Published in final edited form as:

Neuroscience. 2012 June 14; 212: 104–119. doi:10.1016/j.neuroscience.2012.03.040.

Revisiting the hippocampal-amygdala pathway in primates: association with immature-appearing neurons

Julie L. Fudge^{1,2,*}, Danielle M. deCampo¹, and Kyeesha T. Becoats¹

¹Dept. of Neurobiology and Anatomy, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642

²Dept. of Psychiatry, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642

Abstract

Elucidation of the ‘fear circuit’ has opened exciting avenues for understanding and treating human anxiety disorders. However, the translation of rodent to human studies, and vice versa, depends on understanding the homology in relevant circuits across species. Although abundant evidence indicates that the hippocampal-amygdala circuit mediates contextual fear learning, previous studies indicate that this pathway is more restricted in the primate than in rodent. Moreover, cellular components of the amygdala differ across species. The paralaminar nucleus (PL) of the amygdala, a structure that is closely associated with the basal nucleus, is one example, having no clear homologue in rodents. In both human and nonhuman primates, the PL contains a subpopulation of immature-appearing neurons, which merge into the corticoamygdaloid transition area (CTA). To understand whether immature-appearing neurons are positioned to participate in fear circuitry, we first mapped the hippocampal-amygdala projection in the monkey. We then determined whether immature appearing neurons were targets of this path. Retrograde results show that the hippocampal inputs to the amygdala originate in uncus region (CA1’) and the rostral prosubiculum, consistent with earlier studies. The amygdalohippocampal area, ventral basal nucleus, the medial paralaminar nucleus, and its confluence with the CTA are the main targets of this projection. Immature neurons are prominent in the PL and corticoamygdaloid transition area (CTA), and are overlapped by anterogradely labeled fibers from CA1’, particularly in the medial PL and CTA. Hippocampal inputs to the amygdala are more focused in higher primates compared to rodents, supporting previous anatomic studies and recent data from human functional imaging studies of contextual fear. At the cellular level, a hippocampal interaction with immature neurons in the amygdala suggests a novel substrate for cellular plasticity, with implications for mechanisms underlying contextual learning and emotional memory processes.

© 2012 IBRO. Published by Elsevier Ltd. All rights reserved.

*Correspondence to: Phone: 585 273 2028, Fax: 585 756 5334, julie_fudge@urmc.rochester.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

DISCLOSURES AND CONFLICTS OF INTEREST

The authors attest that they have no disclosures or conflicts of interest.

Respective contributions. Julie Fudge was responsible for design of the studies, supervision of data collection and analysis, and wrote the manuscript. Danielle deCampo and Kyeesha Becoats were responsible for collecting and analysis of the anterograde and retrograde tract tracing data. All authors contributed to editing of the manuscript.

Keywords

doublecortin; polysialated neural cell adhesion molecule; CA1; prosubiculum; corticoamygdaloid transition area; paralaminar nucleus

INTRODUCTION

Across species, the hippocampus and amygdala are closely connected structures that influence emotional perception and experience. The path from the hippocampus to the amygdala has received much attention due to its role in contextual fear learning and memory, based on a wealth of rodent behavioral studies (Kim and Fanselow, 1992, Phillips and LeDoux, 1992, Muller et al., 1997, Rosen et al., 1998, Anagnostaras et al., 1999, Goosens and Maren, 2001, Hall et al., 2001, Anglada-Figueroa and Quirk, 2005, Calandreau et al., 2005, Hernandez-Rabaza et al., 2008, Herry et al., 2008). Co-activation of the hippocampus and amygdala in functional imaging studies support the idea that this circuit is also relevant for understanding contextual fear conditioning in humans (LaBar et al., 1998, Grillon et al., 2006, Alvarez et al., 2008, Marschner et al., 2008, Alvarez et al., 2011). More broadly, the recall of a past emotional experiences involves both amygdala and hippocampal activity (Hamann et al., 1999), suggesting that these memories are an internal trigger of emotional states. Delineating the contextual fear circuit in humans is especially important in understanding the pathophysiology of mood and anxiety disorders. In these disorders, environments previously linked to aversive events can subsequently evoke exaggerated or inappropriately persistent fear responses (Phelps, 2004, Indovina et al., 2011). Similarly, in humans, implicit and explicit memories of past events are powerful contributors to emotional and mood states (Lane et al., 1997, Mayberg et al., 1999).

The hippocampus and amygdala in primates are larger, and have a greater cellular complexity, compared to lower species (Reviews by Price (Price et al., 1987), deOlmos ((DeOlmos, 1990) and Rosene (Rosene and Van Hoesen, 1987)). The CA1 region of the hippocampus in primates is greatly expanded at the rostral pole, and is cytoarchitecturally more diverse than in rodents. In particular, the anterior-most portion of CA1 (CA1') forms a significant portion of the 'uncus', a region not found in species lower than the primate (Stephan, 1983). With respect to the amygdala, the most phylogenetically expanded subregion is the basolateral nuclear group (Stephan and Andy, 1977). Compared to the rodent, the primate basolateral nuclei have dramatic cellular gradients (Johnston, 1923, Crosby and Humphrey, 1941, Lauer, 1945, Jimenez-Castellanos, 1949). Another example of discontinuity between species is the paralaminar nucleus (PL), which is the last of the primate amygdala nuclei to form in both human and monkey, arising late in gestational development (Kordower et al., 1992, Ulfing et al., 2003). In the postnatal brain, the PL resides in the remnant of the former lateral ganglionic eminence, merging medially with the corticoamygdaloid transition area (CTA). The PL reaches its largest extent in the human, and has no clear counterpart in the rodent (Review by deCampo (deCampo and Fudge, 2012)). Importantly, in both human and nonhuman primates, the region of the PL contains a subpopulation of immature-appearing neurons (Bedard et al., 2002, Bernier et al., 2002) (Yachnis et al., 2000, Fudge, 2004, deCampo and Fudge, 2012), whose function has yet to be defined. These cells contain proteins associated with neural immaturity, including doublecortin (DCX) and polysialysated neural cell adhesion marker (PSA-NCAM) (Francis et al., 1999, Angata et al., 2007).

Earlier anatomical studies in monkeys indicate that the hippocampal-amygdala pathway differs in primates and rodents (Rosene and Van Hoesen, 1977, Ottersen, 1982, Russchen, 1982, Aggleton, 1986, Amaral, 1986, Saunders et al., 1988, Canteras and Swanson, 1992)

(Reviews Pitkanen (Pitkanen et al., 2000) and McDonald (McDonald, 1998)), being far more restricted in higher primates. Given the important role of the hippocampal-amygdala path in contextual fear learning and memory, and its relevance to human anxiety and mood disorders, a closer examination of this projection in the primate is warranted. We sought first to examine the specificity of the hippocampal-amygdala pathway in the monkey using relatively small injections of retrograde tracers in specific subregions of the amygdala. We then analyzed the relationship of the projection relative to the distribution of immature neurons using anterograde tracer injections into the hippocampus. Our hypothesis was that hippocampal inputs would be positioned to influence the immature-appearing neurons in the amygdala, suggesting novel mechanisms for the acquisition and consolidation of contextual fear associations in primates. Since immature neurons in other brains respond in unique ways to excitatory signaling (Wang et al., 2000; Schmidt-Hieber et al., 2004; Ge et al., 2007), we were interested in exploring afferent influences on immature neurons in the amygdala.

MATERIALS AND METHODS

INJECTION SITES

We placed small injections (40 nL) of Lucifer Yellow conjugated to dextran amine (LY; 10% Molecular Probes, Eugene, OR), fluororuby conjugated to dextran amine (FR; 4%, Molecular Probes); and fluorescein conjugated to dextran amine (FS: 10%, Molecular Probes) into various nuclei of the amygdala, using methodology described previously (Fudge and Tucker, 2009). Injections of LY, FR, FS, Cascade Blue (CB, 10%, Molecular Probes), Phaseolus vulgaris leucoagglutinin (PHA-L) (2%, Sigma, St. Louis, MO), and tritiated amino acids (AA; 1:1 ratio of tritiated proline and leucine: Perkin Elmer, Boston, MA) were then placed into the hippocampal complex. While previous studies have indicated that there is no cross reactivity of antibodies to FR, FS, and LY, preliminary studies carried out in rats indicated that there is cross-reactivity between antibodies to CB and LY (not shown). Therefore, in the animal containing a CB injection, we did not use LY.

SURGERIES

Eleven Old world primates (*Macaca fascicularis* and *Macaca nemestrina*) weighing between 3.0 and 9 kg, ages 2-5 years were used for these studies (Labs of Virginia, Yemassee, SC; Three Springs Laboratories, Pekaski, PA, Worldwide Primates, Tallahassee, FL). All experiments were carried out in accordance with National Institutes of Health guidelines. Experimental design and techniques were aimed at minimizing animal use and were reviewed by the University of Rochester Committee on Animal Research. Initial anesthesia was administered by an intramuscular injection of ketamine hydrochloride (10 mg/kg), after which animals were intubated, and a deep plane of anesthesia was induced by isoflurane gas anesthesia, maintained as needed during surgery. Small deposits (40-50 nL) of FS, FR, or LY were pressure-injected over 10 minutes into the amygdala using a 0.5 μ L Hamilton syringe. One injection of each tracer was used per animal. Injections of FR, CB, LY, FS, PHA-L (40-50 nL) and AA (200 nL) were also injected into various subregions of the hippocampal complex to confirm retrograde results. Following injection placement for all animals, the bone flap was replaced, and the overlying musculature and skin sutured.

