Published in final edited form as:

Neuroscience. 2004 ; 127(2): 539–556.

Bcl-2 IMMUNOREACTIVE NEURONS ARE DIFFERENTIALLY DISTRIBUTED IN SUBREGIONS OF THE AMYGDALA AND HIPPOCAMPUS OF THE ADULT MACAQUE

J. L. Fudge^{a,b*}

^aDepartment of Psychiatry, University of Rochester Medical Center, Rochester, NY 14642, USA

^bDepartment of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642, USA

Abstract

The amygdala and hippocampus are key limbic structures of the temporal lobe, and are implicated in the pathology of mood disorders. Bcl-2, an intracellular protein, has recently been identified in the primate amygdala and hippocampus, and is now recognized as an intracellular target of mood stabilizing drugs. However, there are few data on the cellular phenotypes of bcl-2-expressing cells, or their distribution in specific subregions of the amygdala and hippocampus. We used a number of histochemical markers to define specific subregions of the primate amygdala and hippocampus, and examined phenotype-specific distributions of bcl-2 immunore-active cells within each subregion. Immature-appearing bcl-2 labeled neurons, which co-contain class III β -tubulin immuno-reactivity, are found in distinct subregions in each structure. In the amygdala, bcl-2 positive neurons with an immature morphology are densely distributed in the paralamina nucleus and intercalated cell islands, the parvocellular basal nucleus, and the ventral periamygdaloid cortex and amygdalohippocampal area. In the hippocampus, immature-appearing bcl-2-labeled cells are confined to the polymorph layer (subgranular zone), and base of the granule cell layer in the dentate gyrus. Well-differentiated neurons also express bcl-2. In the amygdala, labeled cells with mature phenotypes are concentrated in the parvocellular basal nucleus, the accessory basal nucleus, and the periamygdaloid cortex. The medial nucleus and central extended amygdala also contain many well-differentiated bcl-2 positive cells. In the hippocampus, the dentate gyrus and Ammon's horn contain many bcl-2 immunoreactive nonpyramidal cells. These are preferentially distributed in the rostral hippocampus. CA3 and CA2 contain relatively higher concentrations of bcl-2-labeled cells than CA1 and the subiculum. Bcl-2 is thus important in intrinsic circuitry of the hippocampus, and in amygdaloid subregions modulated by the hippocampus. In addition, the extended amygdala, a key amygdaloid output, is richly endowed with bcl-2 positive cells. This distribution suggests a role for bcl-2 in circuits mediating emotional learning and memory which may be targets of mood stabilizing drugs.

Keywords

paralamina nucleus; intercalated islands; dentate gyrus; class III β -tubulin; extended amygdala; mood disorder

The amygdala is involved in higher emotional processing (Ledoux, 1992; Meunier et al., 1999; Nishijo et al., 1988; Parkinson et al., 2001), and is modulated by the hippocampus which conveys sensory information associated with past emotional experiences (Mishkin, 1982;

*Correspondence to: J. L. Fudge, Department of Psychiatry, University of Rochester Medical Center, Rochester, NY 14642, USA. Tel: +1-585-273-2028; fax: +1-585-756-5334. E-mail address: julie_fudge@urmc.rochester.edu (J. L. Fudge).

Squire, 1986). The major mood disorders, which manifest as impaired emotional processing, are characterized by structural and functional abnormalities of both the amygdala and hippocampus (Altshuler et al., 2000; Drevets, 1999; Drevets et al., 2002; Sheline et al., 1996; Stoll et al., 2000; Strakowski et al., 1999). New insights into the actions of drugs used to treat mood disorders show that they stimulate intracellular cascades involved in neuronal growth and repair. It is proposed that these actions may ameliorate structural abnormalities in specific brain regions (Coyle and Duman, 2003; Holden, 2003; Manji et al., 2000). Bcl-2, an intracellular protein with antiapoptotic and trophic properties (Behl et al., 1993; Chen et al., 1997; Farlie et al., 1995; Garcia et al., 1992; Middleton et al., 1998; Suzuki and Tsutomi, 1998), has emerged as an important target of mood stabilizing drugs (Chen et al., 1999a,b), and is expressed in the amygdala and hippocampus of adult primates (Bernier et al., 2002; Bernier and Parent, 1998; Fudge, 2002; Yachnis et al., 2000).

While the amygdala and hippocampus are implicated as substrates of human mood disorders, both structures are highly complex. The amygdala is composed of multiple nuclei, and is divided into several subdivisions. The 'baso-lateral' nuclear group (BLNG) is the site where emotional meaning is attached to sensory cues through plasticity-dependent processes (Campeau and Davis, 1995; Huang and Kandel, 1998; LeDoux et al., 1990; Maren, 1999; Muller et al., 1997; Parkinson et al., 2000; Rogan et al., 1997). In contrast, the central amygdaloid nucleus receives strong inputs from the BLNG, and mediates motoric and autonomic responses to conditioned and unconditioned emotional stimuli through outputs to the brainstem and hypothalamus (Parkinson et al., 2000; Aggleton, 1985; Fudge and Haber, 2000; Gallagher et al., 1990; Holland and Gallagher, 1993; Killcross et al., 1997; Price and Amaral, 1981). The central nucleus has recently been considered part of a larger macrostructure known as the 'central extended amygdala,' which includes the central nucleus, the bed nucleus of the stria terminalis, and the cellular islands that bridge the two structures (Alheid and Heimer, 1988; deOlmos and Ingram, 1972; Heimer et al., 1999).

The hippocampus is also composed of several subregions, and mediates the storage and retrieval of contextual information associated with past emotional events (Mishkin, 1982; Squire, 1986). Complex sensory association inputs from the entorhinal cortex and the amygdala enter the hippocampus at the dentate gyrus and CA3, and are sequentially processed through CA3, CA2, and CA1 (Aggleton, 1986; Amaral and Cowan, 1980; Saunders et al., 1988; Witter and Amaral, 1991; Witter et al., 1989). The net relay and storage of this contextual information is dependent on feed-forward inhibitory processing by non-pyramidal (GABAergic) cells in each of the subfields (Buzsaki et al., 1992; Csicsvari et al., 1999). Hippocampal outputs, including those back to the amygdala, derive mainly from CA1 and the adjacent subiculum (Saunders et al., 1988; Aggleton et al., 1980).

Bcl-2 expression is potently upregulated by mood stabilizing drugs in rodent models (Chen et al., 1999a,b), raising the question of whether its trophic or neuroprotective actions mediate mood stabilizers' therapeutic properties in humans (Manji et al., 2003). An important issue, therefore, is the role of bcl-2 in functionally relevant pathways in the normal primate brain. We mapped the specific distribution of bcl-2 positive cells within discrete subregions of the amygdala and hippocampus of normal monkeys, identifying phenotypic characteristics of bcl-2 positive cells within each subregion. Given bcl-2's role in neuronal development, we also examined its co-localization with class III β -tubulin, a cytoskeletal protein associated with differentiation and migration in immature neurons (Easter et al., 1993; Luduena et al., 1988).

EXPERIMENTAL PROCEDURES

Tissue preparation

Four adult male Macaque nemestrina obtained from Three Springs Laboratories (Pekaski, PA, USA) were used in these studies (cases J1, J2, J3, and J5). The animals ranged in age from 2 years old (J1) (juvenile) to 8 years old (J3; young adult). All experiments were carried out in accordance with National Institutes of Health guidelines. Experimental design and techniques were aimed at minimizing animal use and suffering, and were reviewed and approved by the University of Rochester Committee on Animal Research. Prior to perfusion, animals were initially sedated with an intramuscular injection of ketamine (10 mg/kg), and then anesthetized with intravenous pentobarbital to achieve a deep plane of anesthesia (loss of deep tendon and corneal reflexes). The animals were then perfused through the heart with saline, followed by a 4% paraformaldehyde solution in 0.1 M phosphate buffer (pH 7.4). The brains were removed and cryo-protected in 4% paraformaldehyde overnight, and then placed in increasing gradients of sucrose (10%, 20%, and finally 30%). Serial sections of 50 μ m were cut on a freezing microtome into cryoprotectant solution. Adjacent sections were processed individually for Bcl-2 protein and class III β -tubulin immunoreactivity. Additional sections were selected for double-labeling experiments.

Subregions of the amygdala, central extended amygdala, and hippocampus

With the exception of the central nucleus, we describe the primate amygdala using established nomenclature (Amaral and Basset, 1989; Price et al., 1987). The deep amygdaloid nuclei include the lateral, basal, and accessory basal nuclei (Fig. 1A and B). The basal nucleus is subdivided into the parvocellular (Bpc), intermediate (Bi), and magnocellular (Bmc) subdivisions, which are distinguished cytoarchitecturally, and by an increasing gradient of acetylcholinesterase (AChE) staining. The accessory basal nucleus is divided into parvocellular (ABpc) magnocellular (ABmc), and sulcal (ABs) subdivisions. The ABmc and ABs subdivisions have intermediate AChE staining in contrast to the parvocellular subdivision, which has little AChE activity. The medial, or 'superficial,' amygdaloid structures include the anterior cortical nucleus, the medial nucleus, posterior cortical nucleus, and the periamygdaloid cortex (PAC). The PAC is a poorly differentiated three-layered cortex, which merges caudally with the amygdalohippo-campal area (AHA) and rostral entorhinal cortex. The AHA bridges the caudal periamygdaloid cortex, sulcal subdivision (PACs) and rostral hippocampus (Fig. 1B). The intercalated nuclei are clusters of small, heterogeneous cells which are embedded in fiber tracts surrounding the major amygdaloid nuclei. The paralaminar nucleus also contains numerous small cells, and surrounds the basal nucleus at both its rostral and caudal poles.

