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Connections and Function in the Basolateral Nucleus of the Amygdala

Named after the Latin word for almond, *Amygdalum* (Simpson, 1969), the amygdala is an almond shaped structure composed of several nuclei located in the midtemporal lobe (Sah et al., 2003). Over the past 50 years a growing body of research has identified the amygdala as an essential element in the neural circuitry underlying fear conditioning; this research has also implicated the amygdala in the broad role of assigning emotional significance to sensory information (Sah et al. 2003). The basolateral nucleus of the amygdala has also been implicated in memory consolidation and long-term potentiation (Ikegaya, Saito and Abe, 1995; Quirarte, Roozendaal and McGaugh, 1997).

Researchers had begun to explore the functions and connectivity of the amygdala to other cortical and subcortical areas before a complete understanding of the anatomical and morphological characteristics of the amygdaloid nuclei had been fully established. Studies of the anatomy, morphology, and cell types found in the basolateral amygdaloid nuclei provide a basis for understanding how the nuclei found in the amygdala are able to modulate and regulate output to other cortical and subcortical areas.

Anatomy and Morphology

The amygdaloid nuclei can be divided into three main groups (Fig. 1), the basolateral nuclei (includes lateral, basal and accessory basal nucleus), centromedial nuclei (includes medial and central nuclei), and cortical nuclei (includes cortical nuclei and the lateral olfactory tract

nuclei) (Sah et al., 2003). Each main group is further subdivided based on cellular structure, connections, and electrophysiological properties. The lateral, basal, and accessory basal nucleus of the basolateral nuclei are further subdivided into the accessory basal magnocellular subdivision, accessory basal parvocellular subdivision, basal nucleus magnocellular subdivisions, lateral amygdala medial subdivision, lateral amygdala medial subdivision, and lateral amygdala ventrolateral subdivision (Sah et al., 2003).

Some researchers have proposed that the current classification system dividing the amygdaloid nuclei is arbitrary, and instead suggest a classification system based on connectivity and function. These researchers classify the amygdaloid nuclei into four functional systems, frontotemporal, autonomic, main olfactory, and accessory olfactory systems (Swanson and Petrovich, 1998). In the functional classification system, the frontotemporal system consists of basolateral nuclei. Studies of the neuronal morphology and cell types of the basolateral nuclei have the potential to aid in the study of connections and functional significance of the amygdaloid nuclei by suggesting novel ways in which the variety of cell types present may modulate the electrical and neurochemical activity of the region (McDonald, 1982).

Neuronal Types of the Basolateral Amygdala

Several studies have provided a description of neuronal types in the basolateral amygdaloid nuclei, in these studies researchers have identified three main neuronal classes distinguished by perikeryal, dendritic, and axonal morphology (McDonald, 1982; Braak and Braak, 1983). Using the Golgi-Kopsch and rapid Golgi technique in the rat, McDonald (1982) described three neuronal classes; Class I cells are large spiny neurons that have few axon collaterals, while Class II and III neurons are smaller, spine sparse, and have dense local axonal

arborizations. Class I neurons (Fig. 2) were the most commonly stained cells by the Golgi methods used. These neurons are characterized by their pyramidal or piriform perikarya; in the anterior division of the basolateral nucleus measurements of the soma range from 17-22 μm long and 15-17 μm wide, while in the posterior division measurements of the soma range from 15-18 μm long and 13-16 μm wide (McDonald, 1982).

Class II neurons (Fig. 3) can be further subdivided into three distinct types based on their perikaryal, dendritic, and axonal morphology. Based on perikaryal and dendritic morphology neurons may be classified as multipolar, bitufted, or bipolar; however amygdaloid chandelier cells are identified by axonal morphology (McDonald, 1982). In Class II neurons, perikarya are classified by size (the sum of length and width) into small (<24 μm), medium (24-28 μm), and large (>28 μm) groups.

Class III neurons are characterized by small cell bodies (10-12 μm in diameter) and have short varicose dendrites which give the cell a neurogliaform appearance (McDonald, 1982). Class III cells have be found to make extensive dendrodendritic connections with Class I cells, but have not been observed making connections to adjacent Class III dendrites (McDonald, 1982). Further studies of Class III neurons are required to provide an adequate description of this cell class in order to determine the unique contributions of this cell type.

Braak and Braak (1983) used the Golgi method to study the structure of the basolateral amygdaloid nuclei in man; this study found that cell types may be divided into two groups, the first group contained the majority of cells stained which are the pyramidal-like spiny neurons which correspond to McDonald's (1982) Class I neurons. The second group of cells identified were spine sparse and homogenous resembling stellate cells from the cortex; however this does not correspond to the remaining Class II or III cells described by McDonald (1982).

Variations on the Golgi method (Golgi-Kopsch, and rapid Golgi) were used to characterize the morphology and neuronal types in the basolateral amygdaloid nuclei of the rat (McDonald, 1982). Extensive investigations into the neurochemistry, connections, and functions of the basolateral amygdaloid nuclei have been conducted previously in the rat, but McDonald was the first to undertake a detailed study of neuronal types in the basolateral amydaloid nuclei in this species. Although the Golgi method provided abundant data for the Class I neurons, the data for Class II, III, as well as the amygaloid chandelier neurons were limited in this study, and more evidence is required to provide a thorough description of the morphology of these cell types.

Despite the thorough descriptions of Class I and II neurons, Class III neurons were rare in this study, and perhaps alternate methods of study should be used to provide a more complete description of this class of neurons. Alternative methods are also required to investigate axonal morphology as well as to provide a quantitative estimate of the distribution and frequency of each class and neuron subtype, as well as their location in the basolateral amygdaloid nuclei of the rat. McDonald's classification system of cells in the basolateral amygdaloid nuclei is used by other researchers who employ alternative methods in order to describe the electrophysiological and pharmacological characteristics of cells in the basolateral nuclei, demonstrating the importance and validity of his research.

An understanding of the cell types and anatomical structure of the basolateral amygdaloid nuclei can contribute to investigations of neurotransmitters by identifying the location of cells within the nuclei, identifying cells that are likely to be excitatory (Class I spiny pyramidal-like cells) and inhibitory (Class II spine sparse interneurons).

Neurotransmitters

In any discussion of neurotransmitters is important to avoid characterizations of neurotransmitters as excitatory or inhibitory, by recognizing that neurotransmitters are capable of binding to several postsynaptic sites, and these different activation sites may result in a wide variety of effects in postsynaptic target neurons. Descriptions of the different neuronal types found in the basolateral amygdaloid nuclei, as well a description of the different neurotransmitters released by these neurons aid in understanding how the individual nuclei of the amygdala are intrinsically connected, and how the connections to cortical and subcortical areas influence the activity in these areas.

Gamma-aminobutyric Acid

Gamma-aminobutyric acid (GABA) releasing neurons activate ionotropic, chloride-permiable $GABA_A$ receptors. GABA-containing interneurons are responsible for primarily inhibitory effects on postsynaptic target neurons; however a subpopulation of parvalbumin-positive interneurons have been identified which display a form of excitation (Woodruff, Monyer and Sah, 2006). This subpopulation of parvalbumin-positive interneurons involved in excitation are located in the basal nucleus of the amygdala, and were studied using mice expressing enhanced green fluorescent protein (EGFP) under the control of a parvalbumin promoter (Woodruff, Monyer and Sah, 2006). Whole cell and voltage-clamp electrophysiological recordings from a subpopulation of interneuron pairs demonstrated that a single action potential in an interneuron can evoke a rapid, biphasic, outward then inward current sequence in a postsynaptic interneuron (Fig. 4). The outward currents were shown to be

monosynaptic GABAergic events, while inward currents were shown to be disynaptic and caused by activation of AMPA receptors, which suggests the presence of glutamatergic synapses.

