Specialized Pathways from the Primate Amygdala to Posterior Orbitofrontal Cortex

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The primate amygdala sends dense projections to posterior orbitofrontal cortex (pOFC) in pathways that are critical for processing emotional content, but the synaptic mechanisms are not understood. We addressed this issue by investigating pathways in rhesus monkeys (Macaca mulatta) from the amygdala to pOFC at the level of the system and synapse. Terminations from the amygdala were denser and larger in pOFC compared with the anterior cingulate cortex, which is also strongly connected with the amygdala. Axons from the amygdala terminated most densely in the upper layers of pOFC through large terminals. Most of these terminals innervated spines of presumed excitatory neurons and many were frequently multisynaptic and perforated, suggesting high synaptic efficacy. These amygdalar synapses in pOFC exceeded in size and specialization even thalamocortical terminals from the prefrontal-related thalamic mediodorsal nucleus to the middle cortical layers, which are thought to be highly efficient drivers of cortical neurons. Pathway terminals in the upper layers impinge on the apical dendrites of neurons in other layers, suggesting that the robust amygdalar projections may also activate neurons in layer 5 that project back to the amygdala and beyond to autonomic structures. Among inhibitory neurons, the amygdalar pathway innervated preferentially the neurochemical classes of calbindin and calretinin neurons in the upper layers of pOFC, which are synthetically suited to suppress noise and enhance signals. These features provide a circuit mechanism for flexibly shifting focus and adjusting emotional drive in processes disrupted in psychiatric disorders, such as phobias and obsessive–compulsive disorder.

Key words: emotion and amygdala; neural circuits; OCD; orbitofrontal; synapse; thalamus

Introduction

Emotions guide decisions and their disruption impairs flexible behavior as in obsessive–compulsive disorder and autism (Bachevalier and Loveland, 2006; Maia et al., 2008). The amygdala has been implicated in emotional processes and, through connections with prefrontal cortex (Porrino et al., 1981; Ghashghaei and Barbas, 2002), likely transmits affective valence that influences executive functions (for review, see Bechara et al., 2000; Murray, 2007).

Pathways from the amygdala terminate most densely in posterior orbitofrontal cortex (pOFC) and anterior cingulate cortex (ACC; Ghashghaei et al., 2007). The pOFC also receives input representing all sensory modalities from high-order sensory association cortices (for review, see Cavada et al., 2000; Barbas et al., 2011). As the most multimodal prefrontal region and biased recipient of amygdalar pathways, the pOFC is poised to integrate signals from the external environment and the internal milieu associated with the affective significance of stimuli. In contrast, the ACC is a biased sender of projections to the amygdala (Ghashghaei et al., 2007), mediating emotional expression through pathways to central autonomic structures (for review, see Devinsky et al., 1995).

Another strong pathway to pOFC is from the mediodorsal thalamic nucleus (MD) and adjacent midline nuclei (Goldman-Rakic and Porrino, 1985; Dermon and Barbas, 1994). The amygdalar and thalamic pathways show opposite laminar innervation in pOFC (Porrino et al., 1981). Amygdalar fibers densely innervate lower layer 1 and layer 2 and to a lesser extent all other layers (Ghashghaei et al., 2007), while MD innervates most densely the middle cortical layers and to a lesser extent the upper layers (McFarland and Haber, 2002). Different layers represent distinct microenvironments. The upper layers (1–3a) include dendrites from pyramidal neurons in layers 2–5 (DeFelipe and Farinas, 1992) and among inhibitory neurons they are populated preferentially with the neurochemical classes of calretinin (CR) and calbindin (CB) neurons. In contrast, the middle layers are preferentially populated with parvalbumin (PV) inhibitory neurons (Gabbott and Bacon, 1996; Dombrowski et al., 2001).

Pathways that terminate in different layers are thought to differ functionally. Sensory thalamic pathways to the middle cortical layers have been called “drivers” because they reliably elicit action...
potentials in postsynaptic neurons, assuring transmission of signals (Sherman and Guillery, 1998). Pathways that terminate in the upper layers form synapses distally on postsynaptic neurons, mildly change their excitability, and thus have been called “modulators” (Sherman and Guillery, 1998). While the predominant termination pattern of amygdalar fibers in upper pOFC layers suggests a modulatory role, there is evidence that the amygdala can drive cortical neurons, at least in mice (Little and Carter, 2013).

Understanding the features of pathways from the amygdala to distinct prefrontal cortices in comparison with parallel thalamocortical pathways is prerequisite to unraveling core cognitive–emotional interactions that are disrupted in psychiatric disorders. Key outstanding questions addressed in this study pertain to the strength and potential efficacy of these pathways and interactions with excitatory and distinct classes of inhibitory neurons in prefrontal cortices. We identified specializations in amygdalar pathways to pOFC, suggesting specificity in the information conveyed by this strong pathway for flexible behavior.

**Materials and Methods**

**Surgery, tracer injections, and tissue processing**

Experiments were conducted on six rhesus monkeys (*Macaca mulatta*) aged 2–3.5 years of both sexes injected with 1–2 distinct neural tracers for 10 cases overall (Table 1). Experiments were conducted according to the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). Experimental methods were approved by the institutional animal care and use committees at Boston University School of Medicine, Harvard Medical School, and New England Primate Research Center. Procedures involving animals were designed to reduce the number of animals needed and minimize animal suffering.

To calculate stereotaxic coordinates for the injection sites, magnetic resonance images were obtained before surgery following animal sedation with ketamine hydrochloride and propofol anesthesia. Experiments were conducted under sterile conditions, and animals were continuously monitored for respiratory rate, oxygen saturation, heart rate, and temperature. For surgery, the animals were placed under isoflurane anesthesia and positioned in stereotaxic apparatus (Kopf 1430M, David Kopf Instruments), and a small opening was made in the skull and dura. We injected 10% dilutions of Lucifer yellow dextran (LY, 4 μL, 10 kDa; Invitrogen), fluorescein (FE, fluorescein dextran, 3 μL, 10 kDa or a mixture of 3 kDa and 10 kDa; Invitrogen), fluororuby (FR, tetramethylrhodamine dextran, 3–4 μL, mixture of 3 kDa and 10 kDa; Invitrogen), biotinylated dextran amine (BDA, 9 μL, 3 kDa or a mixture of 3 kDa and 10 kDa; Invitrogen), or cascade blue dextran (CBL, 6 μL, mixture of 3 kDa and 10 kDa; Invitrogen) into amygdalar or thalamic nuclei using Hamilton syringes (10 μL). After 18 d, the animals were anesthetized with sodium pentobarbital and perfused transcardially with 4% paraformaldehyde, 0.2% glutaraldehyde in 0.1 M phosphate buffer saline (PBS), pH 7.4. The brain was removed, cryoprotected in ascending sucrose solutions (10–30% sucrose w/v in 0.1 M PBS, pH 7.4, with 0.05% sodium azide; Sigma-Aldrich; Rosene et al., 1986), frozen in isopentane (Fisher Scientific), at −80°C, and cut on a freezing microtome (AO Scientific Instruments, Reichert Technologies), in 50 μm coronal sections forming 10 series. Sections were stored free floating at −20°C in a solution of 30% ethylene glycol, 30% glycerol, and 0.05% sodium azide in 0.05 M phosphate buffer, pH 7.4.