The central amygdaloid nucleus has traditionally been considered part of the amygdala. More recently, it has been conceptualized as the caudal pole of a larger macrostructure known as the 'central extended amygdala,' based on histochemical, cytoarchitectural, and connectational features (Heimer et al., 1999; DeOlmos, 1990; Martin et al., 1991). In this concept, the central extended amygdala includes the central amygdaloid nucleus, lateral bed nucleus of the stria terminalis, and the sublenticular extended amygdala (SLEA). We use the nomenclature previously proposed by DeOlmos (1990) in the human, and Martin et al. (1991) in the human and monkey to describe the central extended amygdala subregions. The central nucleus and lateral bed nucleus of the stria terminalis have structurally similar subdivisions: the lateral core subdivisions (CeLcn and BSTLcn, respectively); medial subdivisions (CeM and BSTLP), and transitional zones with the striatum, known as the amygdalostratial area and capsular subdivisions of the bed nucleus of the stria terminalis (Fig. 1C and D). We used neurotensin (NT) immunoreactivity to define the CeM, the SLEA, and the BSTLP (Martin et al., 1991), and calbindin (CaBP)-immunoreactivity to distinguish the globus pallidum (moderate staining) from the adjacent SLEA (low CaBP staining) (Cote et al., 1991).

The hippocampal complex includes the dentate gyrus, the hippocampal subfields, and subicular complex (the subiculum, presubiculum, and parasubiculum). We define these hippocampal subregions according to nomenclature previously put forth (Bakst and Amaral, 1984; Kobayashi Amaral, 1999). The hippocampal subregions are distinguished using established cytoarchitectural and immunohistochemical criteria, including CaBP and nonphosphorylated neurofilament protein (SMI-32) immunoreactivity (Kobayashi Amaral, 1999) and AChE reactivity (Bakst and Amaral, 1984). Representative sections through the hippocampus, processed with Cresyl Violet and SMI-32, are shown in Fig. 2A and B. The AHA in the hippocampal region is composed of small, densely packed cells. We use the term 'AHA' to describe both the hippocampal AHA and amygdaloid AHA, recognizing that there are cytoarchitectural differences between the caudal (hippocampal) and rostral (amygdaloid) regions. The dentate gyrus is composed of three distinct layers: the granule cell layer, the subjacent polymorph layer, and the molecular layer, which extends to the hippocampal fissure. The granular layer is composed of dark, tightly packed cells in Nissl-stained sections, and is AChE negative and CaBP positive. The polymorph layer contains a variety of cells and is also termed the 'subgranular zone' (SGZ). It has positive reactivity for AChE and CaBP. The molecular layer of the dentate gyrus is composed of inner (AChE-enriched) and outer (AChE-poor) layers. The hippocampal subfields also have three main layers. The pyramidal layer (layer II) is situated above the 'stratum oriens' (layer III), which contains interneurons and the basal dendrites of the overlying pyramidal layer. The molecular layer (layer I) is situated above the pyramidal cell layer (toward the hippocampal fissure), and contains the apical dendrites of the pyramidal cells. In CA2 and CA1, the molecular layer has two components: the stratum radiatum and stratum lacunosum-molecular. In primates, CA3 has a relatively dispersed pyramidal layer, which occupies, and extends out of, the hilar region. The adjacent CA2 has a densely packed band of pyramidal cells, and relatively high AChE levels. Both CA3 and CA2 contain fibers positive for non-phosphorylated neurofilament protein H (SMI-32). CA1 has the broadest, most dispersed pyramidal layer, and low SMI-32 and AChE levels. It forms an oblique border with the subiculum, creating a transition zone sometimes referred to as the 'prosubiculum.' A sharp increase in SMI-32 immunoreactivity marks this transition zone between CA1 and the subiculum. The subiculum is distinguished by high levels of nonphosphorylated neurofilament H (SMI-32) in contrast to CA1 (see Fig. 2B, arrowheads).

Immunocytochemistry

Characteristics of the bcl-2 antibody—Immunostaining for bcl-2 protein was performed using a monoclonal antibody to the human bcl-2 protein, made in the mouse (clone 124; Pezzella et al., 1990; Roche Diagnostics, Mannheim, Germany). This antibody (clone 124) has been previously characterized in human and nonhuman primates using Western blot techniques, and recognizes the 26 kD band characteristic of bcl-2 protein in several tissues, including the brain (Bernier and Parent, 1998; Pezzella et al., 1990; Vyas et al., 1997). Moreover, transfection studies in monkey (vero) cells engineered to overexpress either bcl-xl or bcl-2 protein, show that the clone 124 antibody specifically stains bcl-2, but not bcl-xl, expressing cells (Bernier and Parent, 1998).

Bcl-2—Our immunostaining procedure is a modification of the method of Bernier and Parent (1998). Dilutions curves were performed in advance to determine optimal concentrations of antibody in our material, and control sections, in which the primary antibody was omitted, were processed simultaneously. Sections were rinsed overnight in 0.1 M phosphate buffer (PB; pH 7.4) with 0.08% Triton X-100 (PB-T), and then incubated endogenous per-oxidase inhibitor (10% methanol and 3% hydrogen peroxide in PB) for 5 min. After another 90 min of rinsing in 0.08% PB-T, the tissue was preincubated in a 5% solution of normal horse serum for 30 min, and then placed in the primary antibody (see characteristics above) at 1:50 dilution for five nights (approximately 120 h). The Bcl-2 protein was visualized using the avidin-biotin

reaction (Vectastain ABC Elite kit; Vector Laboratories, Burlingame, CA, USA). Control sections in which the primary antibody was omitted did not show any labeling.

Class III β -tubulin—In brain, class III β -tubulin is a neuron-specific isotype of β -tubulin (Lee et al., 1990). Localization of class III β -tubulin antibody used in this study has been previously demonstrated in human and nonhuman primate brain (Katsetos et al., 1993; Kornack and Rakic, 1999; Weickert et al., 2000), as well as in brains of lower species (Easter et al., 1993). Sections processed for class III β -tubulin were first thoroughly rinsed in 0.1 M PB-T (pH 7.4) overnight. All tissue was then treated with endogenous peroxidase inhibitor for 5 min, rinsed for 90 min, and then preincubated in 10% normal goat serum (NGS) diluted with PB-T for 30 min. Tissue was then placed in the primary antisera: anti-class III β -tubulin (mouse monoclonal, IgG2a isotype, clone SDL.3D10; Sigma, St. Louis, MO, USA) diluted 1:15,000 in NGS–PB-T for four nights (approximately 96 h) at 4 °C. Following rinses with PB-T, the class III β -tubulin protein was visualized using the avidin–biotin reaction (Vectastain ABC kit). Staining for all reactions was enhanced by incubating the sections for 1–3 min in 3,3'-diaminobenzidine tetra-hydrochloride (DAB) and 0.01% H₂O₂ intensified with 1% cobalt chloride and 1% nickel ammonium sulfate. Sections were then mounted on gelatin-coated slides, dehydrated, and coverslipped.

CaBP, SMI-32 (neurofilament H), and NT immunostaining—Sections were thoroughly rinsed overnight, treated with endogenous peroxidase inhibitor, and preincubated in blocking serum as described above. Sections were then placed in anti-CaBP 1:10,000 (mouse monoclonal; Sigma), anti-SMI-32 1:5000 (mouse monoclonal, anti-nonphosphorylated neurofilament H; Sternberger Monoclonals, Lutherville, MD, USA), or anti-NT 1:100,000 dilution (rabbit polyclonal; Diasorin, Inc., Stillwater, MN, USA) at 4 °C for four nights. Following rinses with PB-T, the respective antigens were visualized using the avidin–biotin reaction, using nickel intensification described above. Sections were then mounted on gelatin-coated slides, dehydrated, and coverslipped.

Double-labeling for bcl-2/AChE—In sections double-labeled for Bcl-2/AChE, tissue was processed first for Bcl-2 and nickel-intensified to give a blue–black reaction product. It was then stained for AChE using a modified Geneser-Blackstad method (Geneser-Jensen and Blackstad, 1971).

Double-labeling for bcl-2/ CaBP and bcl-2/SMI-32—Tissue was first processed for bcl-2 using a one-step nickel intensification to yield a blue–black reaction product. Sections were then rinsed overnight, and secondarily processed for CaBP or SMI-32 without intensification to give a light brown reaction.

Double immunofluorescent labeling for bcl-2/class III β -tubulin

Control experiments—Dilutions for the bcl-2 antibody (Roche; mouse monoclonal IgG1 isotype, clone 124) and human class III β -tubulin antibody (Sigma; mouse monoclonal IgG2a isotype, clone SDL.3D10) and fluorescent-labeled secondary antibodies were first established individually. Since each antibody is made in the same species (mouse), and is of a different isotype, we performed two sets of control experiments to rule out the possibility of antibody cross-reactivity (false positive labeling) in the double labeling experiments. In the first control studies, sections were incubated with either bcl-2 or class III β -tubulin antibody, incubated with the appropriate fluorescent secondary antibody for 4 h, and thoroughly rinsed. Selected sections were then blocked with 10% NGS for 30 min and incubated with anti-mouse antibody tagged with a different fluorescent dye (Cascade Blue) for 4 h. After mounting and coverslipping, examination of this tissue showed no Cascade blue signal, indicating that all of the primary antibody had been recognized in the first labeling experiment. In another set of

experiments, we used isotype-specific secondary antibodies to label each primary antibody (AlexaFluor system; Molecular Probes, Eugene, OR, USA). Sections were processed for bcl-2 (IgG1 isotype) or class III β -tubulin (IgG2b isotype) using the appropriate isotype-specific secondary antibody. Control sections incubated with the secondary fluorescent antibody of the 'other' isotype (e.g. sections incubated with anti-bcl-2 [IgG1] and with goat anti-mouse IgG2a) failed to show positive labeling. Since both sets of control experiments showed no false positive cellular labeling, we used the isotype-specific technique in subsequent experiments. Control experiments in which the primary or secondary antibody was omitted also resulted in no labeling.

