikon Optiphot-2 microscope with a motorized stage controller and Optronics DEI 750 digital camera.

At the injection site, the outline of the section and anatomical landmarks such as sulci, ventricles, and gray and white matter interface were traced using a dark-field illumination that successfully delineates the internal structures. The following guidelines were used to estimate the effective uptake area for each injection site: effective uptake areas, or zone 0, were defined as the areas of mechanically damaged tissue in which tracer remained during the post surgical survival period (Conde 1987). Under fluorescence illumination, the effective uptake area appeared yellow or orange demarcating this area from surrounding tissue (Fig. 3). The cortical injection site and region of effective uptake were verified using adjacent Nissl sections stained with thionin (Fig. 3) as well as homotypical commissural retrograde labeling in the contralateral hemisphere.

Cortical cytoarchitectural analysis

Nissl preparations and Neu-N staining of the tissue provided clear cortical identification of the medial cortical areas investigated in this study (Fig. 3).

Retrosplenial cortex

Areas 29 and 30 were identified by their position on the upper bank of the callosal sulcus, lateral and medial, respectively. There is a transition from allocortex of the indusiem griseum to proisocortex of area 30 that occurs within the retrosplenial cortices (Sanides 1972). Area 29 is periallocortex and has an undifferentiated layer III that is difficult to