**Bright-field and confocal microscopy**

**Immunohistochemistry.** Free-floating sections were incubated for 1 h at 4°C in 0.05 M glycine (Sigma-Aldrich) and preblocked for 1 h at 4°C in 5% normal goat serum (NGS; Vector Laboratories), 5% bovine serum albumin (BSA; Sigma-Aldrich), and 0.2% Triton-X (Sigma-Aldrich) in 0.05M PBS. For cases using BDA tracer, sections were incubated for 1 h at 25°C with avidin-biotin horseradish peroxidase (AB-HRP; Vectastain Elite ABC kit, Vector) at a 1:100 dilution in PBS, followed by rinses in PBS (all rinses are 3 × 10 min unless otherwise indicated), and tracer was visualized with incubation for 1–3 min in diaminobenzidine (DAB substrate kit, Vector). For cases with fluorescent tracer injections, sections were first incubated with avidin-biotin blocking solutions (AB blocking kit, Vector), and then rinsed in PBS and incubated overnight at 4°C with primary antibodies to tracers [FE (Invitrogen, catalog #A889; RRID: AB_221561), FR (Invitrogen, catalog #A6397; RRID: AB_1502299), CBL (Invitrogen, catalog #A5760; RRID: AB_11180100), or LY (Invitrogen, catalog #A5750 RRID:AB_1501344) at 1:800 in 1% NGS, 1% BSA, and 0.1% Triton-X in PBS; all rabbit polyclonal IgG]. Sections were then rinsed in PBS and incubated for 2 h at 25°C with biotinylated secondary antibodies (1:200 in 1% NGS, 1% BSA, and 0.1% Triton-X in PBS; biotinylated goat anti-rabbit IgG; Vector, catalog #BA-1000; RRID: AB_2313606), followed by AB-HRP and DAB, as above. Sections were rinsed in 0.1 M phosphate buffer (PB), pH 7.4, mounted on gelatin-coated glass slides, and dried for ≥10 d. Slides were defatted in a 1:1 chloroform–ethanol mixture for 1 h and then rehydrated in a series of ethanol (100, 70, 50%) and then water, followed by staining with 0.05% thionin and rinsed again in water. Slides were then dehydrated in a series of ethanol (70, 95, 100%), cleared in xylene (Sigma-Aldrich), and coverslipped with Entallan mounting medium (Sigma-Aldrich).

For analysis with confocal microscopy, tissue was incubated in glycine and preblocked as above. Tracers and calcium-binding proteins were bound overnight at 4°C with primary antibodies [FE, FR, CBL, or LY: 1:800 in 1% NGS, 1% BSA, and 0.1% Triton-X in PBS; and CB (Swant, catalog #300, RRID: AB_10000347), CR (Swant, catalog #683, RRID: AB_1000032), or PV (Swant, catalog #235, RRID: AB_10000343) at 1:2000, mouse monoclonal, Swiss Antibodies]. Next, the tissue was rinsed in PBS and incubated overnight at 4°C in secondary antibodies conjugated with fluorescent label [1:100 in 1% NGS, 1% BSA, and 0.1% Triton-X in PBS; Alexa 568 goat anti-rabbit IgG (Invitrogen, catalog #11011, RRID: AB_143157) or goat anti-mouse IgG (Invitrogen, catalog #A11019, RRID: AB_143162) and Alexa 488 goat anti-mouse IgG (Invitrogen, catalog #A11001, RRID: AB_10566289) or goat anti-rabbit IgG (Invitrogen, catalog #A11008, RRID: AB_143165)]. Sections were rinsed in PB, mounted on gelatin-coated glass slides, and coverslipped while damp with Prolong Gold Antifade mounting medium (Invitrogen).

In all cases, primary and secondary antibody penetration was enhanced with 8 min runs (3 min on, 2 min off, 3 min on) in a temperature-
controlled variable-wattage microwave oven (150 W at 4°C, Pelco Biowave with ColdSpot and ThermoCube, Ted Pella) during each incubation. For three cases (BB, BN, and BO), a method of antigen retrieval was used before immunohistochemistry to enhance signal: sections were rinsed in PB, then incubated in 0.01 M sodium citrate buffer, pH 8.5, at 35–45°C for 30 min, followed by incubation in glycine, preblocking, and immunohistochemistry as above (Jiao et al., 1999). In control experiments omission of the primary antibodies and incubating in secondary antibody solutions showed no immunolabeling.

Data analysis. We used thionin-stained tissue to determine cytoarchitectonic and laminar boundaries of area OPrO and area 32 (A32) in prefrontal cortex (Barbas and Pandya, 1989). OPrO is dysgranular, contains a layer 2, and has slightly more prominent deep layers compared with upper layers (Barbas and Pandya, 1989). A32 is also dysgranular, has a broad layer 1, and a greater density of neurons in deep layers compared with upper layers (Dombrowski et al., 2001). All analyses were conducted in area OPrO and A32 in the anterior cingulate. We refer to these areas as pOFC (OPrO) and ACC (A32) for short, and based on their common references in the primate literature by location.

Columns with DAB-labeled amygdalar or thalamic fibers within pOFC or A32 were photographed at 1000× covering equal volumes of cortex in layer 1, layers 2–3a, layers 3b–5a, and layers 5b–6 (Olympus BX51 and DP70, Olympus). The maximum diameter of each labeled bouton was measured using the program Reconstruct (Synapse Web Reconstruct, RRID: nil-0000-23420; Fiala, 2005). At least four columns were analyzed to sample evenly across cortical areas; additional columns were sampled until ≥1000 boutons were identified in each case, which is sufficient to obtain a significant result using a Cohen’s d power analysis for an effect size of 0.2 (Cohen, 1988). Statistical analyses were performed using Statistica software (version 10, StatSoft). Boutons were classified as large or small using a k-means cluster analysis for two clusters in each case and each area (cutoff between small and large ranged from 0.91 to 1.26 μm). We used two-tailed t-tests to compare mean diameters across areas and pathways, and ANOVA with Bonferroni’s post hoc analysis to compare bouton size distributions across areas and pathways.

