Differential activation of anatomically defined neuronal subpopulations in the amygdala during fear conditioning and extinction

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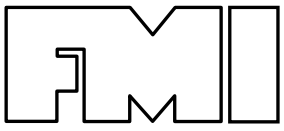

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ABBREVIATIONS

α-DTX:	α-dendrotoxin
AHP_{fast}:	fast afterhyperpolarization
AHP_{medium}:	medium afterhyperpolarization
AHP_{slow}:	slow afterhyperpolarozarion
AMPA:	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic-acid
AMPAR:	AMPA-receptor
AP:	action potential
BA:	basal nucleus of the amygdala
BK-channel:	large conductance Ca^{2+}-dependent potassium channels
BLA:	basolateral complex of the amygdala
bp:	base pairs (DNA)
CA1:	zone of the hippocampus
CA3:	zone of the hippocampus
CE:	central nuclei of the amygdala
CEl:	lateral subdivision of the CE
CEm:	medial subdivision of the CE
CNS:	central nervous system
CS:	conditioned stimulus
dHC:	dorsal hippocampus
EPSC:	excitatory post-synaptic current
EPSP:	excitatory post-synaptic potential
HC:	hippocampus
IbTX:	Iberiotoxin
ICM:	intercalated cell masses
IEG:	immediate early genes

IHC:	immunohistochemistry
KO:	knock-out
LA:	lateral nucleus of the amygdala
LTP:	long-term potentiation
MGm:	medial geniculate nucleus
mPFC:	medial prefrontal cortex
NMDA:	N-mehtyl-D-aspartatic acid
NMDAR:	NMDA-receptor
ON:	over night
PB:	phosphate buffer
PBS:	phosphate buffer saline
PFA:	paraformaldehyde
PTSD:	post-traumatic stress disorder
RB:	retrobeads
RT:	room temperature
s:	second
sEPSC:	spontaneous EPSC

1 Abstract

The amygdala is a key structure of the neuronal circuitry mediating expression and extinction of conditioned fear. However, fear memories are thought to be encoded in a larger network comprising the medial prefrontal cortex (mPFC) and the hippocampus (HC). Thus, amygdala projections to the mPFC and to the HC are likely to be involved in fear learning, yet the cellular substrates of that learning remain unknown. To examine the role of identified amygdala projection neurons in the expression and extinction of conditioned fear responses, I used a combination of in vivo retrograde tracing techniques and analysis of expression of the activity-dependent immediate early genes (IEGs) cFos and Zif268. I show that amygdala neurons projecting to the mPFC or to the HC exhibit differential cFos and Zif268 expression in fear conditioned animals compared with non-conditioned control animals and with animals subjected to extinction. In particular, extinction resulted in a selective induction of cFos in mPFC projecting neurons. A more detailed analysis revealed that neurons projecting to the infralimbic subdivision of the mPFC (IL), but not those projecting to the prelimbic subdivision (PL), account for the specific cFos expression in mPFC-projecting neurons following extinction. To investigate the physiological correlates of fear extinction in anatomically defined subpopulations of amygdala projection neurons I used an electrophysiological ex vivo approach. In these experiments, I recorded from identified BA neurons projecting to PL or IL in slices obtained from mice subjected to extinction. Extinction differentially affects intrinsic properties of PL- and IL-projecting cells. While there was no change in PL-projecting neurons, IL-projecting BA cells showed a learning-related increase in spike half-width and a concomitant decrease in the fast after-hyperpolarization (AHP_{fast}). In control animals, spike half-width and AHP_{fast} were controlled by the activation of voltage-dependent potassium channels (VDPCs) and large-conductance Ca^{2+} dependent potassium channels (BK-channels). After extinction training only VDPCs contribute to the AHP_{fast} in IL-projecitng cells. This indicates a specific modulation of BK-channels in IL-projecting neurons following extinction learning. Our findings suggest that a change in the balance of activity between IL- and PL-projecting BA neurons may be involved in the extinction of conditioned fear.

2 Introduction

2.1 Learning and memory

Memory is an organism's ability to store, retain and retrieve information. It is a pivotal aspect of our lives and ranges from simple associations to complex learning events like playing a piece of music on the piano by heart. While learning to play a piece of music by heart takes training and can be recalled for month or even years after training, other memory processes, such as keeping a telephone number in mind for the process of dialing, involve very time-limited memory that can be gone within seconds or minutes. Thus, memory can be divided into a short-term and a long-term phase. The pivotal aspect of long-term memory is that it involves persistent changes in the underlying neural circuits.

Insight into memory processes has come from the study of mental disorders and the complete or partial loss of memory (amnesia). Different forms of amnesia revealed that considering memory as a single cohesive process is misleading. There are many different kinds of memory and interfering with one can leave another untouched. Memory can be encoded as a habit, fact or emotion and can be associated with different kinds of sensory information. This encoding requires changes in different neural circuits located in brain areas involved in associations, emotion, motor performance and/or sensory perception. Understanding how memory works requires a detailed dissection of these different circuits. A major contribution to this challenge came from Ivan Pavlov who studied how emotionally relevant associations are formed in a dog's brain. In these experiments a dog learned to associate the sound of a doorbell with food. After a few pairings, the sound of the doorbell made the dog salivate in expectation of the food. This form of memory formation, in which an animal learns that a neutral stimulus predicts an emotionally important event is known today as Pavlovian conditioning (or classical conditioning). Pavlovian conditioning has been widely studied to elucidate the neuronal substrates underlying. In the work presented in this thesis, I use an aversive form of Pavlovian conditioning, classical auditory fear conditioning.

2.2 Fear conditioning and extinction

Pavlovian fear conditioning is one of the most widely used paradigms to study the biological basis of emotion, as well as learning and memory: an aversive (often noxious) stimulus, the unconditioned stimulus (US), is presented paired with an emotionally neutral stimulus, the conditioned stimulus (CS). In animal experiments the US is generally a mild footshock and the CS can be a sensory stimulus like a light, smell or tone. After several pairings of the CS and the US the animal learns that the CS predicts the US and starts to show fear responses when exposed to the CS alone (Fig.1) (LeDoux, 1998). Fear responses include changes in heart rate and blood pressure, release of stress hormones, analgesia, and facilitation of reflexes. In rodents, a straightforward and therefore often used way to quantify fear is to measure the freezing response of an animal to an aversive stimulus that can not be avoided. Freezing, observable as an immobile posture, is a defensive behavior since predators usually target moving prey (Fanselow and Poulos, 2005). Pavlovian fear conditioning has a number of advantages as a model for learning: It involves only a small number of stimuli (the CS and the US) and the behavioral output is an innate natural behavior. This signifies the analysis and correlation of the observed changes to single components of the experiment in an easily quantifiable manner.

Conditioned fear responses can be inhibited by extinction training. When the CS is repeatedly presented without the US, the animal learns that the CS does not predict the US any more, and exhibits reduced fear responses (Quirk and Mueller, 2008). Already Pavlov described this phenomenon in his appetitive conditioning experiments with dogs (Pavlov, 1927). His observation that an animal's conditioned response to an extinguished CS can spontaneously recover with time led him to propose that extinction reflects new inhibitory learning rather than the erasure of the conditioned fear memory. It is thought that the brain stores the initial fear memory and the extinction memory in two separate traces. This is confirmed by experiments performed after extinction learning. Presenting the US alone will immediately unveil a fear reaction to the CS a process known as reinstatement. Furthermore, extinction, like FC, is context-dependent, in that not only the cue is associated with presentation (or omission) of the US, but also the

surrounding environment. Placing the animal after extinction back in the context it has been conditioned in will elicit fear behavior. Presentation of the CS in this context will further increase the fear response, a process called renewal (Ji and Maren, 2007; Quirk and Mueller, 2008). Thus, it is widely accepted that extinction represents new inhibitory learning.

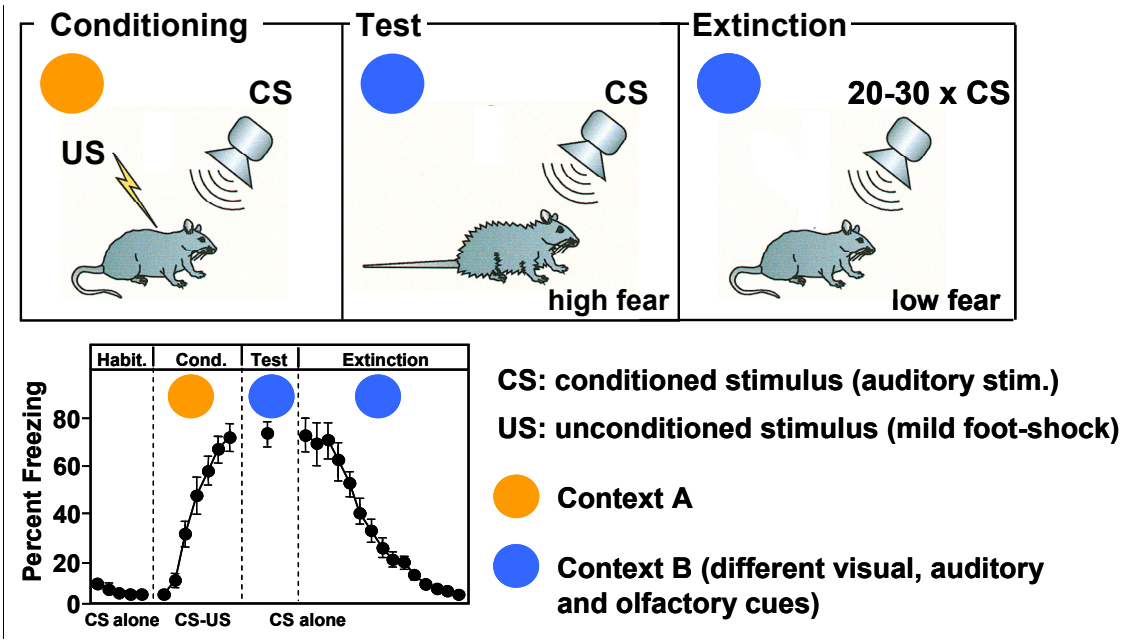


Figure 1: *Pairing a footshock (US) and a tone (CS) in context A leads to conditioned freezing behavior also in context B when the CS is presented (Test). Repeated non-reinforced presentation of the CS in context B will lead to a decrease in fear behavior as response to the tone (Extinction). Defensive fear reactions of mice are measured as percent freezing (lower diagram). Freezing behavior increases after fear conditioning and decreases during extinction.*

Studying fear conditioning and extinction in rodents serves as models for human psychopathology and treatment of those. Too much fear or impaired discrimination between threatening and neutral stimuli leads to diseases like phobias, obsessive compulsive disorder and post-traumatic stress disorder (PTSD) (Kent and Rauch, 2003; Millan, 2003; Uys et al., 2003). In particular PTSD is a disease from which civilians and soldiers who have been exposed to war-time situations often suffer. PTSD patients exhibit a defect in “extinguishing” stress-related situations and it is hoped that by a detailed understanding of the underlying neural substrates potential therapies and drugs can be developed.

2.3 Brain areas involved in the acquisition and extinction of conditioned fear

2.3.1 The amygdala

2.3.1.1 General anatomy

The amygdala is a key brain structure in aversive emotional learning, a brain structure located deeply within the medial temporal lobe (Fanselow and LeDoux, 1999). It is a non-layered structure consisting of different nuclei which differ in their cytoarchitechture, histochemistry and connectivity (Pitkanen et al., 2000a). These nuclei are divided into different groups according to Price *et al* (1987) (1) the basolateral amygdala (BLA), including the lateral (LA), the basal (BA) and the basomedial (BMA) nucleus, (2) the central complex (CE), including the central lateral (CEl), central capsular (CElc) and central medial (CEm) nuclei and (3) more superficial nuclei including the anterior cortical nucleus, bed nucleus of the accessory olfactory tract and others (Fig. 2). The BLA is surrounded by cell clusters the so called intercalated cell masses (ICM), which are comprised solely of GABAergic neurons and could not be classified into the above groups (Pare and Smith, 1993).

The overall flow of information within the amygdala follows the direction of the inter-nuclear projections (Fig.2) (Pitkanen et al., 1997; Sah et al., 2003). The LA serves as the principal sensory interface as it receives multimodal, early sensory information from the thalamus and cortex (Turner and Herkenham, 1991; McDonald, 1998). The CEm serves as the main output station, as its projection neurons target different structures in the brainstem and in the hypothalamus to orchestrate conditioned autonomic and motor responses (Krettek and Price, 1978; Veening et al., 1984; LeDoux et al., 1988). In addition, amygdala nuclei receive unidirectional input from, or are reciprocally connected to cortical and subcortical brain structures (McDonald, 1991; McDonald et al., 1996; McDonald, 1998; Pitkanen, 2000; Pitkanen et al., 2000a). Together with the multitude of inter- and intra-nuclear projections, this suggests that information can be processed both by mechanisms intrinsic to amygdala networks, as well as modified by interactions with other brain structures to integrate sensory inputs,

generate fear response outputs, and modulate fear responses according to circumstances, such as in fear extinction (Pitkanen et al., 1997; Sah et al., 2003).

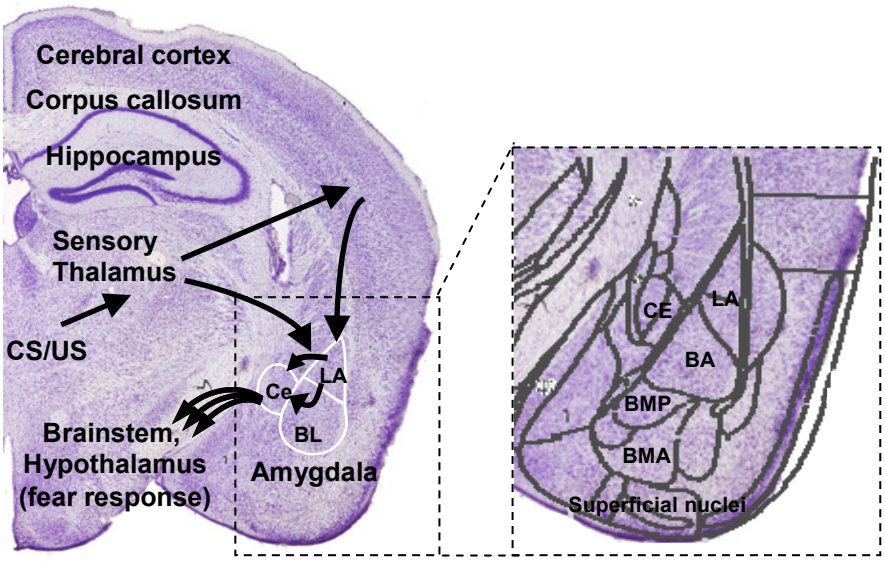


Figure 2: *Structure of the amygdala and surrounding brain-areas. CS-US information is conveyed to the LA via sensory thalamus and cortex. Moreover the CS is processed within the BLA and further conducted to the CE which projects to brainstem and hypothalamus and is thereby capable of eliciting fear responses. LA = lateral nucleus of the amygdala, BA = basal nucleus of the amygdala, BMP = accessory nucleus of the amygdala posterior, BMA = accessory nucleus of the amygdala anterior, CE = central nuclei of the amygdala. (Pictures taken from Paxinos & Franklin, The mouse atlas.)*

2.3.1.2 Intrinsic and extrinsic connectivity of the amygdala

Each nucleus of the amygdala is differentially connected; to areas within and outside of the amygdala. The LA is reciprocally connected to almost all other amygdala nuclei. An exception is the CEl where the LA projects to but does not get input from. Furthermore, the LA is reciprocally connected to external brain structures, including the hippocampus and cortical regions (Pitkanen et al., 2000b). The exception is the thalamus that provides strong input to the LA but does not receive projections (Turner and Herkenham, 1991; Pitkanen et al., 1997). The LA receives dense sensory input of more cortical areas than any other amygdaloid nucleus (McDonald, 1998). Cortical inputs provide information about highly processed visual, somato-sensory, visceral sensory and auditory stimuli. Thus, the LA is the integration site for auditory and somato-sensory inputs (LeDoux et al., 1990; LeDoux, 2000). This is confirmed by physiological data

showing that pairing auditory and nociceptive stimuli enhances the auditory evoked responses recorded in the LA *in vivo* (Quirk et al., 1997; Rogan et al., 1997; Rosenkranz and Grace, 2002). Importantly the LA is also innervated by projections from structures related to other memory systems, including the prefrontal and perirhinal cortical areas, and the hippocampus (HC) (LeDoux, 2000).