Woodruff, Monyer, and Sah (2006) suggest that the delayed fluctuating EPSCs arise from GABAergic excitation of a local glutamatergic neuron which reaches threshold and in turn innervates the postsynaptic interneuron, action potentials activate both feedforward and feedback excitation. The researchers propose that this form of GABAergic excitation plays a role in recruiting homogenous interneuron networks in the basal amygdaloid nuclei. Although the researchers provided arguments to rule out possible explanations for the delayed excitation, they were not able to provide direct evidence to support their hypothesis that principle glutamatergic neurons were the source of the feedback EPSPs observed. In order to determine whether or not the glutamatergic neurons are a source for feedback EPSPs, further studies would need to be conducted, perhaps using more isolated preparations from cell culture.

Substance P

Substance P is a short-chain polypeptide that functions as a neurotransmitter in the basolateral amygdaloid nuclei by activating tachykinin NK1 receptors. It has previously been demonstrated that tachykinin NK1 receptor antagonists have antidepressant and anxiolytic affects in individuals with major depression and anxiety disorders (Rupniak and Kramer, 1999). Based on these findings, Maubach et al. (2001) conducted experiments to determine the physiological role of tachykinin NK1 receptors in the basolateral nucleus of the guinea pig amygdala. Using electrophysiological analysis of the effects of substance P on spiny pyramidal-like and stellate neurons in the presence absence of tachykinin NK1 receptor agonists and antagonists, Maubach et al. found NK1 receptor activation primarily stimulates inhibitory

synaptic activity. The cells investigated in this study would belong to the Class I cells described by McDonald (1982). An examination of NK1 receptor immunoreactivity was found to be limited to a subpopulation of GABA interneurons. The researchers suggest these findings support the physiological role of NK1 receptors in the basolateral amygdaloid nucleus as involved in the control of emotional behavior through inhibitory processes (Maubach et al., 2001). Although it seems clear that the cells of the basolateral amygdaloid nuclei contain inhibitory GABA interneurons, more evidence is required in order to support the role of inhibition in emotional behavior. There is support for the *theory* that the basolateral nuclei are involved in the regulation of emotional behavior, but this theory needs to be tested further in order to support the larger claims pertaining to regulation of emotion.

Glutamate

Glutamate releasing neurons are found in each nuclei of the amygdaloid complex, particularly the basolateral and lateral nuclei. Release of glutamate from projection neurons of the basolateral amygdaloid nuclei has been shown to increase dopamine levels in the nucleus accumbens, which has been implicated in reward (Howland, Taepavarapruk and Phillips, 2002). In a study designed to investigate glutamate receptor-dependent modulation of dopamine efflux in the nucleus accumbens, researchers delivered electrical stimulation (20 Hz, 10 sec, 300 μA) to the basolateral and central amygdaloid nuclei, this induced a long-lasting increase in dopamine efflux in the nucleus accumbens when stimulation was delivered to the basolateral nuclei, but did not result in increased dopamine efflux when stimulation was delivered to the central amygdaloid nucleus (Howland, Taepavarapruk and Phillips, 2002). This study provides support for the division between basolateral and central amygdaloid nuclei based on their respective functional

roles, and demonstrates the ability for the basolateral amygdaloid nuclei to influence the nucleus accumbens through the release of glutamate.

Nitric Oxide

Nitric oxide (NO) is a gaseous neuromodulator which is produced by neurons in the amygdala and other areas of the central nervous system. In a study examining nitric oxide-producing neurons in the rat amygdala, reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPHd) histochemistry was used to identify neurons that produce nitric oxide (Usunoff et al., 2006). Although NADPHd-positive neurons were found throughout the amygdaloid nuclei, very few strongly labeled cells were seen in the basolateral nucleus. The few strongly labeled cells that were observed in the basolateral nuclei were confined to the lateral border. Extensive labeling was observed in the lateral nucleus, in both cases the labeled cells clearly differed from the large pyramidal-like cells found in the basolateral nuclei. This study suggests that nitric oxide exerts its effects locally, although more research needs to be done to determine how the release of nitric oxide effects postsynaptic target neurons within the basolateral amygdaloid nuclei (Usunoff et al., 2006).

Acetylcholine

The basolateral nucleus receives cholinergic input from several areas of the cortex and basal forebrain. It has been proposed that activation of nicotinic acetylcholine receptors (nAChRs) can improve performance in some learning tasks. A recent study has investigated the nicotinic acetylcholine receptors in the basolateral amygdaloid nuclei of the mouse (Jiang and Role, 2008) and found that administration of nicotine elicits facilitation in learning tasks in a

manner that is dependent on the level of cortical input to the basolateral amygdaloid nuclei. Patch-clamp recordings were taken from mouse brain; when nicotine was applied a 40% increase in glutamatergic postsynaptic currents were observed. The application of low frequency stimulation in conjunction with nicotine resulted in activation of 60% of basolateral neurons, and the facilitation lasted for 15 minutes post nicotine washout (Jiang and Role, 2008). This study illustrates the powerful effects exerted by just one of the many neurotransmitters present in the basolateral amygdaloid nuclei, highlighting the ability for individual transmitters to modulate the activity in a specific region.

Intraamygdaloid and Extrinsic Connections

Axonal tracing studies have revealed that the amygdaloid complex has a great number of connections between the individual nuclei, as well as a great number of connections to surrounding cortical and subcortical areas (Sah et al., 2003). Studies of the connections between individual amygdaloid nuclei (intraamygdaloid), as well as connections from cortical and subcortical areas highlight the functional significance of the individual amygdaloid nuclei, by demonstrating how the basolateral nuclei contribute to and modulate the cortico-striatal-pallidal system and limbic system.

Amygdaloid Basolateral Nuclei: Intraamygdaloid Connections

The basolateral complex contains a great number of connections within the lateral, basal and accessory basal nuclei as well as connections between the further cellular subdivisions of each nuclei (Ottersen 1982; Savander, Ledoux and Pitkanen, 1996). Retrograde transport of horseradish peroxidase (HRP) had been used to study the intraamygdaloid projections of the rat.

Ottersen (1982) found an extensive network of intraamygdaloid connections, with the exception the medial nucleus and the central nucleus which were reported not to give rise to intrinsic fibers. Researchers have pointed to the limitations of horseradish peroxidase to highlight connections in nearby nuclei in the amygdaloid complex, such as the difficulty for horseradish peroxidase to detect connections between the basolateral and central nuclei (Smith and Millhouse, 1985). The limitations of horseradish peroxidase point to the need for converging evidence from several different techniques, utilizing different approaches in order to fully characterize the connections within the amygdaloid nuclei. Experiments that used the rapid Golgi method to trace axons from the basolateral to the central nucleus found two different types of axon collateral branching patterns (Smith and Millhouse, 1985). One branching pattern terminates in the central nucleus with axon collaterals making several en passant synapses (Fig. 5). In the other branching pattern, the axon only emits a few collaterals within the central nucleus and continues to the stria terminalis (Smith and Millhouse, 1985).