We determined the density of small and large labeled amygdalar boutons in layers 1, 2–3a, and 3b–5a of pOFC and A32. For comparison, we also include areas 9/46, which are granular areas with well delineated six layers. We determined densities using a semiautomated method of systematic random sampling using a microscope fitted with a motorized stage and controlled by software (Stereoinvestigator 10, MBF Biosciences; Olympus BX60). After pilot studies to determine the optimal sampling rate for each case and layer, we used a counting frame of 50 × 50 μm, disector height of 11 μm, and a counting frame size that varied from 100 × 100 μm to 750 × 750 μm to achieve a Gundersen coefficient of error (m = 1) <0.1. A cutoff of 1 μm was used to distinguish between small and large boutons for all cases and all layers, which is approximately the average of all cutoff values calculated (mean cutoff value, 1.1 μm; n = 12 cases). Densities were normalized to the densest area in each case, and we compared normalized densities across areas using ANOVA with Bonferroni’s post hoc analysis.

We used laser scanning confocal microscopy (Fluoview FV-300, Olympus; LSM-510, Carl Zeiss Microscopy) to study appositions between fluorescently labeled tracer and dendrites labeled for calcium-binding proteins (CB, PV, or CR) using gold-labeled secondary antibodies for tracers [1:200 in 1% NGS, 1% BSA, 0.1% BSA-c, 0.025% Triton X-100 (Roche Applied Science), 0.1% acetylated BSA-c (Aurion), and 3.5% mouse blocking reagent (MOM basic kit, Vector) in PBS]. Sections were then bound overnight at 4°C with antibodies for tracers [FE, FR, or LY: 1:800 in 1% NGS, 1% BSA, 0.1% BSA-c, 0.025% Triton X-100, and 8% MOM protein concentrate (MOM basic kit, Vector) in PBS; rabbit polyclonal IgG, In-vitrogen] and one of three calcium-binding proteins (CB, CR, or PV: 1:2000, mouse monoclonal IgG, Swant). All primary and secondary antibody incubations included an 8 min microwave run as above. Sections were rinsed in PBS then incubated for 6 h at 25°C with biotinylated secondary antibodies for tracers [1:200 in 1% NGS, 1% BSA, 0.1% BSA-c, 0.025% Triton X-100, 8% MOM protein concentrate, and 0.1% cold water fish gelatin (Aurion) in PBS; biotinylated goat anti-rabbit IgG (Vector)] and gold-conjugated secondary antibodies for calcium-binding proteins [1:50 UltraSmall Immunogold F(ab)2 fragment of goat anti-mouse IgG (Aurion, catalog #800.266, RRID: AB_2315632)]. Sections were then postfixed with 3% glutaraldehyde and 1% paraformaldehyde in PB at 4°C. Sections were rinsed in glycine (5 min) and rinsed in PB (2 × 10 min), followed by encephalization conditions solution ECS (1.10, 2 × 10 min; for 1 h at 4°C in 5% NGS, 5% BSA, 0.025% Triton X-100, and 0.1% BSA-c in PB). Sections were incubated overnight at 4°C with antibody for a second calcium-binding protein (CB, PV, or CR: 1:2000 in 1% NGS, 1% BSA, 0.1% BSA-c, 0.025% Triton X-100, and 8% MOM protein concentrate in PB; mouse monoclonal IgG, Swant), followed by rinses in PB and incubation for 1–2 h at 25°C in biotinylated secondary antibody (1:200 in 1% NGS, 1% BSA, 0.1% BSA-c, 0.025% Triton X-100, and 8% MOM protein concentrate in PB; biotinylated goat anti-mouse IgG, Vector), then rinsed in PB and incubated in AB-HRP as above. These calcium-binding proteins were visualized using procedures modified from previous studies (Medalla et al., 2007). tissue sections were incubated as above in 0.01 M sodium citrate, pH 8.5 (30 min at 35°C), 0.05 M glycine (1 h at 4°C), and any BDA tracer was blocked with AB-blocking solutions. Background binding was blocked with 0.1% Triton X-100 for 1 h at 4°C in 5% NGS, 5% BSA, 0.025% Triton X-100 (Roche Applied Science), 0.1% acetylated BSA-c (Aurion), and 3.5% mouse blocking reagent (MOM basic kit, Vector) in PBS. Sections were then bound overnight at 4°C with antibodies for tracers [FE, FR, or LY: 1:800 in 1% NGS, 1% BSA, 0.1% BSA-c, 0.025% Triton X-100, and 8% MOM protein concentrate (MOM basic kit, Vector) in PBS; rabbit polyclonal IgG, In-vitrogen] and one of three calcium-binding proteins (CB, CR, or PV: 1:2000, mouse monoclonal IgG, Swant). All primary and secondary antibody incubations included an 8 min microwave run as above.

Sections were rinsed in PBS then incubated for 6 h at 25°C with biotinylated secondary antibodies for tracers [1:200 in 1% NGS, 1% BSA, 0.1% BSA-c, 0.025% Triton X-100, 8% MOM protein concentrate, and 0.1% cold water fish gelatin (Aurion) in PBS; biotinylated goat anti-rabbit IgG (Vector)] and gold-conjugated secondary antibodies for calcium-binding proteins [1:50 UltraSmall Immunogold F(ab)2 fragment of goat anti-mouse IgG (Aurion, catalog #800.266, RRID: AB_2315632)]. Sections were then postfixed with 3% glutaraldehyde and 1% paraformaldehyde in PB at 4°C. Sections were rinsed in glycine (5 min) and rinsed in PB (2 × 10 min), followed by encephalization conditions solution ECS (1.10, 2 × 10 min; for 1 h at 4°C in 5% NGS, 5% BSA, 0.025% Triton X-100, and 0.1% BSA-c in PB). Sections were incubated overnight at 4°C with antibody for a second calcium-binding protein (CB, PV, or CR: 1:2000 in 1% NGS, 1% BSA, 0.1% BSA-c, 0.025% Triton X-100, and 8% MOM protein concentrate in PB; mouse monoclonal IgG, Swant), followed by rinses in PB and incubation for 1–2 h at 25°C in biotinylated secondary antibody (1:200 in 1% NGS, 1% BSA, 0.1% BSA-c, 0.025% Triton X-100, and 8% MOM protein concentrate in PB; biotinylated goat anti-mouse IgG, Vector), then rinsed in PB and incubated in AB-HRP as above. These calcium-binding proteins were visualized using procedures modified from previous studies (Medalla et al., 2007). tissue sections were incubated in 150 W at 15°C until sample temperature reached 30–35°C (Jensen and Harris, 1989). We conducted control experiments on tissue by omitting primary antibodies and incubating in secondary antibody solutions and no immunolabeling was observed.