The BA receives the strongest intra-amygdaloid input from the LA. In turn it projects to LA, CE and other amygdala nuclei (Savander et al., 1995). Cortical inputs to the BA originate largely in the same areas as those to the LA, but their density is generally lighter (Pitkanen, 2000). This does not apply to projections from the frontal cortex (prefrontal and perirhinal cortex). These are strongly and reciprocally connected to the BA (Berendse et al., 1992; Bacon et al., 1996). Other areas involved in memory formation like the HC are densely interconnected with the BA (McDonald, 1998; Pikkarainen et al., 1999). Additional brain structures the BA is connected to are the thalamus, the basal forebrain and the nucleus accumbens (Pitkanen, 2000).

The CE receives relatively unprocessed visceral sensory inputs directly from some thalamic nuclei, the olfactory bulb, and the nucleus of the solitary tract in the brainstem. Neurons originating from the medial subdivision of the central nuclei (CEm) project to central grey (CG), lateral hypothalamus (LH) and paraventricular hypothalamus (PVN), all of which mediate fear responses (Pitkanen et al., 1997; Medina et al., 2002). Thus the amygdala links sensory inputs from thalamic and sensory cortical regions with hypothalamic and brainstem effector systems to control behavior. The strong interconnections with the HC and the prefrontal cortex suggest that fear memory is stored in a large network.

2.3.1.3 Properties of amygdala neurons

Based on morphological, neurochemical and physiological features, the BLA is a cortex-like structure. Projection neurons (PNs) represent the largest population of neurons in the BLA of around 85% (McDonald and Augustine, 1993; Mahanty

and Sah, 1998). They are large spiny cells, with low firing rates and frequency adaptation (Pare and Gaudreau, 1996). Interestingly, these neurons display a morphological continuity ranging from pyramidal to stellate (Pare et al., 1995; Faber et al., 2001). In general, the somata of projection neurons in the LA are smaller than those of the BA (Millhouse and DeOlmos, 1983).

Aspiny, GABAergic interneurons (INs) form the second group of neurons in the BLA (McDonald and Augustine 1993). INs have smaller somata compared to PNs and form a heterogenous population with regard to their dendritic and axonal arborizations (Carlsen and Heimer, 1988; Pare and Smith, 1998). Like interneurons in cortex and hippocampus, BLA interneurons can be divided into several subtypes based on the expression of a variety of calcium-binding proteins (parvalbumin (PV), calbindin, calretinin) and neuropeptides (somatostatin (SOM), cholecystokinin, neuropeptide Y, vasointestinal polypeptide) (Kemppainen and Pitkanen, 2000; McDonald and Mascagni, 2001; Mascagni and McDonald, 2003).

2.3.1.4 The amygdala in fear conditioning and extinction

The discovery that the amygdala contributes to emotional behavior was made in the 1950s. Bilateral lesions of the amygdala in monkeys led to dull and less fearful behavior (Weiskrantz, 1956). Electrical stimulation of the amygdala elicited high fear responses (Delgado et al., 1956). Today we know that the BLA is a key site for the formation of CS-US associations during fear conditioning and also during extinction learning. This evidence is based on studies using permanent or reversible lesions of the BLA, application of drugs, and electrophysiological recordings of neuronal activity during fear conditioning.

Selective neurotoxic lesions of the BLA before fear conditioning have been shown to impair the formation of CS-US associations (Campeau and Davis, 1995; Cousens and Otto, 1998; LeDoux, 2000). Furthermore, lesions of the BLA made after fear learning have been shown to prevent memory retrieval (Campeau and Davis 1995; Cousens and Otto 1998). However, lesions of the BLA are not useful to study extinction learning, as no fear memory is preserved (Quirk and Mueller, 2008). Better are pharmacological manipulations of the BLA to explore its role in

extinction memory. Formation of long-term memory has been linked to a molecular cascade involving N-methyl-D-aspartate receptor (NMDA-R) mediated calcium entry, activation of protein kinases, gene expression, and protein synthesis (Kandel, 2001). Manipulation of these pathways within the BLA interferes with both, acquisition and extinction of conditioned fear (Falls et al., 1992; Lu et al., 2001; Rodrigues et al., 2001; Schafe et al., 2001; Lin et al., 2003; Desgranges et al., 2008). Furthermore, electrophysiological recordings showed that FC leads to a potentiation of sensory evoked responses to the CS in the BLA (Quirk et al., 1995; Rogan and LeDoux, 1995; Quirk et al., 1997; Rogan et al., 1997; Collins and Pare, 2000). During extinction amygdala output becomes gradually inhibited. This process requires the formation of a new memory trace and includes inhibitory neurons within the amygdala to block CE output. Whether these are local inhibitory networks in the BLA or the intercalated cell masses (ICM) at the interface between BLA and CE is still under debate (Rosenkranz et al., 2003; Berretta et al., 2005; Likhtik et al., 2008)

The overall flow of information within the amygdala follows the direction of the inter-nuclear projections; e.g. from LA to BA, and from BLA to CEA and, within CEA, from the CEl to the CEm (Pitkanen et al., 1997; Sah et al., 2003). Plasticity within the LA potentiates the ability of the CS to excite neurons in the medial subdivision of the central nucleus (CEm) and thereby generates conditioned fear (Davis, 2000; LeDoux, 2000; Malkani and Rosen, 2000a; Blair et al., 2001). Interestingly, formation of long-term memories requires insertion of AMPAR into synapses and interfering with this insertion of new AMPAR into LA synapses prevents fear conditioning (Rumpel et al., 2005). Recently, it has been shown that CREB (adenosine 3`, 5`-monophosphate response element-binding protein) – knockout (KO) mice cannot acquire fear memory, which is completely rescued by intra-LA expression of CREB. Thus, CREB-expression in the LA is necessary and sufficient for fear memory consolidation (Han et al., 2007). Indeed, ablation of the LA neuronal subpopulation that overexpresses CREB after FC, erases the fear memory (Han et al., 2009). These findings resulted in the model that the major site of plasticity and memory storage in FC is the LA, and other amygdala nuclei serve as processing and relay structures.

However, this model is not complete and there is increasing evidence that the LA is not the only site where plasticity happens. Other possible sites of plasticity are the CE (Samson and Paré, 2005; Wilensky et al., 2006) and the BA (Paré et al., 2004; Anglada-Figueroa and Quirk, 2005). The hypothesis that the CE contributes directly to fear conditioning is already supported by anatomical data, showing direct auditory and somatic inputs (Goosens and Maren, 2001). In addition, overtraining of BLA lesioned rats partially rescues fear conditioning which is completely prevented by lesions or pharmacological inactivation of the CE (Zimmerman et al., 2007). Thus, the CE is the probable site that stores the fear memory in the absence of the BLA.

It is not entirely clear how the LA influences CEm output as there are no direct projections to the CEm (Paré et al., 2004). But it is known that the LA projects to the BA which in turn projects to the CEm (Pitkanen et al., 1997; Pare and Smith, 1998). This points to an indirect route of the LA to the CEm via the BA (LeDoux, 1995). Surprisingly, pre-training lesions of the BA had no effect on the acquisition of fear conditioning (Amorapanth et al., 2000; Goosens and Maren, 2001). In contrast, post-training lesions completely abolished the expression of conditioned fear (Anglada-Figueroa and Quirk, 2005). Another recent study from our laboratory shows distinct BA cell populations identified by their CS-response (Herry et al., 2008). Depending if fear or extinction activates these neurons, they were called “fear or extinction cells”. “Extinction cells” become specifically CS-activated during extinction training supporting earlier findings of activity in the BA as a response to extinction learning (Herry and Mons, 2004). These cells were, in contrast to “fear cells”, not found in the LA (Repa et al., 2001). Pharmacological inactivation of the BA impairs behavioral transitions from high to low fear and vice-versa (Herry et al., 2008). Together, this indicates that the BA is actively involved in fear conditioning and extinction and particularly in situations requiring rapid switching between the two converse behavioral states.

2.3.2 Hippocampus

The Hippocampus (HC) is an evolutionary ancient brain structure located in the medial temporal lobe. It is subdivided into the following areas: CA1, CA2 and CA3 fields of the HC, the dentate gyrus (DG) and the subiculum (S) (Fig. 3A). Massive reciprocal connections with the amygdala are formed between ventral CA1 and the subiculum (Pitkanen, 2000). Furthermore the HC is connected to other fear memory-related areas like the mPFC (Ishikawa and Nakamura, 2006).

The HC was put into the spotlight of memory research through studies on the case of patient HM in the 1950s. HM suffered from severe epilepsy originating from both temporal lobes. To control the epilepsy, physicians removed almost the whole HC on both sides. After the surgery HM suffered from acute anterograde amnesia; although his working memory was intact he could not form any new long-term memory (Scoville and Milner, 1957).

Besides general memory formation, the HC plays the major role in spatial memory and navigation. This was primarily investigated in rodents. In 1971 O'Keefe and co-workers recorded from neurons within the HC and demonstrated that activity of specific hippocampal neurons, today known as "place cells", correlates with a specific location of the rat in space (O'Keefe and Conway, 1978). Inactivation of the HC in mice leads to a severe deficit in spatial and contextual learning (Corcoran and Maren, 2001). Therefore, it is believed that that the HC is critical for using contextual information to guide behavior (Fanselow, 2000; Maren and Holt, 2000). Until today the ultimate test for HC performance are spatial tests (Richardson et al., 2002; Herrera-Morales et al., 2007; Pawlowski et al., 2009).

FC and extinction learning are, as mentioned earlier, strongly context-dependent (Bouton et al., 1993; Bouton and Nelson, 1994). This context-dependency can be disrupted by inactivation of the dorsal HC (dHC) (Corcoran and Maren, 2001; Hobin et al., 2006). Recently, it was shown that context-dependent activity to a conditioned tone in the LA is modulated by the dHC (Maren and Hobin, 2007). The authors inactivated the dHC by muscimol application while recording single-units from the LA. Inactivation led to impairment of contextual extinction and a

concomitant loss of context-driven activity in the LA. In general it is believed that the HC is more involved in contextual modulation of Pavlovian FC. However, inactivation of the ventral HC (vHC) interferes with both cued and contextual aspects of FC and extinction, suggesting differing roles for the dHC and the vHC (Maren, 1999; Maren and Holt, 2004).

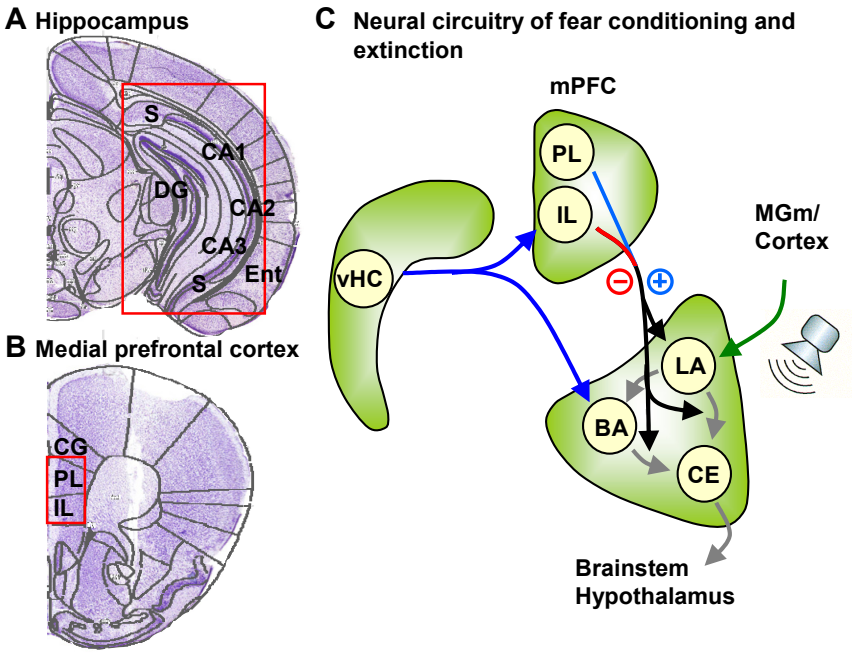


Figure 3: A: *Structure and subareas of the ventral hippocampus (HCv). CA1, CA2, CA3 subfields of the HC, S = subiculum, DG = dentate gyrus, Ent = entorhinal cortex.* ***B:*** *Structure and subareas of the medial prefrontal cortex (mPFC). CG = anterior cingulate cortex, PL = prelimbic cortex, IL = infralimbic cortex.* ***C:*** *During fear conditioning the PL excites amygdala output, which is inhibited after extinction learning by the IL. Both learning paradigms are modulated by hippocampal activity. LA = lateral nucleus of the amygdala, BA = basal nucleus of the amygdala, CE = central nucleus of the amygdala. MGm = medial geniculate nucleus.*

2.3.3 Medial prefrontal cortex (mPFC)

The mPFC is part of the prefrontal cortex that constitutes the anterior part of the frontal lobes of the brain. It consists of three different subdivisions: The anterior cingulate (CG), the infralimbic (IL) and the prelimbic cortex (PL) (Fig 3B) (Groenewegen et al., 1990; Uylings and van Eden, 1990). Since the famous case of Phineas Gage, who suffered from frontal lobe lesions caused by an accident in 1884, the prefrontal cortex has been associated with emotional regulation (Damasio et al., 1994). After the accident Phineas Gage exhibited characteristics

he did not show before: He was quick-tempered, irritable and impatient, but his cognitive abilities were unchanged.

Today, detailed knowledge about the function of the prefrontal cortex in emotional processes comes from animal studies, mostly in rodents (Davidson and Irwin, 1999). Concerning the relevance of the mPFC in FC and extinction, however, previous behavioral studies have reported conflicting results. In rats, lesions of the mPFC led to an increase (Morgan et al., 1993), a decrease (Rosen et al., 1992; Lacroix et al., 2000), or to no change in fear responses (Holson, 1986; Rosen et al., 1992; Gewirtz et al., 1997).