Double labeling protocol—Sections were thoroughly rinsed in 0.1 M PB with 0.08% Triton-X and pre-incubated in 10% NGS diluted in 0.1 M PB with 0.08% Triton-X. Sections were then incubated for 4 days in pooled primary antibodies (anti-class III β -tubulin, IgG2b isotype, 1:7500 and anti-Bcl-2, IgG1 isotype 1:10). Sections were then thoroughly rinsed and preincubated in 10% NGS diluted in 0.1 M PB with 0.08% Triton-X. Tissue was then incubated in the dark in Alexafluor 546-anti-mouse IgG1 and Alexafluor 488-IgG2b, respectively, at 1:200 for 4 h, rinsed, mounted, and coverslipped in aqueous medium (Vectashield; Vector Laboratories). Fluorescent-labeled secondary antibodies were then 'reversed' in a second set of experiments (AlexaFluor 546-antimouse IgG2b and Alexafluor-antimouse IgG1) to establish that similar results were obtained, and that 'bleed-through' was not a factor.

Analysis—Charting of bcl-2 immunoreactivity was done using single labeled sections, visualized at 40 $\times$ through the light microscope, and digitally captured using a CCD camera attached to the microscope. Image capturing and tiling of individual images was done in the program ImagePro 5.0 (Objective Imaging Ltd., Cambridge, UK). Photomontages of each section were then imported into the drawing program Canvas 5.0 (Deneba Systems, Inc., British Columbia, Canada), and labeled cells were charted in a separate layer using the macro tool. Adjacent sections stained for Cresyl Violet or AChE, or immunostained for NT, CaBP and SMI-32 according to the region of interest were similarly captured, montaged, and imported into Canvas 5.0. These sections were aligned with master charts of bcl-2 positive cells, using landmarks such as blood vessels and fiber tracts, and placed in the background. Nuclear and cortical boundaries were then drawn into a separate layer based on the distribution these markers. The distribution of bcl-2 labeled cells within specific subregions was then confirmed in double labeled sections. Double immunofluorescent-labeled sections were visualized using the appropriate fluorescent cubes using 10 $\times$, 20 $\times$, and 40 $\times$ objectives.

RESULTS

Bcl-2 in amygdaloid subregions

Amygdaloid cells containing bcl-2 fall into two general categories, consistent with previous observations (Bernier and Parent, 1998). One is an immature appearing neuronal population with intense immunostaining (Fig. 3D, smallest arrows, and 3E). The distribution of these immature appearing cells is depicted by the small black dots in Fig. 3A–C. A second population of bcl-2 positive cells has morphologic features characteristic of mature neuronal phenotypes found in various amygdaloid nuclei (Fig. 3A–C, large gray dots). Well-differentiated, labeled cells contain a relatively light, granular cytoplasmic staining compared with immature phenotypes (Fig. 3D, large and medium arrows). The relative densities of these two cell types varied according to the amygdaloid subregion as described below.

Basal nucleus/paralamina nucleus

Densely immunoreactive 'immature-appearing' Bcl-2 positive cells are highly concentrated in and around the parvicellular basal nucleus (Fig. 3A–C, small black dots). These labeled cells

are small (6–10 μm), and have round to bipolar soma with scant cytoplasm, and a thin process directed away from the ventricular zone (Alvarez-Buylla and Garcia-Verdugo, 2002; O'Rourke et al., 1995) (Fig. 3E, Fig. 4A–C). Small bcl-2 labeled cells are also oriented along fiber tracts, which encircle and penetrate the Bpc. Individual cells are frequently linked by a single process to passing fibers. Aggregates of labeled cells have the appearance of 'daisy chains' since they are attached to the same fiber bundle, with their spiraling processes creating a tubular shaft around it (Fig. 4C). High concentrations of labeled cells with this immature phenotype form a continuous stream from the medial subventricular area along the remnant of the lateral ganglionic eminence, into the paralamina nucleus and ventral Bpc (Fig. 3B, C). Labeled cells along this stream extend dorsally into the lateral and basal nuclei, and medially into the PAC and AHA.

In single-labeled adjacent sections, class III β -tubulin positive cells are also found in the paralamina nucleus and Bpc, and continue into the PACs. The majority of class III β -tubulin-containing cells are small round to bipolar cells with one or two processes (Fig. 4D). Class III β -tubulin immunoreactivity is confined to the cytoplasm and neuronal processes. Thick, class III β -tubulin-positive fiber bundles, resembling those seen in bcl-2 labeled sections, are also seen in the same vicinity. Double immunofluorescent labeling confirms that the majority of immature-appearing bcl-2 positive cells in the paralamina nucleus and adjacent Bpc co-contain class III β -tubulin (Fig. 4E, F).

Bcl-2 reaction product is also found in pyramidal and nonpyramidal (NP) cells in the basal nucleus, mainly within the parvicellular division (Fig. 3D and Fig. 4B, large and medium arrows, respectively). These cells are in close proximity to the immature appearing cells, overlapping them and extending deeper into the Bpc, particularly at caudal levels (Fig. 3A–C). There is lighter concentration of mature labeled cells in the Bi, Bmc, and rostral Bpc. In contrast to the strong, uniform immuno-reactivity of immature-appearing bcl-2 positive cells, pyramidal and NP cells contain light, granular cytoplasmic staining. Labeled pyramidal neurons are 25–30 μm ., and labeled NP cells are 15–20 μm with a round to stellate morphology (Fig. 3D, Fig. 4B, large and medium arrows, respectively). Both have a granular bcl-2 reaction product in the soma and proximal dendrites.

Intercalated islands

Immature appearing bcl-2 positive cells comprise a sub-population of the intercalated cell islands, which are interposed between the basal and accessory basal nucleus, the accessory basal nucleus and medial nucleus, and in the corticoamygdaloid transition area (see Fig. 3A–C, 5B black arrows). Intercalated islands of bcl-2-labeled cell clusters are also prominent between the lateral nucleus and external capsule, and between the lateral nucleus and amygdalostratial area. Labeled cells in all the intercalated islands are morphologically similar to the immature-appearing neurons in the paralamina nucleus, and co-contain class III β -tubulin. However, not all intercalated cells are bcl-2 positive, indicating cellular heterogeneity within the islands (Fig. 5B, white arrow).

Accessory basal nucleus

There are few to no immature-appearing Bcl-2 positive cells in the accessory basal nucleus, with the exception of the sulcal subdivision (ABs) which contains a moderate concentration. However, there is a light to moderate distribution of well-differentiated labeled pyramidal and non pyramidal cells which aggregate at periphery of the nucleus in close proximity to the intercalated islands (Fig. 3A–C). The majority of differentiated labeled cells are mainly in the ABmc and ABs. Labeled pyramidal cells are round, with several short dendrites, and light granular immunoreactivity (Fig. 5C, arrows). The ABpc contains relatively few labeled cells.

Lateral nucleus

Immature-appearing bcl-2 immunoreactive cells are found in the paralamina and intercalated islands, at the border of the ventrolateral lateral nucleus and external capsule (see above). However, there are relatively few immature-appearing labeled cells in the lateral nucleus proper. Well-differentiated labeled cells are faintly immunoreactive, and are lightly concentrated mainly in the ventrolateral lateral nucleus.

Medial nucleus

The medial nucleus contains many small well-differentiated cells with low levels of bcl-2 immunoreactivity (Fig. 3A–C, Fig. 5A). The majority of labeled cells are triangular-shaped, small cells, and are concentrated mainly in layers II and III, with relatively few labeled cells in layer I. Layer I is characterized by moderate levels of bcl-2 immunoreactivity in the neuropil. There are no bcl-2 positive cells with an immature phenotype in the medial nucleus.

Anterior and posterior cortical nucleus

There is a moderately heavy concentration of Bcl-2 labeled cells in the anterior cortical nucleus, which were similar morphologically to those in the medial nucleus. In contrast, there were few to no bcl-2 positive cells in the posterior cortical nucleus.

PAC

High concentrations of immature-appearing labeled cells which co-contain class III β -tubulin are seen in the PACs. By comparison, the concentration of these immature bcl-2 positive cells is markedly reduced in PAC3, PAC2 and PAC1. PAC3 and PACs contain relatively high concentrations of lightly stained pyramidal cells, mainly in layer II. PACs contain the highest concentration of well-differentiated bcl-2 cells, which intermingle with immature-appearing bcl-2 labeled cells, extending into this transition zone from paralamina nucleus (Fig. 3B). In contrast, the rostral PAC subdivisions (PAC1 and PAC2) contain only scattered pyramidal-like bcl-2 positive cells mainly in layer II.

AHA

High densities of immature-appearing bcl-2 labeled cells extend from the PACs into the AHA. Well-differentiated pyramidal-type labeled neurons similar to those in the PACs also continue into the rostral AHA. However, at more caudal aspects of the AHA, small cells with relatively low levels of immunoreactivity are homogeneously dispersed throughout the area. Further caudal, in the cortical bridge between the amygdala and hippocampus, the superficial cell layer contains labeled pyramidal-type neurons. These are surrounded by smaller labeled cells and neuropil in the molecular and deep cell layers. Labeled small cells continue into the uncus portion of the rostral hippocampus (see below).