Ten to thirteen days after surgery, animals were deeply anesthetized and killed by perfusion through the heart with 0.9% saline containing 0.5 mL of heparin sulfate, followed by 4% paraformaldehyde in a 0.1 M phosphate buffer/30% sucrose solution. The brain was removed, placed in fixative overnight, and sunk in increasing gradients of sucrose (10%, 20%, and 30%). Brains were cut in the coronal plane throughout their rostrocaudal extent on a freezing microtome (50 μ m sections) and stored in cryoprotectant solution at -20°C .

IMMUNOCYTOCHEMISTRY

Tracer labeled cells and/or fibers resulting from FR, CB, FS, PHA-L, and LY injections were detected using immunocytochemical techniques. In order to identify the location of injection sites within specific amygdala and hippocampus subregions, adjacent sections were processed with several histologic stains. In monkeys, AChE is particularly useful for identifying subnuclei of the amygdala (Amaral and Bassett, 1989), and also differentiates the prosubiculum from the subiculum and CA1 (Rosene and Van Hoesen, 1987). Additionally, we immunostained adjacent sections through the hippocampus for SMI-32 (nonphosphorylated neurofilament protein) in order to distinguish the boundary between CA1, prosubiculum, and subiculum (Kobayashi and Amaral, 1999).

Single label immunocytochemistry for tracer—For most cases, we selected every eighth section through the brain to immunostain. Sections were thoroughly rinsed in 0.1 M phosphate buffer (pH 7.2) with 0.3% Triton-X (PB-TX). After treatment with endogenous peroxidase inhibitor for 5 minutes, followed by more rinses, sections were pre-incubated in a blocking solution of 10% normal goat serum in 0.1M PB-TX (NGS-PB-TX) for 30 minutes. Tissue was then placed in primary antisera to LY (1:2000, Molecular Probes, rabbit), FS (1:2000, Molecular Probes, rabbit), CB (1:1000, Molecular Probes, rabbit), and FR (1:1000, Molecular Probes, rabbit) for approximately 96 hours (four nights) at 4 °C. After thorough rinsing with 0.1 M PB-TX, and preincubation with 10% NGS-PB-TX, sections were then incubated in biotinylated secondary anti-rabbit antibody. Tracers were visualized using the avidin-biotin reaction (Vector ABC Standard kit, Burlingame, CA). For visualization of PHA-L, tissue was similarly processed but Tris-NaCl-Triton was used, and tissue was incubated for approximately 144 hours (6 nights) (rabbit anti-PHA-L 1:500, Sigma, St. Louis, MO). Additional compartments for each case were processed for tracer, enhanced with nickel intensification (3, 3'-diaminobenzidine tetrahydrochloride (DAB) with 1% nickel ammonium sulfate and 1% cobalt chloride, catalyzed by 0.03% hydrogen peroxide for 1-2 minutes) and counterstained with AChE (Geneser-Jensen and Blackstad, 1971) or cresyl violet.

SMI-32 immunoreactivity (IR)—Sections were thoroughly rinsed, and preincubated in 10% NGS-PB-TX as described above, and then incubated for 96 hours in SMI-32 (1:5,000, Sternberger Monoclonals, Lutherville, MD, mouse) antisera. Sections were then using the avidin-biotin reaction.

Autoradiography: Autoradiography was used to visualize labeled fibers following the tritiated amino acid injection. Sections for autoradiography were mounted on chrome-alum gelatin-coated slides and cleared in xylene for 48 hours. Slides were then dipped in Kodak NTB2 photographic emulsion (Kodak, Rochester, NY) and exposed for 4-6 months in a light-tight box at -20°C. Slides were then developed in cold Kodak D19 developer (15 °C) for 2.5 minutes, fixed, washed, and counterstained with cresyl violet.

Double immunofluorescence studies—To visualize the relationship between tracer-labeled fibers and DCX-IR and PSA-NCAM-IR neurons in the same section, we employed double immunofluorescent-labeling. We also used this technique to determine co-localization of neural markers. We first determined optimal dilutions for primary antibodies in preliminary single labeling experiments using fluorescent secondary antibodies. Control experiments were also performed in which 1) the primary antibody for each antigen was omitted, 2) primary antibodies against tracer were run in tissue without tracer injections, and 3) secondary antibodies for each primary antibody were 'switched', to rule out cross-reactivity of secondary antibodies. In all cases, there was no labeling. Then, a case containing a tracer injection in the subiculum (J14CB) based on DAB-processed

experiments (above) was thoroughly rinsed in PB-TX overnight, pre-incubated in 10% NGS-PB-TX, and then placed in a solution of pooled antisera to CB (1:1000, Molecular Probes, rabbit) and DCX (1:2500, Santa Cruz Biotechnology, Santa Cruz, CA, goat) or PSA-NCAM (1:50, B.D. Biosciences, Franklin Lakes, NJ, rat). Sections were incubated at 4°C for four days, then again thoroughly rinsed in PB-TX, and pre-incubated with 10% NGS-PB-TX. Sections were then incubated for four hours in the dark at room temperature in a 1:200 solution of fluorescent secondary antibodies (Alexafluor 488 anti-rabbit IgG for CB and Alexafluor 596 anti-goat IgG for DCX detection, or Texas red anti-rat IgM (Genway Biotech Inc, San Diego, CA) for PSA-NCAM detection) in 10% NGS-PB-TX. Sections were then rinsed for an hour in 0.1M phosphate buffer, mounted onto gelatin-coated slides, air-dried, and coverslipped using Vectashield aqueous medium (Vector Labs, Burlingame, CA).

Additional experiments examining co-expression of PSA-NCAM-IR and DCX-IR, and PSA-NCAM-IR and class III β -tubulin-IR, a neuron-specific marker also known as TUJ1 (Easter et al., 1993, Katsetos et al., 1993, Weickert et al., 2000)(1:7500, Sigma, St. Louis, MO, mouse), were conducted. We reversed the fluorescent-tag used in the original experiments to ensure that similar results were obtained, and 'bleed through' was not a problem.

ANALYSIS

The distribution of retrogradely labeled cells in the hippocampus (DAB single label experiments) following amygdala injections were charted under bright-field illumination using the software program Neurolucida 7.0 (Microbrightfield, Inc., Rutland, VT) in conjunction with a video camera (Microfire Optronics, Goleta, CA) attached to the microscope (Olympus AX 70). The boundaries of hippocampal subregions, along with identifying landmarks such as blood vessels and fiber tracks, were drawn from adjacent sections stained with cresyl violet, AChE, or SMI-32, which were then aligned over maps of labeled cells matching landmarks such as blood vessels and fiber tracts for reference. To further analyze the overall location of retrogradely labeled neurons in the rostrocaudal plane of the hippocampus, we created a template of an unfolded map of the hippocampus (Blatt and Rosene, 1998). Additional sections labeled for tracer and counterstained with one of the histologic stains were used to confirm these relationships.

The location of anterograde injection sites in specific hippocampal subregions was determined using adjacent sections stained for AChE, cresyl violet or SMI-32, as well as counterstained sections. The distribution of labeled fibers in the amygdala was charted using a 20x objective using camera lucida techniques, and scanned into the computer using Adobe Photoshop CS2. The distribution of labeled fibers in specific amygdala nuclei was evaluated using adjacent sections stained for AChE or cresyl violet. Sections of adjacent compartments processed for anterograde tracer were then immunoreacted for DCX and PSA-NCAM proteins, and also charted and scanned into the computer. Scanned images were aligned over the charts of anterogradely labeled fibers in adjacent sections, matching landmarks, to allow examination of the relationship between immature-appearing neurons and afferent fibers.

Additional sections double immunofluorescent labeled for 1) DCX-or PSA-NCAM-IR and tracer-IR, 2) DCX/PSA-NCAM-IR and 3) class III β -tubulin-IR/PSA-NCAM-IR were first examined under epifluorescent microscopy and then under a confocal laser microscope (FV1000 Olympus confocal with SIM scanner). Regions of overlap between fluorescently labeled fibers and immature-appearing neurons were first identified using traditional fluorescent microscopy at 20 and 40x magnification. Then, identified regions of interest were scanned using confocal laser microscopy. At the confocal microscope, we used a 60x magnification with oil immersion, sequentially scanned in 2 or 4 μ m increments through the

Z plane (Kalman 10, resolution 1024). Images were merged to examine the relationship between labeled afferent fibers from the hippocampus and PSA-NCAM-IR cells, and the co-localization of PSA-NCAM-IR with DCX-IR and class III β -tubulin-IR in morphologically similar cells in the amygdala.

RESULTS

Anatomy of the primate amygdala (Fig. 1A-B)

The primate amygdala is a heterogeneous collection of nuclei that we describe using the nomenclature of Price, Amaral, and colleagues (Price et al., 1987, Amaral et al., 1992). For reasons noted below, however, we use the original terminology of Crosby and Humphrey for the corticoamygdaloid transition region (CTA) (Crosby and Humphrey, 1941, Rosene and Van Hoesen, 1987). The ‘deep’ nuclear group of the amygdala includes the lateral, basal, and accessory basal nuclei. The lateral nucleus is divided into dorsal, ventral intermediate, and ventral subdivisions with differing cellular features and AChE staining. The accessory basal nucleus consists of the magnocellular (ABmc) and parvicellular (ABpc) subdivisions, which have high and low levels of AChE, respectively. Additionally, there is also a ‘sulcal’ subdivision of the accessory basal nucleus (ABs) that is medial and posterior relative to the other subdivisions. The basal nucleus is subdivided into magnocellular (Bmc), intermediate (Bi) and parvicellular subdivisions (Bpc), which have a decreasing gradient of cell size and AChE staining.

