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Animal studies of amygdala function in fear and uncertainty: Relevance to human research

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Abstract

This article reviews research in both animals and humans on the considerable progress made in elucidating a brain circuitry of fear, particularly the importance of the amygdala in fear conditioning. While there is considerable agreement about the participation of the amygdala in fear in both animals and humans, there are several issues about the function of the amygdala raised by the human research that have not been addressed by or may be answered by animal research. Three of these are addressed in this article: (1) is the amygdala involved in or necessary for both fear learning and unconditioned fear? (2) Does the amygdala code for intensity of fear? (3) Is the amygdala preferentially involved in fear, or is it also activated when there are no overt fear or aversive stimuli, but where the situation can be described as uncertain? We present evidence indicating that the rodent amygdala is involved in some types of fear (conditioned fear), but not all types (unconditioned fear), and may therefore have significance for a differential neurobiology of certain anxiety disorders in humans. Further, similar to the human amygdala, the rodent amygdala responds to varying intensities of aversive stimulation. Finally, it is suggested that, similar to humans, the rodent amygdala is involved in the evaluation of uncertainty. We conclude that progress on elucidating the role of the amygdala in fear is facilitated by corroboration of findings from both animal and human research.

Keywords: Amygdala; Fear; Fear conditioning; Unconditioned fear; *egr-1*; Predator; Uncertainty

In the last several decades, great strides have been made in understanding neural and psychological mechanisms of fear. There has been an intense research effort to study fear in both animals and humans at all levels of inquiry—neuroanatomy, cellular biology, physiology, pharmacology, behavior, psychology, and cognitive neuroscience. The neural and psychological processes during times of fear, or during encounters with fear-associated stimuli, have shown remarkable consistency across both human and animal research. For example, the amygdala has been found to be central to psychological processes such as the perception of fear in humans and the generation of learned fear behavior in animals ranging from rodents to monkeys to humans. However, there is still much to be learned about the basic mechanisms of fear that will undoubtedly have important implications for understanding normal and psychopathological fear, anxiety and depression. In general, findings from animal research inform and direct questions for investigation in humans. Case in point, animal research has consistently demonstrated that the amygdala is crucial for learning and memory in fear conditioning paradigms; subsequent research in humans indicates that the amygdala parallels this role in human fear (Dolan, 2002; LaBar et al., 1995, 1998; Phelps and Anderson, 1997). In a complementary fashion, human research also informs and facilitates questions for animal research. While the parallels in rodent and human fear conditioning are quite remarkable, there are some issues raised by research in humans about the role of the amygdala in fear that remain unanswered or not demonstrated in rodents, but rodent research may help answer some of the issues. These include: (1) the role of the amygdala in learned vs. non-learned fear; (2) whether the amygdala codes for intensity of fear; and (3) if the amygdala is activated in uncertain situations that do not reach the level of overt fear. This paper intends to address these issues about the nature of the amygdala's role in fear raised by human neuroimaging research that we think can be answered by research in animals. These issues will be addressed following a brief review of rodent, non-human primate, and human research on the amygdala, and then a review of the general role of the

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amygdala in fear. We will then address the questions raised by the human research and provide some new data derived from rodent research using amygdala lesions and gene expression in conditioned and unconditioned fear paradigms to help clarify the role that the amygdala plays in the perception, learning and expression of fear.

1. Neuroanatomy of the rat amygdala

The amygdala is comprised of 13 nuclei each having numerous subnuclei (Pitkanen, 2000). From numerous neuroscience methods, some of these are known to be important for conditioned fear (Davis and Whalen, 2001; LeDoux, 2000; Maren and Quirk, 2004; Rodrigues et al., 2004). These include the basolateral complex (BLA; consisting of the lateral, basal and accessory basal nuclei) and the central nucleus of the amygdala (CeA; subdivided into the capsular, lateral and medial divisions). These nuclei sit at an interface between sensory input and motor output important for learning and memory of fear and behavioral responses to fear (Fig. 1). The nuclei of the BLA are richly innervated by neocortical and subcortical uni- and polymodal sensory regions and receive most of their sensory input from the thalamus and cortex. Within the BLA, the lateral nucleus receives auditory and visual information. Input from the hippocampus and prefrontal cortex innervates in varying degrees all nuclei of the BLA. The flow of information through the BLA is primarily from lateral to medial aspects, however with extensive intra-amygdala communication and reciprocity (Pitkanen et al., 1997). The BLA's major role in fear is thought to be in the evaluation of sensory

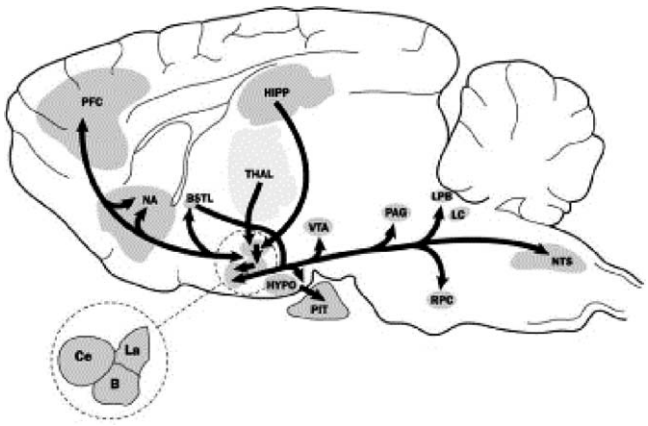

Fig. 1. Schematic drawing of a neuroamatomical circuit of fear. Major areas and pathways are described but not all are included. The amygdala (circle with the lateral (La), basal (B), and central (Ce) nuclei labeled) plays a central role in the circuit. As explained in the text, the lateral nucleus receives most sensory input via the thalamus (THAL) and cortex. The basal nucleus receives input from the lateral nucleus, hippocampus (HIPP), and cortex. The basal nucleus also sends efferents to the lateral portion of the bed nucleus of the stria terimalis (BSTL), nucleus accumbens (NA), and prefrontal cortex (PFC). The central nucleus receives input form the lateral and basal nuclei and has extensive output to diencephelon, midbrain and brainstem. This includes the hypothalamus (HYPO), ventral tegmental area (VTA), periaqueductal gray (PAG), lateral parabrachial nucleus (PB), locus ceureleus (LC), reticularis pontis caudalis (RPC), and the nucleus of the solitary tract (NTS). As can be seen many of these areas send reciprocal connects to the amygdala.