Central extended amygdala

Overall, there are relatively few immature appearing bcl-2 positive cells in the extended amygdala. Labeled cells with an immature morphology are mainly seen in clusters scattered through the SLEA and in the transitional zones between the central extended amygdala and striatum (amygdalostratial area, Fig. 3C, and dorsal capsular subdivision of the bed nucleus, not shown). In contrast, well-differentiated bcl-2 immunoreactive cells are prominent in the central extended amygdala, mainly in the lateral core regions (BSTLcn and CeLcn, respectively; Fig. 6B, B' and C, C'). Unlike other well-differentiated bcl-2 positive cells in the amygdala, those in the BSTLcn and CeLcn contain a dense granular reaction product, which is distributed mainly in the cytoplasm and proximal dendrites (Fig. 6C'). The medial subdivisions (BSTLP and CeM) contain relatively lower concentrations of well-differentiated

labeled cells, with lower bcl-2 levels. Labeled cells in the BSTLP and CeM are heterogeneous and oriented along the fibers passing through the region, extending into the SLEA. The SLEA, amygdalostriatal area, and capsular subdivision of the bed nucleus contain a moderate distribution of lightly labeled bcl-2 positive cells resembling medium spiny neurons (Fig. 3C; see also Fudge and Haber, 2002).

Bcl-2 in hippocampal subregions

Bcl-2 immunoreactivity characterizes both immature-appearing and mature cells in the hippocampus. Immature appearing cells containing bcl-2 are confined to dentate gyrus, mainly in the polymorph layer (SGZ) and base of the granule cell layer. In contrast, bcl-2 immunoreactive cells with mature phenotypes are much more broadly distributed throughout the hippocampus, and are mainly NP neurons. The relative distribution of these phenotypes in specific subregions is described below.

Dentate gyrus (Fig. 7 and Fig. 8)—The density of labeled cells is highest in the rostral dentate gyrus (Fig. 7A), but can be seen throughout its entire rostrocaudal extent. Heterogeneous bcl-2 labeled cells are highly concentrated in the polymorph layer, in the SGZ (Fig. 7B–D). Labeled fusiform cells in the polymorph layer send processes toward the molecular layer. Many bcl-2 positive cells in the polymorph layer co-contain class III β -tubulin; however, occasional bcl-2 positive cells are class III β -tubulin negative (Fig. 8A, B). While there are few labeled cells within the granule cell layer proper, the deepest granule cells contain bcl-2 and class III β -tubulin immunoreactivity (Fig. 7C, Fig. 8A, B). Bcl-2 immunoreactivity in the granule layer is also occasionally seen in immature-appearing, elongated cells which co-contain class III β -tubulin. The morphology of these elongated cells resembles that of migrating neurons (Fig. 8C, D; Nowakowski and Rakic, 1981; O'Rourke et al., 1992). The molecular layer of the dentate gyrus contains relatively mature appearing bcl-2 positive cells, with a stellate morphology. There is a relatively higher distribution of labeled cells in the inner molecular layer compared with the outer molecular layer (Fig. 7B, C).

AHA (Fig. 9A)—The hippocampal portion of the AHA is caudal to the pyramidal-like cells in the amygdaloid AHA, and makes up the medial part of the hippocampal uncus. The hippocampal AHA contains many small, moderate to lightly labeled bcl-2 positive cells, embedded in bcl-2 positive neuropil.

Hippocampal subfields (Fig. 9A–C)—Bcl-2 labeled cells are found along the rostrocaudal extent of the hippocampal subfields, with generally higher concentrations of labeled cells at rostral levels. Overall, CA3 contains the highest concentration of bcl-2 positive cells, with a more moderate distribution in CA2 and CA1. The majority of bcl-2 labeled cells in CA3 are NP cells with a stellate morphology (Fig. 9D). In CA2 and CA1, there are relatively few labeled cells in the pyramidal layers, with the majority of labeled cells concentrated in the stratum oriens and inner molecular layer (Fig. 9F). In the pyramidal layer, scattered labeled NP cells are frequently in close apposition to pyramidal cells. Labeled cells in the stratum oriens are typically multipolar cells with thin, varicose dendrites. The molecular layer contains a somewhat more dispersed distribution of labeled cells compared with the stratum oriens. Bcl-2-labeled cells in the molecular layer are mainly round to stellate; however, labeled fusiform and diamond-shaped cells are also seen. Labeled cells are relatively more concentrated in the stratum radiatum than in the stratum molecular.

Subicular complex—Bcl-2 labeled cells are relatively sparse in the subicular complex compared with other hippocampal subregions. There is a sharp decline in the density of labeled cells in the transitional zone with the subiculum, compared with concentrations in CA1. The molecular layer, pyramidal layer, and stratum oriens of the subiculum proper contain a very

light distribution of bcl-2-labeled cells. The adjacent presubiculum and parasubiculum have a more moderate distribution of labeled cells than the subiculum, mainly in the superficial limit of layer II. Layer I and deep layer II of the presubiculum and parasubiculum contain a relatively light distribution of labeled cells. Most labeled cells in both regions were generally small round to oval cells, with occasional medium size fusiform labeled cells.

Summary

Amygdala (Table 1)—Across cases, the distribution of immature appearing bcl-2 positive cells was high in specific subzones of the amygdala: the parvicellular subdivision of the basal nucleus, the paralamina nucleus, the intercalated cell islands, the ventral subdivisions of the PAC and the AHA. There was no detectable difference in the relative concentrations of immature appearing bcl-2-labeled cells in these subregions across animals. Labeled cells with a mature phenotype were also concentrated in parvicellular basal nucleus, the ventral PAC and AHA, where their distribution overlapped that of immature-appearing labeled cells. In contrast, high concentrations of mature appearing labeled cells in the medial nucleus and lateral core of the central nucleus were not associated with bcl-2-labeled cells of the immature phenotype. The relative concentration of mature labeled cells within each subregion was also consistent across all four animals.

Hippocampus (Table 2)—The hippocampus contains a broad distribution of bcl-2 positive cells which are mainly of a mature, NP phenotype. Labeled NP cells are relatively more concentrated in the rostral hippocampus compared with central and caudal levels. Within this rostrocaudal distribution, the dentate gyrus and CA3/CA2 contain relatively higher concentrations of bcl-2-labeled cells than the CA1 subfield. The subicular complex contains relatively few bcl-2-labeled cells. The relative concentration of labeled cells within each subregion was similar across all animals. Immature-appearing labeled cells in each case are restricted to the polymorph zone and deep granule cell layer of the dentate gyrus.

DISCUSSION

Bcl-2's dual role

The present results show that both immature-appearing and well-differentiated neurons of the amygdala and hippocampus contain bcl-2. The localization of bcl-2 in both phenotypes is consistent with bcl-2's dual functions in the CNS. Bcl-2 is found on several intracellular membranes including the nuclear envelope, the mitochondrial membrane, and endoplasmic reticulum (Baffy et al., 1993; Lam et al., 1994; Reed, 1994) and plays a well-established role in mitigating against apoptosis in the face of glutamate and other stressors (Behl et al., 1993; Farlie et al., 1995; Jacobson and Raff, 1995; Lawrence et al., 1996; Yang et al., 1998; Zhong et al., 1993). Along with other homologous proteins, bcl-2 influences membrane permeability to control calcium influx, thereby protecting organelles from the toxic effects of excessive calcium associated with high glutamate (Reed, 1997). This action of bcl-2 may be important in the survival of both immature-appearing and mature phenotypes.

Bcl-2 also has direct, independent actions on neuronal differentiation and axonal outgrowth (Chen et al., 1997; Middleton et al., 1998; Suzuki and Tsutomi, 1998; Hanada et al., 1993; Oh et al., 1996; Zhang et al., 1996). In this study, immature-appearing bcl-2 positive cells are preferentially distributed in putative sites of neural proliferation (i.e. the subventricular zone and SGZ), suggesting a trophic function in these cells. Manipulating bcl-2 expression in neural progenitors regulates their rate of differentiation, including neurite extension and expression of neuron-specific markers (Middleton et al., 1998; Suzuki and Tsutomi, 1998; Zhang et al., 1996). Studies in bcl-2 deficient embryos show that bcl-2's influence on cell differentiation occurs after expression of the neural phenotype (Middleton et al., 1998). This is consistent with

our finding that many bcl-2-positive immature appearing cells co-contain class III β -tubulin, indicating that bcl-2 is highly expressed in committed neurons in both adult and embryonic brain (see also Yachnis et al., 2000).

Distribution of bcl-2 positive cells: immature phenotypes

Comparison with previous studies—Bcl-2-labeled cells have been previously recognized in the primate amygdala and hippocampus (Bernier et al., 2002; Bernier and Parent, 1998; Yachnis et al., 1997, 2000; Merry et al., 1994). While Merry et al. (1995) failed to detect a bcl-2 positive neuronal phenotype in adult rhesus monkeys (Merry et al., 1994), this departs from subsequent studies, including the present results (Bernier et al., 2002; Bernier and Parent, 1998; Yachnis et al., 1997, 2000). The inability to detect neuronal bcl-2 immunoreactivity in that study may have been due to the relatively old age of the animals (16–18 years) and/or the use of neurofilament H to define the neuronal phenotype. Subsequent primate studies have shown a significant decrease in bcl-2 positive neurons in senescence (Bernier and Parent, 1998; Yachnis et al., 2000). Other studies indicate that class III β -tubulin, but not neurofilament H, is co-localized in immature appearing bcl-2 positive cells (Yachnis et al., 1997; also present results, not shown). Taken together, these studies indicate that in young adult animals, bcl-2 immunoreactivity in immature appearing cells is associated with a neuronal phenotype.