Savander, Go, Ledoux, and Pitkanen (1995, 1996) carried out a series of experiments using *Phaseolus vulgaris* leucoagglutinin in order to study the projections of the basal and accessory basal nuclei. Contrary to previous findings, the researchers demonstrated that the basal nucleus was divided into magnocellular, parvicellular, and intermediate areas. The magnocellular division is reciprocally connected to the lateral portion of the parvicellular division. Whereas the intermediate division only weakly projects to the parvicellular division, the medial parvicellular division sends extensive projections to the intermediate area. Researchers also found that the major projections from the basal nuclei were to the nucleus of the lateral olfactory tract, medial and capsular divisions of the central nucleus, anterior cortical nucleus, and amygdalohippocampal area (Savander, Go, Ledoux, and Pitkanen, 1995; 1996).

These experiments were the first to provide a detailed description of the connections projecting from the basal and accessory basal nuclei, and to demonstrate a degree of topographical organization that had not been demonstrated previously further contributing to the overall understanding of the intraamygdaloid connections and associated external projections.

Afferent Connections

The amygdaloid nuclei receive afferent input from several areas within the cortex as well as subcortical areas. Several different studies of the amygdaloid connections have used horseradish peroxidase to study afferent connections in the rat and cat (Ottersen, 1980,1982; Ono et al., 1985). In a series of studies investigating the connections of the rat amygdala, Ottersen (1982) demonstrated that the basolateral nuclei receive input from the anterior cingulate area, posterior agranular insular area, ventral agranular area, prepyriform cortex, perirhinal region, and dorsal part of the lateral entorhinal area. This study was the first to describe afferent projections from the temporal two thirds of CA1 to the basolateral nucleus, lateral nucleus, and cortical nuclei. In previous experiments (Ottersen, 1980), horseradish peroxidase was used to trace the afferents from the hypothalamus and the basal telencephalon to the amygdaloid complex. The basolateral nuclei were found to receive afferents from the ventral pallidum, ventral part of the globus pallidum, substantia innominata, and lateral olfactory tract (dorsal subdivision). The hypothalamo-amygdaloid projections found in this study terminated for the most part in the medial nuclei, however a few fibers from the ventromedial nucleus of the hypothalamus extended to the basolateral nuclei. The basolateral nuclei were also found to receive restricted projections from the supramammillary nucleus and lateral hypothalamic area. Although horseradish peroxidase has been used extensively to trace the connections from the amygdala to

cortical and subcortical areas each technique has inherent limitations, it would be interesting to see if the same results would be obtained by other tracing methods such as using fluorescent dyes.

In order to test the amygdala's role in processing sensory information thalamo-amygdaloid projections in the rat were investigated using autoradiographic tract-tracing methods (Turner and Herkenham, 1991). The basolateral amygdaloid nuclei were found to receive input from the central medial, interanteromedial, and paraventricular thalamic nuclei, which are the relay stations for sensory information from the thorax and abdomen; these findings suggest that the basolateral amygdaloid nuclei play a role in the processing of sensory information, but the exact mechanisms are unclear. Turner and Herkenham (1991) proposed four amygdaloid systems corticomedial, lateral-basomedial, central, and basolateral; in this classification system the lateral-basomedial combines input from a number of sensory modalities and relays the information to the medial basal forebrain and hypothalamic areas. This study contributed to the known afferents of the basolateral amygdaloid nuclei by the addition of sensory information via the thalamus, the addition of multiple sensory afferents provides a clearer picture of the complexity found in the connections between the amygdala and other cortical and subcortical areas.

Efferent Connections

The basolateral nuclei project to several cortical and subcortial areas. Studies have used HRP conjugated with wheat germ agglutinin to investigate the projections from the basolateral nuclei to the caudatoputamen, nucleus accumbens, and related striatal-like areas of the rat brain. Fluorescent dyes have also been utilized to study the organization of the projections from the

amygdaloid nuclei to prefrontal cortex and associated striatal areas of the rat (McDonald, 1991). Fluorescent dyes True Blue and Diamidino Yellow were injected into the prefrontal cortex and associated striatal region of Sprague-Dawley rats and allowed 4 to 7 days for transport before analysis. Medial and lateral portions of the basolateral nuclei were found to project to the medial and lateral regions of the prefrontal cortex and associated medial and lateral striatal regions. Many cells in the basolateral nuclei were double labeled, these neurons projected to both areas by sending collaterals to both the prefrontal cortex and associated striatal region (Fig. 6). It was previously established that the amygdaloid complex had strong connections to the cortex and striatum, but McDonald (1991) was the first to analyze the cortical, striatal, and pallidal connections to the amygdaloid nuclei, and highlight the potential role for the amygdaloid nuclei in modulating information processing in the cortico-striatal-pallidal system.

The basolateral amygdala and ventral subiculum of the hippocampal formation both project to the nucleus accumbens, which is considered the limbic-motor interface (French, Hailstone and Totterdell, 2003). Although convergence of the basolateral nuclei and hippocampus at the level of the nucleus accumbens can be demonstrated by electrophysiological methods, it is not possible to determine whether there is monosynaptic convergence from the basolateral nuclei and hippocampus to the neurons of the nucleus accumbens. To investigate this question, researchers injected biotinylated dextran amine into the posterior basolateral nucleus; the labeled fibers were examined using electron microscopy to identify their postsynaptic targets. The results suggested that the projections from the basolateral amygaloid nuclei preferentially innervate spiny subiculum neurons which are the presumed pyramidal projection neurons of the nucleus accumbens (French, Hailstone and Totterdell, 2003). Combining tracing techniques with electron microscopy allowed the researchers to not only trace the connections from the

basolateral nucleus, but also allowed them to determine where in the nucleus accumbens these neurons were terminating. The data obtained in this study contributes to the overall knowledge of how the basolateral amygdala can modulate activity in the limbic system.

The organization of projections from the amygdala to the hypothalamus were further examined in a study using retrograde transport of horseradish peroxidase (Ono et al., 1985). Although the majority of afferents to the hypothalamus originated in medial and central nuclei, substantial labeling was observed in the basolateral complex. Topographical organization was observed such that the later half of the amygdaloid nuclei project to the lateral hypothalamic area, and ventromedial hypothalamic nuclei received projections from the entire length of the amygdaloid nuclei with the exception of projections from the posterior cortical nuclei. Ono et al. (1985) were able to provide a detailed description of the projections from the amygdaloid nuclei to the hypothalamus; this research not only demonstrated the organization of afferent connections from the basolateral amygdaloid nuclei to the hypothalamic nuclei, but this study is also an important prerequisite step for researchers who were seeking to understand hypothalamic contributions to behavior.

Functions

The basolateral amygdaloid nuclei shares conections with many cortical and subcortical areas, but once the anatomy and morphology, the cell types and neurotransmitters, and connections have all been described we are left with questions concerning the functions of these connections and how the basolateral amygaloid nuclei interact with these other structures in life to create complex behaviors. A large body of literature suggests that the amygdala is part of the

limbic system involved in emotion, and specifically that the basolateral amygdaloid nuclei are involved in fear conditioning.