The PL is intimately associated with the basal nucleus, forming a thin sheet of densely packed cells that surrounds the entire basal nucleus rostrally and caudoventrally. Although the PL has frequently been grouped with the basal nucleus in humans and monkeys, or ignored, cytoarchitectural and neurochemical studies support its recognition as a separate nuclear zone (Review(deCampo and Fudge, 2012)). Medially, the PL widens and merges imperceptibly with the Bpc in the ‘corticoamygdaloid transition area’ (CTA) (Crosby and Humphrey, 1941, Rosene and Van Hoesen, 1987) near the semiannular sulcus (Fig. 1A and B, boxed areas). At the semiannular sulcus, the PAC is highly undifferentiated, breaking up into islands and disorganized partial layers. Here, cells of the Bpc, PL and deep periamygdaloid cortex (PAC) are intermingled, making these nuclei indistinguishable from one another. We refer to this entire region as the CTA (the ‘sulcal PAC’ of Price (Price et al., 1987), and the amygdalopiriform area of deOlmos (DeOlmos, 1990)) to emphasize the lack of boundary between this rudimentarily layered cortex and the more nuclear Bpc and PL (Crosby and Humphrey, 1941, Rosene and Van Hoesen, 1987, Sims and Williams, 1990). Behind the CTA, the amygdalohippocampal area (AHA) emerges as a three-layered cortex that forms the medial wall of the lateral ventricle.

The ‘superficial nuclei’ of the amygdala include the anterior and posterior cortical nuclei (CoA and CoP, respectively), and the medial nucleus. Beneath them, the primitive 2-3 layered paleocortex known as the periamygdaloid cortex (PAC) forms the medial amygdala. In the dorsal amygdala, the central nucleus is divided into medial and lateral central core subdivisions. The central nucleus is separated from the striatum by a relatively broad, heterogeneous amygdalostriatal area (Fudge and Tucker, 2009). Finally, small clusters of heterogeneous cells known as the intercalated cell islands are lodged in fibers surrounding the major nuclei.

Anatomy of the primate hippocampus (Fig. 1C-E.)

The primate hippocampus occupies a large rostro-caudal extent of the medial temporal lobe and is comprised of three-layered allocortex that includes the dentate gyrus, the hippocampal subfields, and subicular complex. The ‘uncal’ region forms the rostral pole of the

hippocampus, and is only present in primates (Fig 1 C-D, with enlargement of the uncus in E). The uncal hippocampus contains the hippocampal-amygdaloid transition region (HATA), and the uncal portion of CA1 (CA1'). Both structures form a transitional 'bridge' between the body of the hippocampus and caudal amygdala. The HATA is composed of relatively small, very tightly packed cells that gradually give way to the adjacent CA1' (Fig. 1E). Unlike HATA, CA1' contains the classic three layers typical of Ammon's horn. However, the pyramidal cells of CA1' are smaller and much more densely packed than in the ventrolateral and caudal CA1. HATA and CA1' also have distinct patterns of AChE staining that distinguish them from the rest of the hippocampus, and from each other (not shown, see (Rosene and Van Hoesen, 1987, Saunders et al., 1988)).

The dentate gyrus first appears at the caudal end of the uncus and rostral CA1'. It is composed of a granule cell layer, the subjacent polymorph layer, and a molecular layer. The main hippocampal subfields (CA1, CA2, and CA3) surround the dentate gyrus, and also have three main layers. Using the nomenclature of Kobayashi and Amaral, which does not distinguish between CA4 and CA3 in monkey (Kobayashi and Amaral, 1999), CA3 is identified by a relatively dispersed pyramidal layer, which occupies, and extends out of, the hilar region. Adjacent CA2 has a densely packed band of pyramidal cells, and is distinguished by relatively high AChE levels. Both CA3 and CA2 are identified by fibers positive for nonphosphorylated neurofilament protein H (SMI-32), which is low in the CA1 subfield. The classic, ventrolateral CA1 also has low AChE levels throughout its rostrocaudal extent, and forms an oblique border with the transitional 'prosubiculum'. These features, along with a dispersed pyramidal layer, set CA1 apart not only from its CA2 neighbor, but from the uncal CA1' described above. The prosubiculum is most easily distinguished from CA1 by its increasing immunoreactivity for SMI-32-IR, which reaches its highest levels in the subiculum.

Retrograde results

Injection site placement—There were a total of 20 injections of bidirectional tracers into the amygdala (schematically represented in Fig. 2A-B). Some injection sites have been previously presented in other studies (Fudge et al., 2005, Fudge and Tucker, 2009). All injection sites were analyzed with respect to their location in specific amygdala nuclei and subnuclei using counterstains. Eight injection sites were located in the basal nucleus (Fig. 2 dark and light purple): three were centered in the magnocellular subdivision (J12LY, J16LY, and J12FR) and five were in the parvicellular subdivision (J15LY, J20LY, J15FS, J14FS, J14FR). Injection sites involving the medial parvicellular subdivision of the basal nucleus (cases J20LY, J15FS, and J14FS, light purple, boxed areas, in Fig. 2A-B, also Fig. 2D, and E) that involved the medial PL or the irregular cell islands deep to the PAC, were considered to be in the 'corticoamygdaloid transition area' as described above. The injection sites in cases J14FS and J15FS were relatively caudal compared to the other medial Bpc sites. We analyzed these latter cases for evidence of tracer spread into the entorhinal cortex. While many afferent/efferent paths are similar between the rostral entorhinal cortex and basal nucleus (Saunders and Rosene, 1988, Witter and Amaral, 1991), only the entorhinal cortex receives projections from the septum and mammillary nucleus (Insausti et al., 1987). Entorhinal cortex is also defined by its inputs to the dentate gyrus, which originate mainly in layer II, but also layers V/VI (Witter and Amaral, 1991). We found that none of the medial Bpc cases resulted labeled cells in the septum or peri-mammillary area, or had anterogradely labeled fibers in the dentate gyrus. Together these observations suggest a lack of significant entorhinal cortex involvement in the sites. Five injection sites were centered in the accessory basal nucleus (Fig. 2, green): two were relatively confined to the magnocellular subdivision (J12FS, J8LY), two were centered in the parvicellular subdivision of the accessory basal nucleus but spread into the magnocellular subdivision (J21FR and J20FS), and one injection

site was confined to the parvicellular subdivision (J18FR) with slight encroachment on the periamygdaloid cortex. One large injection included the ventral two-thirds of the lateral nucleus throughout its rostrocaudal extent, with slight spread laterally and ventrally into surrounding white matter structures (J17LY, light pink). Four small injection sites were placed in the central amygdaloid nucleus (Fig. 2, yellow): two were confined to the caudal lateral core subdivision (J1LY and J9FS), one was confined to the medial subdivision (J9LY), and one included both medial and lateral core subdivisions (J1FR). There were two small injection sites confined to the medial nucleus, at slightly different rostrocaudal levels (Fig. 2, dark pink): J19LY and J16FR. The injection site in case J16FR was caudal to that in J19LY.

Overview of retrograde results—Injection sites encompassing the basal, accessory basal, and medial nuclei resulted in retrogradely labeled cells in the anterior hippocampus. Injection site placement within specific subregions of the basal nucleus predicted the density, and the rostrocaudal extent, of labeled cells in CA1' and prosubiculum (Table 1). Cases involving injection sites in the central and lateral nuclei did not result in labeled cells in the hippocampus. The distribution of labeled cells following injections into specific nuclei is described below.

Medial nucleus—Both medial nucleus injections (cases J19LY and J16FR) resulted in a moderate number of labeled cells in HATA, with no labeling in other hippocampal subregions.

Accessory basal nucleus, magnocellular and parvicellular subdivisions—All injections into the accessory basal nucleus resulted in labeled cells in CA1', with scattered labeled cells in the prosubiculum (Figure 3). Injection sites that included the parvicellular subdivision also resulted in scattered labeled cells in HATA. No labeled cells were found in other hippocampal regions.

Basal nucleus, magnocellular subdivision—The distribution of labeled cells in all cases was confined to a light concentration of labeled cells in CA1', with scattered labeled cells in the prosubiculum, seen mainly in rostral hippocampus. Other hippocampal areas were devoid of labeled cells.

Basal nucleus, parvicellular subdivision—Cases with injections into the CTA, encompassing the medial parvicellular subdivision of the basal nucleus (cases J20LY, J14FS, and J15FS, Table 1, asterisks), resulted in the highest concentration of labeled cells in the hippocampus. Labeled cells were equally concentrated in both CA1' and the prosubiculum. In contrast, injection sites that were in the Bpc proper (lateral to the CTA) (cases J15LY and J14FR), resulted in relatively fewer labeled cells overall. In these cases, labeled cells were mainly found in the rostral prosubiculum (Fig. 4).

Overlap of anterogradely labeled fibers and immature-appearing neurons

Immature-appearing profiles containing DCX- and PSA-NCAM markers were found almost exclusively in the PL along its medial-lateral and rostrocaudal extent, and in the CTA (Fig. 5A-C). Tightly packed aggregates of PSA-NCAM- and DCX-positive cells occupied the entire expanse of the PL, dispersing into streams of cells in the CTA (Fig. 5B, boxed area) where they were oriented perpendicular to the medial surface of the brain. The majority of labeled cells had a round or bipolar morphology with a long leading process, detected by both traditional single-label immunocytochemistry (Fig. 5D-G), and double-immunofluorescent techniques (Fig. 5H-J). Some labeled cells had bifurcated processes. Almost all DCX-IR cells co-contained PSA-NCAM-IR (Fig. 5H-J) and class III β -tubulin

(not shown). All three proteins were localized to the cytoplasm and thin processes emanating from the cell, with no expression in the nucleus. Leading processes from nearby cells frequently aggregated and spiraled together around passing fibers (Fig. 5F, double arrowheads), creating the appearance of thick coils (Fig. 5F and G, asterisks).