information in the dimensions of emotional valence, vigilance and arousal (Davis and Whalen, 2001; Rosen and Schulkin, 1998) that then influences other amygdala nuclei and brain regions for integrated responses to fearful stimuli. The lateral nucleus projects to the basal and accessory basal nuclei of the complex, and has direct efferent projections to the CeA as well. The basal and accessory basal nuclei also project to the CeA. The CeA, in turn, sends extensive projections to numerous nuclei in the midbrain and brainstem to orchestrate the rapid and primary behavioral, autonomic and endocrine responses to threat and danger (Davis, 1992; Holstege, 1995). The cholinergic basal forebrain neurons that project to wide areas of cortex also are innervated by the CeA allowing for the amygdala to influence arousal and multimodal sensory processing at the level of the cortex (Davis and Whalen, 2001; Kapp et al., 1992). In addition, the prefrontal cortex innervates the CeA to modulate expression of already learned behavior (Quirk et al., 2003). The CeA also receives visceral information from brainstem sites that include the solitary and parabrachial nuclei (Ricardo and Koh, 1978) and reciprocally projects to these brainstem regions. Thus, in addition to the CeA's pivotal role in producing an integrated behavioral fear response, autonomic/visceral information can also influence amygdala activity through the CeA.

Another output pathway of the amygdala important for fear is a direct basal nucleus efferent to the nucleus accumbens. Identification of this pathway led Nauta and Domesick (1984) to suggest an anatomical route by which motivation and motor control are linked in organized active behavior (also see, Amorapanth et al., 2000; Gray, 1999; Swanson, 2000; Swanson and Petrovich, 1998; Yim and Mogenson, 1982). Thus, the CeA, via its projections to lower brain, orchestrates reactive behavioral, autonomic and endocrine responses to fear, while efferents of the basal nucleus of the amygdala participate in active avoidance behaviors to fear (Amorapanth et al., 2000), likely through nucleus accumbens, striatum and thalamus. Furthermore, in addition to indirect regulation of hypothalamic–pituitary–adrenal axis by the CeA through connections of the bed nucleus of the stria terminalis, the medial nucleus of the amygdala was shown recently to regulate endocrine responses via direct projections to the hypothalamus (Dayas et al., 1999).

2. Lesions of the primate amygdala and fear

The amygdala has been linked to the emotion of fear since the pioneering studies of Kluver and Bucy in the late 1930s (Kluver and Bucy, 1939), who found that monkeys with anterior temporal lobe lesions (including the amygdala, hippocampus and surrounding cortices) had several changes in behavior and psychological processing. These changes included visual agnosia, hypermetamorphosis (increased examination of objects), hypersexuality, tameness, and reduced fearfulness. Additional research found that monkeys with lesions of the anterior temporal lobe displayed inappropriate social behavior leading to an inability to maintain dominance in the colony hierarchy, increased social isolation and repeated attacks by other monkeys (Kling and Brothers, 1992). Subsequent studies

demonstrated that surgical or cytotoxic lesions restricted to the monkey amygdala produced the tameness and a reduction in fear (for review, see Aggleton and Young, 2000). A recent study of chemical lesions of the amygdala that destroyed cells, but not axons passing through the amygdala, demonstrated that trait-like anxiety remained normal in lesioned monkeys but acute fear and vigilance to a snake and unfamiliar threatening conspecifics were significantly diminished (Kalin et al., 2001). Thus, studies consistently demonstrate that lesions of the non-human primate amygdala diminish behavioral displays of fear and vigilance to threat, and suggest that the amygdala functions in evaluation of information related to threat and danger.

An early seminal study by Weiskrantz (1956) demonstrated that monkeys with amygdala lesions had a slower rate of acquisition of aversive learning (avoidance) and rapid extinction of conditioned avoidance. Weiskrantz concluded that the amygdala is not responsible for fear, per se, or emotion in general, but is critical for assessing the reinforcing properties of stimuli (both positive and negative), acquiring response contingencies, and forming associations between neutral and emotionally significant unconditioned stimuli. Subsequent amygdala lesion studies led to the same conclusions (Aggleton, 1993; Aggleton and Passingham, 1981).

Similar to non-human primates, lesions of the human amygdala have demonstrated that the amygdala is important for a number of emotions, but particularly for fear. Numerous studies in humans following removal of the amygdala, primarily for intractable epilepsy, have reported deficiencies in recognizing negative emotional stimuli such as facial expression (Adolphs et al., 1998, 1999; Anderson and Phelps, 2000a; Broks et al., 1998; Sato et al., 2002). Calcification of the amygdala as a result of Urbach-Wiethe disease has also demonstrated that these patients without a functional amygdala lack the ability to identify and recreate facial expressions of fear (Adolphs et al., 1994, 1995) and other emotions (Siebert et al., 2003). Furthermore, a patient with Urbach-Wiethe disease and two other patients with encephalitis damaging the amygdala and the surrounding area identified threatening faces as trustworthy (Adolphs et al., 1998). Data from these patients and others with removal or damage to the amygdala suggest that the human amygdala is critical for linking facial representations to the emotion of fear (Adolphs et al., 1995; Anderson and Phelps, 2000a). Classical fear conditioning in humans with either unilateral or bilateral amygdala damage show impaired conditioned responding, as measured by skin conductance responses (Bechara et al., 1995; LaBar et al., 1995). Therefore, the human amygdala may be responsible for producing behavioral responses to threat as well as involved in production of the perception and subjective experience of fear.