Sections were rinsed in PB (3 × 20 min) and postfixed for 15 min in 1% osmium tetroxide (Electron Microscopy Sciences) with 1.5% potassium ferrocyanide (Electron Microscopy Sciences) in PB with a microwave oven (100 W at 12°C; 2 min on, 2 min off; 2 min on) and rinsed in PB (3 × 2 min) and water (3 × 2 min). Sections were then rinsed in 50% ethyl alcohol (3 × 5 min), stained with 1% uranyl acetate (30 min in 70% ethanol; Electron Microscopy Sciences), and dehydrated in a series of ethanol (90, 95, 100%; 3 × 5 min each). For embedding, sections were
infiltrated with propylene oxide (2 × 7 min; Electron Microscopy Sciences), and then a 1:1 mixture of Araldite (Electron Microscopy Sciences) and propylene oxide (1 h). Sections were infiltrated with propylene oxide (2 min) and then a 1:1 mixture of Araldite (Electron Microscopy Sciences) and embedding in Araldite in aclar (Ted Pella). Aclar-embedded tissue was cured for 48 h at 60°C. Small columns of tissue (500–750 μm) were sectioned at 50 nm using an ultramicrotome (Leica Ultracut UCT, Leica Microsystems) and collected on pioloform-coated copper slot grids, to form series of 75–150 sections. Using 80 kV transmission electron microscope at 16,000–26,000× (JEOL 100CX, Jeol), we exhaustively sampled a few sections to identify ~10–30 labeled boutons in each series. Each bouton was photographed in serial sections through the entire bouton plus five additional sections before and after. We analyzed labeled boutons using Reconstruct to trace bouton profiles and postsynaptic sites that were complete in the series were reconstructed in 3D to determine volumes and area of the postsynaptic density (PSD). Models in 3D were generated in Reconstruct and import to 3Ds Max for rendering (version 14, Autodesk). We determined extracellular background level of gold labeling in each piece of tissue (Muly et al., 2009; Medalla and Barbas, 2012). In one series for each piece of tissue, enhanced gold particles in mitochondria were counted, and this value was divided by the total volume of mitochondria in the piece of tissue, enhanced gold particles in mitochondria were counted, and this value was divided by the total volume of mitochondria in the piece of tissue. The threshold for gold-positive elements was set to twice the density of gold in mitochondria. We compared mean diameters, bouton and spine volumes, and PSD areas across pathways using hierarchically nested mixed-model ANOVA for comparisons including boutons from multiple cases, and proportions of synaptic specializations using Fisher exact tests. We used linear regression to analyze correlation between bouton volume and PSD area or spine volume.

We also used systematic random sampling to analyze unlabeled boutons in the neuropil surrounding labeled amygdalar boutons. In one of every 20 series in case BN-R, we measured the PSD area for all unlabeled asymmetric synapses that were complete in the series, using Reconstruct. We compared the PSD area of unlabeled synapses and labeled amygdalar synapses using a two-tailed t test.

Results

Injection sites in the amygdala and MD

The pathways studied with neural tracers are shown in Figure 1 and listed in Table 1. We used cases with large injections in the amygdala (Ghashghaei et al., 2007) to study the overall pattern of amygdalar pathways, and smaller injections (Fig. 1A–D) to study specific aspects of amygdalar pathways at the level of the system and the synapse. The tracer injections in amygdalar nuclei are listed in Table 1; they include the basal and cortical nuclei that project extensively to pOFC in rhesus monkeys (Porrino et al., 1981; Barbas and De Olmos, 1990).

For comparison, we studied the pattern of thalamocortical pathways to pOFC and their synaptic interactions with light, confocal, and electron microscopy. Targeted small injections of tracer in two cases were mainly localized in MD magnocellular, including its junction in one case with MD parvicellular (MDpc; Fig. 1E–G), and in another case with part of the paraventricular (Pa) nucleus (Fig. 1H, I). A third small injection included caudal MDpc (Fig. 1J, K). All of these segments of MD and Pa are connected strongly with pOFC (Dermon and Barbas, 1994). In the third case, the tracer spread to part of the habenula (Fig. 1J, K), which projects mainly to the brainstem (Shelton et al., 2012); we used this case to study thalamocortical pathways with light microscopy.

Amygdalar terminals were densest in pOFC

First we studied the density of amygdalar terminations in pOFC compared with the density in prefrontal A32 and areas 9/46 (Fig. 2A–J). Like pOFC, A32 in the ACC has a dysgranular structure and is phylogenetically old, but the two areas have different functions (for review, see Barbas, 1997). On the other hand, dorsolateral prefrontal areas 9/46 differ markedly from either pOFC or ACC by having a six-layer (eulaminate) structure (Barbas and Pandya, 1989) and a role in cognitive operations (Miller and Cohen, 2001; Wallis et al., 2001).
We found that amygdalar terminals were consistently most dense in pOFC (n = 3 cases for each of three areas; ANOVA with Bonferroni’s post hoc test, F(2,6) = 5.5, p = 0.05 between pOFC and areas 9/46; Fig. 2J), confirming and extending previous findings (Ghashghaei et al., 2007). Each case showed the same density distribution pattern, with pOFC having the highest density of amygdalar terminals, areas 9/46 the lowest, and A32 intermediate density. In each area, a higher proportion of boutons was found in the upper layers compared with the middle layers, and there were no significant differences among areas in this pattern.

**Amygdalar terminals were larger in pOFC than in other prefrontal areas**

After measuring the major diameter of amygdalar boutons in pOFC and A32 in a large population (n = 31,420 boutons in pOFC; n = 16,967 boutons in A32), we performed a k-means cluster analysis, which separated boutons into two groups by diameter: large and small. We used a cutoff of 1 μm to distinguish between small and large boutons for all cases and all layers, which is approximately the average of all cutoff values calculated (mean cutoff value, 1.1 μm; n = 12 cases). The rationale is based on evidence that bouton size is highly correlated with the number of synaptic vesicles, and with the synaptic efficacy of the pathway (Germuska et al., 2006). We then compared the density of large amygdalar boutons (≥1 μm in diameter) in the three prefrontal sites. We found that pOFC also contained the highest density of large amygdalar boutons (n = 3 cases in each of three areas; ANOVA with Bonferroni’s post hoc test, F(2,6) = 8.5, p = 0.02 between pOFC and areas 9/46; Fig. 2K).