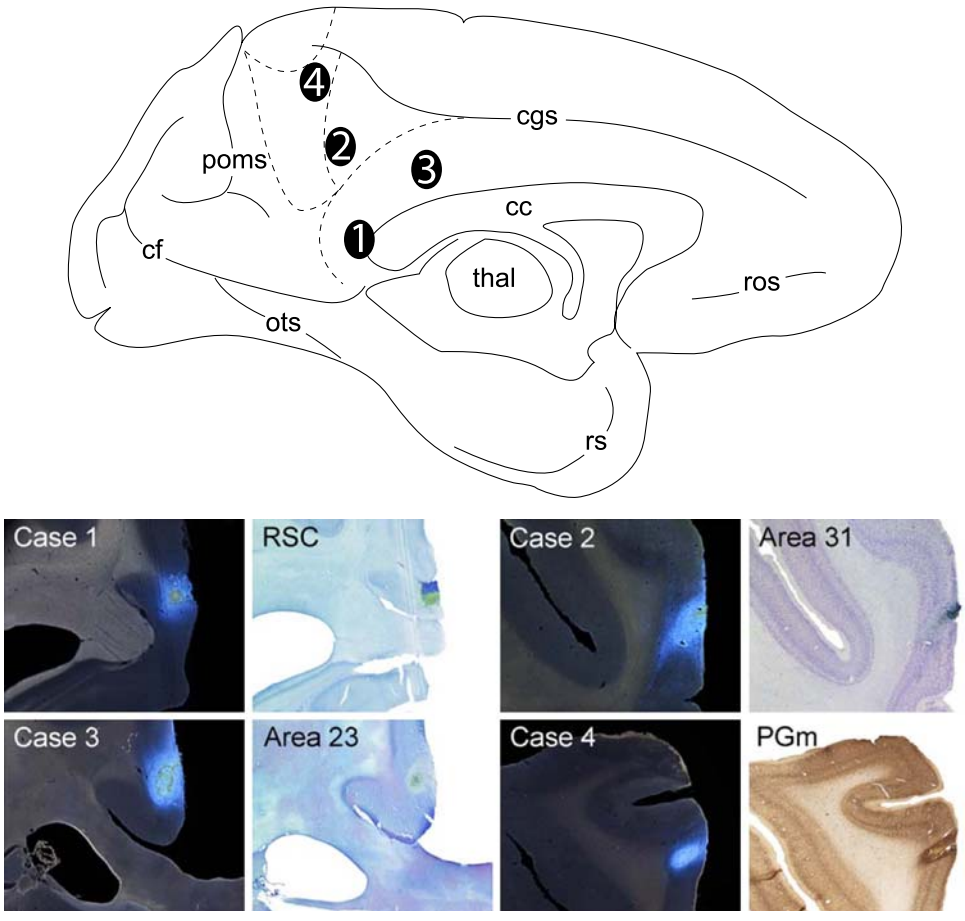

Fig. 3 Injection site locations in each case. (*Top*) medial surface map depicting location of all four injection sites, adapted from (Morecraft et al. 2004). (*Bottom*) epi-fluorescent photographs of coronal sections through the injection sites of all four cases with adjacent cytoarchitectural stains. The case number and determined injection sites are as follows: (*Case 1*) right RSC; (*Case 2*) left area 31; (*Case 3*) right area 23; (*Case 4*) right area PGm. *cc* corpus callosum; *cf* calcarine fissure; *cgs* cingulate sulcus; *ots* occipito-temporal sulcus; *poms* parieto-occipital medial sulcus; *ros* rostral sulcus; *rs*, rhinal sulcus; *thal* thalamus

distinguish from layers II and IV, and is divided into medial and lateral segments (Vogt et al. 1995). In contrast, layer III can be distinguished from layer IV dysgranular area 30, although distinction from layer II is still difficult. Area 30 abuts area 23a of the posterior cingulate and the transition is recognized by the subtle differentiation of layer II and the presence of medium-sized pyramidal neurons in layer Va in 23a. In the non-human primate, areas 29 and 30 extend from the depths of the callosal sulcus onto the medial surface posterior to the splenium for a short distance.

Area 31

Area 31 is located on the medial surface between parietal area PGm and posterior cingulate area 23. Area 31 is often positioned between the cingulate and splenial sulci at the level of, or posterior to, the splenium. Area 31 is differentiated from area 23 by a more defined layer II, although layer II in area 31 is not as well defined as in area PGm. Additionally, there are medium-sized pyramidal cells in layer IIIc in area 31, with larger pyramidal cells scattered throughout the layers. Layer IV is moderate in size, larger than area 23 and contains small granule cells. Layer V contains medium-sized pyramidal neurons and layer VI shows an increased density of small cells. The laminar width of layers is increased in area 31 creating a greater cortical width than area 23. Overall, well-differentiated layers, with a particularly broad layer III with large pyramidal neurons in layer IIIc (Vogt et al. 1995) and a cell dense layer IV identify area 31 and distinguish this area from area PGm.

Area 23

Moderately defined cortical layers, differing from the allocortex of the adjacent retrosplenial cortex, identify area 23. Layer II is not as conspicuous in area 23 as it is in adjacent cortices of areas 31 and PGm and it blends into the superficial layers substrata of layer III. Layer III has small to moderate pyramidal cells in layer IIIc, although the definition of layer II substrata is not clear. Layer IV is small and consists of small granule cells. Small and medium-sized pyramidal cells are found in layer Va, with the occasional presence of a larger pyramidal neuron. Layer VI is not dense and has a few small pyramidal neurons.

Area PGm

Area PGm is distinguished from area 23 of the posterior cingulate by highly differentiated layers, but the transition from area 31 to PGm is not as easy to identify. Granular layers II and IV are very conspicuous in area PGm and are clearly demarcated from layers III and V. Layer III contains small, medium and large-sized pyramidal cells, with the smaller cells occupying the more superficial substrata and larger cells intermingled in the deeper substrata, layer IIIc. Layers V and VI contain medium-sized pyramidal cells with a few smaller pyramidal cells in layer VI. Layer V is difficult to divide into substrata although it is clearly demarcated from layer VI. The most noticeable characteristic of area PGm is an evident thinning of the cortex; consequently, the individual layers appear more densely packed.

Identification of basal nucleus of the amygdala

Nuclei within the amygdala were identified using cytoarchitectural and immunohistochemical techniques. Calcium-binding proteins, calbindin and parvalbumin, AchE, and myelin preparations in addition to cytoarchitectural preparations with Nissl and Neu-N, were utilized in nuclear identification (Fig. 2).