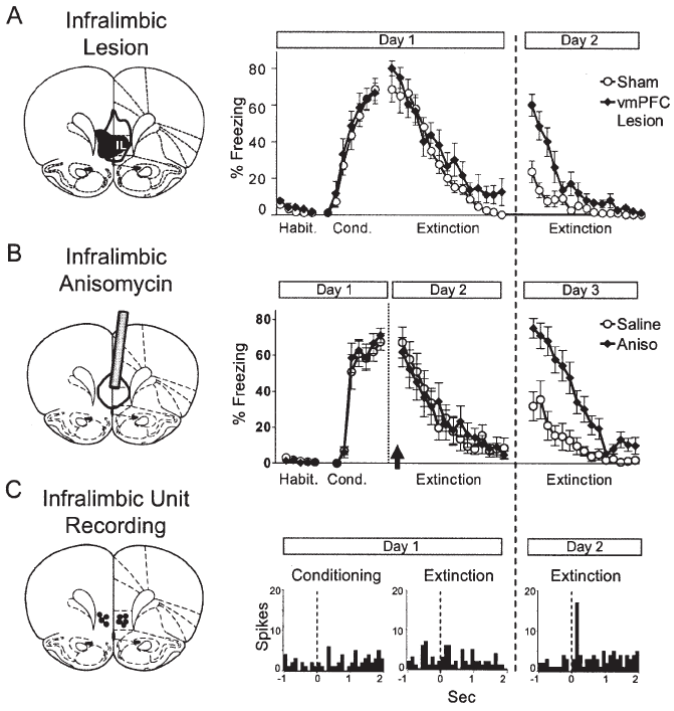


Figure 4: *The IL is functionally involved in retrieval of extinction.* ***A:*** *Lesions of IL do not prevent extinction but interfere with retrievall of extinction the following day (modified from Quirk et al 2000).* ***B:*** *Infusing the protein synthesis inhibitor anisomycin (Aniso) into the IL just before extinction (arrow) has no effect on extinction learning but blocked retrieval of extinction the following day (modified from Santini et al 2004).* ***C:*** *Single unit recording shows that IL neurons respond to the CS only during retrieval of extinction, suggesting that IL tone responses are responsible for low fear after extinction (modified from Milad and Quirk, 2002). These and other data suggest that extinction induced changes in prefrontal neuronal activity is necessary for fear extinction. vmPFC, ventral medial prefrontal cortex; Habit., habituation; Cond., conditioning. From: (Quirk et al., 2006).*

These results may be a consequence of targeting different subdivisions of the mPFC. Indeed, there is increasing evidence for an opposing role of the PL and

the IL in acquisition and extinction of conditioned fear (Fig. 4) (Sierra-Mercado et al., 2006). During trace fear conditioning, a protocol in which CS and US presentation is separated by a certain time interval, PL neurons show a learning-related increase in activity to the CS presentation, whereas IL neurons decrease their activity (Gilmartin and McEchron, 2005). In contrast, IL neurons show extinction learning related increases in activity as a response to the CS (Fig. 4 C) (Milad and Quirk, 2002). Microstimulation of the PL reduces, and IL-stimulation facilitates, extinction learning (Vidal-Gonzalez et al., 2006). Furthermore, microinfusion of the activity-blocker tetrodotoxin (TTX) in the PL reduces freezing to both a tone and a context that were paired with a footshock (Corcoran and Quirk, 2007). In contrast, excitotoxic lesions of the IL, as well as local application of the protein synthesis inhibitor anisomycin, allow for normal acquisition of fear but impaired extinction learning (Fig. 4A, B) (Quirk et al., 2000). Thus, the PL seems to be more involved in fear learning, whereas the IL has a key function in extinction learning (Fig. 4).

It is thought that during extinction activity in the IL (but: not all studies differentiate between IL and PL) inhibits activity and prevents plasticity in LA neurons, probably by activation of local inhibitory networks or by recruitment of intercalated cell masses (Rosenkranz et al., 2003; Berretta et al., 2005; Likhtik et al., 2008). Less is known about the influence the BLA exerts upon the mPFC. BLA stimulation leads to two different kinds of responses in separate populations of mPFC neurons: monosynaptic, excitatory responses and more often inhibition of spontaneous firing (Floresco and Tse, 2007). Furthermore, within the mPFC input from the amygdala and the HC converges and interacts (Ishikawa and Nakamura, 2003).

2.4 Immediate-early genes

Memory can be divided into a short- and long-term phase. A short-term memory does not result in any long lasting neural changes. In contrast long-term memory processes induce enduring neural changes that allow information storage for

weeks, month, or even years. An important feature distinguishing short- from long-term memory is that only the latter depends on mRNA and protein synthesis. Interfering with transcription or translation immediately after training disrupts long-term memory formation in experimental animals, while leaving short-term memory intact (Nader et al., 2000; Schafe and LeDoux, 2000). This labile phase is transient, lasting a couple of hours. Long-term memory becomes stable and insensitive to disruption with time. However, reactivation of a memory can bring it back to a labile state, a process called reconsolidation that is again protein synthesis dependent (Alberini, 2005). Since protein synthesis depends on transcription of DNA to mRNA, important regulators of memory formation are transcription factors (TFs). TFs that play an important role during memory consolidation belong largely to a group of proteins which are activated rapidly and transiently as a response to neuronal activity. These are so called immediate early genes (IEGs). In addition to TF IEGs, there are also effector IEGs that are more directly involved in functional processes at the synapse.

IEGs were identified in the 1960s and 1970s in cancer research. Later it was shown that they are present in the genome of all animals and that they are inducible by a multitude of stimuli, including growth factors or mitogens. Since it became evident that they are induced in the brain following for example seizures, kindling and other activity changes, IEGs were investigated as potential mediators of the cellular changes in memory formation. An important feature of IEG induction is it's transience making IEGs a powerful tool to map activity in the brain (Guzowski et al., 2005). Primarily the TF cFos has been widely used to detect activated brain areas (Herry and Mons, 2004; Berretta et al., 2005; Reijmers et al., 2007).

2.4.1 Zif268

Expression levels of the TF Zif268, also called Egr-1, NGFI-A, Krox-24, TIS8 and ZENK, increase in defined brain-structures upon cellular activation. Following activity eliciting stimulation *zif268* mRNA is expressed in a time window between 20 min and 60 min and Zif268 protein levels are highest between 1 – 2h after

activation (Gass et al., 1992; Malkani and Rosen, 2000a, b; Zangenehpour and Chaudhuri, 2002). In the amygdala, Zif268 is expressed following fear conditioning and extinction, as well as other anxiety related and drug seeking behavior (Malkani and Rosen, 2000a; Thomas et al., 2003; Herry and Mons, 2004; Rosen et al., 2005). Increases in cellular activity within neuronal tissue are known to activate Zif268, likely through an elevation in cytosolic Ca^{2+} (Murphy et al., 1991; Condorelli et al., 1994; Ghosh et al., 1994). An increase in intracellular calcium levels activates different signal transduction pathways and some of them have been implicated in Zif268 induction (Knapska and Kaczmarek, 2004).

As Zif268 is rapidly and transiently induced in activated brain regions and mediates transcription and thereby protein-synthesis it has long been hypothesized that it plays a role in the protein-synthesis dependent processes of long-term memory and LTP. Indeed, recent studies could show a prominent role of Zif268 in both. In the amygdala, administration of the anti-anxiety drug diazepam not only blocks fear conditioning but also Zif268 induction (Malkani and Rosen, 2000b). Furthermore, microinfusion of a *zif268* antisense oligodeoxynucleotide to the LA disrupts Zif268 expression and interferes with contextual fear memory formation (Malkani et al., 2004). Studies on $Zif268^{-/-}$ mice indicate a role for Zif268 in late but not in early phases of LTP. In addition, $Zif268^{-/-}$ mice are impaired in spatial learning tasks and in amygdala-dependent conditioned taste aversion (Jones et al., 2001). Moreover, low expression of Zif268 in the mPFC and the amygdala is associated with resistance to extinction (Herry and Mons, 2004). A recent study could link Zif268 overexpression in the amygdala to strengthening of an aversive memory together with resistance to extinction of that memory trace (Baumgartel et al., 2008). In summary, transcription factor Zif268 is likely to be involved in the consolidation of memory and in the formation of LTP.

2.4.2 cFos

The TF cFos is part of the AP-1 complex which is capable of DNA-binding and transcription initiation. cFos is an ubiquitous protein, present in almost all cell-

types and it is induced by a variety of stimuli including cytokines, stress, growth factors, bacterial and viral infections, and oncoproteins (Herrera and Robertson, 1996). In the brain cFos is rapidly and transiently expressed in activated brain areas with a peak-expression after ~ 2h; basal expression levels are low (Gass et al., 1992; Hughes et al., 1992). A multitude of stimuli in a huge variety of brain structures lead to cFos expression. Seizures can induce cFos throughout the brain (Dragunow et al., 1987; Dragunow and Robertson, 1987; Morgan et al., 1987); sensory stimulation, cerebral ischemia, stroke and axotomy can induce cFos (Kogure and Kato, 1993; Hope, 1998; Coggeshall, 2005; Van der Gucht et al., 2005). Single whisker deprivation in mice leads to cFos positive nuclei in the barrel cortex only in the barrel corresponding to the spared whisker and fear conditioning induces cFos expression in the amygdala and in the mPFC (Barth et al., 2004; Herry and Mons, 2004). Thus, because cFos is reliably induced by activity-changes, its expression has been widely used as tool to identify brain areas activated under specific circumstances. Like Zif268, cFos levels increase as a response to elevated intra-neuronal free calcium (Bading et al., 1993). The calmodulin pathway appears to link the elevated intracellular calcium to gene induction.

Despite the fact that activity induces cFos expression in the brain the role of cFos in learning and memory is still unclear. In mice, fear conditioning induces cFos in the amygdala (Herry and Mons, 2004). Furthermore, resistance to fear extinction is correlated with increased cFos expression in the mPFC and the amygdala (Herry and Mons, 2004). Some studies also show a requirement for cFos expression in learning paradigms. Passive avoidance training does not only induce cFos expression in the amygdala, it also requires cFos expression for the formation of the memory as demonstrated by infusion of antisense oligonucleotides (Lamprecht and Dudai, 1996). Additionally, mice deficient for cFos specifically exhibit selective deficits in hippocampus-dependent learning, like contextual fear conditioning (Fleischmann et al., 2003). Thus, cFos expression is induced in the brain during memory formation and is also necessary for some forms of learning.

In contrast to Zif268, assessment of the role of cFos in in neuronal plasticity led to conflicting results. In anesthetized animals where LTP was elicited by electrical stimulation, no cFos induction could be observed in the dentate gyrus (Douglas et al., 1988). However, very strong stimuli are able to induce cFos expression (Abraham et al., 1993). Mice deficient for cFos in the CNS displayed a reduced magnitude of hippocampal LTP (Fleischmann et al., 2003). In summary, cFos seems to be critically recruited during the activation of neuronal circuits. However, in contrast to Zif268, whose expression could be directly linked to plasticity and memory retrieval, the link of cFos expression to plasticity is conflicting.

2.5 Electrophysiological correlates of fear learning and extinction

In the previous chapters I have discussed the brain's capacity of storing information at a systemic level: I presented the brain structures contributing to different aspects of fear conditioning and extinction and introduced IEGs as modulators of long-term changes in the brain. In this chapter I will discuss changes in cellular physiology which could underlie associative learning in general and in fear learning and extinction in particular. The idea that memory storage results from activity-dependent changes in synaptic strength was postulated by Hebb (1949), who proposed that connections between two cells could be strengthened if both cells were active simultaneously. This process was later on indeed discovered, first in HC, later in other brain areas and is called long-term potentiation (LTP). LTP is one of the best cellular models for information storage associated with learning and memory (Sourdet and Debanne, 1999; Abbott and Nelson, 2000; Bi and Poo, 2001; Sjostrom and Nelson, 2002). However, synaptic plasticity is not the only persistent change that can happen on a cellular level. Additionally or alternatively, plasticity of intrinsic electrical properties such excitability, regulated by ion channels in nonsynaptic structures of neurons has been described, and intensely investigated over the last years (Daoudal and Debanne, 2003; Zhang and Linden, 2003; Disterhoft and Oh, 2006).

2.5.1 Synaptic plasticity

LTP, and the related phenomenon long-term depression (LTD), have been described at many synapses in different brain regions. They were most intensively studied at glutamatergic synapses in the hippocampus (Martin et al., 2000). The current model of LTP involves activation of AMPA-R and the concomitant recruitment of NMDA-R most often being coexpressed at glutamatergic synapses (Malenka and Bear, 2004). Upon activation by glutamate release AMPA-Rs open at resting membrane potential and mediate fast excitatory postsynaptic potentials (EPSPs), through sodium influx (Hestrin et al., 1990). Whereas AMPA-Rs are responsible for basal synaptic transmission, NMDA-Rs function as coincidence-detectors of concerted neuronal activity. At the resting membrane potential NMDA-Rs are blocked by intracellular Mg^{2+} (Nowak et al., 1984). Upon depolarization NMDA-Rs become unblocked and permeable to cations including Ca^{2+} ions when activated by glutamate. The resulting rise in intracellular Ca^{2+} activates a cascade of biochemical changes that leads to persistent changes in synaptic strength (Malenka and Bear, 2004). These changes can either increase (LTP) or decrease synaptic strength (LTD).

The exact nature of the link between LTP and memory formation is still under debate. A particularly useful system to investigate this link is associative FC combined with the search for cellular correlates in the amygdala. Today, there is considerable evidence supporting the notion that LTP at sensory afferents to the basolateral amygdala underlies acquisition of FC (Sah et al., 2008).

Pharmacological and molecular manipulations that block LTP *in vivo* and *in vitro* under some conditions, also block the acquisition of fear conditioning (Bauer et al., 2002; Goosens and Maren, 2004; Rumpel et al., 2005; Humeau et al., 2007). Patch clamp recordings of lateral amygdala neurons show that inputs from cortical and thalamic areas converge in the amygdala and are highly plastic (LeDoux et al., 1991; Romanski and LeDoux, 1993). Fear conditioning enhances synaptic transmission at these afferents to the amygdala *ex vivo* (McKernan and Shinnick-Gallagher, 1997; Tsvetkov et al., 2002). This applies also to CS-evoked responses measured *in vivo* (Quirk et al., 1997; Rogan et al., 1997; Goosens et al., 2003). Furthermore, LTP can be induced at sensory afferents to the amygdala

both *in vivo* and *in vitro* (Rogan and LeDoux, 1995; Huang and Kandel, 1998; Doyere et al., 2003). It has therefore been proposed that LTP at synapses in the amygdala underlie the acquisition of conditioned fear.

2.5.2 Intrinsic plasticity

Synapses are not the only neuronal elements that contribute to long-term activity changes in networks. Neuronal mechanisms contribute to intrinsic neuronal excitability (Daoudal and Debanne, 2003). The excitability of a neuron is determined by the properties and distribution of ion channels in its membrane. It can also be defined as the ability of a neuron to generate an action potential (output) upon stimulation (input). At the mechanistic level, this involves opening of voltage- and Ca2+-dependent ion channels located on the dendrites, soma and axon of a cell.