Because our retrograde results showed that the CA1' was strongly labeled following injections into amygdala regions containing immature neurons, we examined the distribution of anterogradely labeled fibers following two tracer injections at different rostrocaudal levels of this region (cases J14CB and J4AA, Fig. 2J-K.). J14CB was confined to CA1' at the rostral-most pole of the hippocampus, whereas J4AA was situated at a more caudal level and included a small amount of laterally adjacent CA2 in the site (Fig. 2J). Three control injections were centered in CA3 with varying amounts of spread into the dentate gyrus (cases J10LY, J4PHAL, and J10FS). One control injection (case J10FR) was in the rostral presubiculum.

We first compared the distribution of DCX- and PSA-NCAM-positive cells in sections adjacent to those labeled for tracer. In both cases where CA1' (J14CB and J4AA) was injected, the distribution of labeled fibers in the amygdala was similar. Anterogradely labeled fibers were densely concentrated in the CTA, PAC, ABs, and ABpc (Fig. 6A-C, black and 7A-C, green) and AHA. A moderate distribution of labeled fibers overlapped the medial PL and Bpc lateral to the CTA, and there were only scattered labeled fibers in the Bmc and ABmc. Few to no labeled fibers were seen in other nuclear subregions. Overlap of labeled fibers from the CA1' injection sites and immature-appearing neurons occurred in the CTA and medial PL, based on the analysis with adjacent sections.

We then examined sections double-labeled for tracer and doublecortin-IR, or tracer and PSA-NCAM-IR. Confocal microscopic examination of the medial PL showed that fine labeled fibers containing boutons (green) (Fig. 7D) formed neuropil in which immature-appearing cell profiles (red) (Fig. 7E) were embedded. Many immature-appearing cells had small, round soma with a single thin process, with a high nucleus/cytoplasm ratio, similar to the morphology seen in biotin-labeled cases (Fig. 5). Small areas of overlap (yellow, arrows) were seen on the cytoplasmic rim of the immature appearing cells, suggesting axonal contacts (Fig. 7F).

DISCUSSION

Using multiple retrograde injections in specific amygdala subregions, we found that hippocampal inputs to the amygdala originate mainly in CA1' and the rostral subiculum, and target the ventromedial amygdala (Fig. 8A). Anterograde studies focusing on CA1' uncinal inputs confirmed the retrograde findings and further demonstrate overlap with immature-appearing neurons in the primate amygdala. These are the first anterograde studies focusing on the output of the uncinal CA1', a hippocampal subregion that is unique to primates, including human. Our retrograde studies further demonstrated that efferents from CA1' and the prosubiculum were organized in longitudinal strips along the hippocampus' anterior-posterior axis (Fig. 4 and Fig. 8B). This organization resembles the previously reported topography of efferents to several other limbic regions, including the entorhinal, perirhinal and parahippocampal (Saunders and Rosene, 1988, Blatt and Rosene, 1998, Yukie, 2000), and ventromedial prefrontal cortex (areas 25, 24/32, and 14) (Barbas and Blatt, 1995, Insausti and Munoz, 2001). Compared to previously documented projections to limbic cortex in Old world monkeys, hippocampal projections to the amygdala are relatively restricted to the anterior-most pole of CA1'/prosubiculum. Our data, taken together with previous work (Saunders and Rosene, 1988, Barbas and Blatt, 1995, Blatt and Rosene, 1998, Insausti and Munoz, 2001), indicate that the hippocampal-amygdala projection is more

restricted than, and embedded within, broader projections to cortical areas that are themselves closely connected to the amygdala.

Comparison with previous connectional studies

The finding that the hippocampal-amygdaloid path is remarkably specific in nonhuman primates was noted in early anterograde studies (Rosene and Van Hoesen, 1977, Aggleton, 1986, Saunders et al., 1988). Using small retrograde and anterograde injections, we extend these studies by showing that this trajectory originates in a limited rostral sector of CA1' and prosubiculum. That data show the important role of the uncal, which is found only in primates (Stephan, 1983), in sending inputs to rostrally adjacent, highly 'transitional' regions of the amygdala, including the CTA and contiguous PL. Like the uncal CA1', these amygdala target regions are mainly found in the primate and regress in lower orders; indeed the existence of the PL in rodents is uncertain (Price et al., 1987, deCampo and Fudge, 2012). Small retrograde injections in the central nucleus, and a large, extensive injection into the lateral nucleus, gave no evidence for a direct hippocampal input to these regions, again confirming important species differences noted by other investigators (Aggleton, 1986, Saunders et al., 1988). We also identified a small, but potentially significant input pathway from HATA to the medial nucleus of the amygdala, not previously described. Overall this topography supports the idea that memories of past experience from the hippocampus can directly influence specific subcircuits through the amygdala (Fig. 8). In contrast, in rats, the temporal end of CA1 and the subiculum, which are considered the nearest homologue of these hippocampal areas, project relatively broadly throughout the amygdala, innervating the posterior basomedial (accessory basal) nucleus, posterior basolateral nucleus, and contiguous amygdalopiriform area, as well as the caudal central nucleus and lateral nucleus (Veening, 1978, Ottersen, 1982, Canteras and Swanson, 1992, Kishi et al., 2006, Cenquizca and Swanson, 2007) (Reviews by Pitkanen (Pitkanen et al., 2000) and McDonald (McDonald, 1998)). Moreover, these projections appear to emanate from a relatively broad rostrocaudal extent of the hippocampus in rodents (Kishi et al., 2006) in contrast to the situation in the primate.

Functional considerations

Classic anatomical and physiologic studies in rodents indicate that the dorsal hippocampus (which is widely considered analogous to posterior hippocampus in primate) is relatively tuned to spatial cues, while the ventral hippocampus (anterior hippocampus in primates) is more involved in fear responses (Henke, 1990, Kjelstrup et al., 2002, Fanselow and Dong, 2010). Data in humans indicate that the hippocampus mediates spatial memory, but also has a role in encoding and recalling memories of autobiographic events in the past. In humans, there is not currently clear evidence of whether or how these functions are distributed across the hippocampus' anterior-posterior axis. The CA1 subfield, including the uncal area containing CA1', appears strongly linked to autobiographical memory i.e. vivid memory for personal events of an individual's life. Compelling evidence for this comes from studies of patients suffering spells of transient global amnesia. In these patients, brief ischemic episodes result in highly circumscribed epochs of autobiographic memory loss that correlate with pinpoint lesions in CA1 (Bartsch et al., 2011). Functional imaging studies show that CA1 activation at all rostrocaudal levels is highly correlated with the vividness of remembered events. However, there is some evidence that anterior/uncal CA1 is relatively activated by recent events, while vividly-recalled remote events are associated with activations more evenly distributed along the hippocampus' rostrocaudal axis (Gilboa et al., 2004, Viard et al., 2007).

Recent physiologic work in both human and monkey complement the autobiographic memory studies by showing that the hippocampus is important in coding the temporal

context (Paz et al., 2010, Naya and Suzuki, 2011), as well as spatial context (Squire, 1993), of past events. In tasks going forward, the hippocampus appears to convey an ‘incremental timing signal’ against which future events can be compared (Naya and Suzuki, 2011). Our anatomic data, together with these functional studies, suggest that the anterior CA1’/prosubiculum continuum is an efferent hub by which highly personal memories, embedded in both temporal and spatial context, can influence amygdala responses to incoming stimuli. Thus, the temporal or spatial context of highly personal life events conveyed by the hippocampus can color the emotional experience of ‘here and now’ events, and may also influence subjective emotional experience regarding the future, such as anticipation, longing, and expectancy. Herry and colleagues have shown that even a neutral sensory cue, presented in an unpredictable sequence, correlates with increased anterior hippocampal and amygdala activity in both species (Herry et al., 2007). Increased hippocampal and amygdala co-activation, in turn, correlate strongly with anxiety responses in both human (assessed by increased attentional bias to threat information) and mouse (assessed by changes in anxiety-like and avoidance behavior). Thus the experience of temporal unpredictability of a cue (that, by itself, is not aversive) engages the hippocampus and amygdala, with consequences for anxiety-like behaviors going forward.

Pairing an unpredictable shock with a specific environment forms the basis classic contextual fear conditioning paradigms employed both in rodent and human studies. In humans, the conditioned context (a virtual scene) elicits activation of the anterior (uncal) hippocampus and amygdala (Morris et al., 2001, Grillon et al., 2006, Herry et al., 2007, Alvarez et al., 2008, Marschner et al., 2008, Davis et al., 2010), similar to previous results from rat studies (Herry et al., 2008) (Anagnostaras et al., 1999, Hernandez-Rabaza et al., 2008) (Kim and Fanselow, 1992, Phillips and LeDoux, 1992, Muller et al., 1997, Rosen et al., 1998) (Goosens and Maren, 2001) (Hall et al., 2001, Anglada-Figueroa and Quirk, 2005, Calandreau et al., 2005). In humans, path analysis of contextual fear conditioning data shows that rostral hippocampal activity is tightly coupled with signal in the medial, but not lateral, amygdala subregion when subjects view the aversely conditioned context (Alvarez et al., 2008), a result that coincides with the focused anatomy of the hippocampal-amygdala subcircuit (Fig. 8).