3. Human brain imaging and the amygdala

In the 1990s the use of human brain imaging technology (e.g., PET, fMRI) to explore the function of the amygdala exploded and continues at a rapid rate today. Activity in the amygdala is predominantly measured during two basic paradigms: fear conditioning and presentation of faces, but has expanded to other emotional stimuli of various modalities. Fear conditioning has confirmed the human amygdala is activated during fear learning. LaBar et al. (1998) found that increases in amygdala activity (fMRI) were limited to early acquisition and early extinction of fear conditioning to a visual stimulus, indicating a role for the amygdala in encoding situational emotional meanings. Findings of other studies support a role for the amygdala in the acquisition of fear (Buchel and Dolan, 2000; Buchel et al., 1998; Cheng et al., 2003; Knight et al., 2004; Morris et al., 2001). Interestingly, however, is the finding that activity in the amygdala is limited to early conditioning or early extinction, when response contingencies change (Knight et al., 2004). Whalen (1998) has credited the amygdala with interpreting situations that contain some ambiguity or uncertainty, supported by the finding that activity within the amygdala, as measured by fMRI, habituates after subsequent presentations of stimuli-response contingencies. This parallels electrophysiological recordings in the lateral nucleus of the amygdala during fear conditioning in the rat (Quirk et al., 1995, 1997), and suggests that the amygdala is involved in learning new associations about danger or threat. An additional parallel between human (fMRI) and animal fear conditioning (electrophysiology) is that the amygdala is preferentially activated by conditioned stimuli that are paired with an aversive stimulus (CS+) compared to a lack of activation during a non-conditioned stimulus (CS−) (Buchel et al., 1998; LaBar et al., 1998).

These imaging studies complement findings demonstrating a lack of fear conditioning in humans with lesions of the amygdala (Bechara et al., 1995; LaBar et al., 1995). Interestingly, humans with amygdala lesions can describe the conditions of the contingencies (e.g., blue light will be followed by aversive stimulus, but red light will not), but do not display signs of emotional learning about the stimuli (Bechara et al., 1995). This suggests that the mechanisms of emotional learning can be independent of consciousness (Damasio, 1994; Dolan, 2002; LeDoux, 1996).

Aside from the role of the amygdala in acquiring stimulus contingencies, the amygdala may also be responsible for generating behavioral responses to aversive stimuli. Cheng et al. (2003) found that amygdala activity, as measured by fMRI corresponded to conditioned skin responses during fear conditioning trials. This finding in humans lends support to literature in the rodent amygdala, which finds that the amygdala, specifically the central nucleus, generates conditioned autonomic, behavioral, and endocrine responses (Goldstein et al., 1996). How closely the functional anatomy of the rat amygdala matches the human remains to be revealed.

The second major neuroimaging paradigm for studying the human amygdala is presentation of emotional faces. Many studies demonstrate that the amygdala is preferentially activated during presentation of faces expressing fear. In the majority of studies the amygdala was activated preferentially to fear faces but not happy or neutral faces (Breiter et al., 1996; Whalen et al., 2001); however others find no preference for fear faces (Stark et al., 2003; Winston et al., 2003). The use of other aversive stimuli, such as odors, tastes, and words, or recall of

aversive films, stories and pictures all activate the amygdala (Davis and Whalen, 2001). Most interesting is the finding that when subjects are unaware of seeing the fear faces, by use of a masking protocol, the amygdala still is activated or actually more or differentially activated than when the faces are not masked and the subject is conscious of seeing the faces (Anderson et al., 2003a; Morris et al., 1998; Whalen et al., 1998). This suggests that one role of the amygdala is to detect or appraise stimuli as aversive with preattentive or unconscious processing (Dolan, 2002). Indeed, anatomical studies of the amygdala in rodents have found that incoming sensory information travels to the amygdala via two distinct pathways: a short, thalamic pathway, and a longer, cortical pathway (Romanski and LeDoux, 1993). It has been proposed that the short pathway from the thalamus to the amygdala prepares an animal for a potentially aversive encounter without the need of conscious processing, which occurs later via the cortical route (LeDoux, 1996). It is likely, then, that there exists two pathways in humans similar to the pathways in the rat, and that this thalamic pathway is responsible for detecting fearful expressions in the masking paradigm. Morris et al. (1999) have found evidence for this in the human brain using a PET scan of the amygdala during presentation of fearful faces using a masking paradigm.

Many subjects viewing fear faces explicitly describe the facial expression as fear; yet, they report that they are not afraid of the faces (Whalen, 1998). This suggests that activation of the amygdala does not necessarily induce the emotion of fear, but the amygdala is involved in the perception of facial expressions of fear. Thus, there is a disconnect between behavior, subjective feeling and perception of fear. These findings have led to some interesting conceptual ideas about the function of the amygdala during fear and vigilance. Whalen (1998) has argued that while the amygdala responds to strong emotional stimuli, it also responds to more subtle fear stimuli (pictures of fear faces); therefore, the function of the amygdala may be conceptualized as an affective information processor or relevance detector (Sander et al., 2003). Hence, the amygdala would be important for encounters with stimuli that are ambiguous in their threatening properties to determine if the stimuli are truly dangerous. In this respect, the amygdala would process the stimuli for threat and also, via its extensive connections to systems involved in arousal and vigilance, prepare one for defense.

Human neuroimaging of the amygdala has produced data that largely supports findings from rodent research. The strongest parallel is that both human and animal research indicates that the amygdala is critical for acquisition in fear conditioning paradigms. However, there are several issues about the function of the amygdala raised by the human research that have not been addressed by, but may be answered by, animal research. We would like to address three of these issues by posing the following questions:

1. Is the amygdala involved in or necessary for both fear learning and unconditioned fear?
2. Does the amygdala code for intensity of fear?
3. Is the amygdala preferentially involved in fear, or is it also involved in analysis of potentially threatening situations where there are no overt fear or aversive stimuli, but where the situation can be described as uncertain?

We will discuss these questions and then provide some data that may help resolve the questions derived primarily from rodent research in our laboratory using amygdala lesions and gene expression techniques with conditioned and unconditioned fear paradigms.