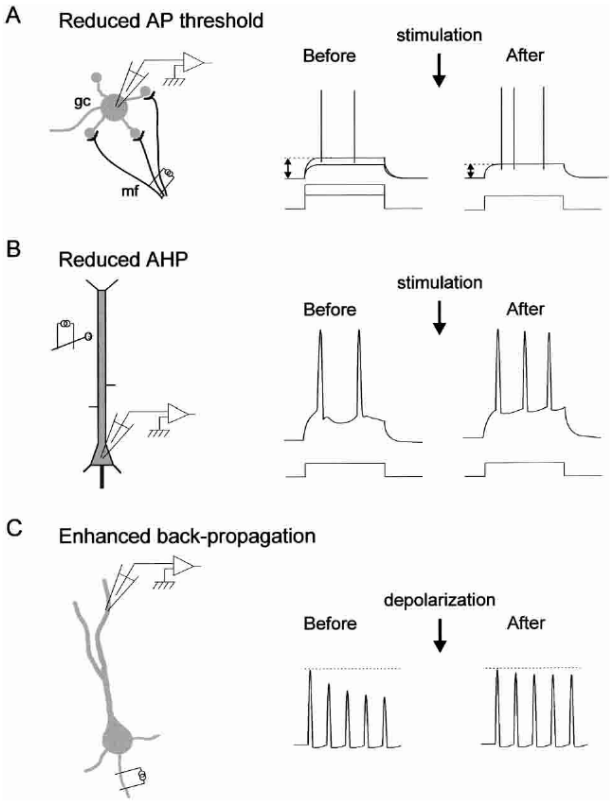


Figure 5: *Different mechanisms for changing cellular excitability.* ***A:*** *A decreased spike threshold causes spiking at smaller depolarization.* ***B:*** *A reduction in the AHP increases the number of spikes elicited by the same depolarizing step.* ***C:*** *Enhanced back-propagation increases depolarization in non-activated cellular compartments. Figure from (Daoudal and Debanne, 2003).*

It has previously been shown that excitability of neurons changes with learning. For example, eye-blink conditioning leads to an increased excitability in hippocampal neurons recorded from conditioned animals compared to naïve or pseudo-conditioned animals (Disterhoft et al., 1986; Schreurs et al., 1997; Saar et al., 1998). Neuronal excitability of mPFC neurons was found to be enhanced following learning of a fear conditioning task in prefrontal cortical neurons (Santini et al., 2008). Different ways to shape a neuron's excitability have been shown (Fig. 5): In cerebellar neurons a reduced AP-threshold increases neuronal excitability upon high frequency stimulation (Fig. 5A) (Armano et al., 2000); Changes in the after-hyperpolarization (AHP) following single AP and bursts of spikes lead to changes in spike frequency and spike frequency adaptation (Fig. 5B) (Disterhoft and Oh, 2006). Finally, enhanced AP back propagation following postsynaptic depolarization increases intrinsic excitability in CA1 pyramidal neurons (Tsubokawa et al., 2000), as well as in entorhinal cortical neurons (Egorov et al., 2002) and in cerebellar neurons (Aizenman and Linden, 2000). At the mechanistic level, synaptic plasticity and intrinsic plasticity share common induction pathways. It has been shown that activation of NMDA-Rs is required for potentiation of intrinsic excitability (Armano et al., 2000; Aizenman et al., 2003). In summary, changes in intrinsic excitability have been identified as a cellular correlate of learning in various brain areas including the mPFC, the HC and the cerebellum. Intrinsic excitability can be shaped in different ways and is determined by expression of Ca^{2+} and voltage dependent ion channels.

2.5.2.1 Afterhyperpolarization (AHP)

The AHP is mediated either by Ca^{2+}- dependent voltage sensitive potassium channels or by voltage-dependent potassium channels, depending on the type of AHP and the cell-type. Three types of AHPs are distinguished by their time-course: fast, medium and slow AHP. The underlying currents are Ic, I_{AHP} and sI_{AHP}, respectively (Lancaster and Nicoll, 1987; Disterhoft and Oh, 2006). The fast and medium AHP contribute to action potential repolarization and spike firing

frequency. The slow AHP is widely acknowledged to be the main determinant of spike frequency adaptation (Faber & Sah, 2002).

The fast AHP follows the downstroke of an AP. It plays not only a role in AP repolarization, but also in determination of spike half-width, thereby influencing the amount of Ca^{2+} entering the cell (Faber and Sah, 2003). It lasts for about 5 ms and in many cell-types it is largely mediated by large conductance Ca^{2+}-dependent potassium channels (BK-channels). BK-channels are gated by Ca^{2+} and blocked by low concentrations of tetraethylammonium ions (TEA) (0.1-1 mM) and by the specific toxins charybdotoxin and iberiotoxin (IbTX). They have single channel conductances greater than 100 pS and are voltage sensitive, opening more easily and longer at depolarized potentials.

However, there is good evidence that, in contrast to other brain structures, the AHP_{fast} in the lateral amygdala is mediated by voltage-dependent potassium channels (VDPCs) (Faber and Sah, 2002). VDPCs open at depolarized potentials and play a role for AP repolarization. VDPCs that mediate AHP_{fast} in LA neurons probably consist of the subunits Kv1.1, Kv1.2 or Kv1.6 as the AHP can be blocked by the snake venom α –dendrotoxin (α-DTX) (Grissmer et al., 1994).

The medium AHP follows single AP and trains of APs, lasts 50-200 msec and is mediated by small conductance potassium channels (SK-channels), which, like BK-channels, are gated by Ca^{2+}. Their single channel conductance is in the 5-20 pS range and they are blocked by high concentrations of TEA (10-20mmol/l) and the bee toxin apamin (Tzounopoulos and Stackman, 2003).

The channel(s) underlying AHP_{slow} are not known. However, the underlying current sI_{AHP} is activated following trains of APs and can last up to 6 seconds. It is insensitive to blockers of SK- and BK-channels, but can be modulated by neuromodulaters, such as acetylcholine and noradrenaline (Disterhoft and Oh, 2006).

All three currents I_c, I_{AHP} and sI_{AHP} have been implicated in shaping intrinsic neuronal plasticity upon learning. In the HC, where the AHP_{fast} is mediated by BK-channels, trace eyeblink conditioning increases the intrinsic excitability of CA1

neurons by decreasing the fast and the slow AHP (Matthews et al., 2008; Matthews et al., 2009). However, blocking BK-channels in HC *in vivo* leads to an unspecific increase in activity also due to increased release probability and impedes acquisition of trace eyeblink conditioning. In the IL, fear conditioning decreases both the AHP_{fast} and the AHP_{slow} and thereby also increases cellular excitability of pyramidal neurons in layer II/III and V (Santini et al., 2008). However, decreases of the AHP_{fast} are not always accompanied by increased excitability. Selective changes in spike frequency adaptation of neurons have been reported as well as no changes in firing behavior of a cell (Gu et al., 2007; Haghdoost-Yazdi et al., 2008).

The slow AHP is increased in hippocampal cells from aging animals (Landfield and Pitler, 1984; Power et al., 2002), and this increase correlates with increased AP accommodation and difficulty in learning hippocampus-dependent tasks such as trace eyeblink conditioning (Moyer et al., 2000) and the Morris water maze (Tombaugh et al., 2005).

2.6 Aim of this study

Although the amygdala is a key component of the neuronal circuitry mediating expression and extinction of conditioned fear, fear memories are thought to be encoded in a larger network comprising the medial prefrontal cortex (mPFC) and the hippocampus (HC). Particularly, two subdivisions of the mPFC, the prelimbic (PL) and the infralimbic (IL) cortex, are assumed to be involved in fear conditioning and extinction, respectively. Thus, amygdala projections to the PL, the IL and to the HC are likely to be involved in fear learning, yet the cellular substrates remain unknown. In my Thesis, I examined the role of identified amygdala projection neurons in the expression and extinction of conditioned fear responses. I used a combination of in vivo retrograde tracing techniques and expression analysis of the activity-dependent IEGs cFos and Zif268. Additionally, I addressed physiological correlates of fear conditioning and extinction in anatomically defined neurons using patch-clamp recordings in an ex vivo electrophysiological approach.

3 Material and methods

3.1 Animals

Male C57BL/6J mice (RCC, Füllinsdorf, Switzerland) or cFos-GFP transgenic mice lines 1-3 and 4-1 (Barth et al., 2004) backcrossed into C57BL/6J (over 10 generations) were used for all experiments. Adult mice of 5 weeks to five month of age were housed separately in a temperature controlled room with a 12/12 hour light/dark cycle with food pellets and water available ad libitum. All procedures were carried out with an approval by the Veterinary Department of the Canton of Basel-Stadt.

3.2 Genotyping cFos-GFP transgenic mice

Genotyping was done from biopsy of the tailtips. Tailtips were kept in lysis buffer containing proteinase K over night at 55 °C and heat-shocked at 95 °C for 5 min. Primers specific to the coding sequence of gfp (Primer 1: 5'GAACTCCAGCAGGACCATGT3', Primer 2: 5'TATATCATGGCCGA CAAGCA3', from Microsynth, Switzerland) plus additional standard reagents (Invitrogen GmbH) were mixed with the DNA samples for the polymerase chain reaction (PCR). For the subsequent gel electrophoresis a 1% agarose gel was stained with with 0.005% ethidium bromide for visualization of DNA with UV-light.

3.3 Behavioral training

Mice were submitted to an auditory fear-conditioning paradigm in which a CS (7.5 kHz, 50 ms, 30 X @ 0.9 Hz, 80 dB), was paired with a US (mild foot shock, 0.6 mA, 1 s). Conditioning took place in a chamber consisting of a shock grid floor made of stainless steel rods placed in a square transparent Plexiglas box. A speaker was positioned on top of the square transparent box. The whole system was placed inside a sound-attenuating wooden cubicle. The shock grid was connected to a current generator and scrambler to deliver the foot-shock. The current generator and scrambler were controlled by a computer running the Tru Scan 99 software for in-time delivery of CS and US (Coulbourn Instruments, Allentown, PA). Extinction training was performed over two days in a different context (same system but different visual and olfactory cues). Freezing behavior

was quantified during each behavioral session using an automatic infrared beam detection system placed on the bottom of the experimental chamber (Coulbourn Instruments, Allentown, PA). Statistical analyses were performed using paired *t* test and one-way ANOVA comparisons at the $p < 0.05$ level of significance. Results are presented as mean SEM.

3.4 Retrograde labeling and adeno-associated virus (AAV) injection

For retrograde labeling of BA neurons projecting to either mPFC or HC we used fluorophore coated latex microspheres (red exc.= 530 nm, em.= 590 nm and green exc.= 460 nm, em.=505 nm) referred to as retrobeads (RBs) (Lumafluor Inc., Naples, FL). RBs were dialyzed with a sucrose solution (0.32 M) to reduce osmotic stress in injected tissues. Polycarbonate membrane filters (Sterlitech Corp., Washington, USA; pore-size 0.01 µm, diameter 25 mm) were placed in a cell culture dish containing ~ 10 ml sucrose solution and incubated ON at RT. Subsequently RBs were stored at 4°C until use.

Mice were anesthetized with initially 3 % Isoflourane (Minrad, Inc., Buffalo, NY, USA) with 2 % during stereotactical surgery. Mice were placed into a stereotactical frame (David Kopf Instruments; Bilaney GmbH, Düsseldorf, Germany) and injected with RBs to the mPFC or the HC using a syringe (600 Series, 5µl, Hamilton Bonaduz AG, Bonaduz, GR, Switzerland) or a picospritzer (Föhr Medical Instruments GmbH, Seeheim-Ober Beerbach, Germany) in combination with glass pipettes (borosilicate glass capillaries, World Precision Instruments, Inc., Sarasota, Florida, USA pulled on a Flaming/Brown micropipette puller P-97, Sutter Instruments, Novato, CA, USA). The injection volumes for the mPFC and the HC were 0.2 and 0.3 µl respectively. Coordinates to target bilaterally the mPFC were originating from the bregma: rostral + 1.9, lateral +/- 0.3 and ventral 2.3 (PL) and 2.5 (IL), respectively; the HC coordinates were caudal – 3.6, lateral +/- 3.6 and ventral 3.7 (all coordinates calculated from the Mouse Brain Atlas by Franklin and Paxinos). During surgery mice were locally treated with lidocaine (Boehringer Ingelheim, Germany) and naropin (Astrazeneca, Germany) for analgesia. Post-surgery treatment of mice involved subcutaneous injection of 0.06ml Metacam (Boehringer Ingelheim, Germany) to reduce pain and inflammation risk.

3.5 Immunohistochemistry

Mice were deeply anaesthetized using urethane 2 h after the completion of the behavioral testing and perfused transcardially with ice-cold solution of 4% paraformaldehyde in phosphate buffer (PFA; pH 7.4). After postfixation overnight in the same fixative at 4 °C, coronal sections (50 μm) were cut on a vibratome (Leica, Nussloch, Germany) and collected in phosphate-buffered saline (PBS). Free-floating sections were rinsed in PBS. After four rinses, sections were incubated in a blocking solution (10% bovine serum albumin (BSA) and 0.2% Triton X-100 in PBS) containing the primary polyclonal rabbit anti-c-Fos (Oncogene Research Products; 1: 20000 dilution) or anti-Zif268 (Santa Cruz; 1: 5000 dilution) over night (ON) at 4 °C. Subsequently, sections were washed with PBS and incubated for 2 h at RT with fluorescent goat antirabbit IgG (Santa Cruz; 1: 1000 in PBS). Finally immuno-labeled sections were rinsed in 3x in PBS, mounted on gelatin-coated slides, dehydrated and coverslipped.

3.6 Confocal microscopy and image analysis

3D z-stacks of BA parts and 2D overview pictures (tiles) of the whole amygdala were acquired using confocal fluorescence microscopes, either a LSM 510 or a LSM 510 meta (Carl Zeiss AG, Germany) and three different lasers (488, 543 and 633 nm). Tiles and z-stacks were acquired using a 40x/1.3 oil immersion DIC lens. Settings for acquisition (photomulitplier assignment and contrast values) were adjusted for different staining batches using a pixel saturation tool on very bright and dark spots. Pinhole was always adjusted to 1. Tiled images from the entire amygdala were collected with an opened pinhole. Confocal z-stack image series were sectioned in 1 µm thin optical planes (325.8 µm x 325.8µm x 10-35µm), tiles were taken in 9 x 9 images (2592.8µm x 2602.2µm). For quantification of IEG induction six tiled overview pictures/mouse and for assessing colocalisation of RBs/IEG five images per animal were analyzed.

Quantification of IEG induction following different behavioral paradigms of the mice were done on tiles and analyzed with Imaris (Bitplane, Zürich, Switzerland) and LSM Image Browser (Carl Zeiss AG, Germany). The different subnuclei of the BLA (BA, LA, BMA) were defined with the Image Browser and then imported to Imaris to automatically count positive nuclei with the spot detection software.

Parameters were kept constant within comparable batches of raw files. The spot diameter was set to 10 µm for every analysis, whereas the detection threshold was changed depending on the experiment. For the RBs/IEG colocalisation, double labeled neurons were counted manually in the LSM Image Browser.

3.7 Mouse brain slice preparation

Brain coronal slices were prepared from six to nine week old male mice injected with RBs into the mPFC. Mice were either transgenic for cFos-GFP (lines 1-3 or 4-1 backcrossed into C57BL/6J background) or littermates. Brains were dissected in ice-cold artificial cerebrospinal fluid (ACSF), and sliced (300 µm thick) with a Microm slicer (model HM 650 V; Walldorf, Germany) at 4ºC. Sapphire blades (Delaware Diamond Knives, USA) were used, to improve slice quality. Slices were recovered for 45 min at 37ºC in an interface chamber containing ACSF equilibrated with 95% O2/5% CO2. The ACSF contained (in mM): 124 NaCl, 2.7 KCl, 2 CaCl2, 1.3 MgCl2, 26 NaHCO3, 0.4 NaH2PO4, 18 glucose, 2.25 ascorbate.

3.8 Electrophysiological recordings

Whole-cell patch-clamp recordings were obtained from RB-labeled projection neurons in the BA at RT in a submerged chamber with a constant perfusion with fresh ACSF.

Neurons were visually identified with infrared video microscopy using an upright microscope equipped with x5 and x40 objectives (Olympus, Hamburg, Germany). Fluorescent cells were identified using a Polychrome V Till imaging system (Till Photonics, Gräfelfing, Germany). Patch electrodes (3-5MΩ) were pulled from borosilicate glass tubing and were filled with an intracellular solution consisting of (in mM): 130 potassium-gluconate, 10 HEPES, 10 phosphocreatineNa_2, 4 Mg-ATP, 0.4 Na-GTP, 5 KCl, 0.6 EGTA (pH adjusted to 7.25 with KOH, ~ 280 - 300mOsm). In voltage- and current-clamp recordings, membrane potential was held at −70mV. Monosynaptic EPSPs were elicited by stimulation of afferent fibers with a bipolar twisted platinum/10% iridium wire (25µm diameter). Bipolar stimulating electrodes were placed on afferent fibers from the internal capsule (containing thalamic afferents), the external capsule (containing cortical afferents)

or ventral to the CE (containing HC and mPFC afferents). All recordings were performed in the presence of 100µM picrotoxin, a $GABA_A$ antagonist.