Fear Conditioning

Fear conditioning has become a predominant paradigm for the study of plasticity, learning and memory; the form of the fear conditioning most often used is based on Pavlovian conditioning (Nithianantharajah and Murphy, 2008). This type of fear conditioning commonly involves associating or pairing a previously neutral stimulus (conditioned stimulus CS), such as a tone, then presenting an unconditioned naturally aversive stimulus (unconditioned stimulus US), such as a foot shock. The response to the aversive stimulus is the unconditioned response (UR). The previously neutral stimulus CS (tone) is repeatedly paired with the aversive stimulus US (foot shock); the CS (tone) must be presented before or in conjunction with the administration of the US (foot shock) for the learning trials to be effective. Through repeated learning trials the CS comes to elicit the UR (pain response) without the administration of the US, in other words through repeated learning trials the tone comes to elicit the response, in this case a response to pain that may include vocalizations and escape behavior.

Nithianantharajah and Murphy (2008) recently investigated the levels of synaptophysin, a synaptic vesicle protein, in the basolateral amygdaloid nuclei following auditory fear conditioning. The conditions for the study included a context or control group that did not receive the tone or foot shock, a tone only, shock only, and combined tone-shock condition. The auditory fear conditioning used in this study consisted of trials in which tone-shock mice were placed into a chamber for two minutes followed by a 30 second tone followed by a 30 second foot shock. Control mice were placed in the chamber for 3 minutes and returned to cages. The

tone only mice were placed in chamber for 2 minutes, presented with 30 second tone and removed after 30 more seconds. The shock only mice were placed in chamber for two minutes and 30 seconds, and the remaining 30 seconds presented with foot shock. Nithianantharajah and Murphy (2008) found higher levels of synaptophysin in mice that had learned to associate the tone with fear, compared to all other non-learning controls. These findings are encouraging in that they show some support for a model of learning where specific learned events may be identified based on physiologically recognizable changes in specific regions of the brain.

Fear conditioning research is particularly applicable to clinical psychology; literature has suggested that fear motivated learning may contribute to the onset of affective and anxiety disorders (Akirav and Maroun, 2006). The fear response (freezing, sweating, increased heart rate and blood pressure) in animal research provides a physiological analogue to the anxiety response in humans (Sah et al., 2003). Research concerning fear conditioning and extinction of the fear response with the aid of pharmacological agents have direct applications to the development of pharmacological treatments for depression and anxiety.

The basolateral and central amygdaloid have been implicated in fear and anxiety related behavior. Corticotropin-releasing factor binds to corticotropin-releasing factor receptors 1 and 2 ($CRF_{1,2}$); CRF_1 has previously been explored as a therapeutic target for treatment of stress-related disorders. In a study designed to investigate CRF_1 receptors in the basolateral amygdala, researchers found there were abundant CRF_1 receptors in the basolateral compared to central nuclei (Hubbard et al., 2007). Microinfusions of DMP696, a selective CRF_1 receptor antagonist, made into the basolateral or central amygdaloid nuclei were found to significantly reduce phosphorylation of cAMP response element-binding protein (pCREB) in the basolateral but not central nuclei. Oral administration of DMP696had no effect on the ability to acquire fear through

conditioning procedures, but reduced contextual freezing in behavioral assays. Microinfusions of DMP696 into the basolateral nucleus were also shown to disrupt freezing behavior when administered between 5 minutes and 3 hours, but not 9 hours, after a fear conditioning trial. This suggests that the basolateral amygdaloid nuclei play a role in the consolidation of contextual fear, which may involve basolateral pCREB induced synaptic plasticity (Hubbard et al., 2007). This study clearly identifies the distinction between the role of the basolateral amygdaloid nucleus in memory consolidation process, compared to the acquisition of conditioning, thus providing support for a very specific memory related function.

Memory Consolidation

Memory consolidation has been shown to be facilitated by emotional arousal and release of adrenal stress hormones; specifically the release of noradrenalin and acetylcholine in the basolateral complex of the amygdaloid nucleus (Paré, 2003). The basolateral nuclei were investigated to determine whether to glucocorticoid-induced effects on memory storage depend on activation of β-andrenergic activation within the amygdala of Sprague-Dawley rats (Quirarte, Roozendaal and McGaugh, 1997). Inhibitory avoidance training was implemented (a rat upon entering a dark chamber receives a foot shock) then basolateral or central nuclei were infused with microinjections of β-andrenergic agonists and antagonists (proranolol, atenolol, zinterol) via a cannula. Injections into the central nuclei had no effects on the inhibitory avoidance training, whereas injections of agonists to the basolateral nuclei produced memory enhancing effects and antagonists were able to reduce the memory enhancing effects resulting from administration of agonists (Fig. 7).

Results from this study provide more support for the role of glucocorticoids in memory modulation, and provide support that the basolateral nuclei is the point of interaction for these two systems. The ability for the basolateral amygdaloid nuclei to enhance inhibitory avoidance training was not observed in the central nucleus, this provides further support for the functional divisions of the amygdaloid nuclei into distinct basolateral, central, and cortical divisions.

Long-term Potentiation

Long-term potentiation (LTP) has become a predominant paradigm for the study of synaptic plasticity and memory and is most commonly studied in the intrinsic connections of the hippocampus; however, inputs from other areas of the brain are likely to affect the process of LTP in vivo. In an electrophysiological study, the basolateral amygdaloid nuclei were investigated to determine the contribution of the basolateral nuclei on LTP induction in the dentate gyrus in vivo (Ikegaya, Saito and Abe, 1995). This study found that a conditioning stimulus alone (100 pulses at 100 Hz) delivered to the ipsilateral or contralateral basolateral nuclei did not change the dentate gyrus synaptic potential, but when the conditioning stimulus was delivered in conjunction with a weak titanic stimulation (20 pulses at 20 Hz) to the perforant path-dentate gyrus synapses, robust LTP was observed but only for ipsilateral and not contralateral basolateral stimulation (Fig. 8).

There are still many unanswered questions concerning the contribution of the basolateral amygdaloid nuclei to LTP concerning where the basolateral neurons terminate, and which neurotransmitters are released at these terminals. However, these results suggest that the ipsilateral basolateral amygdaloid neurons contribute to LTP in the dentate gyrus in vivo. This study provided valuable information concerning the ability of individual amygdaloid nuclei such

as the basolateral to function as independent units, in this case the differences between ipsilateral and contralateral contributions to long-term potentiation highlight the specificity of these connections and how they operate to form complex behaviors such as learning and memory.

Discussion

The basolateral amygdaloid nuclei are involved in several processes from the induction of LTP in the dentate gyrus, to fear conditioning, and emotional significance of memories. The connections of the basolateral nuclei extend to cortical and subcortical areas that clearly implicate the basolateral nuclei in the limbic system as well as the cortico-striatal-pallidal system. In some cases like the cortico-striatal-pallidal system, the efferent and afferent connections maintain an organized topography in which medial and lateral basolateral nuclei connections reach medial and lateral prefrontal cortical and associated striatal areas. Similar topographical organization is also observed in the connections between the thalamus, hypothalamus, and basolateral amygdaloid nuclei. With the amygdala connected to so many different systems, the individual nuclei of the amygdaloid complex may have a range of different functions, and the ability to modulate sensory information, as well as information for other cortical and subcortical areas.