Next we compared directly the size of amygdalar terminals in pOFC and in A32. We found that amygdalar terminals across all layers in pOFC were larger than those in A32, though not significantly (mean diameter ± SE: pOFC, 1.0 ± 0.021 μm, n = 6 cases; A32, 0.97 ± 0.0057 μm, n = 3; two-tailed t test, t(26) = 1.9, p = 0.1). By comparing the frequency of large boutons, we found that the amygdalar projection to pOFC contained significantly more large boutons than A32 (1–1.5 μm diameter; pOFC, n = 6 cases and 8 bins; A32, n = 3 cases and 8 bins; multifactorial ANOVA with Bonferroni’s post hoc test, F(7,56) = 30.6, p = 0.002; Fig. 3A). These data show that pOFC receives denser projections as well as a higher proportion of large terminals from the amygdala compared with A32, another phylogenetically old prefrontal area that is also strongly connected with the amygdala.

**Amygdalar terminals were larger than thalamic terminals in pOFC**

We then compared amygdalar terminals in pOFC with terminals from the thalamic MD nucleus, which is the major source of thalamic input to pOFC (Goldman-Rakic and Porrino, 1985; Dermon and Barbas, 1994). Across all layers of pOFC, amygdalar terminals were larger than those from MD (mean diameter ± SE: amygdalar, 1.0 ± 0.021 μm, n = 6 cases with 31,420 boutons; thalamic, 0.88 ± 0.050 μm, n = 3 cases with 7,169 boutons; two-tailed t test, t(26) = 2.8, p = 0.03). We further characterized the frequency of large boutons and found that compared with

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**Figure 2.** Amygdalar boutons were densest and largest in pOFC. A–C, Areas of study: lateral (A), orbital (B), and medial (C) views of a rhesus monkey brain show areas of interest in areas 9/46, OPro (pOFC), and A32 (ACC). Scale bar, 1 cm. D–F, Nissl-stained columns of cortex in areas A9/46, A32, and pOFC, normalized to the highest density in each case (pOFC). Vertical lines indicate SE. *p ≤ 0.05.
boutons from MD, the amygdalar projection to pOFC contained a significantly higher proportion of large boutons (1–1.5 μm diameter; amygdalar, n = 6 cases and 8 bins; thalamic, n = 3 cases and 8 bins; multifactorial ANOVA with Bonferroni’s post hoc test, F(1, 2771) = 9.5, p = 0.0001; Fig. 3B).

Next we analyzed the middle cortical layers specifically (layers 3b–5a, which are thought to be the primary recipient of driver thalamocortical fibers. We found that amygdalar terminals (mean diameter ± SE, 1.06 ± 0.026 μm, n = 6 cases) were significantly larger than the thalamic even in the middle layers (layers 3b–5a: 0.89 ± 0.047 μm, n = 3; two-tailed t test, t(37) = 3.1, p = 0.02), which is consistent with the pattern shown above.

Analysis of boutons measured at the synaptic level in the electron microscope confirmed the data obtained at the light microscope (mean diameter ± SE, 0.99 ± 0.033 μm, n = 164 amygdalar boutons from two cases; 0.84 ± 0.041 μm, n = 39 thalamic boutons from two cases; hierarchically nested mixed-model ANOVA, F(3, 527) = 10.7 for fixed effect of injection site, p = 0.02; F(2,199) = 0.3 for random effect of case, p = 0.7; Fig. 4A–H). When reconstructed in 3D, amygdalar boutons were larger in volume (mean volume ± SE, 0.33 ± 0.035 μm³, n = 152 boutons from two cases) than the thalamic (0.19 ± 0.024 μm³, n = 36 boutons from two cases; hierarchically nested mixed-model ANOVA, F(1, 206) = 14.6 for fixed effect of injection site, p = 0.001; F(1,183) = 0.1 for random effect of case, p = 0.9; Fig. 4I). These results show that amygdalar boutons in the upper layers of pOFC are even larger than thalamic boutons forming synapses in the middle layers; the latter form a robust pathway in sensory systems that reliably relays information from the thalamus to cortex (Sherman and Guillery, 1998).

Appositions between amygdalar and thalamic projections with inhibitory neurons
A key unanswered question about the amygdalar and thalamic pathways is the nature of their postsynaptic targets in pOFC. With regard to cortical inhibitory neurons, the upper layers (1–3a) of pOFC are populated mostly by two classes of inhibitory neurons: CR-positive (Fig. 5A, green) presumed inhibitory neurons, which form synapses on neighboring inhibitory neurons, at least in the upper layers of some areas, and CB-positive (Fig. 5A, red) inhibitory neurons, which form synapses on the dendrites of nearby pyramidal neurons in layers 2–5 (DeFelipe, 1997). The middle–deep layers (3b–6) contain mostly PV-positive (Fig. 5A, blue) inhibitory neurons, which provide strong inhibition by forming synapses on the soma, axon initial segment, or proximal dendrites of layer 3 and 5 pyramidal neurons (DeFelipe, 1997).

We first studied the prevalence of appositions between amygdalar fibers and PV, CB, and CR inhibitory neurons in pOFC using laser scanning confocal microscopy (n = 376 appositions of 8,035 boutons in two cases; Fig. 5B, C). Of the three classes of presumed inhibitory neurons, amygdalar projections in the upper layers of pOFC formed appositions mostly with CB (8%; Fig. 5F) and CR neurons (8%) and less often with PV neurons (2%). By comparison, in the middle layers of pOFC, amygdalar boutons formed fewer appositions with CB (2%, χ²(1, n = 2322) = 41.9, p < 0.0001; Fig. 5F) or CR neurons (3%, χ²(1, n = 2771) = 13.9 p = 0.0002) and more with PV neurons (94%, χ²(1, n = 3202) = 13.4, p = 0.0003).