The basal nucleus, in coronal sections, is present medial to the lateral nucleus through most of the primate amygdala (Amaral et al. 1992). Although the lateral and basal nuclei are often difficult to distinguish in the primate amygdala, basal nucleus neurons tend to stain darker on Nissl sections, are less densely packed and are separated from lateral nucleus neurons by the lateral fiber bundle (Amaral et al. 1992). The accessory basal nucleus, located medial to the basal nucleus, is clearly separated from the basal nucleus by the intermediate fiber bundle. Three major subdivisions of the primate basal nucleus could be identified: parvicellular, intermediate, and magnocellular (Amaral et al. 1992; Schumann and Amaral 2005). The parvicellular division is the most rostral and ventral of the three, with small, moderately stained, densely packed neurons on Nissl stained sections. The intermediate division of the basal nucleus lies slightly more caudal and dorsal to the parvicellular division with larger, darker neurons on Nissl sections. The magnocellular division makes up the dorsolateral portion of the basal nucleus, with the largest and most darkly stained neurons of the amygdala on Nissl sections.

In sections double stained for calbindin and parvalbumin, immunoreactivity was higher in the mangocelluar division of the basal nucleus, which made it clearly distinguishable from the adjacent central and lateral nuclei (Fig. 2a). A Myelin stain was used to identify the lateral fiber bundle that separates the basal nucleus from the lateral nucleus, as well as the intermediate fiber bundle that separates the basal nucleus from the accessory basal nucleus (Fig. 2b). Neurons throughout the entire basal nucleus intensely stained for Aceytlcholinesterase, which was not present in the adjacent lateral, accessory basal, or central nuclei, and therefore provided a clear outer boundary for the basal nucleus (Fig. 2c). Similar to Nissl-stained sections, the basal nucleus was easily identifiable on the Neu-N stained sections as described above (Fig. 2d).

Results

Case 1—RSC injection

Case 1 was a FB injection of the RSC, immediately posterior to the caudal extremity of the corpus callosum in the right hemisphere (Fig. 3). The effective zone of uptake was determined to encompass cortical area 30, and posterior portions of 23a.

Serial sections through the amygdala revealed consistent labeling that spanned a large portion of the basal nucleus. Labeled cells first appeared at the midrostrocaudal level through the amygdala at approximately the coronal level of the optic chiasm; labeling was absent in the anterior portion of the basal nucleus. Labeling continued through to the caudal extent of the basal nucleus of the amygdala. There was a noticeable absence of labeling in other nuclei of the amygdala, including the neighboring lateral and accessory basal nuclei (Fig. 4).

The labeled cells were mostly concentrated in the intermediate division of the basal nucleus and to a lesser extent in the magnocellular division rostrally and lateral portion of