Several techniques have been utilized to study the connections of the basolateral nuclei including electron microscopy, electrophysiology, and tract tracing studies using retrograde and anterograde transport of fluorescent dyes and horseradish peroxidase. Rapid Golgi and other variations such as the Golgi-Kopsch method have been used to study the morphology and anatomy of the basolateral nuclei, highlighting the neuronal types. Each of these techniques and

methods bring a more complete understanding of the basolalteral amygdaloid nuclei, its distinct neuronal types, connections, and functions within the central nervous system.

Many questions remain regarding the significance of the basolateral amygdaloid nuclei, but the role in emotion and fear conditioning have clearly been established. Research continues to examine aspects of basolateral nuclei function and connectivity, and surely the future will reveal the subtleties of the intraamygdaloid connections, as well as bring us closer to understanding how the amygdaloid nuclei including the basolateral nuclei contribute to behavior.

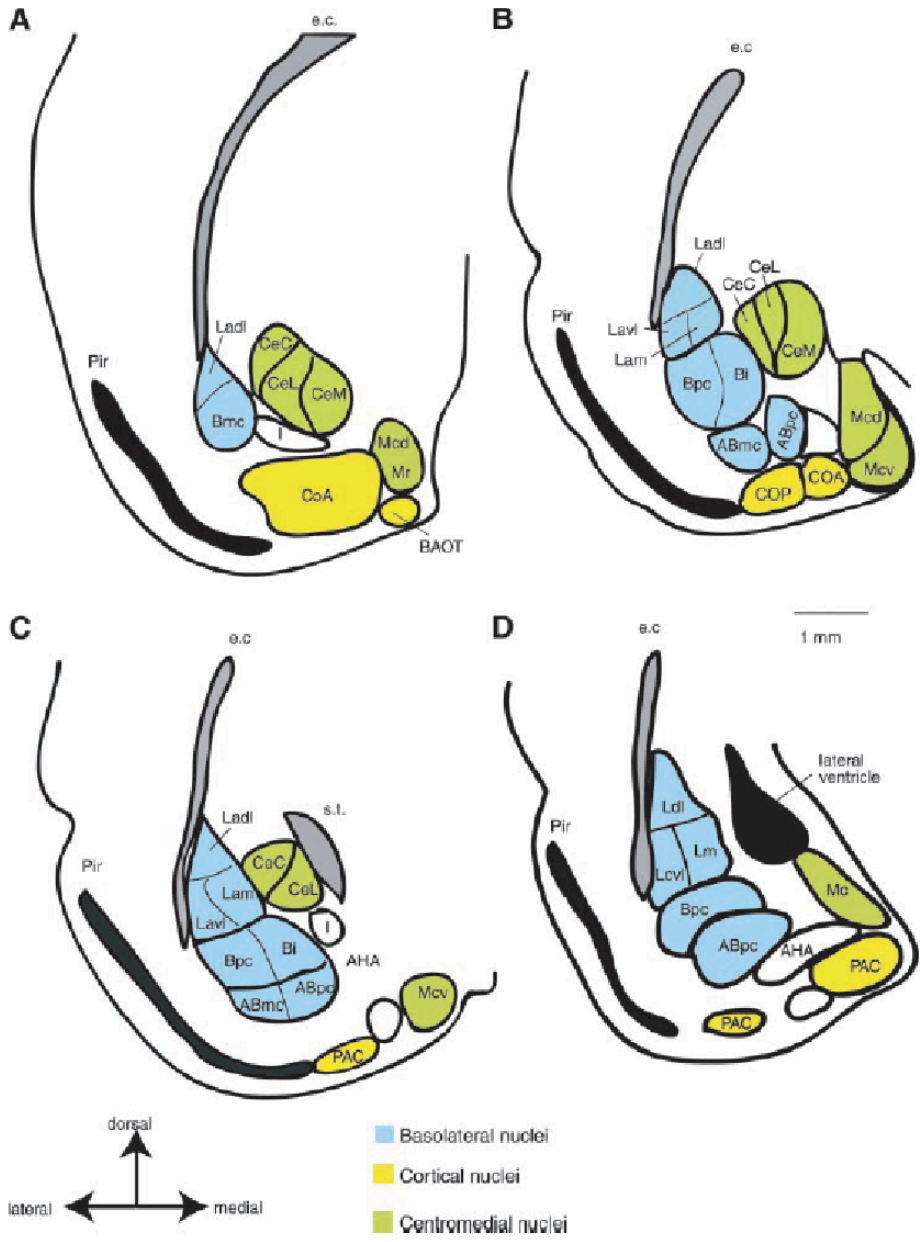

Figure 1.
Nuclei of the rat amygdaloid complex. Coronal sections rostral (A) to caudal (D). The different nuclei are divided into three groups. Blue represents basolateral nuclei, yellow represents cortical nuclei and green represents centromedial nuclei. Abbreviations: ABmc accessory basal magnocellular subdivision, ABpc accessory basal parvocellular subdivision, Bpc basal nucleus magnocellular subdivisions, e.c. external capsule, Ladl lateral amygdala medial subdivision, Lam lateral amygdala medial subdivision, Lavl lateral amygdala ventrolateral subdivision, Med medial amygdala dorsal subdivision, Mr medial amygdala rostral subdivision, Pir piriform cortex, s.t. stria terminalis (Sah et al., 2003).

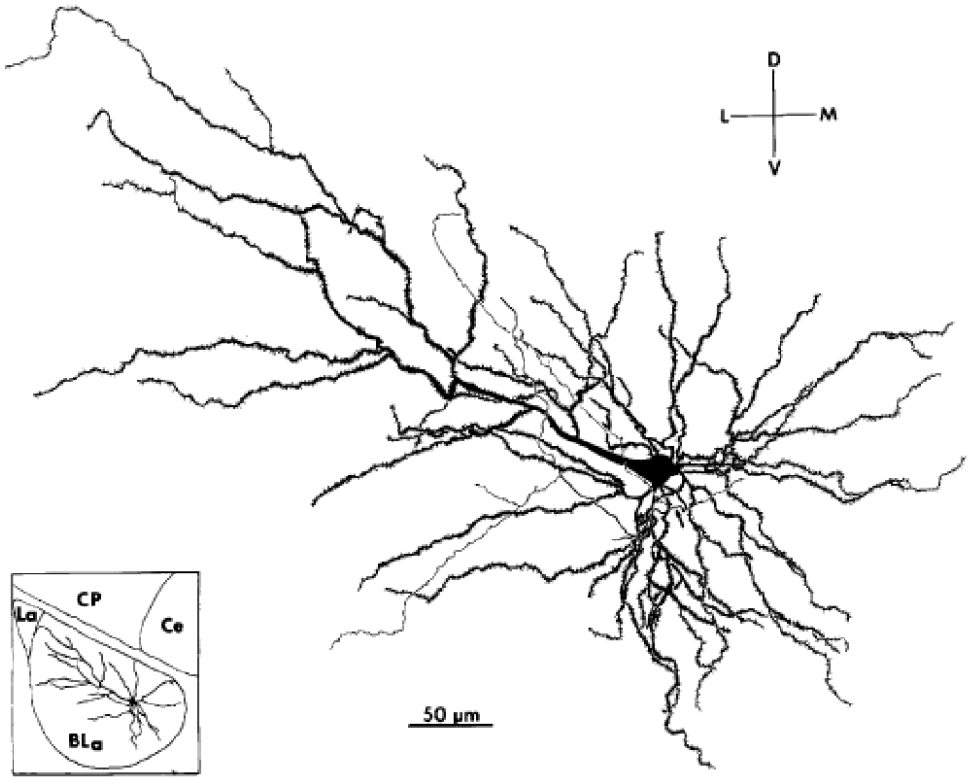

Figure 2

Drawing of a Class I neuron in the anterior division of the basolateral nucleus. The neuron has a pyramidal appearance, one apical dendrite is present, basal dendrites are numerous, and dendrites are spiny. Arrow indicates the axon, inset shows the position of the cell, and the cross indicates orientation (McDonald, 1982).