4. Is the amygdala involved in fear learning and unconditioned fear?

Neuroimaging studies with normal humans and studies of humans with amygdala lesions reliably demonstrate that the amygdala is critical for emotional learning in fear conditioning paradigms (Buchel and Dolan, 2000; Davis and Whalen, 2001; Dolan, 2002; Phelps and Anderson, 1997). Other studies employing non-learning paradigms suggest that the human amygdala is critical for evaluation or interpretation of fear in a number of modalities (for review, see Zald, 2003), whether it is learned or not learned. However, a few studies have questioned whether the amygdala is critical for normal evaluation of facial expressions or whether amygdala damage in humans produces a global deficit in evaluation of emotional stimuli (Anderson and Phelps, 2002; Phelps and Anderson, 1997). For instance, in some studies, humans with amygdala lesions or damage have intact dispositional affect (Anderson and Phelps, 2002), normal subjective, and autonomic responses to affective stimuli (LaBar et al., 1998; Tranel and Damasio, 1989), and unimpaired affective evaluation of negative emotional scenes and words (Cahill et al., 1995; Hamann et al., 1997). Furthermore, meta-analyses of human brain structures activated during emotional states or emotional stimulus presentation find that fear stimuli are associated with amygdala activation; however, there are still many studies that do not demonstrate this relationship (Murphy et al., 2003; Phan et al., 2002). Taken together, results suggest that neural systems that do not include the amygdala may analyze and orchestrate behavioral responses to fear stimuli and negative affect. An interesting notion derived from these failures to demonstrate emotional deficits in amygdala damaged humans is that the age of amygdala injury may be a critical variable (Anderson and Phelps, 2000b; Hamann et al., 1996), with earlier damage causing more severe deficiencies in evaluating emotional stimuli (Anderson and Phelps, 2000b). This suggests that the amygdala may be involved in emotional learning, and those patients with the earliest damage would display the most severe deficits in emotional evaluation (Phelps and Anderson, 1997) because they would have less experience and opportunity to learn to evaluate emotional stimuli before damage to the amygdala occurred. Another interpretation is that the amygdala is involved in emotional learning and memory, but not some unlearned or unconditioned emotion. Whether the amygdala is involved in the learning of emotion but not unconditioned emotion is, of course, very difficult to address in humans because exposure to emotional stimuli and learning

occur throughout life, while testing is done without control of earlier learning or exposure. This however can be addressed in rats raised in the laboratory with no previous exposure to unconditioned fear stimuli. Comparison of the role of the amygdala in conditioned and unconditioned fear is addressed in the following sections.

4.1. Fear learning and memory in rodents

Extensive research in rats on the role of amygdala in fear has delineated clear roles for certain nuclei of the amygdala in the learning and memory of fear. Lesions of the BLA in the rat provide convincing evidence that the lateral and basal nuclei not only process incoming sensory information, but may play a role in the learning and memory of fear conditioning as well. Lesions of the basolateral amygdala up to a year and a half following fear conditioning abolish memory for the conditioned fear (Gale et al., 2004). Lesions of the basolateral amygdala do not produce a specific performance deficit, as overtraining procedures consisting of numerous footshocks will produce fear behavior in lesioned rats that is comparable to rats with intact amygdalae (Cahill et al., 2000; Kim and Davis, 1993; Maren, 1998). Therefore, it is likely that the amygdala is involved not only in the learning of fear, but may act as a permanent storage site for encoding details about an aversive experience (however see Cahill et al., 1999).

In addition to lesions, imaging methods in rodents demonstrate that the amygdala is activated during fear conditioning (electrophysiological recordings also indicate amygdala activation). Postmortem imaging techniques, like gene expression determine the relative activation of neurons in specific brain regions following experimental manipulations by imaging a class of genes, and their protein products, that are very reactive to environmental manipulation (Dragunow, 1996). This class of genes is called immediate-early genes, as they are expressed within minutes of an environmental change in brain regions known to be important for the particular experimental manipulation (for review, see Herdegen and Leah, 1998). The mRNA of these immediate-early genes is induced rapidly and their expression returns to baseline, on average, within an hour. The protein expression begins within 30 min and may last for a couple of hours. Thus, a regionally specific neural record of activity shortly after experimental manipulation can be used to determine possible brain structures and patterns of brain activity important for information processing and behavior. The technique of gene expression imaging in rats is therefore analagous to human functional neuroimaging methods in that activation in discrete areas of brain can be correlated with particular events, stimuli or psychological processes.

We have demonstrated that one of these immediate-early genes, early growth response gene 1 (*egr-1*) is induced by fear conditioning specifically in the lateral nucleus of the amygdala shortly following training (Malkani and Rosen, 2000b; Rosen et al., 1998) (Fig. 2). The lateral nucleus is a site of plasticity during fear learning and possibly site of storage for some aspects of fear memory (Blair et al., 2001; Fanselow and LeDoux, 1999; Rodrigues et al., 2004). Rats that were extensively handled and exposed to the conditioning context, but not fear conditioned, did not display fear behavior or increased *egr-1* in the amygdala (Malkani and Rosen, 2000b; Rosen et al., 1998). Additionally, rats that received footshock temporally arranged so no fear conditioning occurred had much lower levels of *egr-1* expression in the amygdala than the fear conditioned rats (Malkani and Rosen, 2000b; Rosen et al., 1998). These data suggest that while an unconditioned aversive stimulus can increase expression in the amygdala similar to that shown in humans (Morris et al., 2001), fear learning induces significantly more robust expression of *egr-1* gene expression. Other research has found that other immediate-early genes (i.e., *c-fos*) also increase in the amygdala following fear conditioning (Hall et al., 2000; Holahan and White, 2004; Malkani and Rosen, 2000a; Radulovic et al., 1998; Ressler et al., 2002).