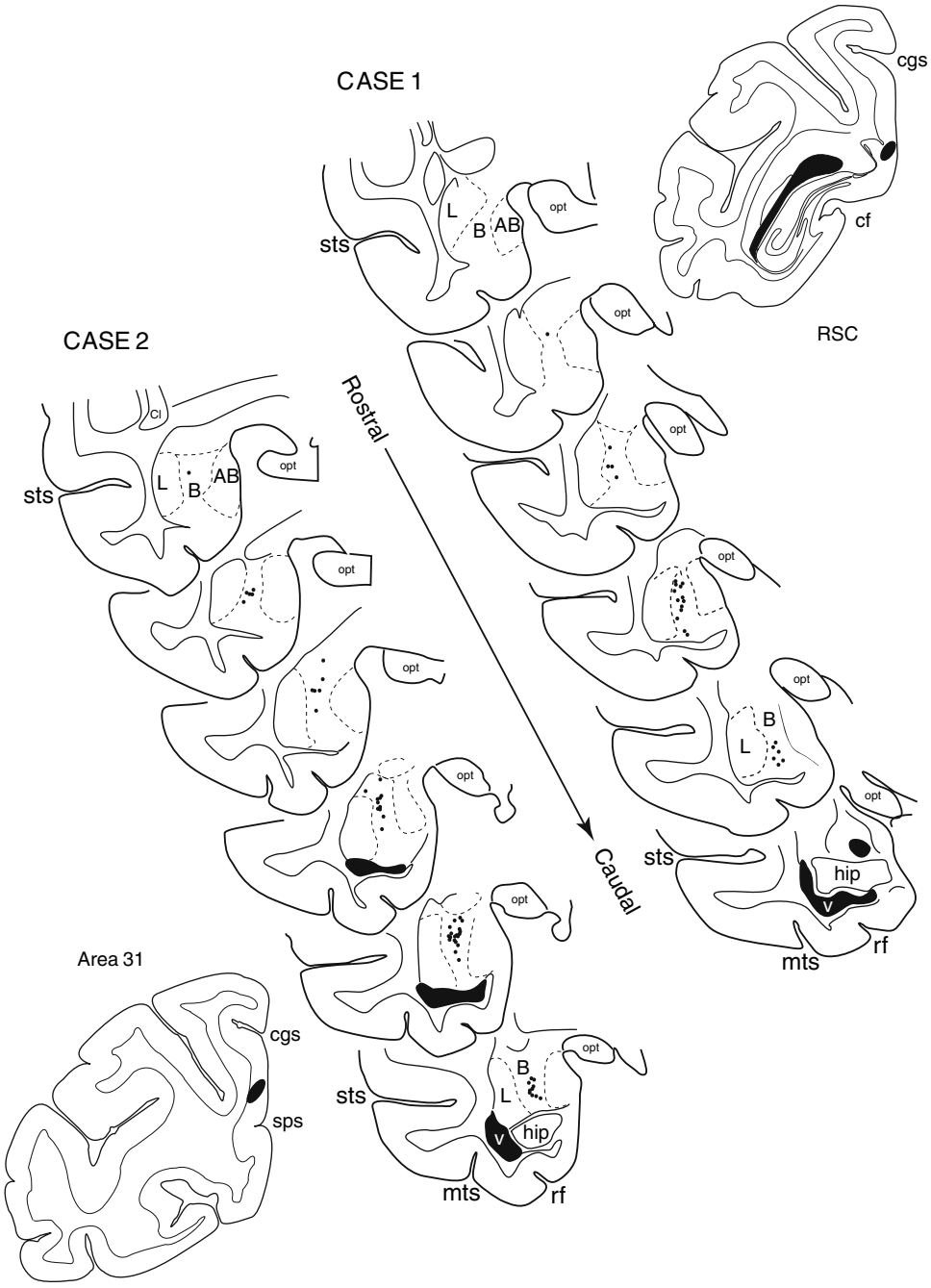

Fig. 4 Neurolucida chartings of labeled cells observed in the amygdala with retrograde injections to RSC (*Case 1*) and area 31 (*Case 2*). *AB* accessory basal nucleus; *B* basal nucleus; *cgs* cingulate sulcus; *cf* calcarine fissure; *cl* claustrum; *hip* hippocampus; *L* lateral nucleus; *mts* medial temporal sulcus; *opt* optic tract; *rf* rhinal fissure; *spl* splenial sulcus; *sts* superior temporal sulcus; *v* temporal horn of lateral ventricle

the parvicellular division caudally (Fig. 4). The labeled cells appeared to be medium-sized pyramidal neurons consistent with the intermediate basal nucleus, although the size of labeled cells varied as some cells were larger dorsally, indicative of the magnocellular division. The pattern of labeling was sparse and scattered, with non-labeled cells interspersed between each labeled cell.