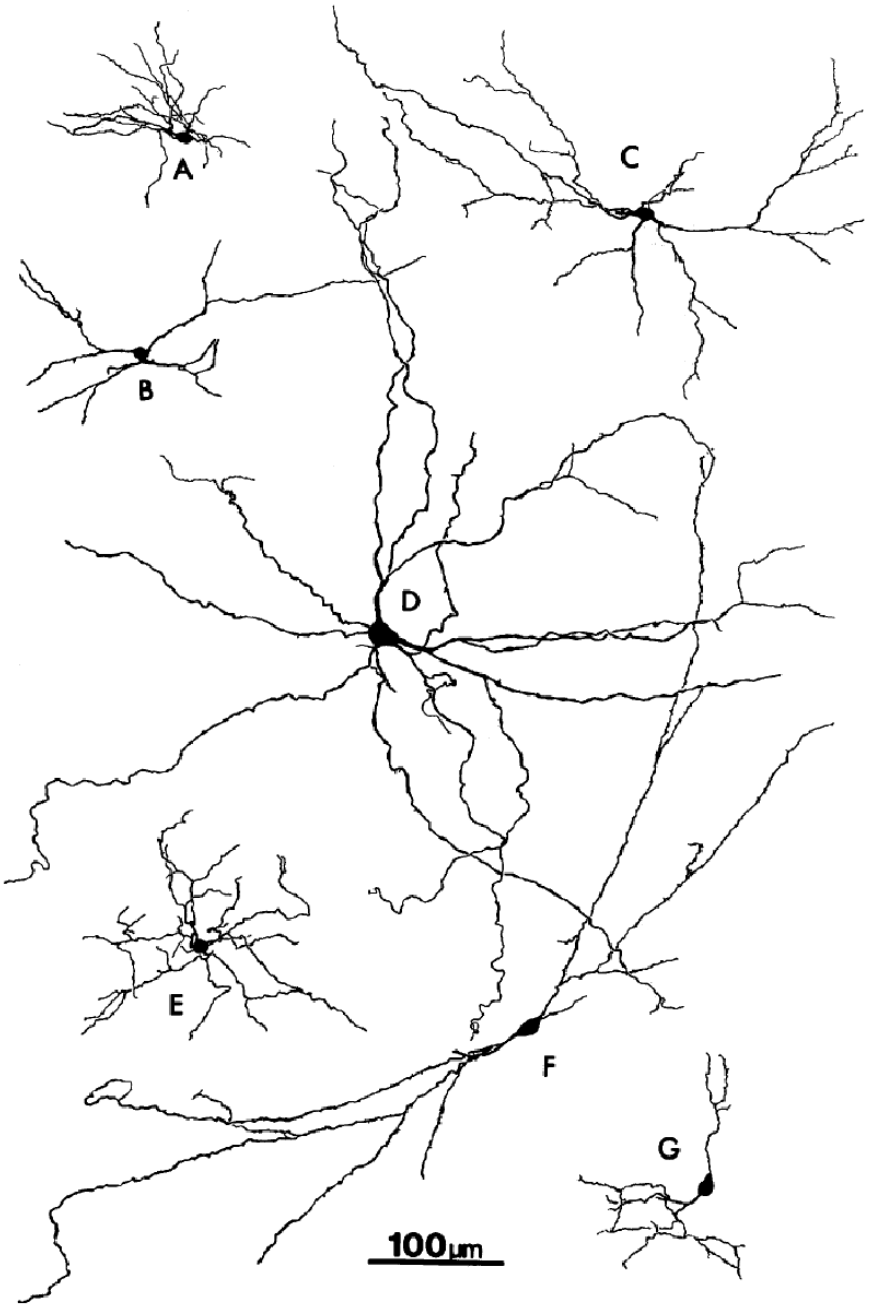

Figure 3
Drawings represent variations in Class II neuronal morphology. A) Small bitufted cells of the posterior division of the lateral nucleus. B) Small multipolar neuron of the posterior division of the basolateral nucleus. C) Medium size bitufted cell of the posterior division of the basolateral nucleus. D) Large multipolar neuron of the anterior division of the basolateral nucleus. E) Small multipolar neuron of the anterior division of the basolateral nucleus (note high degree of branching compared to cell B. F) Large bipolar neuron of the anterior division of the basolateral nucleus. G) Medium-size bipolar neuron of the anterior division of the basolateral nucleus (McDonald, 1982).