Potential importance of immature appearing neurons in the hippocampal-amygdala circuitry

A major objective of our study was to determine whether hippocampal information is able to afferently influence immature-appearing neurons in the amygdala. This arrangement would suggest unique mechanisms by which past events can impact emotional experience in the present, as in contextual fear conditioning. While immature neurons in allocortical and periallocortical cortex are described in many species (Gomez-Climent et al., 2008, Xiong et al., 2008, Luzzati et al., 2009, Zhang et al., 2009), in monkeys and humans, they are densely distributed in the amygdala (Yachnis et al., 2000, Bernier et al., 2002, Fudge, 2004, Zhang et al., 2009). The ontogeny of the immature neurons remains unknown. Two thymidine analogue studies in adult New World monkeys indicate that PSA-NCAM- and DCX-IR are co-expressed in bromodeoxyuracil-positive cells arising the subventricular zone, and sweep in a ‘temporal stream’ through the ventral amygdala, suggesting postnatal proliferation (Bernier et al., 2002, Marlatt et al., 2011). The distribution of DCX/PSA-NCAM/class III beta tubulin neurons described here matches these descriptions, although our emphasis on amygdala anatomy places the amygdala-located cells in the PL and CTA. Our study did not address the age of these immature-appearing neurons, and it remains controversial whether such cells arise *de novo* in the adult, or much earlier in life (i.e. are not newly generated, but are ‘resident’ neurons). Indeed, early anatomists noted long ago the persistence of immature-appearing cells in the region of the CTA in the brains of humans and monkeys (Johnston,

1923, Sanides and Sas, 1970). Regardless of the ontogeny of immature neurons in the primate amygdala, their co-expression of DCX and PSA-NCAM suggest that functions associated with these proteins can be influenced by excitatory hippocampal inputs.

Contextual learning is notoriously resistant to erasure, and is a factor in fear recall (LeDoux, 2000). While adaptive in dangerous environments, this phenomenon may play a role in the intransigence of human fear and anxiety disorders. If immature amygdala neurons participate in contextual fear conditioning, their enrichment in PSA-NCAM and DCX implies a special sensitivity to neural plasticity. The PSA moiety on the NCAM molecule is a necessary permissive factor for the development of long-term potentiation (LTP) in both embryonic and mature neural systems (Muller et al., 1996, Theodosis et al., 1999, Bonfanti, 2006). Relatively high constitutive expression of PSA-NCAM protein in the amygdala suggests a lower threshold for LTP development in this region. In addition, the PSA aggregate of the NCAM protein is critical for tangential migration, regulating neural differentiation, and facilitating neurite outgrowth in young neurons (Ono et al., 1994, Hu et al., 1996, Seki and Rutishauser, 1998, Petridis et al., 2004, Angata et al., 2007). PSA-NCAM may thus support maturational changes in an activity-dependent manner. Doublecortin is responsible for stabilization of microtubules (Gleeson et al., 1999, Koizumi et al., 2006b, Moores et al., 2006), and axonal extension (Friocourt et al., 2003, Bonfanti, 2006), and is required for neural migration (Koizumi et al., 2006a, Friocourt et al., 2007, Bai et al., 2008). Knock-out of DCX arrests neuronal migration into the amygdala in mouse models (Bai et al., 2008). The presence of DCX-IR, coupled with the post-mitotic, migratory appearance (Rivas and Hatten, 1995, Nam et al., 2007) of cells in the hippocampal terminal field of the amygdala raises the possibility that intracellular cascades directing migration and differentiation may also be influenced by these afferent signals. This idea, while highly speculative, does not depend on the concept of 'neurogenesis', in the sense of constant proliferation of immature neurons in the PL or CTA. Hippocampal signals may simply influence activity-dependent elaboration of neural connections and migration in a population of immature neurons present from birth, a phenomenon that is well-described in post-natal development of sensory systems in humans and other species (Katz and Shatz, 1996).

In rodents, immature neurons (identified with PSA-NCAM/DCX-IR) in the hippocampal dentate gyrus participate in contextual fear conditioning, among other functions (van Praag et al., 2002, Jessberger and Kempermann, 2003) (Esposito et al., 2005, Saxe et al., 2006, Kee et al., 2007, Tashiro et al., 2007). Here, immature neurons have a lower threshold for LTP inducibility compared to their mature counterparts (Wang et al., 2000, Schmidt-Hieber et al., 2004, Ge et al., 2007). Ablation of adult-generated new neurons in the dentate gyrus after contextual fear learning in rodents results in loss of the fear memory, suggesting that the immature neurons play a critical role in maintaining specific fear memory traces (Arruda-Carvalho et al., 2011). Contextual information flows into the dentate gyrus, then through CA3 to CA1 to induce glutamate-dependent activation in the amygdala (Maren and Fanselow, 1995, Hall et al., 2001, Herry et al., 2008, Wang et al., 2009). Our anatomic data in the monkey suggests that contextual learning may also engage immature neurons the amygdala node of this circuit in higher primates, possibly stimulating structural change as well as physiologic responses.

Located at the crossroads of circuitry involved in emotional learning and memory, the PL and contiguous CTA of the primate receive complex contextual information, including autobiographical memories, channeled from the anterior-most pole of CA1' and subiculum. This anatomic arrangement suggests that excitatory signals from the hippocampus are uniquely positioned to shape amygdala responses to memories embedded in specific temporal or spatial contexts. Our results suggest that these amygdala responses be shaped by novel mechanisms involving excitatory signaling effects on immature neurons.

Acknowledgments

This work is supported by R01 MH62391 (JF) and the Babigian Fellowship Fund (Department of Psychiatry, University of Rochester Medical Center)(DD).

ABBREVIATIONS

AAA	anterior amygdaloid area
AB	accessory basal nucleus
ABmc	accessory basal nucleus, magnocellular subdivision
ABpc	accessory basal nucleus, parvicellular subdivision
ABs	accessory basal nucleus, sulcal subdivision
AC	anterior commissure
AHA	amygdalohippocampal area
Bi	basal nucleus, intermediate subdivision
Bmc	basal nucleus, magnocellular subdivision
Bpc	basal nucleus, parvicellular subdivision
C	caudate nucleus
CA1'	uncal CA1
CA1	ventral subdivision of CA1 subfield
CeLcn	central nucleus, lateral core subdivision
CeM	central nucleus, medial subdivision
CoA	anterior cortical nucleus
CoP	posterior cortical nucleus
CTA	corticoamygdaloid transition area
Eo	entorhinal cortex, olfactory subdivision
H	hippocampus
HATA	hippocampo-amygdaloid transition area
Ic	Intercalated islands
IC	internal capsule
L	lateral nucleus
M	medial nucleus
P	putamen
PAC	periamygdaloid cortex
PL	paralaminar nucleus
ProS	prosubiculum
S	subiculum
V	ventricle

REFERENCES

- Aggleton JP. A description of the amygdalo-hippocampal interconnections in the macaque monkey. *Exp Brain Res*. 1986; 64:515–526. [PubMed: 3803489]
- Alvarez RP, Biggs A, Chen G, Pine DS, Grillon C. Contextual fear conditioning in humans: cortical-hippocampal and amygdala contributions. *J Neurosci*. 2008; 28:6211–6219. [PubMed: 18550763]
- Alvarez RP, Chen G, Bodurka J, Kaplan R, Grillon C. Phasic and sustained fear in humans elicits distinct patterns of brain activity. *Neuroimage*. 2011; 55:389–400. [PubMed: 2111828]
- Amaral, DG. Amygdalohippocampal and amygdalocortical projections in the primate brain. In: Schwarcz, R.; Yehezkel, A., editors. *Excitatory amino acids and epilepsy*. Plenum Publishing Corporation; 1986. p. 3-17.
- Amaral DG, Bassett JL. Cholinergic innervation of the monkey amygdala: An immunohistochemical analysis with antisera to choline acetyltransferase. *J Comp Neurol*. 1989; 281:337–361. [PubMed: 2703552]
- Amaral, DG.; Price, JL.; Pitkanen, A.; Carmichael, ST. *The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction*. Wiley-Liss, Inc.; 1992. Anatomical organization of the primate amygdaloid complex; p. 1-66.
- Anagnostaras SG, Maren S, Fanselow MS. Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. *J Neurosci*. 1999; 19:1106–1114. [PubMed: 9920672]
- Angata K, Huckaby V, Ranscht B, Tersikh A, Marth JD, Fukuda M. Polysialic acid-directed migration and differentiation of neural precursors are essential for mouse brain development. *Mol & Cell Biol*. 2007; 27:6659–6668. [PubMed: 17682066]
- Anglada-Figueroa D, Quirk GJ. Lesions of the basal amygdala block expression of conditioned fear but not extinction. *J Neurosci*. 2005; 25:9680–9685. [PubMed: 16237172]
- Arruda-Carvalho M, Sakaguchi M, Akers KG, Josselyn SA, Frankland PW. Posttraining ablation of adult-generated neurons degrades previously acquired memories. *J Neurosci*. 2011; 31:15113–15127. [PubMed: 22016545]
- Bai J, Ramos RL, Paramasivam M, Siddiqi F, Ackman JB, LoTurco JJ. The role of DCX and LIS1 in migration through the lateral cortical stream of developing forebrain. *Dev Neurosci*. 2008; 30:144–156. [PubMed: 18075262]
- Barbas H, Blatt GJ. Topographically specific hippocampal projections target functionally distinct prefrontal areas in the rhesus monkey. *Hippocampus*. 1995; 5:511–533. [PubMed: 8646279]
- Bartsch T, Dohring J, Rohr A, Jansen O, Deuschl G. CA1 neurons in the human hippocampus are critical for autobiographical memory, mental time travel, and autonoetic consciousness. *Proc Natl Acad Sci U S A*. 2011; 108:17562–17567. [PubMed: 21987814]
- Bedard A, Levesque M, Bernier PJ, Parent A. The rostral migratory stream in adult squirrel monkeys: contribution of new neurons to the olfactory tubercle and involvement of the antiapoptotic protein Bcl-2. *European J Neurosci*. 2002; 16:1917–1924. [PubMed: 12453055]
- Bernier PJ, Bedard A, Vinet J, Levesque M, Parent A. Newly generated neurons in the amygdala and adjoining cortex of adult primates. *Proc Natl Acad Sci USA*. 2002; 99:11464–11469. [PubMed: 12177450]
- Blatt GJ, Rosene DL. Organization of direct hippocampal efferent projections to the cerebral cortex of the rhesus monkey: projections from CA1, prosubiculum, and subiculum to the temporal lobe. *J Comp Neurol*. 1998; 392:92–114. [PubMed: 9482235]
- Bonfanti L. PSA-NCAM in mammalian structural plasticity and neurogenesis. *Prog Neurobiol*. 2006; 80:129–164. [PubMed: 17029752]
- Calandreau L, Desmedt A, Decorte L, Jaffard R. A different recruitment of the lateral and basolateral amygdala promotes contextual or elemental conditioned association in Pavlovian fear conditioning. *Learn & Mem*. 2005; 12:383–388.
- Canteras NS, Swanson LW. Projections of the ventral subiculum to the amygdala, septum, and hypothalamus: a PHAL anterograde tract-tracing study in the rat. *J Comp Neurol*. 1992; 324:180–194. [PubMed: 1430328]