Case 2—31 injection

Case 2 was a FB injection of cortical area 31, located between the splenial sulcus and posterior cingulate sulcus on the medial surface in the left hemisphere (Fig. 3). The zone of uptake was slightly posterior and dorsal to the injection in Case 1.

Similar to Case 1, labeled cells appeared approximately at the midrostrocaudal level through the basal nucleus of the amygdala, at the level of the optic chiasm, and the anterior amygdala was void of labeled cells. The labeled cells persisted to the caudal extent of the basal nucleus; there were no observable cells in other amygdaloid nuclei (Fig. 4).

Although the pattern of labeling in Case 2 was very similar to Case 1, there was a slight difference in the position of the labeled cells as compared to Case 1. Particularly, labeled cells observed in Case 2 appeared more clustered in dorsal intermediate and magnocellular divisions, whereas the pattern of labeling in Case 1 was more distributed throughout the amygdalar subdivisions (Fig. 4). The morphology of the labeled cells was similar. In both Cases 1 and 2, labeled cells were mostly medium-sized pyramidal neurons, indicative of the intermediate division of the basal nucleus with a few noticeably larger cells of the magnocellular division. Again, a number of non-labeled cells were present between each labeled cell.

Cases 3 and 4—area 23 and PGm

Case 3 consisted of a FB injection to area 23 of the posterior cingulate gyrus in the right hemisphere. The injection primarily involved subdivision 23b with some infringement on 23a, but nonetheless was limited to area 23 (Fig. 3). This injection was anterior to both Cases 1 and 2 at the level of the splenium.

Case 4 was an injection to area PGm of the medial parietal cortex in the right hemisphere. The injection was positioned just below the ascending ramus of the cingulate sulcus posterior and dorsal to the injection in Case 2 (Fig. 3).

Upon analysis of Cases 3 and 4 no labeling was observed in any nuclei of the amygdala.

General results

Analysis of Cases 1 and 2, retrograde injections of RSC and area 31, showed labeled cells predominantly in the intermediate and magnocellular divisions of basal nucleus of the amygdala and establishes a direct connection between the amygdala and RSC and area 31. In both cases the labeled cells were found in medium to large-sized cells within the caudal 2/3 of the basal nucleus of the amygdala. No labeling was found in the rostral segments of the basal nucleus or other amygdaloid nuclei. In contrast, analysis of retrograde injections to cortical areas anterior and posterior to the RSC and area 31, areas 23 and PGm, did not reveal any labeled cells within the amygdala.

Discussion

The present study demonstrates that the RSC and adjacent area 31 receive direct output from the basal nucleus of the amygdala. This study was precipitated by neuroimaging findings suggesting the RSC is involved in processing emotional information (Maddock 1999; Maddock et al. 2003; Piefke et al. 2003; Cato et al. 2004). To address this issue anatomically, retrograde tracer injections were placed in the cortical areas comprising the RSC and adjacent cortical areas of posterior medial cortices. Ensuing analysis of the amygdala revealed retrogradely labeled cells predominantly in the intermediate and magnocellular divisions of the basal nucleus of the amygdala from retrograde tracer injections of the RSC and area 31. Injections of the posterior cingulate (area 23) and medial parietal (area PGm) cortices were void of labeled cells in the amygdala. This finding provides a direct anatomical route for emotionally relevant information from the amygdala to the RSC and area 31, which, in part, supports neuroimaging claims that the RSC is involved in processing emotionally salient information.

Although connectional studies of this cortical region may have been initiated in past, it is possible that the amygdaloid projections described here were not reported because of tracer leakage into the underlying white matter at the injection site or the limited number of labeled cells present within each amygdaloid section. However, the injections presented in the current study are confined to the cortex because of the unique surgical approach from the medial surface of the hemisphere, allowing for little possibility of tracer leakage into the underlying white matter. Moreover, since only a sample of sections were processed for this analysis, the actual number of cells within the amygdala with input to the RSC and area 31 is likely considerably larger. The current study provides evidence that a direct connection exists. A more extensive study with additional cases and stereological neuron counts of the divisions of the basal nucleus that project to the RSC is needed to quantify and fully understand the nature of these direct connections.