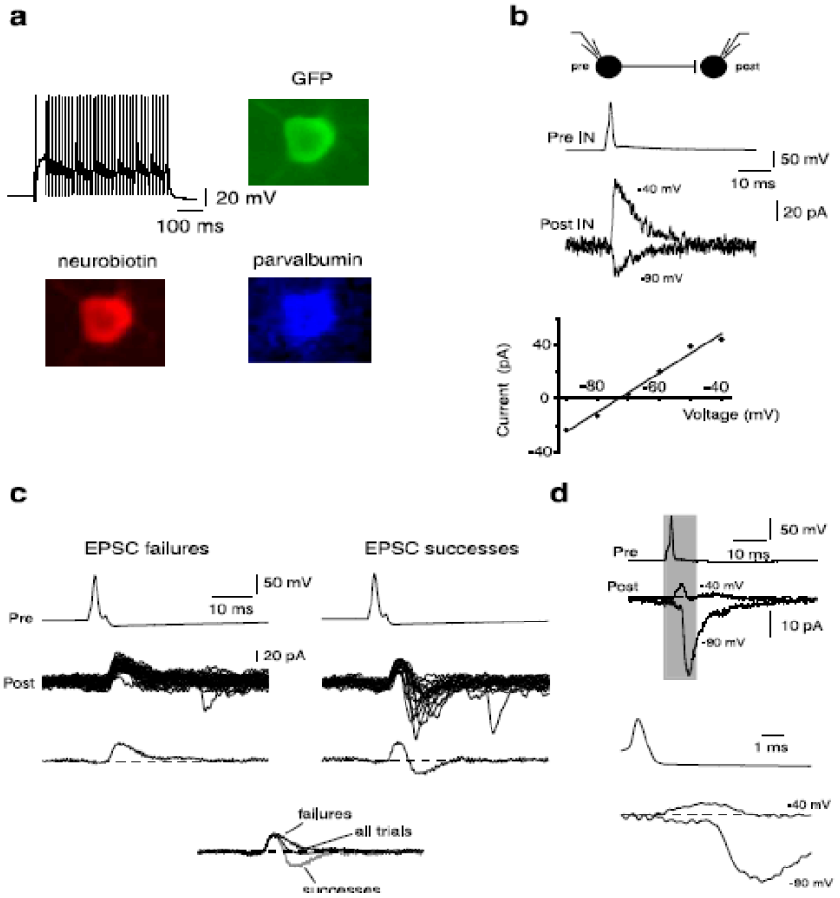

Figure 4. Single interneuron action potentials evoke biphasic currents in postsynaptic interneurons. a, GFP-expressing neurons (green) show electrophysiological responses typical of interneurons. Illustrated is the voltage response to a 600 ms suprathreshold, depolarizing current injection. In some recordings, neurons were loaded with neurobiotin (red). These neurons were positive for parvalbumin (blue), confirming they are parvalbumin-expressing interneurons. b, Paired recordings from two EGFP-positive interneurons (top). Traces below show the current response in the voltage-clamped postsynaptic cell at holding potentials of _40 and _90 mV (bottom traces) to a single action potential in the presynaptic interneuron (top trace). The current–voltage relationship of the postsynaptic current is shown in the bottom panel. c, Interneuron pairs show biphasic currents. Single action potentials evoked in the presynaptic interneuron evoke two types of responses in a voltage-clamped postsynaptic interneuron (Vh, _40 mV). Traces on the left show time-locked outward current responses, whereas traces on the right show responses that are also accompanied by a delayed inward current. The average current in each case is shown in the bottom trace. The bottom panel shows overlay of averaged traces in which there were EPSC failures (thick black), EPSC successes (gray), or the average for all trials (thin black). d, Only the early synaptic current reverses at the chloride equilibrium potential. Averaged postsynaptic responses in a voltage-clamped postsynaptic interneuron to a single action potential in the presynaptic interneuron. Note the dual component response. Hyperpolarization of the postsynaptic neuron to -90 mV reversed the initial component and increased the amplitude of the delayed inward current. The shaded region is expanded at the bottom to demonstrate the delay of the large inward component (Woodruff, Monyer, Sah, 2006).

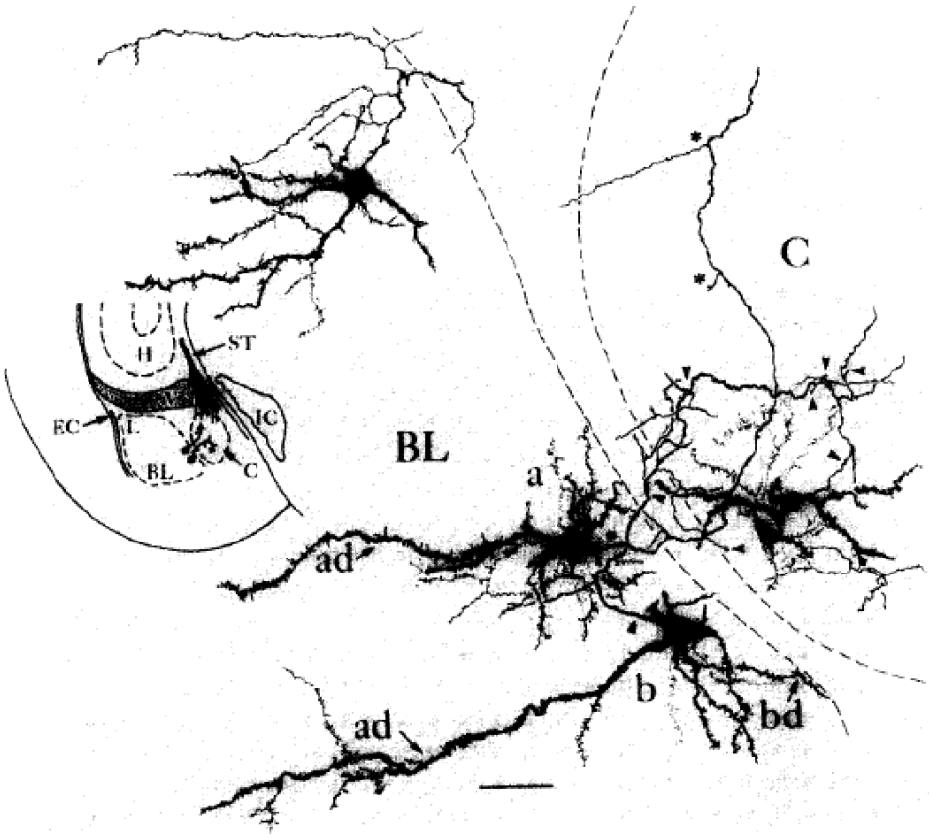

Figure 5.

A single, oblique transverse section. 8-day-old mouse, a and b: pyramidal cells in BL. Asterisks: the origin of the axon of cell a and its collaterals in C. Arrowheads: the origin of the axon of cell b and its collaterals and boutons en passant in C. The filled circles in the inset drawing indicate the location of cells a and b in BL. The arrows leading from the filled circles summarize the course of the two axons. BL, basolateral nucleus: C, central nucleus; EC, external capsule; H, hippocampus; IC, internal capsule: L, lateral nucleus: ST, stria terminalis: ad, apical dendrite; bd, basilar dendrite. Bar = 50 #m (Smith and Millhouse, 1985)

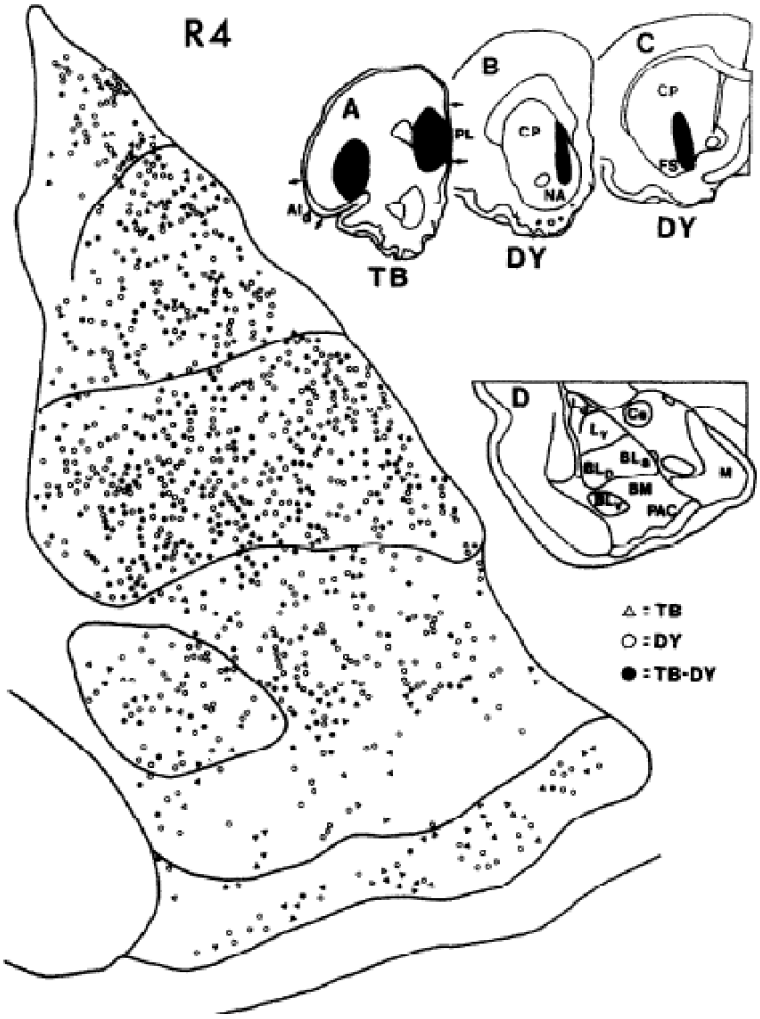

Figure 6.
Drawing illustrating the results of case R4. Injections of TB were made into the PL of the medial PFC and the AI, of the lateral PFC (A). Injections of DY were made into the nucleus accumbens (NA) and fundus striati (FS) (B, C). The distribution of amygdaloid neurons that were double-labeled (closed circles), single-labeled by TB triangles, or single-labeled by DY open circles (McDonald, 1991).