Data were acquired with pClamp9.2 (Axon Instruments, Union City, CA, USA) and recorded with a Multiclamp700B. Voltage-clamp experiments were filtered at 2 kHz and sampled at 5 kHz, whereas all current-clamp experiments were filtered at 10 kHz and sampled at 20 kHz. Series resistance was monitored throughout the experiments by applying a hyperpolarizing pulse. Any modification of the series resistance exceeding 20% was a cause for the exclusion of the data from the analysis. Spiking patterns were assessed by applying two different protocols to the cells in current-clamp; one applying hyperpolarizing currents from -200 pA to depolarizing currents at +250 pA in 50 pA steps, the other ranging from -400 pA to +500pA in 100 pA steps. Paired-pulse ratio was measured from files recorded in voltage-clamp. Stimulation electrodes, placed at the external or internal capsule or ventral to the CE, were programmed to evoke two EPSPs separated by 50 ms. The first EPSP was calibrated to an approximate size of 50 – 100 mV. Spontaneous recordings were achieved in the gap-free modus. Two 5 min files were recorded and before and after series resistance has been checked. All values are expressed as means ± s.e.m. Statistical comparisons were done with paired or unpaired Student's t-test or with a one- or two-way ANOVA followed by a post-hoc Tukey test as appropriate (two-tailed $p < 0.05$ was considered significant).

3.9 Biocytin filling and staining

One neuron per slice was filled with a Neurobiotin/Biocytin mix (approx. 5mg/ml intracellular solution; Vector Laboratories, Inc., Burlingame, CA, USA; Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Subsequently, slices were transferred to 4% PFA and kept for 1 – 3 weeks at 4°C for fixation. Staining procedure as described before using ABC-elite kit (Vector Laboratories, Inc., Burlingame, CA, USA; (McDonald, 1992; Wilson and Sachdev, 2004))

3.10 Disconnecting-inactivation of BA and mPFC by muscimol iontophoresis

Muscimol micro-iontophoresis injection was performed in chronically implanted animals. Single-barrel micropipettes with a tip diameter of 10 to 15 µm were cut at 1 cm length and filled with a solution containing muscimol covalently coupled to a fluorophore (Muscimol-Bodipy-TMR conjugated, Invitrogen; 5 mM in phosphate buffered saline (PBS) 0.1 M, DMSO 40%) or with bodipy alone (Invitrogen; 5 mM in PBS 0.1 M, DMSO 40%). Mice were bilaterally implanted at the following coordinates: one side was targeted to the BA 1.7 mm posterior to bregma; 3.3 mm lateral to midline; and 4 mm to 4.3 mm deep from the cortical surface. The other side was targeted to the IL: 1.9 mm anterior to bregma; 0.3 mm lateral to midline; and 2.4 mm deep from the cortical surface. Chlorided silver wires were inserted in each micropipette and attached to a connector. A third silver wire screwed onto the skull and attached to the connector served as a reference electrode. The entire miniature was secured using cyanoacrylate adhesive gel. After surgery, mice were allowed to recover for 1 day. On the injection day, iontophoretic applications were performed by means of cationic current (+14 µA) for 9 min and 6 min for BA and IL targeting, respectively using a precision current source device (Stoelting). Mice were submitted to the behavioural procedure 5 min after the end of iontophoretic injections and were immediately perfused at the end of the experiments. Brains were collected for further histological analysis. Serial slices containing the amygdala were imaged at X5 using an epifluorescence stereo binocular (Leica), and the location and the extent of the injections were controlled. Mice were included in the analysis only if they presented a injection targeting exclusively the BA and the IL, respectively. Statistical analyses were performed using paired and unpaired Student's t-tests post hoc comparisons at the $P < 0.05$ level of significance. Results are presented as mean ± s.e.m.

3.11 Molecular approach for inactivation of synaptic transmission (MISTs)

3.11.1 AAV serotype testing

To investigate which AAV serotype efficiently infects the amygdala I used pre-made AAVs AAV1-CAG-GFP and AAV2-CAG-GFP (Applied Viromics, USA). Until usage AAVs were kept at -80°C. Prior to injection I mixed the AAV containing solution 50:50 with red fluorescent latex beads for easy identification of the injection site (no retrograde transport; Invitrogen GmbH) and kept the vial at 4°C. Injection was performed as described before for retrograde labeling with a Hamilton syringe. Coordinates for targeting the BLA were 1.7 mm posterior, 3.3 mm lateral and 4.3 mm ventral. Two weeks after injection, mice were transcardially perfused with 4% PFA, brains were sectioned and sections were stained for GFP (GFP-AB, Invitrogen GmbH). Picture analysis was done at a confocal microscope LSM510 (Zeiss, Germany). Both serotypes were able to infect the BLA but AAV1 appeared more to be more efficient (verification by eye).

3.11.2 Cloning procedure to generate AAV for MIST

To generate the adeno-associated virus (AAV) for MISTs I cloned a modified Vamp2, fused to a small protein capable of homodimerization (VampFv2) into a pAAV-CAG-shuttle plasmid (Applied Viromics, CA, USA). Additionally a modified shuttle vector containing the synapsin- instead of the chicken β-actin (CAG) – promoter was generated. A VampFv2-IRES-eGFP original construct was generously provided by the lab of Karel Svoboda (AK-353, pBS Kozak-VampFv2-IRES-EGFP-WPRE; Janelia Farm, USA). Standard cloning procedures were applied as previously described. Kozak-VampFv2-EGFP-WPRE-polyA was amplified from AK-353 to include a NheI cleavage site at the 3' end of the construct using PCR (Primer: VampFv2-NheI 5'GCTTGCTAGCGAATTCTTAATTAAGCCACCATG 3'; VampFv2-SphI 5' CCCAGCATGCCTGCTATTG 3'). The PCR product as well as shuttles pAAV-CAG and pAAV.Syn were cut with restriction enzymes NheI and SphI and ligated to the plasmids pAAV-CAG-VampFv2 and pAAV-Syn-VampFv2 (Fig. 6). To verify accuracy of the two constructs they were sent to the sequencing facility.

Subsequently I sent the verified vectors in for virus (AAV1) production (Applied Viromics, USA).

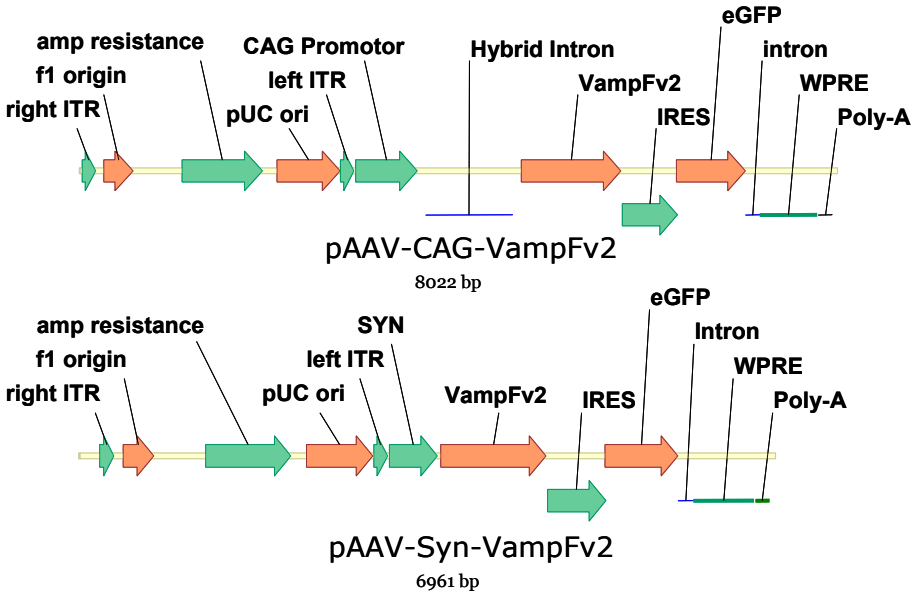


Figure 6: *Viral vectors to express VampFv2 and GFP in neurons. Expression is driven either by the CAG- (upper construct) or the Syn- (lower construct) promoter.*

3.11.3 Testing of AAV-Syn-VampFv2 and AAV-CAG-VampFv2

To test how efficient synaptic transmission is silenced by AAV-Syn/CAG-VampFv2 infection we infected the CA3 region in hippocampal slice cultures (generously provided by the lab of Thomas Oerthner). Infection was obtained by injecting the virus to the target region with a picospritzer (see retrograde labeling). Two to three week after infection whole-cell patch-clamp recordings were obtained from CA1 projection neurons at RT in a submerged chamber with a constant perfusion with fresh cellculture ACSF (ASCFcc). The ACSFcc contained (in mM): 124 NaCl, 2.7 KCl, 4 CaCl2, 4 MgCl2, 26 NaHCO3, 0.4 NaH2PO4, 18 glucose, 2.25 ascorbate, 1 Chloroadenosine, 0.1 Picrotoxin, 0.01 carboxypiperazin-4-yl-propyl-1-phosphonic acid (CPP). The dimerizer FKBP was obtained from ARIAD Pharmaceuticals (USA) and solved in DMSO to 100 µM.

A stimulation electrode was placed on green fluorescent cells in the CA3 region heavily projecting to CA1 neurons. Following acquisition of baseline evoked responses, perfusion was changed to ACSFcc containing FKBP at a 1:1000

dilution (Karpova et al., 2005). Control conditions contained also 0.1% of DMSO. Electrophysiological recordings were performed as described in chapter 3.8.

4 Results

4.1 Different induction of immediate early genes in anatomically defined BA projection neurons after consolidation and extinction of conditioned fear

4.1.1 Immediate early gene expression in the amygdala following acquisition and extinction of conditioned fear

To map the activity induced by fear-related learning in the amygdala I analyzed immediate early gene (IEG) expression in different subnuclei of the amygdala following auditory fear conditioning and extinction. I used the IEGs cFos and Zif268 which are widely employed in activity-mapping paradigms throughout the brain and which are rapidly and transiently induced (Herry and Mons, 2004; Guzowski et al., 2005). Because expression of both IEGs peaks 2h following activity-induction I performed my experiments at this time point (Gass et al., 1992). IEG expression in brain sections from mice subjected to different behavioral training was analyzed (Fig. 7A). Naïve animals and those receiving only auditory stimulation (CS only) served as controls. No associative learning occurred under these conditions and the mice showed low freezing behavior (Fig. 7B). Fear conditioned (FC) mice showed high fear responses to CS presentation 2h and 2 days following acquisition (Fig. 7C, D,: FC $p < 0.001$; Fear consolidation day2 $p < 0.001$, day3 $p < 0.001$, student's T test). Extinction, by repeated non-reinforced presentation of the CS led to a gradual decline of fear responses (Fig. 7E, no CS vs CS day2 $p < 0.001$, CS day2 vs CS day3 $p < 0.001$, student's T test).

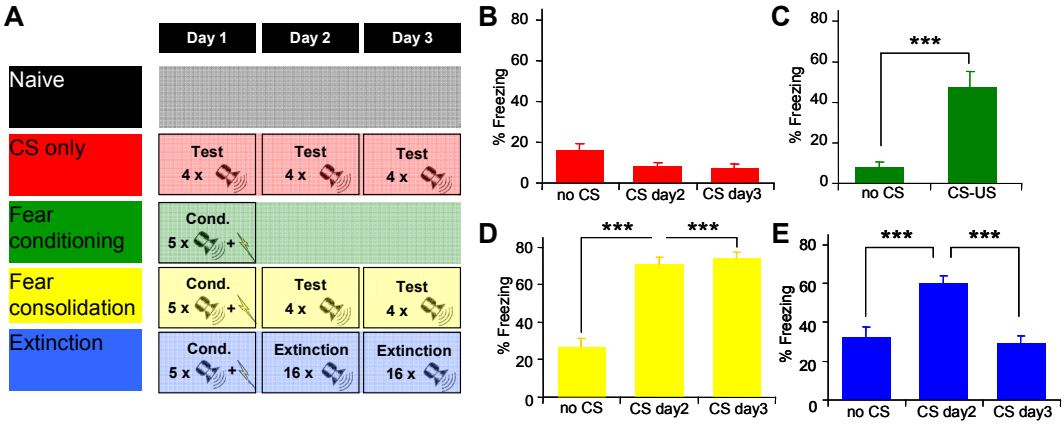


Figure 7: *Behavioral analysis of mice included in the study.* ***A:*** *Naïve animals were habituated to humans by handling. CS only mice were exposed to the tone (CS) on 3 consecutive days (n=21). For FC, mice were exposed to 5 CS-US pairings and either subsequently (fear conditioning, n=12)), or after 2 days of memory consolidation (fear consolidation, 4 CS presentations per session, n=22) or 2 days of extinction (16 CS presentations per session, n=17) sacrificed for IEG expression analysis.* ***B:*** *Control mice showed no elevated freezing response to the CS, compared to baseline levels.* ***C, D:*** *During FC mice acquired high freezing behavior to the tone (C) that persisted in the fear consolidated mice over two consecutive days (D).* ***E:*** *Extinction led to decline of fear behavior.*

The LA, the BA and the BMA at rostro-caudal coordinates from Bregma: -1,22 – 2.18 were analyzed for IEG expression. The number of IEG positive cells was normalized to the number of all neurons in these nuclei. The number of neurons was determined by counting NeuN (neuronal nuclei) positive nuclei in amygdala brain sections of the same rostro-caudal levels from naïve animals (Fig. 8A). The total number of NeuN positive nuclei varied little (LA: 1069 +/- 23 cells/mm^2; BA: 693 +/- 34 cells/mm^2, n = 3). Naive animals expressed undetectable or very low baseline levels of cFos in the amygdala, whereas CS presentation alone induced an increase in cFos expression in all nuclei (Fig. 8B). This is not surprising as it is known that a proportion of amygdala neurons is activated by sensory input like tones (Quirk et al., 1995). FC strongly increased the number of cFos-positive cells, whereas fear consolidation and extinction resulted in a moderate increase of cFos-positive cells only in the LA and the BA. The highest expression of cFos was detected in the BA, where up to 12 +/- 1 % (n = 12) of all neurons expressed cFos after FC. In contrast, in the LA FC induced cFos expression in about 6 +/- 1 % (n = 12) of the cells.

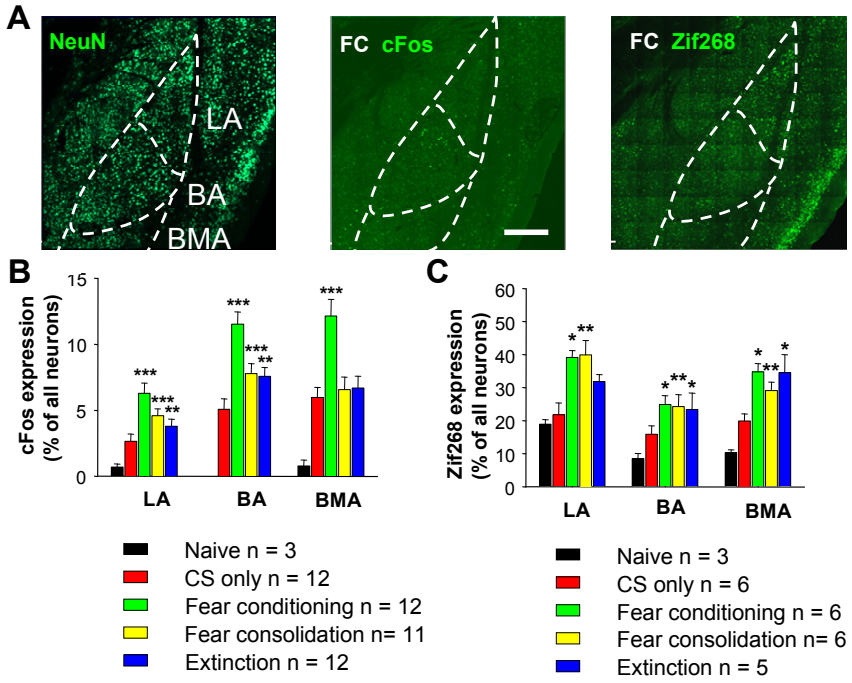


Figure 8: *Fear learning and extinction induce IEG expression in different subnuclei of the amygdala.* ***A:*** *Example for a NeuN Ab (left panel), cFos Ab (middle panel) and Zif268 Ab (right panel (Alexa 488) stained brain slice (section shows amygdala) used for anatomical quantification.* ***B, C:*** *Quantification of cFos and Zif268 expression in all neurons of different amygdala subnuclei as a function of behavioral pradigms. **p < 0.01 ***p < 0.001.*

Robust baseline expression in naïve mice was found for Zif268 (9 +/- 2 % of all neurons, n = 3). In contrast to cFos, expression levels of Zif268 were almost the same between the naïve and the CS only groups (Fig. 8C). Following FC, fear consolidation and extinction Zif268 expression was moderatly upregulated in the LA, the BA and the BMA. While the majority of cFos-positive cells clustered in the anterior part of the BA, Zif268 was predominately found in the LA (Fig. 8A). In summary, expression of different IEGs in the amygdala depends on the specific behavioral state of the animal and thus provides a good tool to dissect functionally distinct neuronal subpopulations within the amygdala.