We then studied appositions between thalamic terminals and inhibitory neurons in the middle layers (3b–5a) of pOFC, which are the predominant layers innervated by thalamic afferents (n = 101 appositions of 1,226 boutons in two cases; Fig. 5D, E). Thalamic terminals, compared with amygdalar terminals, targeted a greater proportion of inhibitory neurons in the middle layers. Specifically, thalamic axons formed more appositions with PV (11%, χ²(1, n = 1496) = 24.5, p < 0.0001; Fig. 5G) or CR neurons (10%, χ²(1, n = 941) = 15.4, p = 0.0001), and a similar proportion with CB neurons (3%).

In a case with a more medial injection of tracer (Fig. 1H, I; case BO-R), thalamic afferents terminated in significant numbers in the upper layers of pOFC as well (n = 123 appositions of 1,156 boutons). Compared with amygdalar axons in the upper layers of pOFC, thalamic axons targeted significantly more CB neurons (14%, χ²(1, n = 1834) = 13.2, p = 0.0003) and a similar proportion of CR neurons (7%). Meanwhile, no boutons formed appositions with PV neurons. Consistent with these findings, midline and paralaminar thalamic nuclei activate feedforward inhibition in upper cortical layers in mice (Cruikshank et al., 2012). These data show that, compared with amygdalar fibers, thalamic fibers targeted a higher proportion of inhibitory neurons in both the upper and middle layers of pOFC.

A few amygdalar axons targeted inhibitory neurons proximally in the upper layers of pOFC, forming significantly more appositions on cell bodies of inhibitory neurons in layers 1–3a (n = 11 of 310 appositions: 7 CB positive, 3 PV positive, 1 CR positive) than thalamic axons, which formed no appositions involving a soma (n = 0 of 123 appositions; χ²(1, n = 433) = 4.5, p = 0.03). On the other hand, in the middle layers of pOFC, both amygdalar and thalamic axons formed appositions with cell bodies of presumed inhibitory neurons (n = 5 of 224 thalamic appositions; n = 1 of 66 amygdalar appositions; all PV positive; Fig. 5D). This suggests that, although amygdalar fibers interacted with relatively fewer inhibitory neurons in the upper layers of pOFC, they targeted inhibitory neurons more proximally than the thalamic pathways.

Postsynaptic targets of amygdalar projections in pOFC
We further investigated at the synaptic level the excitatory and inhibitory targets of amygdalar terminals in pOFC. We reconstructed synapses formed between amygdalar axons and elements of postsynaptic neurons in the upper layers (1–3a) of pOFC using
electron micrographs from uninterrupted serial sections through the synapse photographed in the electron microscope (n = 164 boutons from two cases; Fig. 6A–C). We identified postsynaptic targets by morphology for excitatory neurons, which have spiny dendrites, and by morphology and label for CB, CR, or PV for presumed inhibitory neurons, which have smooth or sparsely spiny dendrites (Peters et al., 1991; DeFelipe, 1997).

The majority of amygdalar boutons innervated single spines of presumed excitatory neurons in the upper layers of pOFC (75%, n = 123 of 164 boutons; Fig. 6D). A smaller proportion...
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(10%, n = 17) of amygdalar boutons formed synapses with single dendrites of presumed inhibitory neurons, and only one terminal formed a synapse with a soma. The remaining boutons were multisynaptic (14%, n = 23), which were more frequent in the amygdalar pathway than the thalamic (3%, n = 1 of 39 boutons; two-tailed Fisher exact test, p = 0.05; Fig. 6D). Of these multisynaptic amygdalar boutons, the majority formed a synapse with one dendrite and ≥1 spine (8%, n = 12 of 164 boutons) or...
formed synapses with multiple spines (5%, \(n = 9\)), and a minority (<1%, \(n = 1\)) formed synapses with multiple dendrites or with a soma and multiple spines (<1%, \(n = 1\)).

Some amygdalar boutons in the upper layers of pOFC formed synapses with dendritic shafts of presumed inhibitory neurons (16% of synapses, \(n = 31\) of 189 synapses total, formed by 164 labeled boutons). Boutons forming synapses with \(\geq 1\) dendritic shaft or soma (presumed inhibitory neurons) were significantly larger than boutons with only excitatory postsynaptic targets (mean diameter \(\pm\) SE, 1.2 \(\pm\) 0.094 \(\mu\)m, \(n = 32\) boutons with inhibitory targets from two cases; 0.95 \(\pm\) 0.033 \(\mu\)m, \(n = 132\) boutons with excitatory targets from two cases; hierarchically nested mixed-model ANOVA, \(F_{1,2,1} = 18.1\) for fixed effect of target, \(p = 0.05\); \(F_{2,160} = 0.4\) for random effect of case, \(p = 0.6\); Fig. 6E). Among labeled dendrites, amygdalar boutons mostly formed synapses with CB-positive dendrites of presumed inhibitory neurons in the upper layers of pOFC. Amygdalar boutons with \(\geq 1\) inhibitory target were larger than amygdalar boutons with only excitatory targets (Fig. 6E).

Among labeled dendrites, amygdalar boutons mostly formed synapses with CB-positive dendrites of presumed inhibitory neurons in the upper layers of pOFC. Amygdalar (blue diamonds, solid line) and thalamic (yellow squares, dashed line) bouton volume was positively correlated with PSD area. Amygdalar (blue diamonds, solid line) and thalamic (yellow squares, dashed line) bouton volume was positively correlated with spine volume. \(* p = 0.05\).

Figure 6. Excitatory and inhibitory postsynaptic targets of amygdalar boutons in pOFC. A–C, Amygdalar boutons (blue) form synapses (yellow) with \(\text{A}\) a smooth dendrite of a presumed inhibitory neuron (red), \(\text{B}\) a smooth dendrite (red) labeled for CB and a spiny dendrite of a presumed excitatory neuron (green), and \(\text{C}\) a spiny dendrite of a presumed excitatory neuron (green). Scale: cubes are 1 \(\mu\)m \(^3\). D, Left, Amygdalar boutons most frequently innervated single spines in the upper layers of pOFC, and a significant proportion were multisynaptic. Right, Thalamic boutons also formed synapses mostly with spines, but thalamic pathways contained fewer multisynaptic boutons in the middle layers of pOFC. E, Amygdalar boutons with \(\geq 1\) inhibitory target were larger than amygdalar boutons with only excitatory targets. F, Among labeled dendrites, amygdalar boutons mostly formed synapses with CB-positive dendrites of presumed inhibitory neurons in the upper layers of pOFC. G, Amygdalar (blue diamonds, solid line) and thalamic (yellow squares, dashed line) bouton volume was positively correlated with PSD area. H, Amygdalar (blue diamonds, solid line) and thalamic (yellow squares, dashed line) bouton volume was positively correlated with spine volume. \(* p = 0.05\).