The labeled cells observed in both Cases 1 and 2 demonstrate that two distinct cytoarchitectural regions, RSC and

area 31, are receiving similar patterns of projections from the basal nucleus of the amygdaloid complex and hence may be processing similar information. It is possible that the site of activation seen in imaging studies, attributed to RSC, in fact, extends to encompass area 31, crossing cytoarchitectural boundaries. Thus, processes often attributed to the RSC in functional imaging studies, such as processing emotional stimuli, may involve the RSC as well as adjacent cytoarchitectural regions.

The projections from the basal nucleus of the amygdala in Cases 1 and 2 appear to be in approximately the same region within the basal nucleus. This study focused on the RSC and adjacent posteromedial cortices and is therefore limited in topographical speculation. However, the absence of basal nuclear projections to the posterior cingulate (area 23), in contrast to the robust, widespread projections to the anterior cingulate (area 24) (Porrino et al. 1981; Van Hoesen 1981; Amaral and Price 1984; Amaral et al. 1992) and somatotopically organized projections to the cingulate motor areas (Morecraft et al. 2007) demonstrates the discontinuous nature of basal nucleus projections to cingulate cortices. Moreover, the overall cortical projection pattern to the cingulate gyrus suggests discrete functional connectivity with the amygdala, specifically that the RSC and area 31 may be involved in processes that the adjacent posterior cingulate cortices are not.

The basal nucleus of the amygdala is the principal source of many amygdala-cortical and subcortical projections that have been described and reviewed in detail elsewhere (Amaral et al. 1992; Freese and Amaral 2007). Intrinsically, the basal nucleus projects extensively to the lateral, accessory basal, and central nuclei of the amygdaloid complex, and receives efferent projections primarily from the lateral nucleus (Pitkanen and Amaral 1991; Pitkanen and Amaral 1998). Extrinsically, the basal nucleus has reciprocal connections with the striate, extrastriate, inferotemporal, orbitofrontal, medial prefrontal, anterior cingulate, cingulate motor and insular cortices (Pandya et al. 1973; Mizuno et al. 1981; Pandya et al. 1981; Tigges et al. 1982; Amaral and Price 1984; Russchen et al. 1985; Barbas and De Olmos 1990; Stefanacci and Amaral 2002; Morecraft et al. 2007). Regions of the inferior temporal lobe with established roles in memory function, such as the hippocampus (Amaral 1986), entorhinal cortex (Pitkanen et al. 2002), and perirhinal and parahippocampal cortices (Stefanacci et al. 1996) are highly interconnected with the basal nucleus of the amygdala. The current study now adds the retrosplenial cortex and area 31 to the extensive list of direct extrinsic projections from the basal nucleus of the primate amygdala.

The measure of retrograde labeling observed within the confines of the basal nuclei in Cases 1 and 2 is less than the labeling examined in other subcortical areas. In all four cases presented in this study, extensive labeling was observed within the entire rostrocaudal extent of the insular claustrum as well as within the smaller temporal lobe component (Parvizi et al. 2006). A thorough examination of the thalamic labeling from these cases also revealed an extensive labeling pattern, particularly within the posterior association nuclei, such as the lateroposterior and pulvinar nuclei (Buckwalter et al. 2007). The relatively lesser amount of labeling observed within the basal nucleus in comparison to other subcortical structures suggests that these direct connections may not be the only or principal means of conveying putative emotional information to the retrosplenial cortices. In fact, it is likely that emotional information from the basal nucleus reaches the retrosplenial cortices via circuits through the thalamus and anterior cingulate cortices. These indirect connections may act in concert with the direct connections from the basal nucleus to provide a modulatory multi-synaptic system for emotional integration within the retrosplenial cortices.