4.1.2 Anatomically defined neurons in the basal amygdala (BA) differentially express cFos and Zif268 following fear conditioning and extinction

In contrast to the cortex or the HC, the amygdala is a non-layered structure. Within the subnuclei, excitatory pyramidal cells and different types of GABAergic

interneurons are intermingled, making it difficult to distinguish subpopulations of cells. To identify different neuronal sub-populations, I performed injections of a fluorescent retrograde tracer (Lumaflour retrobeads, RBs) into the mPFC and into the ventral HC. Both brain areas have been implicated in fear learning and extinction (Quirk et al., 2006; Ji and Maren, 2007). Since the amygdala is strongly connected to these structures, amygdala projections to the mPFC and HC are likely to be involved in fear learning and extinction.

RB labeling allows identification of amygdala neurons with defined anatomical targets (Fig. 9A). mPFC and vHC projecting neurons were mainly found in the magnocellular part of the BA. Thus, my analysis is limited to the BA (Fig. 9B). Double injections into the mPFC and the vHC in the same animals using different RB-fluorophores showed that retrogradely labeled cells were intermingeled within the same region of the BA. Double labeled neurons were observed very rarely, suggesting that individual cells exclusively project either to the mPFC or the HC (Fig. 9A, B).

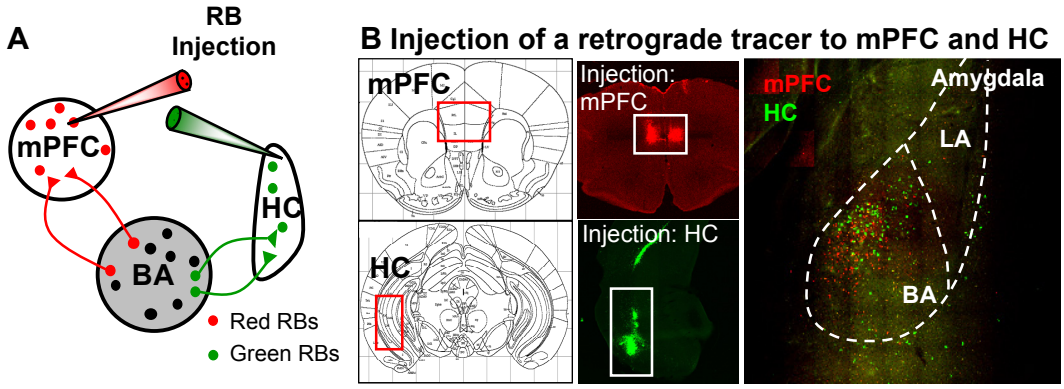


Figure 9: *Injection of Retrobeads into mPFC (red) and HC (green) leads to labeling of anatomically defined cells in the amygdala.* ***A:*** *Schematic drawing.* ***B:*** *representative injections to the mPFC (upper panel, red RBs) and the HC (lower panel, green RBs) and related labeling in the amygdala (right panel). Left panels to illustrate anatomical location of injection from Paxinos and Franklin, mouse brain atlas.*

To address whether IEG expression in different neuronal sub-populations is associated with fear conditioning and extinction *in vivo*, injections of RBs into the mPFC and the HC were combined with IEG stainings (Fig. 10A). In mPFC projecting neurons, a prominent increase in cFos expression occurred in animals from the FC group compared to the CS only group (Fig. 10B; first panel). After

fear consolidation and extinction training, mPFC-projecting cells showed intermediate cFos levels (Fig. 10B). In contrast, HC-projecting neurons showed a moderate increase in cFos expression in behavioral training eliciting high fear, but no increase after fear extinction (Fig. 10B; middle panel, $p < 0.05$, one-way ANOVA, CS only vs. FC $p = 0.055$, CS only vs. fear consolidation p = n.s., post hoc tukey test).

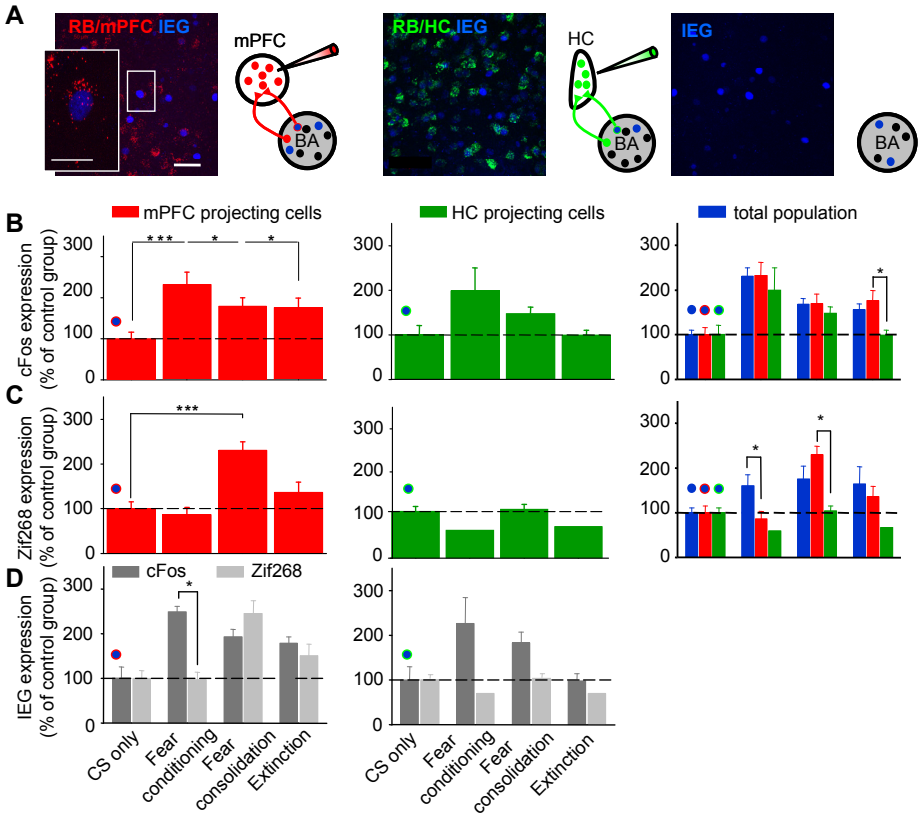


Figure 10: *Colocalization of FC-induced IEG immunoreactivity with RB retrograde tracer.* ***A:*** *IEG expression analysois of mPFC- (left panel), HC-projecting (middle panel) or all (right panel) BA neurons.* ***B:*** *BA cells projecting to the mPFC express cFos highest after FC and also after fear consolidation and extinction (left panel). HC-projecting neurons express cFos in response to high fear behavior but not after extinction (middle panel). Comparison of cFos expression in all, mPFC-projecting and HC-projecting BA neurons (right panel).* ***C:*** *Zif268 expression is specifically induced by fear consolidation in mPFC-projecting neurons (left panel). HC-projecting neurons are not induced for Zif268 expression (middle panel). Comparison of all vs mPFC- and HC-projecting BA neurons reveals high cell-type specificity (right panel).* ***D:*** *Comparing cFos and Zif268 expression shows differences in induction. * $p < 0.05$, *** $p < 0.001$.*

When comparing the normalized increase in cFos positive cells in the BA vs. the increase in mPFC- and HC- projecting subpopulations it becomes clear that the low cFos expression in HC-projecting cells after extinction is highly characteristic (Fig. 10B; right panel). Whereas after FC and fear consolidation cFos induction in mPFC and HC projecting neurons follows the overall trend of highest cFos

expression after fear conditioning and an intermediate increase after fear consolidation, after extinction only mPFC-projecting BA neurons show an increase in cFos. HC-projecting cells are excluded from the overall increase cFos induction after extinction.

Zif268 expression was quite different in mPFC-projecting cells compared to cFos expression. Only after fear consolidation mPFC-projecting BA neurons expressed high Zif268 levels (Fig. 10C; right panel). This is in accord with the postulated role of Zif268 in the consolidation of fear memory (Malkani *et al*., 2004). No upregulation could be found in HC-projecting neurons following different behavioral training (Fig. 10C, middle panel). Comparing the overall induction of Zif268 in the BA with that in mPFC- and HC- projecting neurons revealed a highly cell-type specific expression pattern as a response to different behavioral paradigms (Fig. 10C; right panel). Even though FC induced Zif268 expression in the BA, these neurons did not project to either the mPFC or the HC. Fear consolidation resulted in a slightly over-proportional expression of Zif268 in mPFC-projecting cells and no induction in HC-projecting neurons. Similarly there was no increase in Zif268 expression after extinction in HC-projecting cells, but in mPFC-projecting neurons.

To rule out that colocalisation data between anatomically defined neurons and IEGs were biased due to variability in the number of labeled cells, I counted all cells included in each group. There was no difference in the number of retrogradely labeled cells between groups (Fig. 11).

Comparison of cFos and Zif268 showed IEG-dependent specificity for both the cell-type and the type of behavioral training (Fig. 10D). In particular, acute FC induced cFos expression in a cell-type specific manner mPFC- and HC-projecting neurons (Fig. 10D; left and middle panel). Although literature points to a prominent role of the HC in contextual aspects of fear learning and extinction (Cammarota *et al*., 2005; Maren and Quirk, 2004) no upregulation of cFos and Zif268 could be detected in HC-projecting cells after extinction (Fig. 10D; middle panel). Thus, extinction and fear learning differentially induced cFos and Zif268 in amygdala neurons in a cell-type specific manner.

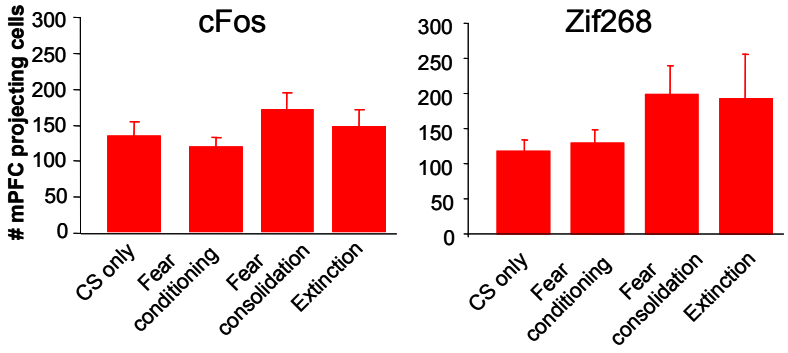


***Figure 11**: Control plots that fluctuations in cell numbers between groups do not account for the results shown in Fig. 9. **A, B:** Total number of mPFC-projecting BA neurons to quantify the overlap between anatomically labeled cells and cFos positive (A)or Zif268 positive (B) cells, respectively.*

4.1.3 Fear learning and extinction differentially induce cFos in neurons projecting to distinct subdivisions of the mPFC

Since the IL and the PL subdivisions of the mPFC are thought to exert opposing effects on fear learning, we hypothesized that neurons within the amygdala projecting to the IL or PL could contribute to fear conditioning and extinction, respectively (Maren and Quirk, 2004). Indeed, analysis of data with precise PL- or IL-injections revealed different expression of cFos and Zif268 (Fig. 12). cFos expression was high in PL-projecting neurons after high-fear behavioral paradigms and low after extinction (Fig. 12A). In contrast, IL-projecting neurons showed increased cFos expression in the extinguished mice and low cFos expression after FC and fear consolidation. Preliminary results for Zif268 expression in IL- and PL-projecting showed no increase in expression for either sub-area after FC (Fig. 12C). Fear consolidation induced Zif268 expression in both the PL- and the IL-projecting cells. In contrast to cFos, Zif268 was not upregulated in IL-projecting neurons after extinction.

These different results were not a consequence of fluctuating cell numbers between groups (Fig. 13). Similar number of neurons was counted for the different behavioral groups (Fig. 13). There was no correlation between the total number of anatomically defined cell number and the extend of colocalization with cFos.

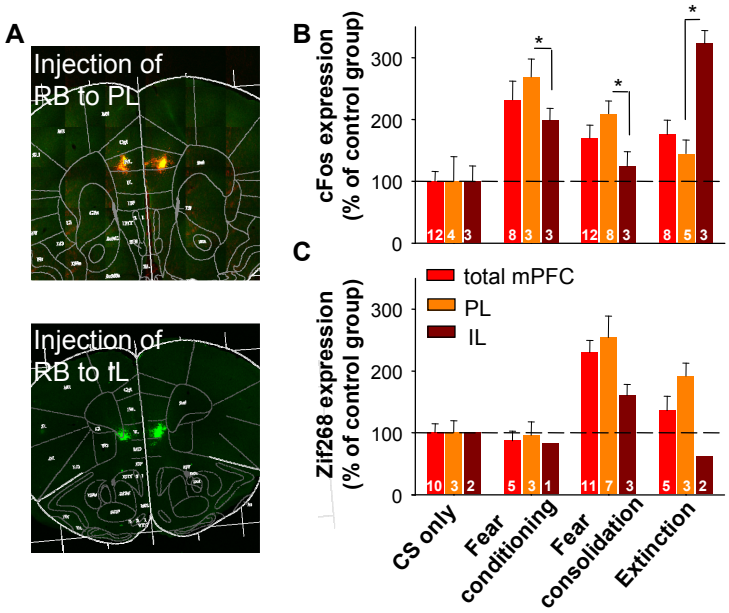


Figure 12: *Colocalization of FC-induced cFos immunoreactivity with RB retrograde tracer injected to PL or IL.* ***A:*** *Examples for RB injection sites in the PL (top, red) and IL (bottom, green) part of the mPFC.* ***B:*** *Neurons projecting to the infralimbic division of the mPFC express cFos specifically after extinction, whereas neurons projecting to the prelimbic division express cFos after FC and consolidation of FC.* ***C:*** *Neurons projecting to the mPFC do not express Zif268 after FC. Following fear consolidation and extinction PL-projecting BA neurons express higher Zif268 levels. * P< 0.05 **P< 0.01 ***P<0.001.*

Together, fear learning and extinction differentially induced cFos in amygdala neurons projecting to the PL and the IL subdivisions of the mPFC, respectively. This suggests that IL-projecting neurons were activated by extinction training, whereas PL-projecting neurons were specifically activated during fear learning. Preliminary results for Zif268 showed that its expression, similar to cFos, was high in PL-projecting neurons after fear consolidation of mice. However, extinction training revealed the mirror-image: Zif268 induction in PL-projecting neurons. This suggests cell-type specificity in the behavior-dependent induction of different IEGs. Furthermore, extinction training induced large and characteristic effects in terms of IEG expression in IL-projecting BA neurons and is therefore a very interesting behavioral paradigm to study in those cells.