Specific distribution in the amygdala—Immature appearing cells with bcl-2 immunoreactivity are highly concentrated in the ventral amygdala, consistent with previous reports (Yachnis et al., 2000; Bernier and Parent, 1998; Bernier et al., 2000). They are specifically concentrated within the paralaminar nucleus and intercalated islands, the Bpc, caudal PAC and AHA. The presence of morphologically similar cells within these contiguous structures has been noted across several species (Crosby and Humphrey, 1941; Humphrey, 1936; Johnston, 1923; Millhouse, 1986). In older anatomical studies, the ‘massa intercalata’ were hypothesized to be immature, possibly migratory, cells, which became ‘trapped’ in fiber bundles during development (Johnston, 1923; Humphrey, 1936). Millhouse’s classic study of the intercalated islands in the rodent (which included the paralaminar nucleus) showed that the majority of these cells were 7–12 μ m with round nuclei and scant cytoplasm, similar to the size and morphology of immature-appearing bcl-2 positive cells in the present study. Our results support the idea that paralaminar/intercalated cells contain a unique subpopulation of immature appearing neurons, based on morphologic and histochemical markers. Their unique arrangement in ‘daisy chains’ along passing fibers adds support to earlier speculations about their migratory nature (Millhouse, 1986; Humphrey, 1936). While the question of neural migration cannot be resolved in our material, the arrangement of immature-appearing bcl-2 positive cells along fibers is reminiscent of the appearance of migratory neurons that move along glial fibers (Rakic, 1971; Rivas and Hatten, 1995). Migratory cells, like the bcl-2 positive cells in the present results, have small and round to bipolar soma which are distal to thin leading processes. These processes contain β -tubulin, and spiral around passing fibers (Rivas and Hatten, 1995), similar to the immature appearing bcl-2/class III β -tubulin labeled cells in the present study. We also found that these immature appearing labeled cells extend into cortico-amygdaloid ‘transition’ zones in the primate (i.e. the ventral Bpc, PACs and AHA) indicating that these cortical regions share a relatively undifferentiated cellular component, and may have a relatively high capacity for plastic change.

Specific distribution in the hippocampus—Immature appearing bcl-2 positive cells are restricted to the polymorph layer (SGZ), and deep granule cell layer of the dentate gyrus. The SGZ in adult primates is a source of newly dividing cells, with 10 to 20% estimated to be immunoreactive for class III β -tubulin (Kornack and Rakic, 1999; Eriksson et al., 1998; Gould et al., 2000). Our results indicate that many immature appearing class III β -tubulin positive cells in the SGZ co-contain bcl-2. Some double-labeled neurons have elongated cell bodies

with truncated leading and trailing processes typical of migrating hippocampal cells (Nowakowski and Rakic, 1981; Eckenhoff and Rakic, 1984), suggesting that in these cells, bcl-2 may play a role in neural differentiation or axonal extension.

Distribution of bcl-2 immunoreactive cells: mature phenotypes—Bcl-2 is expressed at generally lower levels in well-differentiated neurons of both the amygdala and hippocampus. This distribution is consistent with bcl-2's role outside neural differentiation, perhaps protecting more mature cells neurons from excitotoxic injury. In the amygdala, well-differentiated bcl-2 positive cells are densely distributed in close proximity to immature appearing cells in the Bpc, ABs, caudal PAC, and AHA. The medial nucleus and central extended amygdala also contain mature bcl-2 positive cells, which are not associated with immature phenotypes. Abundant bcl-2 immunoreactivity in cells of the lateral core subdivisions of the central extended amygdala stands in contrast to the generally low levels of immunoreactivity in mature cells throughout the rest of the amygdala and hippocampus. This may reflect a high level of glutamatergic stress within these extended amygdaloid subdivisions, which receive inputs from all the other amygdaloid nuclei (Aggleton, 1985; Krettek and Price, 1978).

Bcl-2 is also widely expressed in mature NP cells of the dentate gyrus and Ammon's horn (CA3–CA1), suggesting a high degree of neuroprotection in this pathway. The rostral dentate gyrus and CA3, which are major input regions for excitatory inputs from the perforant path and amygdala (Witter and Amaral, 1991; Amaral, 1986), contain the highest concentrations of bcl-2 immunoreactive cells. CA3 is distinguished by having relatively greater numbers of bcl-2 positive NP cells dispersed throughout its pyramidal layer. In CA2 and CA1, bcl-2 immunoreactive NP cells are mainly concentrated in the stratum oriens and stratum radiatum, which in turn receive the bulk of excitatory inputs from CA3 (Ishizuka et al., 1990; Rosene and Van Hoesen, 1977; Swanson and Cowan, 1977). In the hippocampus, the NP cells synchronize the activity of multiple pyramidal cells through feed-forward inhibition (Csicsvari et al., 1999; Buzsaki and Eidelberg, 1982; Cobb et al., 1995; Buhl et al., 1994), thus controlling how excitatory inputs conveying contextual information are encoded, stored, and retrieved (Klausberger et al., 2003; Moser, 2003). This preferential distribution of bcl-2 in the NP cells indicates that it is an important defense in this modulatory system. The declining concentration of bcl-2 immunoreactive cells from CA3 to CA1, and in the subiculum, may indicate less of a role for bcl-2's protective properties once sensory information is maximally processed by feed-forward inhibitory circuits. However, under pathologic conditions such as hypoxia and seizure, the relative lack of bcl-2 in CA1 and the subiculum may underlie the unique vulnerability of these areas to injury (Bachevalier and Meunier, 1996; Tasker, 2001; Zola-Morgan et al., 1986, 1992).

Functional implications—Bcl-2 is upregulated by mood stabilizing drugs; however, its specific distribution in amygdaloid and hippocampal circuitry of normal primates has not been previously detailed. We found that bcl-2 is distributed in discrete subregions of the amygdala and hippocampus that are interconnected, and together participate in emotional processing (Fig. 10). The amygdala codes the emotional salience of sensory cues, and is an important target of the contextual inputs from the hippocampus (Fanselow and Kim, 1994; Maren and Fanselow, 1995). In primates, hippocampal afferents terminate specifically in the parvocellular basal nucleus, ventral PAC, and paralamina nucleus (Saunders et al., 1988; Rosene and Van Hoesen, 1977; Aggleton, 1986), which contain relatively high concentration of bcl-2 positive cells. This distribution points to a role for bcl-2 in amygdaloid areas that are modulated by contextual inputs. Increasing evidence indicates that contextual learning is a glutamate-dependent event that involves plastic changes in the amygdala (Maren and Fanselow, 1995; Hall et al., 2001; Schafe and LeDoux, 2000). Therefore, high concentrations of bcl-2-containing cells in these zones may contribute to these plastic changes. While the function of

immature-appearing cells in the amygdala is not yet known, one possibility is that they are a cellular reserve to replace mature neurons lost to high glutamate demands in this critical brain region. Bcl-2 may also protect both mature and immature neurons in this critical subregion from excitotoxicity.

Circuitry in which emotional responses are modulated by contextual sensory information may be especially relevant in human mood disorders. For example, in severe depression amygdaloid responses to emotionally relevant sensory stimuli are abnormal (Sheline et al., 2001; Thomas et al., 2001; Drevets, 2001), and drug therapy normalizes amygdaloid responsivity (Sheline et al., 2001). The distribution of bcl-2 in circuits mediating contextually modulated emotional responses in the primate suggests a role for its neuroprotective and/or neurotrophic properties in maintaining this normal emotional function. Furthermore, drugs that stimulate bcl-2 expression may specifically enhance neuronal function in this emotional circuitry.

REFERENCES

- Aggleton JP. A description of intra-amygdaloid connections in old world monkeys. *Exp Brain Res* 1985;57:390–399. [PubMed: 3972039]
- Aggleton JP. A description of the amygdalo-hippocampal interconnections in the macaque monkey. *Exp Brain Res* 1986;64:515–526. [PubMed: 3803489]
- Aggleton JP, Burton MJ, Passingham RE. Cortical and subcortical afferents to the amygdala of the rhesus monkey (*Macaca mulatta*). *Brain Res* 1980;190:347–368. [PubMed: 6768425]
- Alheid GF, Heimer L. New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. *Neuroscience* 1988;27:1–39. [PubMed: 3059226]
- Altshuler LL, Bartzokis G, Grieder T, Curran J, Jimenez T, Leight K, Wilkins J, Gerner R, Mintz J. An MRI study of temporal lobe structures in men with bipolar disorder or schizophrenia. *Biol Psychiatry* 2000;48:147–162. [PubMed: 10903411]
- Alvarez-Buylla A, Garcia-Verdugo JM. Neurogenesis in adult subventricular zone. *J Neurosci* 2002;22:629–634. [PubMed: 11826091]
- Amaral, DG. Amygdalohippocampal and amygdalocortical projections in the primate brain. In: Schwarcz, R.; Yehezkel, A., editors. *Excitatory amino acids and epilepsy*. New York, NY: 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, Cowan WM. Subcortical afferents to the hippocampal formation in the monkey. *J Comp Neurol* 1980;189:573–591. [PubMed: 6769979]
- Bachevalier J, Meunier M. Cerebral ischemia: are the memory deficits associated with hippocampal cell loss? *Hippocampus* 1996;6:553–560. [PubMed: 8953308]
- Baffy G, Miyashita T, Williamson JR, Reed JC. Apoptosis induced by withdrawal of interleukin-3 (IL-3) from an IL-3-dependent hematopoietic cell line is associated with repartitioning of intracellular calcium and is blocked by enforced Bcl-2 oncoprotein production. *J Biol Chem* 1993;268:6511–6519. [PubMed: 8454620]
- Bakst I, Amaral DG. The distribution of acetylcholinesterase in the hippocampal formation of the monkey. *J Comp Neurol* 1984;225:344–371. [PubMed: 6725649]
- Behl C, Hovey L 3rd, Krajewski S, Schubert D, Reed JC. BCL-2 prevents killing of neuronal cells by glutamate but not by amyloid beta protein. *Biochem Biophys Res Commun* 1993;197:949–956. [PubMed: 7903532]
- 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]
- Bernier PJ, Parent A. Bcl-2 protein as a marker of neuronal immaturity in postnatal primate brain. *J Neurosci* 1998;18:2486–2497. [PubMed: 9502809]