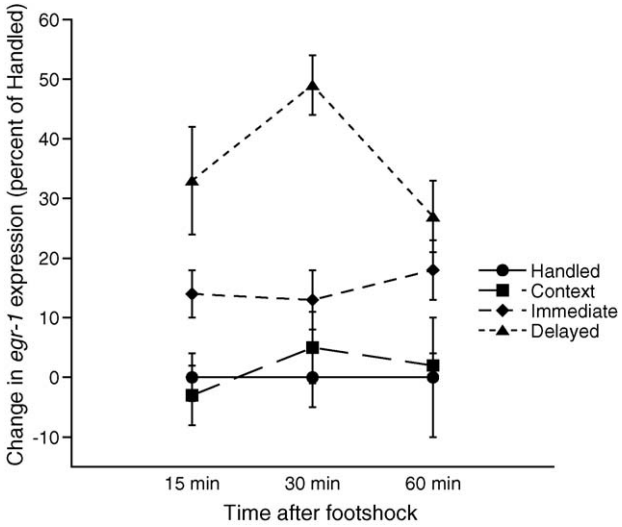

Fig. 2. *egr-1* mRNA is increased in the La following fear conditioning. *egr-1* is significantly increased in the delayed-shock group (the only group demonstrating conditioned fear) compared to the other groups. Expression peaked at 30 min after initiation of learning. Expression is also increased in the immediate-shock group (group received footshock immediately after being placed in training chamber, but did not display any fear conditioning) compared to the handled and context groups that did not receive footshocks suggesting that footshock stress also increases *egr-1* in the LaDL, but not as much as fear conditioning did. (Adapted from Malkani and Rosen, 2000b.)

4.2. Unconditioned fear

The animal literature is also beginning to find nuclei of the amygdala that are important for fear conditioning may not be involved in unconditioned, innate fear. Animal research can address the question about unconditioned fear because rats can be raised in the laboratory without any experience with unconditioned, innate fear stimuli. Most studies use exposure to a predator, usually a cat, or exposure to predator odor as an unconditioned fear stimulus. These are used because they can induce a state of fear without inflicting physical pain.

Initial studies demonstrated that very large and extensive lesions of the amygdala disrupt fear responses to cat exposure

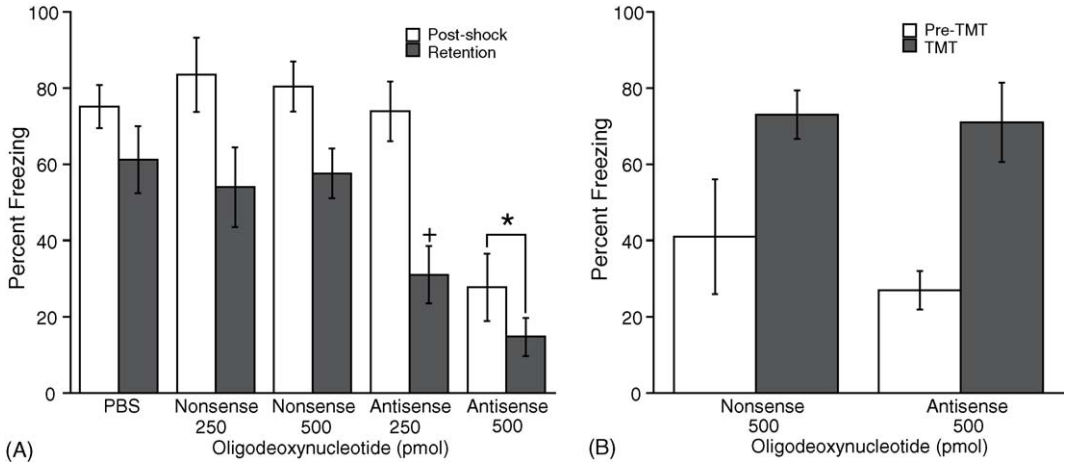

Fig. 3. *egr-1* antisense oligodeoxynucleotide infused into amygdala blocks fear conditioning, but not unconditioned freezing to fox odor. (A) Two doses of the antisense are compared to the vehicle (phosphate buffered-saline (PBS)) and a nonsense oligodeoxynucleotide with the same nucleotides as the antisense but in a scrambled order. The smaller dose of the antisense (250 pmol) significantly reduced freezing in the retention test conducted 24 h after the injections and fear conditioning compared to the control groups (+). The 500 pmol dose interfered with freezing both in the post-shock and retention test compared to the control groups (*). The data indicate that inhibiting *egr-1* activity in a dose-related manner within the amygdala can specifically block long-term memory of fear. (B) A 500 pmol dose of the *egr-1* antisense did not interfere with unconditioned freezing to TMT suggesting that shock-induced freezing and fear conditioning are regulated by *egr-1*, but unconditioned freezing is not. (Adapted from Malkani et al., 2004.)

(Blanchard and Blanchard, 1972; Fox and Sorenson, 1994). However, more recent studies with smaller lesions or temporary inactivation of discrete amygdala nuclei (lateral, basal, or central nuclei) do not find major deficits in the ability to respond to predator odors (Fendt et al., 2003; Li et al., 2004; Rosen, 2004; Wallace and Rosen, 2001). Additional studies also find that a brightly lit test chamber generates unconditioned fear that is not diminished by lesions of the central nucleus of the amygdala (Walker and Davis, 1997). Interestingly, lesioning and inactivating other nuclei in the amygdala or extended amygdala that are not part of the fear conditioning circuit, that is, the medial nucleus of the amygdala and bed nucleus of the stria terminalis, appear to disrupt unconditioned fear to a predator odor (Fendt et al., 2003; Li et al., 2004).

Interestingly, lesions of the basolateral complex, lateral nucleus or central nucleus of the amygdala typically produce a small (about 15–20%), but significant, reduction in fear-related freezing to predator odor (Rosen, 2004; Vazdarjanova et al., 2001; Wallace and Rosen, 2001). This suggests that there is a small component of fear to predator odor that is modulated by nuclei typically associated with fear learning. Thus, fear to predator odor may not be wholly an unconditioned fear, but there may be a smaller conditioning element that engages the amygdala. Teasing out contributions of neural circuits of conditioned and unconditioned fear may have importance for our understanding of these two types of fear and their interaction (Mineka and Ohman, 2002; Rosen, 2004).