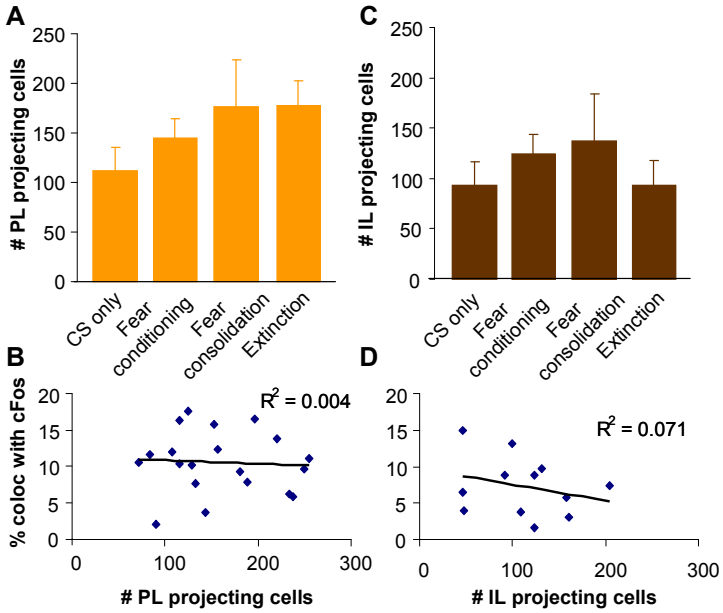


Figure 13: *Control plots that fluctuations in cell numbers between groups do not account for the results shown in Fig. 11.* ***A, C****: Total number of PL- (A) and IL-projecting (C) BA neurons used to quantify the overlap between anatomically labeled cells and cFos positive cells.* ***B, D:*** *Correlation plots showing no correlation between the number of anatomically labeled cells (PL-(B) and IL-projecting (D)) and the colocalization with cFos I analyzed.*

4.2 Physiological correlates of extinction in anatomically defined BA neurons

4.2.1 The synaptic drive in PL/IL-projecting neurons is not altered following extinction training

To further investigate if anatomically defined BA neurons contribute specifically to fear learning and extinction, I characterized IL- and PL-projecting cells physiologically using an *ex vivo* approach. Changes in synaptic strength or intrinsic neuronal plasticity occur upon fear conditioning and extinction in the amygdala and in the mPFC (McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997; Santini et al., 2008). However, properties of mPFC-projecting amygdala neurons have not been investigated so far. The most pronounced differences in cFos expression were observed between IL- and PL-projecting BA neurons after extinction. Therefore, I recorded from identified IL- or PL-projecting neurons in slices obtained from extinction-trained mice to investigate learning related changes (Fig. 14A, B). I analyzed four different groups of neurons: PL- and IL-projecting neurons from CS only control animals (PL-CS only and IL-CS only) and

PL- and IL-projecting cells from animals subjected to extinction training (PL-X and IL-X). First, I examined the passive properties, and found that they did not differ between groups (Table 1, one-way ANOVA across all four groups: V_{Rest} n.s.; R_{series} n.s.; R_{in} n.s.; Tau n.s.; Cap n.s.).

Table 1: *Neurons projecting to PL or IL share the same basic properties.*

	PL-CS only (n=16)	PL-X (n=27)	IL-CS only (n=26)	IL-X (n=10)
Vrest (mV)	**- 71** +/- 1.6	**- 69** +/- 1	**- 69** +/- 2	**- 68** +/- 1.4
Rseries (Mohm)	**14.1** +/- 0.5	**15.1** +/- 0.5	**14.6** +/- 0.3	**15.7** +/- 0.6
Rin (MOhm)	**186** +/- 18	**194** +/- 13	**175** +/- 21	**167** +/- 13
Tau (ms)	**3.2** +/- 0.1	**3.4** +/- 0.2	**3.2** +/- 0.1	**3.5** +/- 0.2
Cap (nF)	**263** +/- 14	**267** +/- 13	**258** +/- 13	**252** +/- 19

To investigate if synaptic changes were induced by extinction training I measured the paired pulse ratio (PPR) of evoked excitatory postsynaptic currents (EPSCs) by stimulating afferent fibers innervating the amygdala (Fig. 14C). The PPR is a correlate for synaptic release probability and is calculated as the ratio of the second event's peak over the first (Fourcaudot et al., 2008). I stimulated inputs from cortical and thalamic areas that reach the amygdala via the external and the internal capsule, respectively. Another site of incoming fibers from the mPFC and the HC to the BA is located ventral to the CE. This pathway has been identified in our laboratory using anterograde or retrograde viral tracing techniques (unpublished data: Ingrid Ehrlich and Steffen Wolff). The PPR in the three input pathways (cortical, thalamic and HC/mPFC) was determined using an inter-stimulus interval of 50 ms (Fig. 14C). None of the examined pathways showed a change in PPR in any of the groups analyzed (Table 2; one-way ANOVA: PPR_{thal} $p = 0.08$; PPR_{ctx} n.s.; $PPR_{HC/mPFC}$ n.s.).

Table 2: *Neurons projecting to PL or IL share the same values for PPR on the different pathways. Data for PPRctx was not included to analysis due to low n.*

	PL-CS only	PL-X	IL-CS only	IL-X
PPRctx	**1.4** +/- 0.09 (n=4)	**1.4** +/- 0.11 (n=8)	**1.3** +/- 0.06 (n=7)	? (n=2)
PPRthal	**1.6** +/- 0.12 (n=10)	**1.4** +/- 0.1 (n=21)	**1.5** +/- 0.1 (n=5)	**1.8** +/- 0.2 (n=9)
PPRHC/mPFC	**1.4** +/- 0.12 (n=14)	**1.3** +/- 0.13 (n=11)	**1.3** +/- 0.15 (n=10)	**1.1** +/- 0.1 (n=5)

To address if overall synaptic drive onto different subpopulations was altered by learning, I recorded spontaneous EPSCs (sEPSCs, Fig. 14D). This allows for an unbiased readout of overall synaptic drive. Changes in the frequency of events suggest changes in the number of connections made onto the recorded cell, while changes in the amplitude point to a strengthening of synaptic connections. Rise- and decay-time are determined by ion-channel composition, but are also shaped by the distance of the synapse to the somatic recording pipette. Analysis of frequency, amplitude, rise- and decay-time of sEPSCs did not reveal any differences between groups (Table 3; one-way ANOVA: frequency n.s., amplitude n.s., rise-time n.s., decay-time n.s.).

Table 3: *IL- and PL-projecting neurons do not differ in terms of frequency, amplitude, rise- and decay-time of spontaneous activity. Frequency is shown as number of events in 5 min.*

	PL-CS only (n=16)	**PL-X (n=27)**	**IL-CS only (n=26)**	**IL-X (n=10)**
Frequency	**1387** +/- 208	**1858** +/- 251	**1938** +/- 447	**1722** +/- 472
Amplitude (pA)	**13** +/- 0.4	**14.9**+/- 0.8	**14.6**+/- 0.8	**13** +/- 0.6
Risetime (ms)	**2.1**+/- 0.04	**2.1**+/- 0.04	**2.1**+/- 0.03	**2.1**+/- 0.09
Decaytime (ms)	**4.1**+/- 0.2	**4.7**+/- 0.2	**4.2**+/- 0.1	**4.2**+/- 0.3

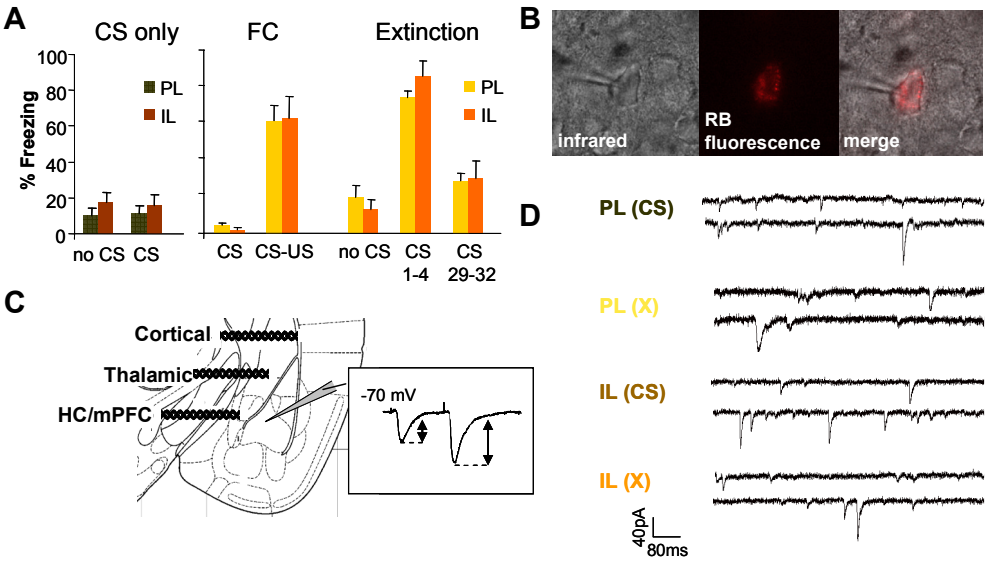


Figure 14: A: *Behavior of CS only and extinguished animals included to physiological analysis of anatomically labeled neurons. Mice with RB injections to PL or IL did not differ in terms of freezing behavior.* ***B****: Infrared DIC and fluorescent images of a living, retrobead labeled BA neuron.* ***C:*** *Schematic drawing (Paxinos and Franklin, mouse brain atlas) indicating stimulation- (cortical, thalamic, HC/mPFC pathway) and recording sites (BA) for PPR measurement (inset).****D:*** *Example traces of spontaneous activity.*

In summary, I did not find significant changes in synaptic properties. However, the IL-X group showed a trend toward an increased PPR in the thalamic and a decreased PPR in the HC/mPFC pathway. Furthermore, a value for PPR_{ctx} is missing because of insufficient numbers of experiments. Clearly, more experiments are needed to draw stronger conclusions about putative changes in release probability in IL-projecting BA neurons upon extinction training of mice.

4.2.2 IL-projecting neurons change intrinsic cellular properties following extinction

Next, I explored whether excitability and spiking properties of BA neurons were altered after extinction learning. To do so, I applied a series of negative and positive current steps in current-clamp mode while holding cells at -70 mV (Fig. 15A). I found that PL-projecting BA neurons did not change their firing or the AP properties following extinction learning (Fig. 15B; Table 4). In contrast, IL-projecting neurons showed a decreased AHP_{fast} at the downstroke of the AP (Fig 15C; Table 4; two-way ANOVA: $p < 0.001$; post hoc Tukey test: PL:CS only-IL:CS only $p < 0.05$, IL:X-IL:CS only $p < 0.01$, PL:X-IL:CS only $p < 0.01$, IL:X-PL:CS only n.s., PL:X-PL:CS only n.s., PL:X-IL:X n.s.) and an increased spike half-width following extinction learning (Fig. 15D; Table 4; two-way ANOVA: $p < 0.01$; post hoc Tukey test: PL:CS only-IL:CS only n.s., IL:X-IL:CS only $p < 0.001$; PL:X-IL:CS only n.s., IL:X-PL:CS only $p < 0.001$, PL:X-PL:CS only n.s., PL:X-IL:X $p < 0.01$). AHP_{fast} was measured in a 5 ms time-window following the first AP, as it is most pronounced after the fist spike. My results are in line with previous findings demonstrating that learning decreases the AHP_{fast} in mPFC neurons following extinction (Santini et al., 2008) and in hippocampal pyramidal neurons after trace eyeblink conditioning (Matthews et al., 2008). Furthermore, the AHP_{fast} underlying current I_c contributes directly to the spike half-width (Gu et al., 2007).

Table 4: *Firing and AP properties of PL- and IL-projecting BA neurons. The intrinsic properties of PL-projecintg BA neurons are not changed following extinction learning. IL-projecting neurons display an increased spike half-width and a decreased AHP_{fast} following extinction learning. Excitability = number of spikes at 250pA current injection, ISI = inter-spike-interval.*

	PL-CS only (n=16)	PL-X (n=27)	IL-CS only (n=26)	IL-X (n=10)
Excitability	**6.9** +/- 1.6	**7** +/- 0.9	**6** +/- 0.8	**6.5** +/- 0.9
Spike-threshold (mV)	**- 42** +/- 1	**- 43** +/- 0.7	**- 41** +/- 0.6	**- 40** +/- 0.8
Spike-amplitude (pA)	**86** +/- 0.8	**86** +/- 0.8	**86** +/- 0.7	**84** +/- 1.4
Spike half-width (ms) *	**1.4** +/- 0.03	**1.5** +/- 0.03	**1.4** +/- 0.04	**1.7** +/- 0.2***
AHP_{fast} (mV) *	**- 2.8** +/- 0.7	**- 2.8** +/- 0.6	**- 5.1** +/- 0.7**	**- 1.5** +/- 1.2
AHP_{medium} (mV)	**3.3** +/- 0.5	**2.9** +/- 0.3	**2.3** +/- 0.6	**3.9** +/- 0.7
AHP_{slow} (mV)	**- 0.6** +/- 0.2	**- 1** +/- 0.1	**- 0.7** +/- 0.1	**0.9** +/- 0.3
ISI (3^{rd} / 1^{st})	**2.1** +/- 0.1	**2.5** +/- 0.2	**2.4** +/- 0.2	**2.9** +/- 0.5

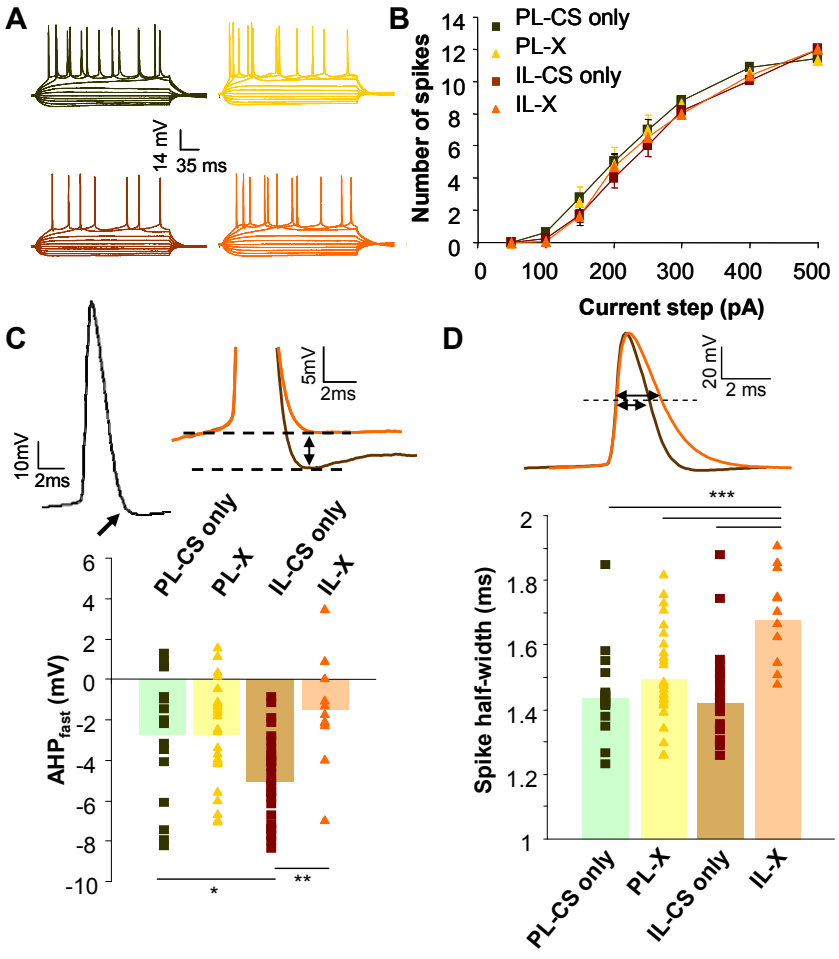


Figure 15: A: *Example traces of hyper- and depolarizing steps applied to neurons to assess intrinsic properties.* ***B:*** *Neurons projecting to PL or IL display the same intrinsic excitability.* ***C, D:*** *IL-projecting neurons decrease AHP_{fast} and increase spike half-width following extinction learning, while PL-projecting neurons do not change these properties.*

In summary, extinction learning did not result in changes in synaptic properties of IL- and PL- projecting BA neurons. Interestingly, intrinsic neuronal properties of IL-projecting neurons were altered upon extinction training. The AHP_{fast} was markedly reduced and the spike half-width was broadened. This decreased AHP_{fast} and the increased spike half-width may allow for larger Ca^{2+} influx into the neuron during spiking, which, in turn, could lead to downstream biochemical changes in the cell. This could account for the increased cFos expression in IL-projecting BA neurons following extinction.