- Cenquizca LA, Swanson LW. Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. *Brain Res Rev.* 2007; 56:1–26. [PubMed: 17559940]
- Crosby EC, Humphrey T. Studies of the vertebrate telencephalon: the nuclear pattern of the anterior olfactory nucleus, tuberculum olfactorium and the amygdaloid complex in adult man. *J Comp Neurol.* 1941; 74:309–347.
- Davis FC, Johnstone T, Mazzulla EC, Oler JA, Whalen PJ. Regional response differences across the human amygdaloid complex during social conditioning. *Cereb Cortex.* 2010; 20:612–621. [PubMed: 19556348]
- deCampo DM, Fudge JL. Where and what is the paralaminar nucleus? A review on a unique and frequently overlooked area of the primate amygdala. *Neurosci Biobehav Rev.* 2012; 36:520–535. [PubMed: 21906624]
- DeOlmos, JS. Amygdala. In: Paxinos, G., editor. *The Human Nervous System.* Academic Press; San Diego: 1990. p. 583-710.
- Easter SS Jr, Ross LS, Frankfurter A. Initial tract formation in the mouse brain. *J Neurosci.* 1993; 13:285–299. [PubMed: 8423474]
- Esposito MS, Piatti VC, Laplagne DA, Morgenstern NA, Ferrari CC, Pitossi FJ, Schinder AF. Neuronal differentiation in the adult hippocampus recapitulates embryonic development. *Journal of Neuroscience.* 2005; 25:10074–10086. erratum appears in *J Neurosci.* 2005 Dec 7;25(49):table of contents. [PubMed: 16267214]
- Fanselow MS, Dong HW. Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron.* 2010; 65:7–19. [PubMed: 20152109]
- Francis F, Koulakoff A, Boucher D, Chafey P, Schaar B, Vinet MC, Friocourt G, McDonnell N, Reiner O, Kahn A, McConnell SK, Berwald-Netter Y, Denoulet P, Chelly J. Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. *Neuron.* 1999; 23:247–256. [PubMed: 10399932]
- Friocourt G, Koulakoff A, Chafey P, Boucher D, Fauchereau F, Chelly J, Francis F. Doublecortin functions at the extremities of growing neuronal processes. *Cereb Cortex.* 2003; 13:620–626. [PubMed: 12764037]
- Friocourt G, Liu JS, Antypa M, Rakic S, Walsh CA, Parnavelas JG. Both doublecortin and doublecortin-like kinase play a role in cortical interneuron migration. *J Neurosci.* 2007; 27:3875–3883. [PubMed: 17409252]
- Fudge JL. Bcl-2 immunoreactive neurons are differentially distributed in subregions of the amygdala and hippocampus of the adult macaque. *Neuroscience.* 2004; 127:539–556. [PubMed: 15262342]
- Fudge JL, Breitbart MA, Danish M, Pannoni V. Insular and gustatory inputs to the caudal ventral striatum in primates. *J Comp Neurol.* 2005; 490:101–118. [PubMed: 16052493]
- Fudge JL, Tucker T. Amygdala projections to central amygdaloid nucleus subdivisions and transition zones in the primate. *Neuroscience.* 2009; 159:819–841. [PubMed: 19272304]
- Ge S, Yang CH, Hsu KS, Ming GL, Song H. A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. *Neuron.* 2007; 54:559–566. [PubMed: 17521569]
- Geneser-Jensen FA, Blackstad TW. Distribution of acetyl cholinesterase in the hippocampal region of the guinea pig. *Z Zellforsch.* 1971; 114:460–481. [PubMed: 5550728]
- Gilboa A, Winocur G, Grady CL, Hevenor SJ, Moscovitch M. Remembering our past: functional neuroanatomy of recollection of recent and very remote personal events. *Cereb Cortex.* 2004; 14:1214–1225. [PubMed: 15166099]
- Gleeson JG, Lin PT, Flanagan LA, Walsh CA. Doublecortin is a microtubule-associated protein and is expressed widely by migrating neurons. *Neuron.* 1999; 23:257–271. [PubMed: 10399933]
- Gomez-Climent MA, Castillo-Gomez E, Varea E, Guirado R, Blasco-Ibanez JM, Crespo C, Martinez-Guijarro FJ, Nacher J. A population of prenatally generated cells in the rat paleocortex maintains an immature neuronal phenotype into adulthood. *Cereb Cortex.* 2008; 18:2229–2240. [PubMed: 18245040]
- Goosens KA, Maren S. Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. *Learn & Mem.* 2001; 8:148–155.
- Grillon C, Baas JM, Cornwell B, Johnson L. Context conditioning and behavioral avoidance in a virtual reality environment: effect of predictability. *Biol Psych.* 2006; 60:752–759.

- Hall J, Thomas KL, Everitt BJ. Cellular imaging of zif268 expression in the hippocampus and amygdala during contextual and cued fear memory retrieval: selective activation of hippocampal CA1 neurons during the recall of contextual memories. *J Neurosci*. 2001; 21:2186–2193. [PubMed: 11245703]
- Hamann SB, Ely TD, Grafton ST, Kilts CD. Amygdala activity related to enhanced memory for pleasant and aversive stimuli. *Nat Neurosci*. 1999; 2:289–293. [PubMed: 10195224]
- Henke PG. Hippocampal pathway to the amygdala and stress ulcer development. *Brain Res Bull*. 1990; 25:691–695. [PubMed: 2289157]
- Hernandez-Rabaza V, Hontecillas-Prieto L, Velázquez-Sánchez C, Ferragud A, Pérez-Villaba A, Arcusa A, Barcia J, Trejo J, Canales J. The hippocampal dentate gyrus is essential for generating contextual memories of fear and drug-induced reward. *Neurobiol Learn Mem*. 2008; 90:553–559. [PubMed: 18644245]
- Herry C, Bach DR, Esposito F, Di Salle F, Perrig WJ, Scheffler K, Luthi A, Seifritz E. Processing of temporal unpredictability in human and animal amygdala. *J Neurosci*. 2007; 27:5958–5966. [PubMed: 17537966]
- Herry C, Ciocchi S, Senn V, Demmou L, Muller C, Luthi A. Switching on and off fear by distinct neuronal circuits. *Nature*. 2008; 454:600–606. [PubMed: 18615015]
- Hu H, Tomasiewicz H, Magnuson T, Rutishauser U. The role of polysialic acid in migration of olfactory bulb interneuron precursors in the subventricular zone. *Neuron*. 1996; 16:735–743. [PubMed: 8607992]
- Indovina I, Robbins TW, Nunez-Elizalde AO, Dunn BD, Bishop SJ. Fear-conditioning mechanisms associated with trait vulnerability to anxiety in humans. *Neuron*. 2011; 69:563–571. [PubMed: 21315265]
- Insausti R, Amaral DG, Cowan WM. The entorhinal cortex of the monkey: III. Subcortical afferents. *J Comp Neurol*. 1987; 264:396–408. [PubMed: 3680636]
- Insausti R, Munoz M. Cortical projections of the non-entorhinal hippocampal formation in the cynomolgus monkey (*Macaca fascicularis*). *Eur J Neurosci*. 2001; 14:435–451. [PubMed: 11553294]
- Jessberger S, Kempermann G. Adult-born hippocampal neurons mature into activity-dependent responsiveness. *Eur J Neurosci*. 2003; 18:2707–2712. [PubMed: 14656319]
- Jimenez-Castellanos J. The amygdaloid complex in monkey studies by reconstructional methods. *J Comp Neurol*. 1949; 91:507–526. illust. [PubMed: 15402613]
- Johnston JB. Further contributions to the study of the evolution of the forebrain. *J Comp Neuro*. 1923; 35:337–481.
- Katsetos CD, Frankfurter A, Christakos S, Mancall EL, Vlachos IN, Urich H. Differential localization of class III, beta-tubulin isotype and calbindin-D28k defines distinct neuronal types in the developing human cerebellar cortex. *J Neuropathol & Exp Neurol*. 1993; 52:655–666. [PubMed: 8229085]
- Katz LC, Shatz CJ. Synaptic activity and the construction of cortical circuits. *Science*. 1996; 274:1133–1138. [PubMed: 8895456]
- Kee N, Teixeira CM, Wang AH, Frankland PW. Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. *Nat Neurosci*. 2007; 10:355–362. see comment. [PubMed: 17277773]
- Kim JJ, Fanselow MS. Modality-specific retrograde amnesia of fear. *Science*. 1992; 256:675–677. [PubMed: 1585183]
- Kishi T, Tsumori T, Yokota S, Yasui Y. Topographical projection from the hippocampal formation to the amygdala: a combined anterograde and retrograde tracing study in the rat. *J Comp Neurol*. 2006; 496:349–368. [PubMed: 16566004]
- Kjelstrup KG, Tuvnes FA, Steffenach HA, Murison R, Moser EI, Moser MB. Reduced fear expression after lesions of the ventral hippocampus. *Proc Natl Acad Sci U S A*. 2002; 99:10825–10830. [PubMed: 12149439]
- Kobayashi, Y.; Amaral, DG. Chemical neuroanatomy of the hippocampal formation and the perirhinal and parahippocampal cortices. In: Bloom, FE., et al., editors. *The Primate Nervous System, Part III*. Vol. 15. Elsevier Science B.V.; Amsterdam: 1999. p. 285–402.