Imaging of gene expression also demonstrates that the amygdala nuclei important for fear conditioning do not ‘‘light up’’ following exposure to a cat or predator odors (Day et al., 2004; Dielenberg et al., 2001; McGregor et al., 2002, 2004; Rosen et al., 2005). One study also found that inhibition of *egr-1* in the amygdala by injection of an antisense oligodeoxynucleotide reduced fear conditioned freezing behavior, but did not diminish unconditioned freezing to a predator odor (Malkani et al., 2004) (Fig. 3). Other studies suggest that the central nucleus of the amygdala, while necessary for conditioned fear, is not crucial for fear behavior to unconditioned stimuli like predator odors and brightly lit environments (Fendt et al., 2003; Li et al., 2004; Rosen, 2004; Walker and Davis, 1997). Taken together, the lesion, inactivation, and gene expression data indicate the amygdala circuitry critical for learning and memory of fear may not be necessary for expression of unconditioned, innate fear. However, other amygdala nuclei (such as the medial nucleus, bed nucleus of the stria terminalis) and circuitry through the amygdala and related structures may be involved in innate fear (Fendt et al., 2003; Li et al., 2004; Rosen, 2004; Walker and Davis, 1997). In addition to theoretical implications for differential fear learning and non-learned fear circuitries may have on ideas of functional evolution and development of brain fear and affective systems, this dissociation may have practical significance for different types of anxiety disorders. Activation of the amygdala is associated with post-traumatic stress disorder, which has a strong learning component, while activation is not seen with specific phobias of animals (e.g., spiders), which may be more related to unconditioned, innate fear (Rauch et al., 2003), however see (Carlsson et al., 2004). If these distinctions are further substantiated, they may have profound implications for therapeutic approaches to these disorders.

5. Does the amygdala code for intensity of fear?

Emotional stimuli carry at least two types of information: valence and intensity. While the strongest support of the role of the amygdala has been with the negative emotion of fear, there is now sufficient evidence that the amygdala is also involved in positively valenced emotion in both humans and animals (Baxter and Murray, 2002). Recent studies in humans also suggest that the amygdala codes for intensity of emotion, but

possibly not valence (Anderson et al., 2003b; Small et al., 2003). Two recent studies in humans, one using odor and the other taste as emotional cues, found that the fMRI signal in the amygdala increased with the subjective increase in intensity of the stimuli, but did not change as the positive and negative qualities of the stimuli were varied (Anderson et al., 2003b; Small et al., 2003; however, see Winston et al., 2005). While the lack of change in the amygdala with perception of positive and negative cues appears to be counter to the view that the amygdala is involved in appraisal of emotional qualities of stimuli (Davis and Whalen, 2001), the increase correlated with intensity clearly aligns with the well documented role of the amygdala in arousal and modulation of learning and memory of emotionally arousing events (McGaugh, 2004). Further, increases or decreases in display of fear behavior thought to be dependent on the amygdala are routinely used to measure the strength of learning and memory in rodents (Fanselow and Bolles, 1979; Sigmundi et al., 1980). Nevertheless, changes in rodent amygdala activity associated with different levels of emotional intensity, as has been found in humans, are rarely investigated.

To test whether the rat amygdala displays varying degrees of activation that can be associated with different levels of fear, we measured gene expression of *egr-1* in the amygdala following a one-trial fear conditioning procedure with a no shock, low shock and high shock condition. Rats were placed in the testing chamber for 3 min and then either given no shock or a single shock. Rats in the low-shock condition were given a 1 s, 0.6 mA footshock, while the high-shock condition rats were given a 1 s, 1.5 mA footshock. Post-shock freezing behavior was measured for 4 min and rats were sacrificed 30 min after the shock. The brains were processed for expression of *egr-1* mRNA in the lateral nucleus of the amygdala. Shown in Fig. 4, rats displayed more freezing as the footshock current increased. Furthermore, *egr-1* expression in the lateral nucleus also increased with stronger footshock.

The results of this experiment can be interpreted in at least two ways. First, because fear (freezing behavior) appears to increase with increasing amounts of shock and the activation of the amygdala corresponds to the increase in fear, the result is a demonstration that the amygdala codes for intensity of fear and arousal, just as the human amygdala does. Second, the amygdala can merely be responding to the amount of footshock delivered, thus the amount of gene expression is a measure of the intensity of the unconditioned stimulus, and not a measure of the level of fear the animal is experiencing. The first is a richer, more psychologically satisfying interpretation, whereas the second is a more parsimonious explanation but relegates the amygdala to a pain detection apparatus.

An interesting behavioral paradigm, the immediate-shock deficit paradigm, has been developed that may help to elucidate the function of the amygdala in this case. During contextual fear conditioning, if rats are given a few minutes to acclimate to the test chamber before a shock is given, as is typically done, the rats learn to be afraid of the test chamber. However, if rats are not given time to acclimate and a shock is given immediately upon being placed in the test chamber, the rats display a deficit in fear conditioning. When returned to the test chamber for a memory test, the rats do not display any fear behavior in the chamber. This immediate-shock deficit in fear learning has been explained as an inability to form a representation of the context with such as short pre-shock exposure to the contextual cues that there is no CS representation for an association with the shock to occur (Fanselow, 1986, 1990; Rudy et al., 2004). Thus, although the same amount of shock is given to rats receiving immediate shock as those receiving delayed shock, no learning occurs. An alternative explanation is that the US is processed differently in immediate-shock rats (Lattal and Abel, 2001).

Expression of *egr-1* in the lateral nucleus of the amygdala has been examined following the immediate-shock deficit paradigm (Malkani and Rosen, 2000b; Rosen et al., 1998). In comparison to rats given a single footshock after being in the test chamber for 3 min, immediate-shock rats have significantly less *egr-1* mRNA expression in the amygdala. Expression of *egr-1* increased about 50% in the fear-conditioned group compared to a handled control group, while expression increased only 15% in the immediate-shock group. Thus, although rats receive the same level of footshock in the two