- Bernier PJ, Vinet J, Cossette M, Parent A. Characterization of the subventricular zone of the adult human brain: evidence for the involvement of Bcl-2. *Neurosci Res* 2000;67–78. [PubMed: 10802345]
- Buhl EH, Halasy K, Somogyi P. Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites [erratum appears in *Nature* 1997 387:106]. *Nature* 1994;368:823–828. [PubMed: 8159242]
- Buzsaki G, Eidelberg E. Direct afferent excitation and long-term potentiation of hippocampal interneurons. *J Neurophysiol* 1982;48:597–607. [PubMed: 6290613]
- Buzsaki G, Horvath Z, Urioste R, Hetke J, Wise K. High-frequency network oscillation in the hippocampus. *Science* 1992;256:1025–1027. [PubMed: 1589772]
- Campeau S, Davis M. Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *J Neurosci* 1995;15:2301–2311. [PubMed: 7891168]
- Chen DF, Schneider GE, Martinou JC, Tonegawa S. Bcl-2 promotes regeneration of severed axons in mammalian CNS. *Nature* 1997;385:434–439. [PubMed: 9009190]
- Chen G, Hasanat KA, Bechuk JM, Moore GJ, Glitz D, Manji HK. Regulation of signal transduction pathways and gene expression by mood stabilizers and antidepressants. *Psychosom Med* 1999a; 61:599–617. [PubMed: 10511011]
- Chen G, Zeng WZ, Yuan PX, Huang LD, Jiang YM, Zhao ZH, Manji HK. The mood-stabilizing agents lithium and valproate robustly increase the levels of the neuroprotective protein bcl-2 in the CNS. *J Neurochem* 1999b;72:879–882. [PubMed: 9930766]
- Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyi P. Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* 1995;378:75–78. [PubMed: 7477292]
- Cote P-Y, Sadikot AF, Parent A. Complementary distribution of calbindin D-28k and parvalbumin in the basal forebrain and midbrain of the squirrel monkey. *Eur J Neurosci* 1991;3:1316–1329. [PubMed: 12106229]
- Coyle JT, Duman RS. Finding the intracellular signaling pathways affected by mood disorder treatments. *Neuron* 2003;38:157–160. [PubMed: 12718851]
- 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.
- Csicsvari J, Hirase H, Czurko A, Mamiya A, Buzsaki G. Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving Rat. *J Neurosci* 1999;19:274–287. [PubMed: 9870957]
- DeOlmos, JS. Amygdala. In: Paxinos, G., editor. *The human nervous system*. San Diego: Academic Press; 1990. p. 583–710.
- deOlmos JS, Ingram WR. The projection field of the stria terminalis in the rat brain. *J Comp Neurol* 1972;146:303–333. [PubMed: 5086675]
- Drevets WC. Prefrontal cortical-amygdalar metabolism in major depression. *Ann N Y Acad Sci* 1999;877:614–637. [PubMed: 10415674]
- Drevets WC. Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. *Curr Opin Neurobiol* 2001;11:240–249. [PubMed: 11301246]
- Drevets WC, Price JL, Bardgett ME, Reich T, Todd RD, Raichle ME. Glucose metabolism in the amygdala in depression: relationship to diagnostic subtype and plasma cortisol levels. *Pharmacol Biochem Behav* 2002;71:431–447. [PubMed: 11830178]
- Easter SS Jr, Ross LS, Frankfurter A. Initial tract formation in the mouse brain. *J Neurosci* 1993;13:285–299. [PubMed: 8423474]
- Eckenhoff MF, Rakic P. Radial organization of the hippocampal dentate gyrus: a Golgi, ultrastructural, and immunocytochemical analysis in the developing rhesus monkey. *J Comp Neurol* 1984;223:1–21. [PubMed: 6707248]
- Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH. Neurogenesis in the adult human hippocampus. *Nat Med* 1998;4:1313–1317. [PubMed: 9809557]
- Fanselow MS, Kim JJ. Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid to the basolateral amygdala. *Behav Neurosci* 1994;108:210–212. [PubMed: 7910746]

- Farlie PG, Dringen R, Rees SM, Kannourakis G, Bernard O. Bcl-2 transgene expression can protect neurons against developmental and induced cell death. *Proc Natl Acad Sci USA* 1995;92:4397–4401. [PubMed: 7753817]
- Fudge, J. Program No. 305.17, 2002 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience; 2002. Bcl-2 expression in specific subregions of the macaque temporal lobe.
- Fudge J, Haber S. Defining the caudoventral striatum in primates: cytoarchitectural and histochemical features. *J Neurosci* 2002;22:10078–10082. [PubMed: 12451107]
- Fudge JL, Haber SN. The central nucleus of the amygdala projection to dopamine subpopulations in primates. *Neuroscience* 2000;97:479–494. [PubMed: 10828531]
- Gallagher M, Graham PW, Holland PC. The amygdala central nucleus and appetitive Pavlovian conditioning: lesions impair one class of conditioned behavior. *J Neurosci* 1990;10:1906–1911. [PubMed: 2355257]
- Garcia I, Martinou I, Tsujimoto Y, Martinou J-C. Prevention of programmed cell death of sympathetic neurons by the bcl-2 protooncogene. *Science* 1992;258:302–304. [PubMed: 1411528]
- Geneser-Jensen FA, Blackstad TW. Distribution of acetyl cholinesterase in the hippocampal region of the guinea pig. *Z Zellforsch* 1971;114:460–481.
- Gould E, Tanapat P, Rydel T, Hastings N. Regulation of hippocampal neurogenesis in adulthood. *Biol Psychiatry* 2000;48:715–720. [PubMed: 11063968]
- 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]
- Hanada M, Krajewski S, Tanaka S, Cazals-Hatem D, Spengler BA, Ross RA, Biedler JL, Reed JC. Regulation of Bcl-2 oncoprotein levels with differentiation of human neuroblastoma cells. *Cancer Res* 1993;53:4978–4986. [PubMed: 8402688]
- Heimer, L.; De Olmos, JS.; Alheid, GF.; Person, J.; Sakamoto, N.; Shinoda, K.; Marksteiner, J.; Switzer, RC. The human basal forebrain: part II. In: Bloom, FE.; Bjorkland, A.; Hokfelt, T., editors. *Handbook of chemical neuroanatomy, Vol. 15: the primate nervous system, part III*. Amsterdam: Elsevier; 1999. p. 57-226.
- Holden C. Future brightening for depression treatments. *Science* 2003;302:810–813. [PubMed: 14593164]
- Holland PC, Gallagher M. Amygdala central nucleus lesions disrupt increments, but not decrements, in conditioned stimulus processing. *Behav Neurosci* 1993;107:246–253. [PubMed: 8484890]
- Huang YY, Kandel ER. Postsynaptic induction and PKA-dependent expression of LTP in the lateral amygdala. *Neuron* 1998;21:169–178. [PubMed: 9697861]
- Humphrey T. The telencephalon of the bat. *J Comp Neurol* 1936;65:603–711.
- Ishizuka N, Weber J, Amaral DG. Organization of intrahippocampal projections originating from CA3 pyramidal cells in the rat. *J Comp Neurol* 1990;295:580–623. [PubMed: 2358523]
- Jacobson MD, Raff MC. Programmed cell death and Bcl-2 protection in very low oxygen. *Nature* 1995;374:814–816. [PubMed: 7536895]
- Johnston JB. Further contributions to the study of the evolution of the forebrain. *J Comp Neurol* 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]
- Killcross S, Robbins TW, Everitt BJ. Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. *Nature* 1997;388:377–380. [PubMed: 9237754]
- Klausberger T, Magill PJ, Marton LF, Roberts JD, Cobden PM, Buzsaki G, Somogyi P. Brain-state- and cell-type-specific firing of hippocampal interneurons in vivo. *Nature* 2003;421:844–848. [PubMed: 12594513]
- Kobayashi, Y.; Amaral, DG. Chemical neuroanatomy of the hippocampal formation and the perirhinal and parahippocampal cortices. In: Bloom, FE.; Bjorklund, A.; Hokfelt, T., editors. *The primate nervous system, handbook of chemical neuroanatomy, part III, Vol. 15*. Amsterdam: Elsevier Science B.V.; 1999. p. 285-402.