- Koizumi H, Higginbotham H, Poon T, Tanaka T, Brinkman BC, Gleeson JG. Doublecortin maintains bipolar shape and nuclear translocation during migration in the adult forebrain. *Nat Neurosci*. 2006a; 9:779–786. [PubMed: 16699506]
- Koizumi H, Tanaka T, Gleeson JG. Doublecortin-like kinase functions with doublecortin to mediate fiber tract decussation and neuronal migration. *Neuron*. 2006b; 49:55–66. see comment. [PubMed: 16387639]
- Kordower JH, Piecinski P, Rakic P. Neurogenesis of the amygdaloid nuclear complex in the rhesus monkey. *Brain Res Dev Brain Res*. 1992; 68:9–15.
- LaBar KS, Gatenby JC, Gore JC, LeDoux JE, Phelps EA. Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *Neuron*. 1998; 20:937–945. [PubMed: 9620698]
- Lane RD, Reiman EM, Ahern GL, Schwartz GE, Davidson RJ. Neuroanatomical correlates of happiness, sadness, and disgust. *Amer J Psych*. 1997; 154:926–933.
- Lauer EW. The nuclear pattern and fiber connections of certain basal telencephalic centers in the Macaque. *J Comp Neurol*. 1945; 82:215–254.
- LeDoux JE. Emotion circuits in the brain. *Ann Rev Neurosci*. 2000; 23:155–184. [PubMed: 10845062]
- Luzzati F, Bonfanti L, Fasolo A, Peretto P. DCX and PSA-NCAM expression identifies a population of neurons preferentially distributed in associative areas of different pallial derivatives and vertebrate species. *Cereb Cortex*. 2009; 19:1028–1041. [PubMed: 18832334]
- Maren S, Fanselow MS. Synaptic plasticity in the basolateral amygdala induced by hippocampal formation stimulation in vivo. *J Neurosci*. 1995; 15:7548–7564. [PubMed: 7472506]
- Marlatt MW, Philippens I, Manders E, Czeh B, Joels M, Krugers H, Lucassen PJ. Distinct structural plasticity in the hippocampus and amygdala of the middle-aged common marmoset (*Callithrix jacchus*). *Exp Neurol*. 2011; 230:291–301. [PubMed: 21605555]
- Marschner A, Kalisch R, Vervliet B, Vansteenwegen D, Buchel C. Dissociable roles for the hippocampus and the amygdala in human cued versus context fear conditioning. *J Neurosci*. 2008; 28:9030–9036. [PubMed: 18768697]
- Mayberg HS, Liotti M, Brannan SK, McGinnis S, Mahurin RK, Jerabek PA, Silva JA, Tekell JL, Martin CC, Lancaster JL, Fox PT. Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. *Amer J Psych*. 1999; 156:675–682.
- McDonald AJ. Cortical pathways to the mammalian amygdala. *Prog Neurobiol*. 1998; 55:257–332. [PubMed: 9643556]
- Moore CA, Perderiset M, Kappeler C, Kain S, Drummond D, Perkins SJ, Chelly J, Cross R, Houdusse A, Francis F. Distinct roles of doublecortin modulating the microtubule cytoskeleton. *EMBO Journal*. 2006; 25:4448–4457. [PubMed: 16957770]
- Morris JS, Buchel C, Dolan RJ. Parallel neural responses in amygdala subregions and sensory cortex during implicit fear conditioning. *Neuroimage*. 2001; 13:1044–1052. [PubMed: 11352610]
- Muller D, Wang C, Skibo G, Toni N, Cremer H, Calaora V, Rougon G, Kiss JZ. PSA-NCAM is required for activity-induced synaptic plasticity. *Neuron*. 1996; 17:413–422. [PubMed: 8816705]
- Muller J, Corodimas KP, Fridel Z, LeDoux JE. Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli. *Behav Neurosci*. 1997; 111:683–691. [PubMed: 9267646]
- Nam SC, Kim Y, Dryanovski D, Walker A, Goings G, Woolfrey K, Kang SS, Chu C, Chenn A, Erdelyi F, Szabo G, Hockberger P, Szele FG. Dynamic features of postnatal subventricular zone cell motility: a two-photon time-lapse study. *J Comp Neurol*. 2007; 505:190–208. [PubMed: 17853439]
- Naya Y, Suzuki WA. Integrating what and when across the primate medial temporal lobe. *Science*. 2011; 333:773–776. [PubMed: 21817056]
- Ono K, Tomasiewicz H, Magnuson T, Rutishauser U. N-CAM mutation inhibits tangential neuronal migration and is phenocopied by enzymatic removal of polysialic acid. *Neuron*. 1994; 13:595–609. [PubMed: 7917293]
- Ottersen OP. Connections of the amygdala of the rat. IV: Corticoamygdaloid and intraamygdaloid connections as studied with axonal transport of horseradish peroxidase. *J Comp Neurol*. 1982; 205:30–48. [PubMed: 7068948]

- Paz R, Gelbard-Sagiv H, Mukamel R, Harel M, Malach R, Fried I. A neural substrate in the human hippocampus for linking successive events. *Proc Natl Acad Sci U S A*. 2010; 107:6046–6051. [PubMed: 20231430]
- Petridis AK, El-Maarouf A, Rutishauser U. Polysialic acid regulates cell contact-dependent neuronal differentiation of progenitor cells from the subventricular zone. *Dev Dynamics*. 2004; 230:675–684.
- Phelps EA. Human emotion and memory: interactions of the amygdala and hippocampal complex. *Curr Opin Neurobiol*. 2004; 14:198–202. [PubMed: 15082325]
- Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Beh Neurosci*. 1992; 106:274–285.
- Pitkanen A, Pikkarainen M, Nurminen N, Ylinen A. Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. *Ann N Y Acad Sci*. 2000; 911:369–391. [PubMed: 10911886]
- Price, JL.; Russchen, FT.; Amaral, DG. The limbic region. II. The amygdaloid complex. In: Hokfelt, BT.; Swanson, LW., editors. *Handbook of Chemical Neuroanatomy*. Elsevier; Amsterdam: 1987. p. 279-381.
- Rivas RJ, Hatten ME. Motility and cytoskeletal organization of migrating cerebellar granule neurons. *J Neurosci*. 1995; 15:981–989. [PubMed: 7869123]
- Rosen JB, Fanselow MS, Young SL, Sitcoske M, Maren S. Immediate-early gene expression in the amygdala following footshock stress and contextual fear conditioning. *Brain Res*. 1998; 796:132–142. [PubMed: 9689463]
- Rosene, D.; Van Hoesen, G. The hippocampal formation of the primate brain. In: Jones, E.; Peters, A., editors. *Cerebral Cortex: Further Aspects of Cortical Function, Including Hippocampus*. Vol. VI. Plenum Press; New York: 1987. p. 345-456.
- Rosene DL, Van Hoesen GW. Hippocampal efferents reach widespread areas of cerebral cortex and amygdala in the rhesus monkey. *Science*. 1977; 198:315–317. [PubMed: 410102]
- Russchen FT. Amygdalopetal projections in the cat. I. Cortical afferent connections. A study with retrograde and anterograde tracing techniques. *J Comp Neurol*. 1982; 206:159–179. [PubMed: 7085926]
- Sanides F, Sas E. Persistence of horizontal cells of the Cajal foetal type and of the subpial granular layer in parts of the mammalian paleocortex. *Z Mikrosk Anat Forsch*. 1970; 82:570–588. [PubMed: 5501158]
- Saunders RC, Rosene DL. A comparison of the efferents of the amygdala and the hippocampal formation in the rhesus monkey: I. Convergence in the entorhinal, prorrhinal, and perirhinal cortices. *J Comp Neurol*. 1988; 271:153–184. [PubMed: 2454246]
- Saunders RC, Rosene DL, Van Hoesen GW. Comparison of the efferents of the amygdala and the hippocampal formation in the rhesus monkey: II. Reciprocal and non-reciprocal connections. *J Comp Neurol*. 1988; 271:185–207. [PubMed: 2454247]
- Saxe MD, Battaglia F, Wang JW, Malleret G, David DJ, Monckton JE, Garcia AD, Sofroniew MV, Kandel ER, Santarelli L, Hen R, Drew MR. Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. *Proc Natl Acad Sci U S A*. 2006; 103:17501–17506. [PubMed: 17088541]
- Schmidt-Hieber C, Jonas P, Bischofberger J. Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature*. 2004; 429:184–187. [PubMed: 15107864]
- Seki T, Rutishauser U. Removal of polysialic acid-neural cell adhesion molecule induces aberrant mossy fiber innervation and ectopic synaptogenesis in the hippocampus. *J Neurosci*. 1998; 18:3757–3766. [PubMed: 9570806]
- Sims KS, Williams RS. The human amygdaloid complex: a cytologic and histochemical atlas using Nissl, myelin, acetylcholinesterase and nicotinamide adenine dinucleotide phosphate diaphorase staining. *Neuroscience*. 1990; 36:449–472. [PubMed: 1699167]
- Squire LR. The hippocampus and spatial memory. *Trends Neurosci*. 1993; 16(2):56–57. [PubMed: 7680500]
- Stephan H. Evolutionary trends in limbic structures. *Neurosci Biobehav Rev*. 1983; 7:367–374. [PubMed: 6669324]