Functional implications

In non-human primates, damage to the amygdala produces abnormal fear behavior (Zola-Morgan et al. 1991; Emery et al. 2001; Prather et al. 2001) and an impaired ability to correctly evaluate dangerous versus benign stimuli (Bauman et al. 2004). Extensive experimental studies with rats suggest that the amygdala is critically involved in acquiring and retaining memories of emotional experiences (McGaugh 2004 for review). Specifically, the basolateral amygdala in the rat (basal nucleus in the primate) modulates the consolidation of memories via efferent connections to many cortical and subcortical areas (LeDoux 2000; Pitkanen et al. 2000; McGaugh 2004). As mentioned above, the basal nucleus of the amygdala has extensive reciprocal connections with the hippocampus (Amaral 1986) and lesions to the basolateral nucleus in the rat interfere with contextual fear conditioning (LeDoux 2000). Human patients with amygdala lesions appear to have normal declarative memory (Bechara et al. 1995), which involves the hippocampus, but have impairments in long-term memory for emotionally arousing stimuli (Adolphs et al. 1997; Adolphs et al. 2001). The basal nucleus of the amygdala, via its direct reciprocal connections with the hippocampus and other cortical areas (Amaral 1986), appears to influence or modulate how strongly an event may be stored in memory by evaluating the degree of emotional salience (Cahill and McGaugh 1998; Packard and Cahill 2001).

In rats, lesions of RSC result primarily in spatial navigation (Harker and Whishaw 2002; Aggleton and Vann 2004; Harker and Whishaw 2004) and spatial memory (Vann et al. 2003; van Groen et al. 2004) impairment. In humans, lesions of the RSC are rare and almost always involve

adjacent cortical areas, and as such, make it difficult to interpret their functional significance. However, reports show that human subjects, similar to rats, have deficits mainly in spatial navigation and memory, clinically termed topographical disorientation (Alsaadi et al. 2000; Maguire 2001), or amnesia (Valenstein et al. 1987; Saito et al. 2003). These spatial navigation and memory deficits are likely due to deafferentation of the RSC from other spatially relevant medial temporal structures (Kobayashi and Amaral 2003; Parvizi et al. 2006). In such cases, there are no reports of emotional disturbances.

Taken together, these studies also suggest that RSC functions may be lateralized, with spatial information processing primarily in the right RSC and memory processing in the left RSC (Kobayashi and Amaral 2000; Maguire 2001; Kobayashi and Amaral 2003). However, our studies suggest that there is no specific lateralization of the output from the basal nucleus of the amygdala to the RSC and adjacent cortex. In both Case 1, an injection of the left hemisphere, and Case 2, an injection of the right hemisphere, labeled neurons were observed in the basal nucleus, suggesting that the function of the amygdalo-restrosplenial connection is not lateralized.

Given the wealth of data that links the RSC with spatial navigation and memory and the emerging notion that these cortices play some role in emotional processing, it is possible that the RSC may be a site of cortical integration for spatial memory and emotional information. In particular, the RSC is a putative site for spatial memory retrieval (Andreasen et al. 1995), hence causing memory deficits when insulted. Direct projections from the basal nucleus of the amygdala to the RSC and area 31, in addition to amygdalo-hippocampal interconnectivity, provide a potential mechanism for incorporating spatial memories in the RSC with current emotional output from the amygdala.

Conclusions

The results of this study show that the basal nucleus of the amygdala directly projects to the RSC and area 31 of the posteromedial cortices. These projections provide a direct mechanism for emotion-related information to be integrated with spatial memory in the RSC and adjacent cortices, supporting imaging studies which demonstrate activation of these cortices with the presentation of emotionally salient stimuli (Maddock 1999; Maddock et al. 2003; Piefke et al. 2003; Cato et al. 2004). In addition to the need for a more extensive anatomical study of the connections demonstrated here, a functional co-activation study that correlates the activity of the amygdala and the RSC and adjacent cortices will further validate the functional and anatomical linkage of these two areas.

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