- Kornack DR, Rakic P. Continuation of neurogenesis in the hippocampus of the adult macaque monkey. *Proc Natl Acad Sci USA* 1999;96:5768–5773. [PubMed: 10318959]
- Krettek JE, Price JL. Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. *J Comp Neurol* 1978;178:225–254. [PubMed: 627625]
- Lam M, Dubyak G, Chen L, Nunez G, Miesfeld RL, Distelhorst CW. Evidence that BCL-2 represses apoptosis by regulating endoplasmic reticulum-associated Ca^{2+} fluxes. *Proc Natl Acad Sci USA* 1994;91:6569–6573. [PubMed: 8022822]
- Lawrence MS, Ho DY, Sun GH, Steinberg GK, Sapolsky RM. Overexpression of Bcl-2 with herpes simplex virus vectors protects CNS neurons against neurological insults in vitro and in vivo. *J Neurosci* 1996;16:486–496. [PubMed: 8551333]
- Ledoux JE. The amygdala: neurobiological aspects of emotion, memory and mental dysfunction. New York, NY: Wiley-Liss, Inc.; 1992. *Emotion and the amygdala*; p. 339–351.
- LeDoux JE, Cicchetti P, Xagoraris A, Romanski LM. The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. *J Neurosci* 1990;10:1062–1069. [PubMed: 2329367]
- Lee MK, Rebhun LI, Frankfurter A. Posttranslational modification of class III beta-tubulin. *Proc Natl Acad Sci USA* 1990;87:7195–7199. [PubMed: 2402501]
- Luduena RF, Zimmermann HP, Little M. Identification of the phosphorylated beta-tubulin isotype in differentiated neuroblastoma cells. *FEBS Lett* 1988;230:142–146. [PubMed: 3350148]
- Manji HK, Moore GJ, Chen G. Clinical and preclinical evidence for the neurotrophic effects of mood stabilizers: implications for the pathophysiology and treatment of manic-depressive illness. *Biol Psychiatry* 2000;48:740–754. [PubMed: 11063971]
- Manji HK, Quiroz JA, Sporn J, Payne JL, Denicoff K, NAG, Zarate CA Jr, Charney DS. Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. *Biol Psychiatry* 2003;53:707–742. [PubMed: 12706957]
- Maren S. Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. *J Neurosci* 1999;19:8696–8703. [PubMed: 10493770]
- 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]
- Martin LJ, Powers RE, Dellovade TL, Price DL. The bed nucleus-amygdala continuum in human and monkey. *J Comp Neurol* 1991;309:445–485. [PubMed: 1918444]
- Merry DE, Veis DJ, Hickey WF, Korsmeyer SJ. bcl-2 protein expression is widespread in the developing nervous system and retained in the adult PNS. *Integrat Ann Indexes* 1994;120:301–311.
- Meunier M, Bachevalier J, Murray EA, Malkova L, Mishkin M. Effects of aspiration versus neurotoxic lesions of the amygdala on emotional responses in monkeys. *Eur J Neurosci* 1999;11:4403–4418. [PubMed: 10594668]
- Middleton G, Pinon LG, Wyatt S, Davies AM. Bcl-2 accelerates the maturation of early sensory neurons. *J Neurosci* 1998;18:3344–3350. [PubMed: 9547242]
- Millhouse OE. The intercalated cells of the amygdala. *J Comp Neurol* 1986;247:246–271. [PubMed: 2424941]
- Mishkin M. A memory system in the monkey. *Phil Trans R Soc Lond B Biol Sci* 1982;298:85–95.
- Moser EI. Neurobiology: interneurons take charge. *Nature* 2003;421:797–799. [PubMed: 12594494]
- 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]
- Nishijo H, Ono T, Nishino H. Single neuron responses in amygdala of alert monkey during complex sensory stimulation with affective significance. *J Neurosci* 1988;8:3570–3583. [PubMed: 3193171]
- Nowakowski RS, Rakic P. The site of origin and route and rate of migration of neurons to the hippocampal region of the rhesus monkey. *J Comp Neurol* 1981;196:129–154. [PubMed: 7204662]
- O'Rourke NA, Dailey ME, Smith SJ, McConnell SK. Diverse migratory pathways in the developing cerebral cortex. *Science* 1992;258:299–302. [PubMed: 1411527]
- O'Rourke NA, Sullivan DP, Kaznowski CE, Jacobs AA, McConnell SK. Tangential migration of neurons in the developing cerebral cortex. *Development* 1995;121:2165–2176. [PubMed: 7635060]

- Oh YJ, Swarzenski BC, O'Malley KL. Overexpression of Bcl-2 in a murine dopaminergic neuronal cell line leads to neurite outgrowth. *Neurosci Lett* 1996;202:161–164. [PubMed: 8848256]
- Parkinson JA, Crofts HS, McGuigan M, Tomic DL, Everitt BJ, Roberts AC. The role of the primate amygdala in conditioned reinforcement. *J Neurosci* 2001;21:7770–7780. [PubMed: 11567067]
- Parkinson JA, Robbins TW, Everitt BJ. Dissociable roles of the central and basolateral amygdala in appetitive emotional learning. *Eur J Neurosci* 2000;12:405–413. [PubMed: 10651899]
- Pezzella F, Tse AG, Cordell JL, Pulford KA, Gatter KC, Mason DY. Expression of the bcl-2 oncogene protein is not specific for the 14;18 chromosomal translocation. *Am J Pathol* 1990;137:225–232. [PubMed: 2201196]
- Price JL, Amaral DG. An autoradiographic study of the projections of the central nucleus of the monkey amygdala. *J Neurosci* 1981;1:1242–1259. [PubMed: 6171630]
- Price, JL.; Russchen, FT.; Amaral, DG. The limbic region II. The amygdaloid complex. In: Hokfelt, BT.; Swanson, LW., editors. *Handbook of chemical neuroanatomy*. Amsterdam: Elsevier; 1987. p. 279–381.
- Rakic P. Guidance of neurons migrating to the fetal monkey neocortex. *Brain Res* 1971;33:471–476. [PubMed: 5002632]
- Reed JC. Bcl-2 and the regulation of programmed cell death. *J Cell Biol* 1994;124:1–6. [PubMed: 8294493]
- Reed JC. Double identity for proteins of the Bcl-2 family. *Nature* 1997;387:773–776. [PubMed: 9194558]
- Rivas RJ, Hatten ME. Motility and cytoskeletal organization of migrating cerebellar granule neurons. *J Neurosci* 1995;15:981–989. [PubMed: 7869123]
- Rogan MT, Staubli UV, LeDoux JE. Fear conditioning induces associative long-term potentiation in the amygdala [published erratum appears in *Nature* 1998 391:818]. *Nature* 1997;390:604–607. [PubMed: 9403688]
- 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]
- 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]
- Schafe GE, LeDoux JE. Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. *J Neurosci* 2000;20:1–5. [PubMed: 10627575]
- Sheline YI, Barch DM, Donnelly JM, Ollinger JM, Snyder AZ, Mintun MA. Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. *Biol Psychiatry* 2001;50:651–658. [PubMed: 11704071]
- Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW. Hippocampal atrophy in recurrent major depression. *Proc Natl Acad Sci USA* 1996;93:3908–3913. [PubMed: 8632988]
- Squire LR. Mechanisms of memory. *Science* 1986;232:1612–1619. [PubMed: 3086978]
- Stoll AL, Renshaw PF, Yurgelun-Todd DA, Cohen BM. Neuroimaging in bipolar disorder: what have we learned? [erratum appears in *Biol Psychiatry* 2001 49.]. *Biol Psychiatry* 2000;48:505–517. [PubMed: 11018223]
- Strakowski SM, DelBello MP, Sax KW, Zimmerman ME, Shear PK, Hawkins JM, Larson ER. Brain magnetic resonance imaging of structural abnormalities in bipolar disorder. *Arch Gen Psychiatry* 1999;56:254–260. [PubMed: 10078503]
- Suzuki A, Tsutomi Y. Bcl-2 accelerates the neuronal differentiation: new evidence approaching to the biofunction of bcl-2 in the neuronal system. *Brain Res* 1998;801:59–66. [PubMed: 9729279]
- Swanson LW, Cowan WM. An autoradiographic study of the efferent connections of the hippocampal formation in the rat. *J Comp Neurol* 1977;172:49–84. [PubMed: 65364]
- Tasker RC. Hippocampal selective regional vulnerability and development. *Dev Med Child Neurol* 2001;6–7.
- Thomas KM, Drevets WC, Dahl RE, Ryan ND, Birmaher B, Eccard CH, Axelson D, Whalen PJ, Casey BJ. Amygdala response to fearful faces in anxious and depressed children. *Arch Gen Psychiatry* 2001;58:1057–1063. [PubMed: 11695953]

- Vyas S, Javoy-Agid F, Herrero MT, Strada O, Boissiere F, Hibner U, Agid Y. Expression of Bcl-2 in adult human brain regions with special reference to neurodegenerative disorders. *J Neurochem* 1997;69:223–231. [PubMed: 9202314]
- 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]
- Witter MP, Van Hoesen GW, Amaral DG. Topographical organization of the entorhinal projection to the dentate gyrus of the monkey. *J Neurosci* 1989;9:216–228. [PubMed: 2913203]
- Yachnis AT, Powell SZ, Olmsted JJ, Eskin TA. Distinct neuro-developmental patterns of Bcl-2 and Bcl-x expression are altered in glioneuronal hamartias of the human temporal lobe. *J Neuropathol Exp Neurol* 1997;56:186–198. [PubMed: 9034373]
- 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 Exp Neurol* 2000;59:113–119. [PubMed: 10749100]
- Yang L, Matthews RT, Schulz JB, Klockgether T, Liao AW, Martinou JC, Penney JB Jr, Hyman BT, Beal MF. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity is attenuated in mice overexpressing Bcl-2. *J Neurosci* 1998;18:8145–8152. [PubMed: 9763461]
- Zhang KZ, Westberg JA, Holtta E, Andersson LC. BCL2 regulates neural differentiation. *Proc Natl Acad Sci USA* 1996;93:4504–4508. [PubMed: 8633098]
- Zhong LT, Sarafian T, Kane DJ, Charles AC, Mah SP, Edwards RH, Bredesen DE. bcl-2 Inhibits death of central neural cells induced by multiple agents. *Proc Natl Acad Sci USA* 1993;90:4533–4537. [PubMed: 8506295]
- Zola-Morgan S, Squire LR, Amaral DG. Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. *J Neurosci* 1986;6:2950–2967. [PubMed: 3760943]
- Zola-Morgan S, Squire LR, Rempel NL, Clower RP, Amaral DG. Enduring memory impairment in monkeys after ischemic damage to the hippocampus. *J Neurosci* 1992;12:2582–2596. [PubMed: 1613549]