- Stephan H, Andy OJ. Quantitative comparison of the amygdala in insectivores and primates. *Acta Anat (Basel)*. 1977; 98:130–153. [PubMed: 404833]
- Tashiro A, Makino H, Gage FH. Experience-specific functional modification of the dentate gyrus through adult neurogenesis: a critical period during an immature stage. *J Neurosci*. 2007; 27:3252–3259. [PubMed: 17376985]
- Theodosis DT, Bonhomme R, Vitiello S, Rougon G, Poulain DA. Cell surface expression of polysialic acid on NCAM is a prerequisite for activity-dependent morphological neuronal and glial plasticity. *J Neurosci*. 1999; 19:10228–10236. [PubMed: 10575020]
- Ulfing N, Setzer M, Bohl J. Ontogeny of the human amygdala. *Ann N Y Acad Sci*. 2003; 985:22–33. [PubMed: 12724145]
- van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. *Nature*. 2002; 415:1030–1034. [PubMed: 11875571]
- Veening JG. Subcortical afferents of the amygdaloid complex in the rat: an HRP study. *Neurosci Lett*. 1978; 8:197–202. [PubMed: 19605158]
- Viard A, Piolino P, Desgranges B, Chetelat G, Lebreton K, Landeau B, Young A, De La Sayette V, Eustache F. Hippocampal activation for autobiographical memories over the entire lifetime in healthy aged subjects: an fMRI study. *Cereb Cortex*. 2007; 17:2453–2467. [PubMed: 17204823]
- Wang S, Scott BW, Wojtowicz JM. Heterogenous properties of dentate granule neurons in the adult rat. *J Neurobiol*. 2000; 42:248–257. [PubMed: 10640331]
- Wang SH, de Oliveira Alvares L, Nader K. Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation. *Nat Neurosci*. 2009; 12:905–912. [PubMed: 19543280]
- Weickert CS, Webster MJ, Colvin SM, Herman MM, Hyde TM, Weinberger DR, Kleinman JE. Localization of epidermal growth factor receptors and putative neuroblasts in human subependymal zone. *J Comp Neurol*. 2000; 423:359–372. [PubMed: 10870078]
- Witter MP, Amaral DG. Entorhinal cortex of the monkey: V. Projections to the dentate gyrus, hippocampus, and subicular complex. *J Comp Neurol*. 1991; 307:437–459. [PubMed: 1713237]
- Xiong K, Luo DW, Patrylo PR, Luo XG, Struble RG, Clough RW, Yan XX. Doublecortin-expressing cells are present in layer II across the adult guinea pig cerebral cortex: partial colocalization with mature interneuron markers. *Exp Neurol*. 2008; 211:271–282. [PubMed: 18378231]
- Yachnis AT, Roper SN, Love A, Fancey JT, Muir D. Bcl-2 immunoreactive cells with immature neuronal phenotype exist in the nonepileptic adult human brain. *J Neuropathol Exper Neurol*. 2000; 59:113–119. [PubMed: 10749100]
- Yukie M. Connections between the medial temporal cortex and the CA1 subfield of the hippocampal formation in the Japanese monkey (*Macaca fuscata*). *J Comp Neurol*. 2000; 423:282–298. [PubMed: 10867659]
- Zhang XM, Cai Y, Chu Y, Chen EY, Feng JC, Luo XG, Xiong K, Struble RG, Clough RW, Patrylo PR, Kordower JH, Yan XX. Doublecortin-expressing cells persist in the associative cerebral cortex and amygdala in aged nonhuman primates. *Front Neuroanat*. 2009; 3:17. [PubMed: 19862344]

Highlights

1. Immature neurons are a feature of specific amygdala nuclei in primates
2. Hippocampal inputs to the amygdala are restricted in primate compared to rat
3. Hippocampal inputs overlap immature neurons in primate amygdala
4. The hippocampal-amygdala path is unique at both systems and cellular levels in primates

Fig. 1.
Coronal sections through the amygdala (A-B) and hippocampus (C-D). Bar = 1 mm. E. Nissl stained section of the 'uncus' at higher power. Bar = 100 μ m

Fig. 2.

A-B. Schematic of the placement of retrograde injection sites in the rostrocentral (A) and caudal (B) amygdala. The region of the CTA, which includes the medial PL, medial Bpc and periamygdaloid cortex is depicted by the box. Injections are color-coded by location, see text. C. Low power photomicrograph of an injection into the medial nucleus, case J19LY. D-F and G-I. Injection sites in cases J20LY and J14FS, respectively, shown in brightfield illumination at very low power, followed by higher power images (darkfield) and AChE-stained adjacent sections. Arrows point the center of injection site. J. Schematic of injection sites in the hippocampus, with sections arranged in rostral to caudal order. K. Low power photomicrograph of an injection into CA1' in case J14CB. Bars = 1mm.

Fig. 3. Photomicrographs of labeled cells in CA1' following a caudal injection confined to ABmc. A. Low power image of the uncus hippocampus immunostained for fluorescein and counterstained with cresyl violet. Arrows point to labeled cells throughout CA1'. B. Higher power photomicrograph of HATA, with no labeled cells. C. Labeled cells in CA1', occupying layer II. Bars= 100 μ m.

Fig. 4.

The distribution pattern of labeled cells in the hippocampus following injections into the medial Bpc including CTA (A-C, J20LY), medial Bpc outside of the CTA (D-F, J15LY), and the lateral Bpc (G, J14FR). H-I. Photomicrographs showing the distribution of labeled cells in case J15LY relative to the boundaries of CA1, prosubiculum, and subiculum determined with SMI-32 immunoreactivity in an adjacent section (I.) Bars = 100 μ m

Fig. 5.

A-C. Camera lucida drawn charts of the distribution of PSA-NCAM positive cells in the amygdala of a young adult animal. D-E. Photomicrographs of DCX- and PSA-NCAM-labeled cells in the paralamina nucleus at 10x magnification. Note the small round cell bodies with thin leading processes. F-G. Higher power images of DCX- and PSA-NCAM-labeled cells (40x). Arrows point to cell bodies with high nuclear to cytoplasm ratio. Double arrows show examples of thin leading processes apposed to passing fibers, which appear to be ensheathed in DCX-positive fibers. Asterisk indicates passing fiber. Scale bars = 50 μm. H-J. Double labeling of immature neurons in the corticoamygdaloid transition area with DCX-ir (green) and PSA-NCAM-ir (red). Scale bars = 10 μm.

Fig. 6.
A-C. The distribution of anterogradely labeled fibers (black) and doublecortin-IR cells (orange) in adjacent sections through the amygdala, following a tritiated amino acid injection into CA1' in the uncus (case J4AA). Overlap occurs mainly in the medial PL and CTA.

Fig. 7.

A-C. The distribution of anterogradely labeled fibers (green) and doublecortin-IR cells (pink) in the amygdala as charted in adjacent sections following an injection into CA1', case J14CB. A lack of SMI-32-IR at the level of the injection site, in conjunction with Nissl staining, confirmed injection placement in CA1' rather than prosubiculum. Boxed areas show region of highest overlap between anterogradely labeled fibers and immature-appearing neurons. D-F. High power confocal images from the boxed area in B. Overlap of anterogradely labeled terminals (D.) in close proximity to PSA-NCAM-labeled cells (E.), suggesting putative synaptic contacts (F, arrows). Scale bars= 10 μm.

Fig. 8.

Schematics showing the organization of the hippocampal amygdala pathway. A. Inputs from CA1', prosubiculum, and HATA terminate in an overlapping pattern in the ventromedial amygdala, dissipating in more lateral and dorsal regions. The ventromedial amygdala, particularly the CTA/medial PL, receives the strongest inputs from the hippocampus. This region is endowed with immature-appearing neurons. B. Unfolded map of the hippocampus showing the continuity of the subfields in the rostrocaudal axis, and the rostrocaudal extent of amygdala projections. The dentate gyrus is not shown.

Table 1

Relative distribution of labeled cells in the hippocampus following retrograde injections in amygdala

Amygdala Injection site	Case	HATA	CA1'	ProS
Medial n.	J16FR	++	–	–
	J19LY	++	–	–
ABpc/PAC	J18FR	+	++	+
ABmc/ABpc	J21FR	+	++	+
	J20FS	–	++	++
ABmc	J12FS	–	++	+
	J8LY	–	+++	+
Bpc	J15 FS *	–	+++	+++
	J20 LY *	–	+++	+++
	J14FS *	–	+++	+++
	J15LY	–	+	+++
	J14FR	–	–	+
Bmc	J8FR	–	+	+
	J16LY	–	+	+
	J12LY	–	+	+
CeN	J1LY, J1FR, J9FS, J9LY	–	–	–
Lateral n.	J17LY	–	–	–

* Medial injections including CTA