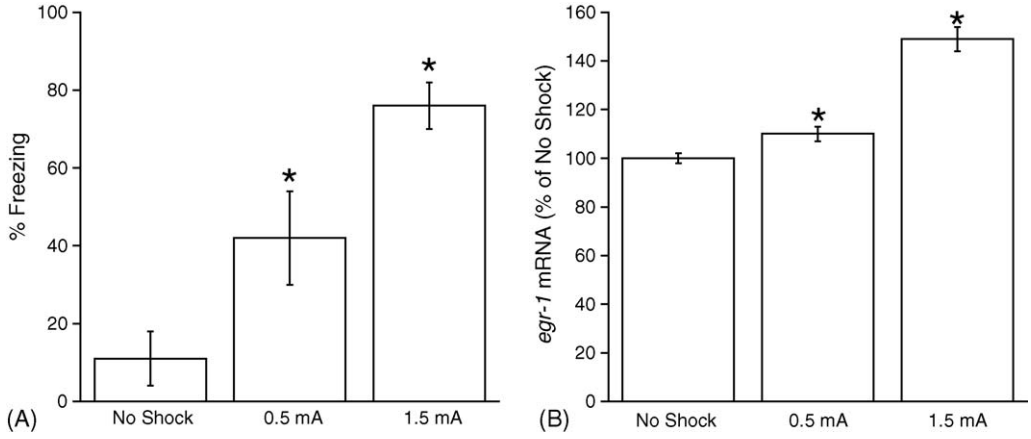

Fig. 4. Increased freezing and *egr-1* mRNA in the amygdala to more intense footshock. (A) Rats freeze more with increased current of a 1-s footshock. (B) Increases in *egr-1* mRNA expression in the lateral nucleus of the amygdala with increased current of a 1-s footshock. Expression was measured 30 min after footshock. (*) Denotes group is statistically different from respective no shock group.

groups, amygdala *egr-1* is more robustly increased in the fear-conditioned rats compared to those showing no learning. This demonstration strongly suggests that the lateral nucleus of the amygdala is not merely responding to footshock, but to the contingencies that support fear learning and memory. Furthermore, the experiments with different levels of shock and the immediate-shock deficit paradigm suggest that the amygdala cannot be merely a pain detector or shock intensity indicator, but is likely involved in the processing of subjective levels of fear.

6. Does the amygdala detect uncertainty or ambiguity?

The previous two examples of amygdala activation in rats using gene expression as a measure of activity emphasized the amygdala's involvement in explicit conditioning of fear. However, the role of the amygdala is thought not to be limited and preferentially involved in processing fear, but to play a role in appraisal during times of uncertainty and heightened vigilance (Davis and Whalen, 2001; Rosen and Schulkin, 1998; Whalen, 1998). As indicated earlier, this notion has exquisitely been refined not simply in terms of novelty and unfamiliarity that warrant vigilance, but to the idea of ambiguity (Whalen, 1998). Whalen suggests that the amygdala is ''engaged most readily by biologically relevant, associative ambiguity, defined as learning situations in which stimuli have more than one possible interpretation, leading to more than one prediction of subsequent biologically relevant events'' (Whalen, 1998, p. 181).

In humans, fearful emotional faces may be an example of ambiguous stimuli (Whalen, 1998). Compared to angry faces, which provide information on both the presence and source of threat, fearful faces provide only the presence, but not the source, of threat. Fearful faces therefore are more ambiguous than angry faces because they provide less information to make appraisals about a probable threat. Both studies of humans with amygdala lesions and neuroimaging in intact humans demonstrate that the amygdala is more important for and engaged in the processing of fear faces compared to angry faces (Whalen et al., 2001). For example, amygdala lesioned humans have a severe deficit in identifying and responding emotionally to fearful faces, but still respond appropriately to angry faces (Adolphs et al., 1994). Further, in an fMRI study, the amygdala of normal humans responded more strongly to fearful faces than angry faces (Whalen et al., 2001). The hypothesis that the amygdala is required during times of ambiguous stimuli and events, when more information processing about biologically relevant stimuli is necessary, is supported by these studies.

A similar idea about the function of the amygdala that goes beyond the confines of fear, is the view of the ''amygdala as a 'relevance detector' would integrate the 'fear module' hypothesis with the concept of an evolved neural system devoted to the processing of a broader category of biologically relevant stimuli'' (Sander et al., 2003).

Another functional schema for the amygdala may be in terms of uncertainty. Uncertainty has been conceptualized as at least two types: expected and unexpected uncertainty (Yu and Dayan, 2003, 2005). Expected uncertainty can be defined as known unreliability of predictive cues within a context, whereas unexpected uncertainty occurs when unsignaled context switches produce strongly unexpected observations. These ideas of uncertainty may further refine the notions of the amygdala as an ambiguity or relevance detector, into a system that responds when in an environment or context where unexpected change occurs, but not necessarily in contexts when change is expected to occur.

Although several lesion and imaging studies in humans support the role of the amygdala in associative ambiguity or uncertainty (Whalen, 1998), there is little empirical data in the rat to support the hypothesis of the amygdala as an ambiguity or uncertainty detector. The rat amygdala does not seem to be necessary during exposure to unambiguous or certain fear stimuli as shown by studies demonstrating that gene expression in the amygdala fear circuit does not increase in response to predators and predator odors, and that lesions or inactivation of these nuclei do not disrupt fear responses to predator odors (Fendt et al., 2003; Li et al., 2004; Wallace and Rosen, 2001). However, recent gene expression research from our laboratory does begin to support the notion that the amygdala may, in fact, be engaged differentially in situations of expected and unexpected uncertainty.

While our data on gene expression indicate that the lateral nucleus of the amygdala is involved learning and memory in explicit fear conditioning situations (Malkani and Rosen, 2000b; Rosen et al., 1998), according to a postulated role of the amygdala in unexpected uncertainty, the amygdala should also be involved in assessment of environmental cues as one faces a new situation. In a typical fear conditioning experiment to assess gene expression, we handle our rats for a week or so to familiarize them to the experimental environment but without being placed in the testing chamber or receiving fear conditioning. Gene expression in rats that are only handled is very low and consistent. We also typically include a group of rats that is handled, and then on the experimental day, gets placed in the test chamber but does not receive footshock. These rats display gene expression levels in the amygdala that are not increased compared to the handled only group. Another group that receives fear-conditioning displays increased gene expression in the amygdala demonstrating that gene expression is related to fear learning. What is a bit curious about our results is that the second group that gets placed in a new environment but does not receive shock also does not have increased gene expression. If, according to the associative ambiguity hypothesis or unexpected uncertainty, being put in a new test chamber is possibly dangerous and should activate the amygdala. Indeed, a number of studies have found that gene expression does increase in the amygdala of rats and mice when placed in a new environment (Hall et al., 2000; Radulovic et al., 1998). Why some studies find placement in a new environment engages the amygdala and others do not is not clear.