Abbreviations

ABmc, accessory basal nucleus, magnocellular subdivision
 ABpc, accessory basal nucleus, parvicellular subdivision
 ABs, accessory basal nucleus, sulcal subdivision
 AC, anterior commissure
 AChE, acetylcholinesterase
 AHA, amygdalohippocampal area
 Astr, amygdalostriatal area
 Bcl-2, B cell lymphocyte protein-2
 Bi, basal nucleus, intermediate subdivision
 BLNG, ‘basolateral’ nuclear group
 Bmc, basal nucleus, magnocellular subdivision
 Bpc, basal nucleus, parvicellular subdivision
 BSTLcn, lateral bed nucleus of the stria terminalis, lateral core subdivision
 BSTLP, lateral bed nucleus of the stria terminalis, posterior subdivision
 C, caudate nucleus
 CaBP, calbindin-D 28k
 CeLcn, central nucleus, lateral core subdivision
 CeM, central nucleus, medial subdivision
 CoA, anterior cortical nucleus
 CoP, posterior cortical nucleus
 GC, granule cell layer

H, hippocampus
IC, intercalated islands
L, lateral nucleus
M, medial nucleus
Mi, inner molecular layer
Mo, outer molecular layer
NGS, normal goat serum
NP, nonpyramidal cells
NT, neurotensin
P, putamen
PAC, periamygdaloid cortex
PACs, periamygdaloid cortex, sulcal subdivision
ParaS, parasubiculum
PB, phosphate buffer
PB-T, phosphate buffer with Triton X-100
PL, paralaminar nucleus
PreS, presubiculum
SGZ, subgranular zone
SLEA, sublenticular extended amygdala
SMI-32, nonphosphorylated neurofilament protein
V, ventricle

Fig. 1. Representative sections through the amygdala and extended amygdala. (A, B) AChE-stained coronal sections through the mid- and caudal levels of the amygdala, respectively. (C, D) Midlevel and caudal levels of the extended amygdala, in coronal sections immunostained for NT. NT immunoreactivity is seen in the CeM, the SLEA, and the BSTLP.

Fig. 2. Representative sections through the hippocampus at the level of the uncus, stained with Cresyl Violet (A) and immunoreacted for SMI-32 (B). The transition between CA1 and the subiculum is best seen with SMI-32 immunoreactivity (arrow).

Fig. 3. (A–C) Schematic of the distribution of bcl-2 positive cells at three rostrocaudal levels of the amygdala. Immature-appearing bcl-2 positive cells are depicted by small black dots (one cell/dot), and mature bcl-2 positive cells are depicted by large gray dots (three to four cells/dot). (D) Photomicrograph of bcl-2 labeled cells (boxed area in B). Large arrows show pyramidal cells, medium double arrows show NP cells, and small arrows show immature-appearing labeled cells. Scale bar = 100 μ m. (E) High power images of undifferentiated bcl-2-positive cells (arrows), counterstained with Cresyl Violet. Scale bar = 25 μ m.

Fig. 4.

(A) Photomicrograph of bcl-2 positive cells in the parvocellular basal nucleus (boxed area in schematic). Arrows indicate thick bcl-2 positive fiber bundles that course through the region. Scale bar=100 μm. (B) Higher power view showing darkly immunoreactive immature-appearing cells (small arrows) and less densely distributed, mature cells (large and medium arrows) with lower bcl-2 levels. Scale bar=50 μm. (C) High power view of immature bcl-2 immunoreactive cells tethered to a thick fiber bundle by single processes. Scale bar=50 μm. (D) Single-labeled class III β-tubulin positive cells with an immature phenotype in the parvocellular basal nucleus (arrows). Note thick coils of labeled fibers that resemble bcl-2-labeled fiber bundles in B and C (case J2). Scale bar=50 μm. (E and F) Double

immunofluorescent labeled cells in the parvicellular basal nucleus (boxed area in schematic), labeled for bcl-2 (E) and class III β -tubulin (F) Arrows show examples of double-labeled cells. Asterisks mark blood vessels. Scale bar=100 μ m.

Fig. 5. (A–C) Examples of bcl-2-labeled cells in specific subregions of the amygdala. (A) Well-differentiated bcl-2 positive cells in the medial nucleus. (B) Intercalated islands immunoreacted for bcl-2 and counterstained with Cresyl Violet. Bcl-2 positive cells with an immature phenotype (arrow) form a subpopulation of the intercalated cells, some of which are not labeled (open arrow). (C) Well-differentiated pyramidal-like cells in the ABs. Bars in A, B=25 μm, bar in C=50 μm.

Fig. 6. (A–C) Schematic of the distribution of mature bcl-2 positive cells in the central extended amygdala (three to four cells/dot). (A') Labeled cells in the rostral BSTLcn and transition zone between the bed nucleus of the stria terminalis and the ventral caudate nucleus. (B') Macroscopic view of the labeled cells in the lateral core subdivision of the bed nucleus of the stria terminalis. (C') High power view of labeled neurons containing dense reaction product in the lateral core subdivision of the central nucleus. Scale bar=50 μ m.

Fig. 7. Distribution of bcl-2 positive cells in the rostral hippocampus (A), with enlargement through portions of the dentate gyrus in B, C, D. Photomicrographs of bcl-2 labeled sections show that labeled cells are relatively concentrated in the polymorph layer (SGZ; C, D). Scale bar=50 μm.

Fig. 8. Double immunofluorescent labeling for bcl-2 (A, C) and class III β -tubulin (B, D) in the dentate gyrus. Images were converted to grayscale from color. Many bcl-2 positive cells co-contain class III β -tubulin (white arrows A–D), but not all (open arrows in A and B). A bcl-2 positive cell in the granule layer has an elongated morphology reminiscent of migrating cells, and is class III β -tubulin positive (arrows in C, D). Scale bar=100 μ m.

Fig. 9. (A–C) Schematic of labeled cells through rostral to central-caudal hippocampus. (D) Labeled NP cells in the CA3 (hilar region, boxed). Scale bar=25 μ m. (E) Labeled NP cells in the molecular layer of the dentate gyrus (boxed area). Scale bar=25 μ m. (F) Section through CA1 double labeled for bcl-2 and CaBP (boxed area in C, rotated 90°). Note the relatively higher density of bcl-2 positive cells in the stratum radiatum (small arrows) compared with the pyramidal layer. Scale bar=50 μ m.

Fig. 10. Functional circuitry. Bcl-2 immunoreactivity is mainly found in hippocampal interneurons, which modulate net excitatory flow through the hippocampus. Hippocampal targets in the amygdala (parvocellular basal nucleus, paralamina nucleus, and PACs) also contain high concentrations of bcl-2 positive cells, as does the extended amygdala, a major output path.

Table 1Relative distribution of bcl-2 labeled cells in the amygdala and extended amygdala subregions in all animals^a

	Immature phenotype	Mature phenotype
<i>Amygdaloid subregion</i>		
Basal nucleus		*
Magnocellular subdivision		***
Intermediate subdivision		
Parvicellular subdivision	****	****
Paralaminar nucleus	****	
Intercalated islands	****	
Accessory basal nucleus		**
Magnocellular subdivision		***
Sulcal subdivision	**	*
Parvicellular subdivision		*
Lateral nucleus		****
Medial nucleus		***
Anterior cortical nucleus		*
Posterior cortical nucleus		*
Periamygdaloid cortex 1		*
Periamygdaloid cortex 2		***
Periamygdaloid cortex 3	*	****
Periamygdaloid cortex, sulcal	****	****
Amygdalohippocampal area	****	****
Anterior amygdaloid area		*
<i>Central extended amygdala</i>		
Central nucleus		**
Medial subdivision		****
Lateral core subdivision		***
Amygdalostriatal area	**	***
Bed nucleus of the stria terminalis		**
Posterolateral subdivision		****
Lateral core subdivision		***
Capsular subdivision	**	***
Sublenticular extended amygdala	*	**

^aNo asterisk depicts few to no labeled cells

* light distribution

** moderate distribution

*** moderately heavy distribution

**** heavy distribution

Table 2Relative distribution of bcl-2 labeled cells in the hippocampal subregions in all animals^a

Hippocampal subregion	Immature phenotype	Mature phenotype
Dentate gyrus	****	****
Polymorph layer	*	
Granule cell layer		****
Molecular layer		****
AHA		
CA3		
Stratum oriens		****
Pyramidal layer		****
Molecular layer		**
CA2		
Stratum oriens		***
Pyramidal layer		*
Molecular layer		***
CA1		
Stratum oriens		**
Pyramidal layer		*
Molecular layer	**	
Subiculum		
Stratum oriens		*
Pyramidal layer		*
Molecular layer		*
Presubiculum		
layer III		*
pyramidal layer		**
molecular layer		*
Parasubiculum		
Layer III		*
Pyramidal layer		**
Molecular layer		*

^aNo asterisk depicts few to no labeled cells

* light distribution

** moderate distribution

*** moderately heavy distribution

**** heavy distribution