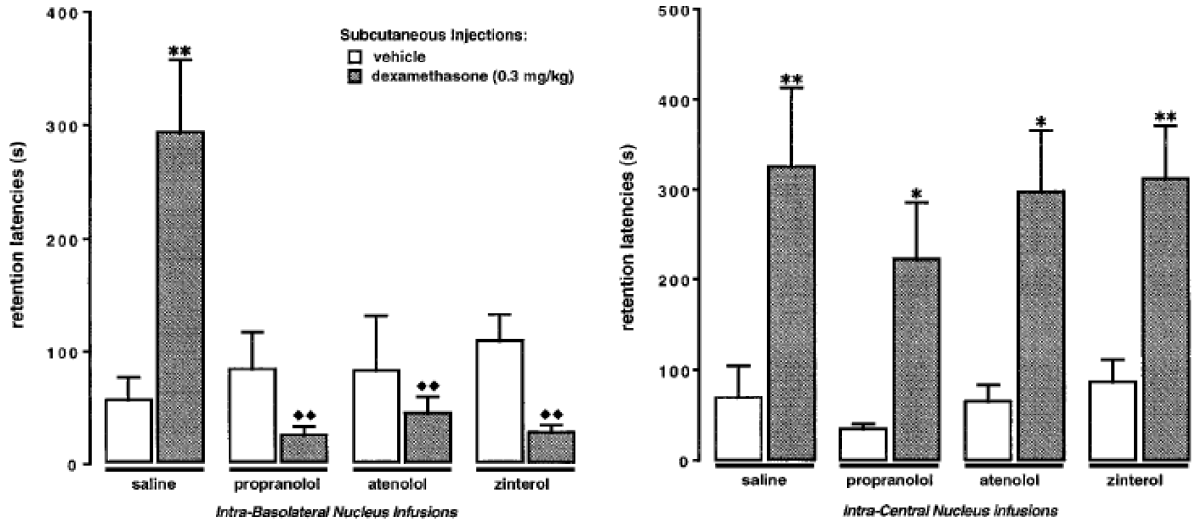

Figure 7.
Inhibitory avoidance retention latencies of animals that received pretraining infusions of either the nonspecific b-adrenergic antagonist propranolol (0.5 mg), the b1-adrenergic antagonist atenolol (0.5mg), or the b2-adrenergic antagonist zinterol (0.5mg) into the BLA or CEA and immediate posttraining subcutaneous injections of dexamethasone (0.3 mgykg). Bars represent mean (6SEM) latency in seconds. p, P , 0.05; pp, P , 0.01 as compared with the corresponding vehicle group; rr, P , 0.01 as compared with the vehicledexamethasone group (n 5 8–14group).

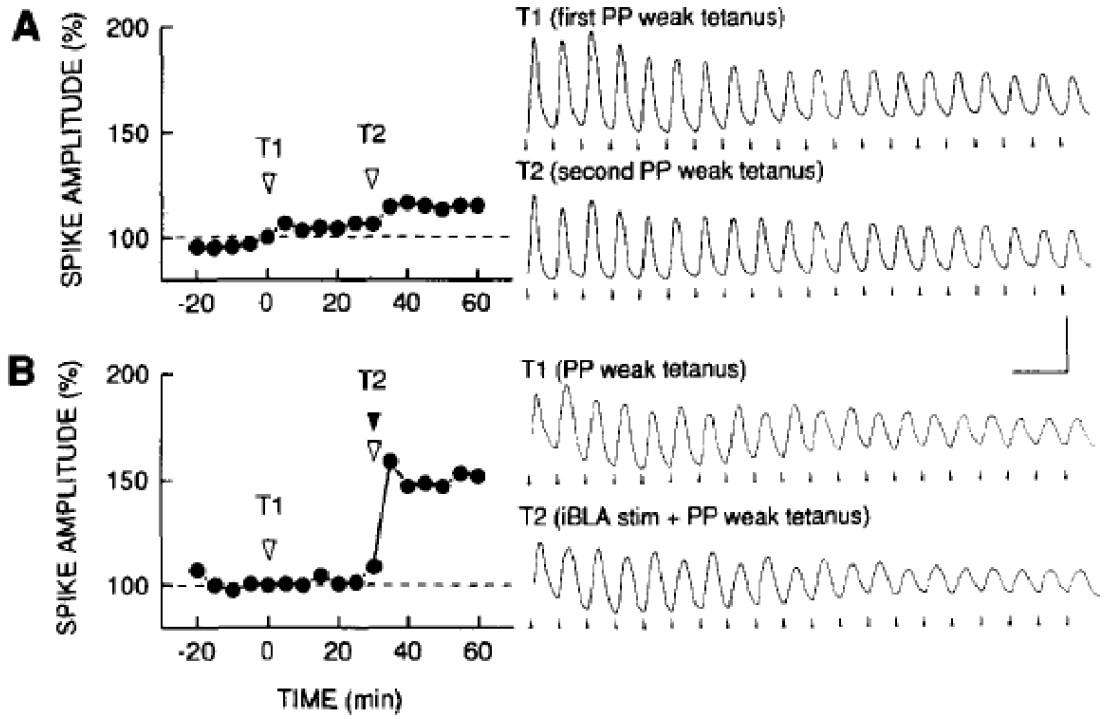

Figure 8.
Effect of the ipsilateral BLA stimulation on synaptic responses during PP weak titanic stimulation. The PP weak tetanic stimulation (20 pulses at 20 Hz) was repeatedly applied within the same rat, and the synaptic responses during the first and second tetani were compared. A: the PP weak tetanus was applied twice at an interval of 30 min (TI and T2). In the example shown here, the responses seem to be slightly potentiated after T2, but the changes are not statistically significant. The mean population spike amplitude 30 min after T2 of five rats was 107.7 4- 2.6%. B: at the first trial (T l, 0 min), the PP weak tetanus alone was applied, and at the second trial (T2, 30 min), the ipsilateral BLA stimulation (100 pulses at 100 Hz) and the PP weak tetanus were simultaneously applied. The right traces are the synaptic responses during TI and T2. Calibration bars: vertical 5 mV, horizontal 100 ms (Ikegaya, Saito, Kazuho and Abe, 1995).

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