Synaptic specializations of amygdalar and thalamic boutons

The majority of synapses formed by amygdalar boutons were macular (Peters and Kaiserman-Abramof, 1969), with the postsynaptic density forming a complete disk (Fig. 4A–C). Some amygdalar synapses were perforated with a discontinuous PSD (21%, \(n = 40\) of 189 synapses; Fig. 4D), most of which were on spines (80%, \(n = 32\) of 40); the remainder were on dendrites. This was similar to the proportion of thalamic boutons forming per-
forated synapses in the middle layers (13%, n = 5 of 40 synapses, all axosomatic). Among amygdalar and thalamic boutons, PSD area was significantly correlated with bouton volume (linear regression: amygdala, n = 178 boutons, $R^2 = 0.24, F_{(1,176)} = 54.1, p < 0.0001$; thalamus, n = 36 boutons, $R^2 = 0.36, F_{(1,34)} = 18.8, p = 0.0001$; Fig. 6G). The lower slope of the correlation between amygdalar bouton volume and PSD area may be due to the greater proportion of multisynaptic amygdalar boutons, compared with the thalamic pathway.

The volume of postsynaptic spines was also significantly correlated with bouton volume (linear regression: amygdala, n = 145 boutons, $R^2 = 0.45, F_{(1,143)} = 117.5, p < 0.0001$; thalamus, n = 26 boutons, $R^2 = 0.42, F_{(1,24)} = 17.3, p = 0.003$; Fig. 6H). Thalamic boutons in the middle layers targeted a significantly higher proportion of spines containing a spine apparatus (SA) or smooth endoplasmic reticulum (SER, 55%, n = 16 of 29 spines; two-tailed Fisher exact test, $p = 0.02$) compared with amygdalar boutons (32%, n = 50 of 156 spines; Fig. 4C). SER is found in dendrites and some spines, which at times forms an SA; these components are associated with stable spines (Ostroff et al., 2010). Additionally, a minority of amygdala-targeted spines in pOFC (5%, n = 7 of 156 spines; Fig. 4C,D) also received a symmetric synapse, presumably from a local inhibitory neuron. These results show that amygdalar boutons formed perforated synapses, larger synapses, and targeted larger spines.

**Comparison of amygdalar synapses with surrounding neuropil**

We next investigated whether the prevalence of large amygdalar synapses differs from nonlabeled synapses in the neuropil. This analysis revealed that amygdalar boutons formed synapses that were significantly larger than those in the surrounding neuropil (mean area ± SE, 0.11 ± 0.0064 μm², n = 178 amygdalar synapses from two cases; 0.84 ± 0.0052 μm², n = 165 unlabeled synapses from one case; two-tailed $t$ test, $t_{(341)} = 2.6, p = 0.01$).

**Discussion**

Novel findings from the system to the synapse revealed that the pathway from the amygdala to pOFC is stronger than to other prefrontal cortices or even the thalamocortical pathway (Fig. 7). This conclusion is based on evidence that the amygdalar pathway to pOFC is denser (Ghashghaei et al., 2007), has more large terminals, is largely excitatory, and is distinguished for unique synaptic specializations among other pathways. In aggregate, these features suggest that the amygdala can exert a powerful influence on pOFC.

**Specialized amygdalar pathways target preferentially the pOFC: comparison with ACC**

We found that pathways from large or restricted amygdalar sites consistently innervated most robustly the pOFC than areas 9/46 or even ACC, which is also strongly linked with the amygdala (Ghashghaei et al., 2007). The amygdalar pathway to pOFC had a significant proportion of large terminals. Large terminals have a higher content of vesicles and mitochondria (Germuska et al., 2006; Zikopoulos and Barbas, 2007b), and are associated with greater synaptic efficacy and highly active synapses (Reichova and Sherman, 2004; Zikopoulos and Barbas, 2007b).

Among prefrontal areas, the pOFC is distinguished for receiving extensive projections from sensory association cortices representing each modality (for review, see Barbas, 2000). Coupled with robust input from the amygdala, the pOFC is poised to link external cues with internal valuations, reliably convey the salience of task-specific stimuli, and quickly form associations related with reward and update them as conditions change (Tremblay and Schultz, 1999; Wallis and Miller, 2003; Simmons and Richmond, 2008; Morrison and Salzman, 2011; Luk and Wallis, 2013; Wilson et al., 2014). The above studies show that responses of neurons in orbitofrontal cortex vary based on task context, suggesting flexible integration of information. It has been hypothesized that processing flexibly the state of a task applies to diverse tasks that test function in orbitofrontal cortex across species (Wilson et al., 2014).

**Figure 7.** Summary of features in amygdalar pathway to pOFC, ACC, and areas 9/46. **A,** Thickness of arrows depicts the relative density and terminal size of amygdalar pathways to pOFC, ACC, and areas 9/46. **B,** Amygdalar terminals were larger and formed synapses more often with excitatory neurons in the upper layers of pOFC compared with thalamic axons in the middle layers. Among inhibitory neurons, amygdalar axons targeted CB and CR neurons in the upper layers, while thalamic axons targeted more PV and CR neurons in the middle layers. Large amygdalar synapses may drive excitatory neurons in pOFC, while synapses on CB and CR neurons may reduce background excitation, or noise. Excitatory spiny stellate neurons (SS) are a major target of thalamic pathways. BL, Basolateral; BM, basomedial; Co, cortical; mc, magnocellular sector; P, pyramidal neuron; pc, parvicellular sector.
The ACC differs in several ways from the pOFC. While the pOFC is a stronger “receiver” of pathways from the amygdala, the ACC is a stronger “sender” of pathways to the amygdala (Ghashghaei et al., 2007). The emphasis in ACC appears to be in processing the value of actions and outcomes (Kennerley et al., 2006), consistent with its connections with cingulate motor areas (Barbas and Pandya, 1987; Morecraft and Van Hoesen, 1998). Furthermore, the most robust projections of ACC are motor related and innervate brainstem and spinal autonomic centers (An et al., 1998; Rempel-Clower and Barbas, 1998; Barbas et al., 2003). These efferent autonomic pathways mediate emotional expression, including vocalizations and distress calls in primates (for review, see Vogt and Barbas, 1988; Devinsky et al., 1995; Hadland et al., 2003).