Some new data (Donley and Rosen, submitted for publication) from our laboratory might clarify things. It was reasoned that if rats were handled in an environment with unpredictable changes in noise, they would be encountering many new, but non-threatening stimuli. These rats may therefore interpret additional

new experiences with expected uncertainty and respond as if they expect change. Thus, when put in a new chamber, they may expect that nothing threatening or harmful should happen. Conversely, if rats are handled in a quiet, static environment and acclimated to this quiet environment, then any change would not be expected and may signal the possibility of danger (i.e., unexpected uncertainty).

In our experiment to test this hypothesis, rats were handled for 1 week in either a very quiet room or in a room with a television playing a loud movie. On fear conditioning day, rats were divided into three groups: handled only, context only, or fear conditioned. Handled rats were sacrificed without testing. For the other two groups, testing was done in a quiet room with only a 70 dB white noise coming from an audio speaker in each test chamber. The context group was placed in the test chambers for 7 min and then sacrificed 30 min later. The footshock group received a 1 s shock 3 min after being placed the chambers and then remained in the chambers for an additional 4 min. The rats were sacrificed 30 min later. The brains were processed for *egr-1* expression in the amygdala.

The different handling regimens had no effect on freezing behavior, either in the context groups or footshock groups. As can be seen in Fig. 5, the footshock groups displayed high levels of fear as measured by freezing, whereas the context groups did not. However, in the measure of amygdala activity, gene expression was increased in the footshock groups as expected, but the context groups displayed differential activity in the amygdala. The group handled in the noisy environment displayed low levels of expression, as we have found before in this group (Malkani and Rosen, 2000b; Rosen et al., 1998). In contrast, handling in the static, quiet environment produced expression levels in the context group as large as the footshock group. Thus, the handling experience, either noisy or quiet, had a large effect on activation of the amygdala.

We believe that these experiments indicate that the amygdala is not only activated during fear conditioning, but also during times of unexpected uncertainty. The rats handled in the noisy environment experienced expected uncertainty, where known unreliable changes in noise was predictable, and therefore being placed in a new environment was just another change that did not activate the amygdala. In contrast, being placed in a new context following handling in the quiet environment was an unexpected change that activated the amygdala. Thus, our interpretation is, that in addition to the amygdala being important of learning about fear, the amygdala is involved in emotional processing of information of uncertain quality only when there is sufficient surprise associated with it (i.e., has unexpected uncertainty). While other interpretations are possible – the rats with experience in a noisy environment could be undergoing habituation, experiencing learned irrelevance or latent inhibition – the notion of unexpected uncertainty might be a rubric for a number of different phenomena, including novelty, unfamiliarity and ambiguity.

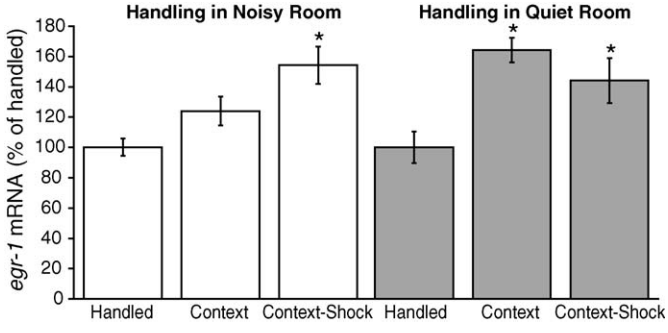

Fig. 5. Loud, unpredictable noise during handling compared to quiet handling reduces *egr-1* mRNA expression in the amygdala during exposure to a new environment. (*) Denotes group is statistically different from the respective handled group. Data from Donley and Rosen (submitted for publication).

7. Conclusions

In this article, we have described some parallels in the role of the human and rodent amygdala in fear. Fear conditioning experiments in both humans and rodents complement each other, where lesions and activity measures (PET, fMRI in humans; gene expression in rodents) demonstrate that the amygdala plays a crucial role in this type of learning. Other types of negative affect that are not explicitly learned (presentation of negatively valenced stimuli, affect disposition) are not consistently affected by amygdala lesions nor consistently induce activation in the amygdala of humans. We find converging evidence from lesion and activity measure studies of gene expression during both fear conditioning and the expression of unconditioned fear that suggest amygdala fear circuits are important for learning of fear and the expression of long-term memory of fear, but other circuits instantiate expression of unconditioned fear. Thus, the rodent experiments support the idea generated from human studies that the role of the amygdala is to learn about emotional stimuli, but other types of fear or mood may occur independently of the amygdala. The distinction between learned and unlearned fear may have importance for understanding the role of the amygdala in anxiety disorders, where it has been shown that the amygdala is highly activated by experiencing the particular anxiety or fear in PTSD, social phobic and panic disorder patients that has been learned (Bouton et al., 2001), but not in animal phobias that may be innate and not leaned (Rauch et al., 2003).

Rodent studies further complement data from humans indicating that the amygdala codes for intensity of the emotional perception or response. Higher intensity footshocks induce greater activation of the amygdala and also produce greater learning and memory of the fear conditioning. Whether the amygdala codes of emotional valence is not clear from the human data, but rodent studies suggest that the amygdala may code for both intensity and valence, and for both positive and negative emotional learning.

Finally, our studies in rodents suggests that, similar to human studies, the amygdala may not just be involved in fear learning, but also during determination of whether a stimulus or situation is threatening or dangerous. Thus, during times of uncertainty or when the degree of threat is unclear and does not reach the level of overt fear, the amygdala is still activated. This parallels findings from studies presenting fear and other emotional faces to human subjects. While the fear faces activate the amygdala, the subjects report that they do not feel afraid of

the faces. The complementary findings in rodents and humans suggest that using subtler types of emotional stimuli or experiences (particularly in rodent studies) may provide a more complete picture of the role of the amygdala in emotion.

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