Specialized amygdalar drivers to the upper layers of pOFC: comparison with the thalamic

The predominance of dense and potentially efficient pathways from the amygdala to the upper layers of pOFC is also unusual and different from thalamic relay pathways. In the latter, upper-layer terminations are thought to have a modular role (Sherman and Guillery, 1998). Dense pathways from thalamic relay nuclei target most densely the middle cortical layers (layers 3b–5a), a pattern seen also for MD to prefrontal cortex (Giguere and Goldman-Rakic, 1988). These thalamocortical pathways are described as drivers, capable of eliciting action potentials in postsynaptic neurons (Freund et al., 1989; Sommer and Wurtz, 2004). In contrast, amygdalar terminations in the upper layers were not only denser but also larger than the thalamic in the middle layers.

Synaptic specializations further suggest higher strength in the amygdalar pathway than the thalamic to pOFC. This is exemplified by amygdalar innervation of large spines, which are associated with large synapses and large EPSCs (Harris and Stevens, 1989; Matsuzaki et al., 2001). Multisynaptic amygdalar boutons were more frequent than the thalamic in pOFC; such boutons increase in number after long-term potentiation and learning (Jones et al., 1997; Muller et al., 2000; Geinisman et al., 2001). Finally, some amygdalar terminals formed perforated synapses, which are enriched with AMPA receptors and are thought to participate in more efficient computations (Geinisman, 2000; Nicholson et al., 2006; Nava et al., 2014).

The strong amygdalar pathway to the upper layers provides a circuit mechanism for extended influences in pOFC, by impinging on the apical dendrites and tufts of pyramidal neurons from layers 2, 3, and 5, as well as calbindin and calretinin inhibitory neurons (DeFelipe, 1997; Callaway, 1998; Douglas and Martin, 1998). The amygdalar pathway to the upper layers of pOFC may thus affect the activity of the entire cortical column, including neurons that project back to the amygdala, as shown in mouse medial prefrontal cortex (Little and Carter, 2013). In rhesus monkeys, neurons in layer 5 and to a lesser extent in layer 3 project to the amygdala (Ghashghaei et al., 2007), and pOFC uniquely targets the purely inhibitory intercalated masses (IMs) of the amygdala (Ghashghaei and Barbas, 2002). In turn, IM neurons project to the central amygdalar nucleus, a key output of the amygdala to brainstem and hypothalamic autonomic centers (for review, see Price, 2003; Barbas et al., 2011). The pOFC projects as well to the central nucleus, albeit to a lesser extent, and through these dual pathways the pOFC may flexibly adjust autonomic drive, depending on the circumstances (Ghashghaei and Barbas, 2002). When activated by the robust amygdalar pathways, the pOFC may modify the activity of the amygdala as conditions change (Simmons and Richmond, 2008), adding necessary flexibility in reward learning (John et al., 2013).

Amygdalar drivers and feedforward inhibition: a mechanism for gain control

Like other long-distance pathways in primates, the amygdalar pathway to orbitofrontal cortex is excitatory (Miyashita et al., 2007). We found that the amygdala also innervates a higher proportion of excitatory postsynaptic sites than the thalamic, while the opposite applies for inhibitory postsynaptic targets. Among the small proportion of synapses with presumed inhibitory neurons, amygdalar axons innervated mostly CB and CR neurons in pOFC. CB neurons form synapses on distal dendrites of neighboring pyramidal neurons and mildly modulate them, while CR neurons modulate other inhibitory neurons in the upper layers (DeFelipe et al., 1989; DeFelipe, 1997). This circuit mechanism can enhance signal and reduce weak activity at the fringes of columns of neurons engaged in working memory tasks in monkey lateral prefrontal cortex (Constantindis and Goldman-Rakic, 2002; Wang et al., 2004). By analogy, amygdalar axons may increase the signal-to-noise ratio by activating CB and CR inhibitory neurons and simultaneously drive neurons in pOFC through large and efficient synapses on excitatory postsynaptic targets (Fig. 7). This circuit mechanism may control gain by enhancing activity by relevant signals and reducing noise to help focus attention on stimuli with affective import. Interestingly, the amygdala, pOFC, and MD have in common robust and overlapping projections to the inhibitory thalamic reticular nucleus (TRN), which is thought to be the brain’s vanguard for attention (for review, see Zikopoulos and Barbas, 2007a). All three pathways extend to the sensory sectors of TRN and may gate sensory stimuli with emotional significance (Zikopoulos and Barbas, 2012). The pathway to TRN provides an alternative route through which the amygdala may influence pOFC via the thalamic MD, to which it also projects (Russchen et al., 1987).

Circuits for flexible behavior and their disruption in psychiatric diseases

Dynamic integration of external sensory stimuli and internal valuations in orbitofrontal cortex based on context (Simmons and Richmond, 2008; Wilson et al., 2014) is likely disrupted in a variety of psychiatric diseases, including obsessive–compulsive disorder (OCD) and phobias (Barbas et al., 2011; John et al., 2013). Specifically, pathways connecting the amygdala and orbitofrontal cortex have been implicated in the pathology of OCD, characterized by recurrent intrusive thoughts and impulses, leading to repetitive compulsive behaviors (for review, see Chamberlain et al., 2005; Maia et al., 2008; Arnsten and Rubia, 2012). OCD is thought to arise from excess activation of loops connecting the basal ganglia, the amygdala, ACC, and orbitofrontal cortex (Huey et al., 2008; Maia et al., 2008; Haber and Heilbronner, 2013). Specifically, the orbitofrontal cortex has reduced volume and shows increased activation in OCD patients experiencing symptoms (Breiter and Rauch, 1996; Szeszko et al., 1999; Adler et al., 2000; Kang et al., 2004). Lesions of the connections among orbitofrontal cortex, the thalamus, and basal ganglia have been used with some success as therapy for OCD (Maia et al., 2008; Greenberg et al., 2010). Further, the therapeutic effects of deep brain stimulation in a rat OCD model have been attributed to ultimate excitation of presumed inhibitory neurons and decreased firing rates of orbitofrontal neurons (McCracken and Grace, 2007).

The precise circuit neuropathology of OCD is not clearly understood. But based on the above studies, our findings suggest a
possible pathway mechanism, mediated through a strong excitatory amygdalar pathway to POFC. Increased excitation from the amygdala in OCD may override the comparatively few synapses on the modulatory CB and CR neurons in pOFC, and compromise the ability to disambiguate the contextual significance of stimuli. Reduced inhibition in orbitofrontal cortex may decrease the ability to focus only on relevant stimuli and respond also to multiple other stimuli that richly impinge on pOFC.

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