4.2.3 AHP_{fast} and spike half-width in IL-projecting BA neurons is mediated by VDPC and BK-channels

To examine which channels mediate the observed changes in excitability I pharmacologically interfered with their function *in vitro*. The channels mediating the AHP_{fast} in neurons were identified in most brain structures as BK-channels (Matthews et al., 2008). However, in the LA, it was demonstrated that BK-channels are rather implicated in spike half-width determination than in the AHP_{fast} (Faber and Sah, 2003). The channels largely responsible for the AHP_{fast} are the VDPC subtypes Kv1.1, Kv1.2 and Kv1.6 (Faber and Sah, 2002). The BA has never been examined in this respect. BK-channels are known to be specifically blocked by the scorpion toxin iberiotoxin (IbTX), whereas the VDPC subtypes Kv1.1, Kv1.2 and Kv1.6 are blocked by α-dendrotoxin (α-DTX), a snake toxin. To investigate which channels mediate the pronounced AHP_{fast} in IL-projecting BA neurons in control conditions I recorded from IL-projecting neurons in slices obtained from CS only mice and blocked either BK-channels, VDPCs or both. Bath application of either DTX or IbTX alone reduced the AHP_{fast}, with a greater effect for DTX (Fig. 16A; DTX: reduction in AHP_{fast} = 4.49 mV +/- 0.44, $p < 0.001$; IbTX: reduction in AHP_{fast} = 2.66 mV +/- 1.05, $p < 0.05$; paired students t test). Similarly, the spike half-width depended on both channels as washin of either DTX or IbTX prolonged the spike (Fig. 16B; DTX: increase in spike half-width = 0.096 ms +/- 0.034, $p < 0.05$; IbTX: increase in spike half-width = 0.18 ms +/- 0.05, $p < 0.01$; paired students t test). Sequential bath application of both blockers sequentially resulted in a 59 +/- 4.6 % reduction of the AHP_{fast} by DTX

and a 41 +/- 4.6 % reduction by IbTX (IbTX washin in the presence of DTX, n = 1; DTX washin in the presence of IbTX, n = 2). In summary, my data support the notion that both BK-channels and VDPCs underlie the AHP_{fast} and the spike repolarization in IL-projecting BA neurons.

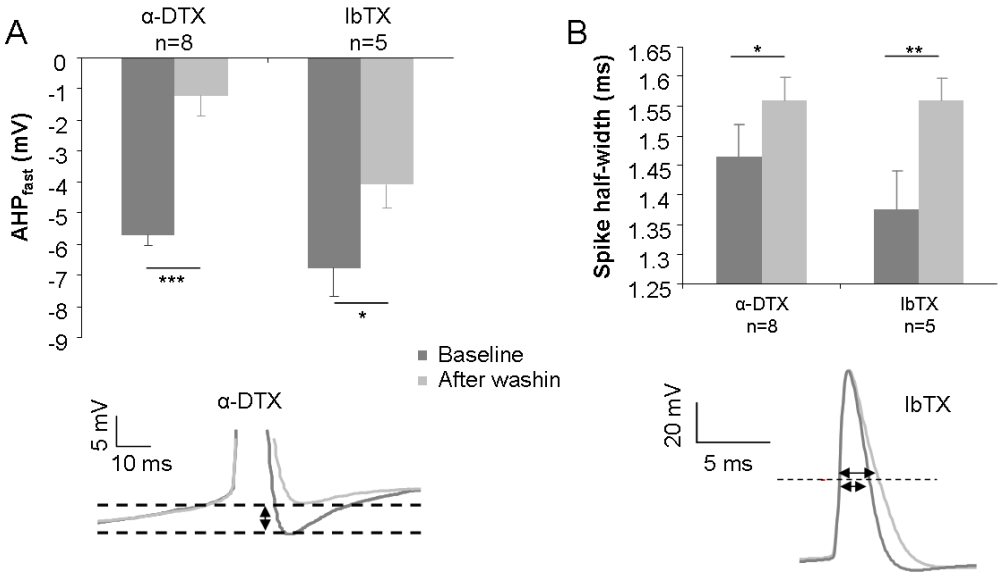


Figure 16: *Currents mediated by voltage-dependent potassium channels and BK-channels are involved in action potential repolarization and AHPfast in IL-projecting BA neurons.* ***A:*** *A specific blocker of voltage-dependent potassium channels (α-DTX, 100nM) and a BK-channel blocker (IbTX, 50nM) partially block the AHP_{fast}.* ***B:*** *Bath application of DTX and IbTX increase spike half-width.*

4.2.4 The decrease in the AHP_{fast} in IL-projecting neurons following extinction learning might be mediated by a functional downregulation of BK-channels

After extinction, IL-projecting neurons displayed a decreased AHP_{fast} and an increased spike half-width. This suggests a down-regulation of the underlying channels. As both parameters are mediated by BK-channels and VDPCs, either one of these potassium channels, or both, could contribute to this effect. Because applying α-DTX and IbTX to IL-projecting neurons in control conditions mimicked the changes in AHP_{fast} and spike half-width that were observed after extinction training, I analyzed if other parameter changes would be mimicked by drug application. Comparing the excitability of IL-projecting neurons before and after extinction revealed no change in the cellular excitability and spiking pattern even though the AHP_{fast} was decreased (Fig. 17 A, left panel). Application of α-DTX increased excitability in control IL-projecting cells (Fig. 17 A, right panel). In

contrast, blockade of BK-channels by IbTX mimicked the spiking property changes observed following extinction (Fig. 17A, lower panel): While the AHP_{fast} was decreased and the spike half-width was increased the excitability remained unchanged. This indicates a functional down-regulation of BK-channels in IL-projecting neurons following extinction.

To further investigate which channels were modulated by extinction, I applied IbTX and α-DTX to IL-projecting BA neurons recorded from mice subjected to fear extinction. Preliminary data showed that application of α-DTX blocked the AHP_{fast}, whereas wash-in of IbTX had no effect (Fig. 17B). This further supports our hypothesis of a functional down-regulation of BK-channels following extinction training. However, the spike half-width was still increased by application of both drugs. More experiments are required to confirm these preliminary results.

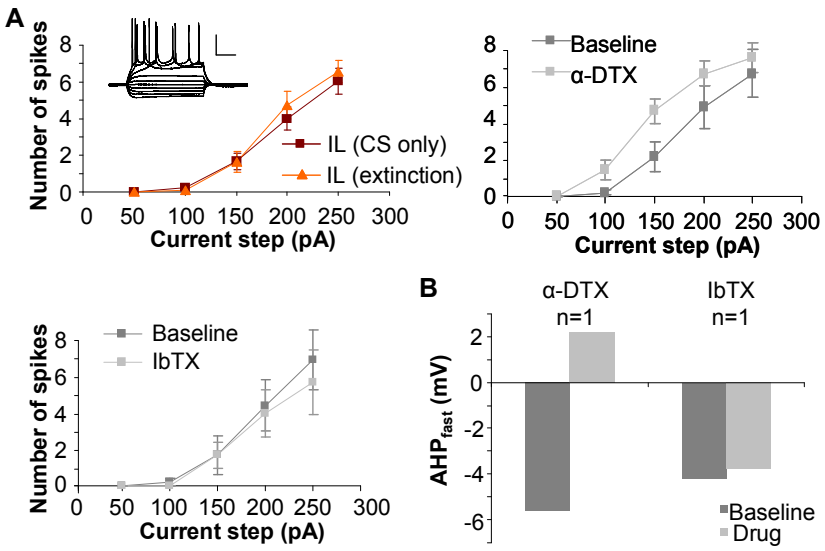


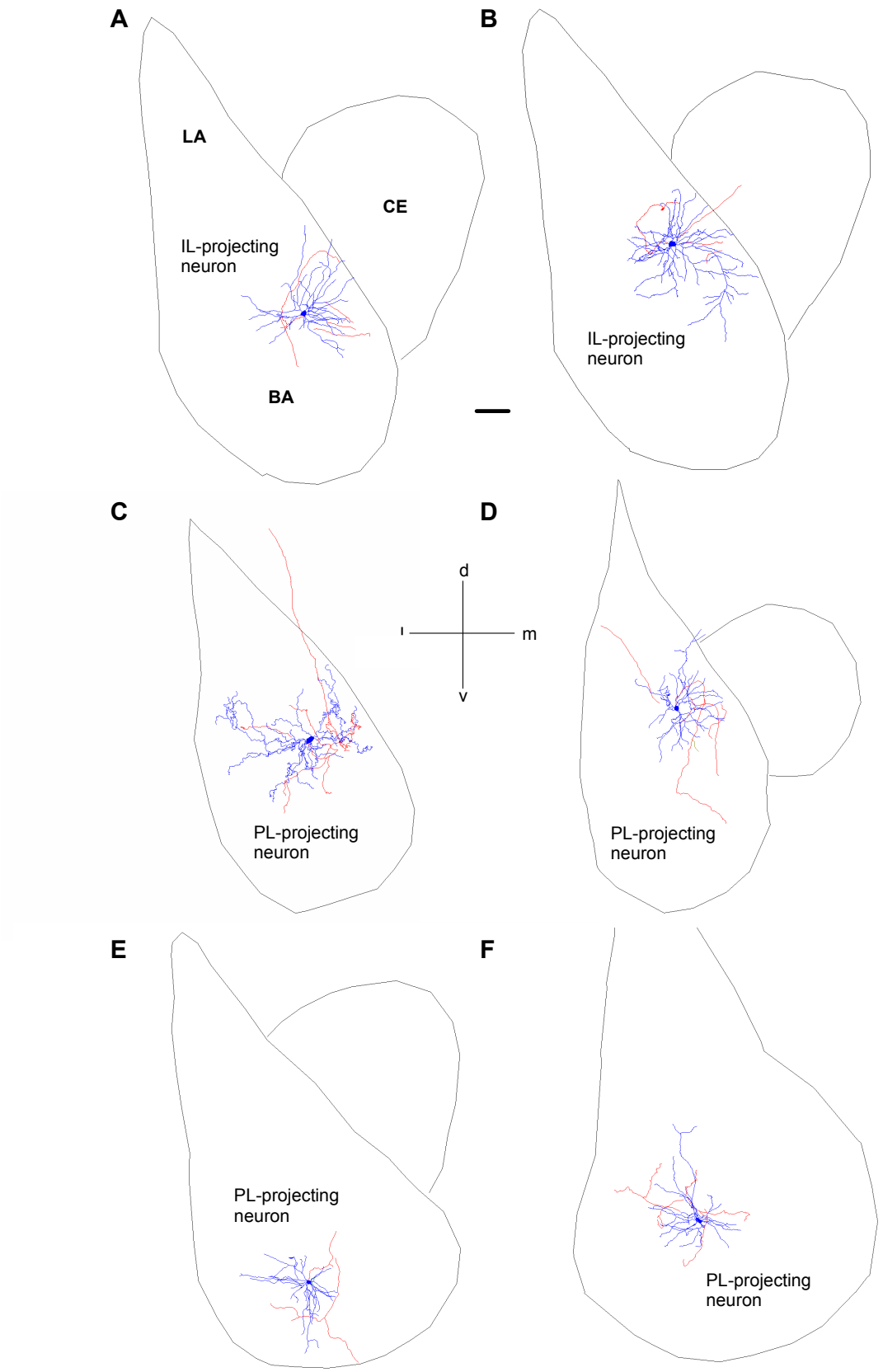
Figure 17: *The decrease in AHPfast and increase in spike half-width in IL-projecting neurons following extinction might be mediated by a functional down-regulation of BK-channels.* ***A:*** *Excitability of IL-projecting neurons was not changed by behavioral manipulation (left panel). Application of α-DTX to IL-projecting neurons in control conditions increased excitability (right panel, p < 0.001 paired student's t test), while IbTX left excitability unchanged (lower panel).* ***B:*** *Application of α-DTX to IL-projecting neurons following extinction training blocked the AHP_{fast}, while IbTX had no effect.*

4.2.5 Anatomical reconstructions of PL- and IL-projecting neurons

To investigate if neurons that project to PL or IL show different local axonal projections or dendritic arborization, recorded neurons were filled with a Neurobiotin/Biocytin mix for reconstruction. Eight PL-projecting and two IL-

projecting BA neurons were reconstructed (Fig. 18). In four of the reconstructed neurons the axon was cut close to the cell body and therefore no conclusions could be drawn on local axonal projections (Fig. 18G-J). In two cells, one projecting to the PL and one to the IL, the axon could traced beyond the BLA (Fig. 18B, C). Interestingly, the IL-projecting neuron sent its axon to the CE, most likely to its lateral part (CEl) (Fig. 18B). The PL-projecting cell projected to the striatum (Fig. 18C). In another IL-projecting neuron I could follow the axon to the border of the BLA to the central amygdala (Fig. 18A). Three more PL-projecting BA neurons exhibited axons locally in the BLA, but never came close to the CE (Fig. 18D-F).

The dendritic arborization varied between individual neurons with a clear difference between PL- and IL-projecting neurons. While some cells had an even distribution of the dendrites around the cell body (Fig. 18A, C, D, G, I), others were polarized, displaying one prominent dendritic branch sampling a specific part of the BLA (Fig. 18B, H and J). Three of the PL-projecting neurons seemed to reach for input from the border of the BLA, where fibers from cortical areas run and the intercalated cell masses are located (Fig. 18D, H and J). Interestingly, a dendritic branch from another PL-projecting neuron was located in the central amygdala (Fig. 18G). In summary, IL-projecting neurons can locally project to the CEl, which was not found in eight neurons projecting to the PL. This suggests some specificity of local axonal projections in neurons projecting to PL and IL. The two main subnuclei of the CE the CEl and the medial division of the CE (CEm), consist of inhibitory cells. The CEm is the output nucleus of the amygdala sending projections to brainstem and hypothalamus to elicit fear responses. The CEl projects to the CEm, thereby inhibiting and disinhibiting the CEm to suppress or gate fear behavior. This suggests that excitatory connections from IL-projecting BA neurons to the CEl could have an important impact on CEm output by regulating activity in the CEl. PL-projecting neurons can project to the striatum. Dendritic trees are highly variable across the two classes. However, the number of reconstructed IL/projecting neurons is still low. To clarify whether different axonal projection patterns co-vary with long-range projections, and whether there is specificity in the dendritic arborization patterns, more neurons need to be reconstructed.


A
LA
CE
IL-projecting
neuron
BA
B
IL-projecting
neuron
C
d
l
m
v
PL-projecting
neuron
D
PL-projecting
neuron
E
PL-projecting
neuron
F
PL-projecting
neuron

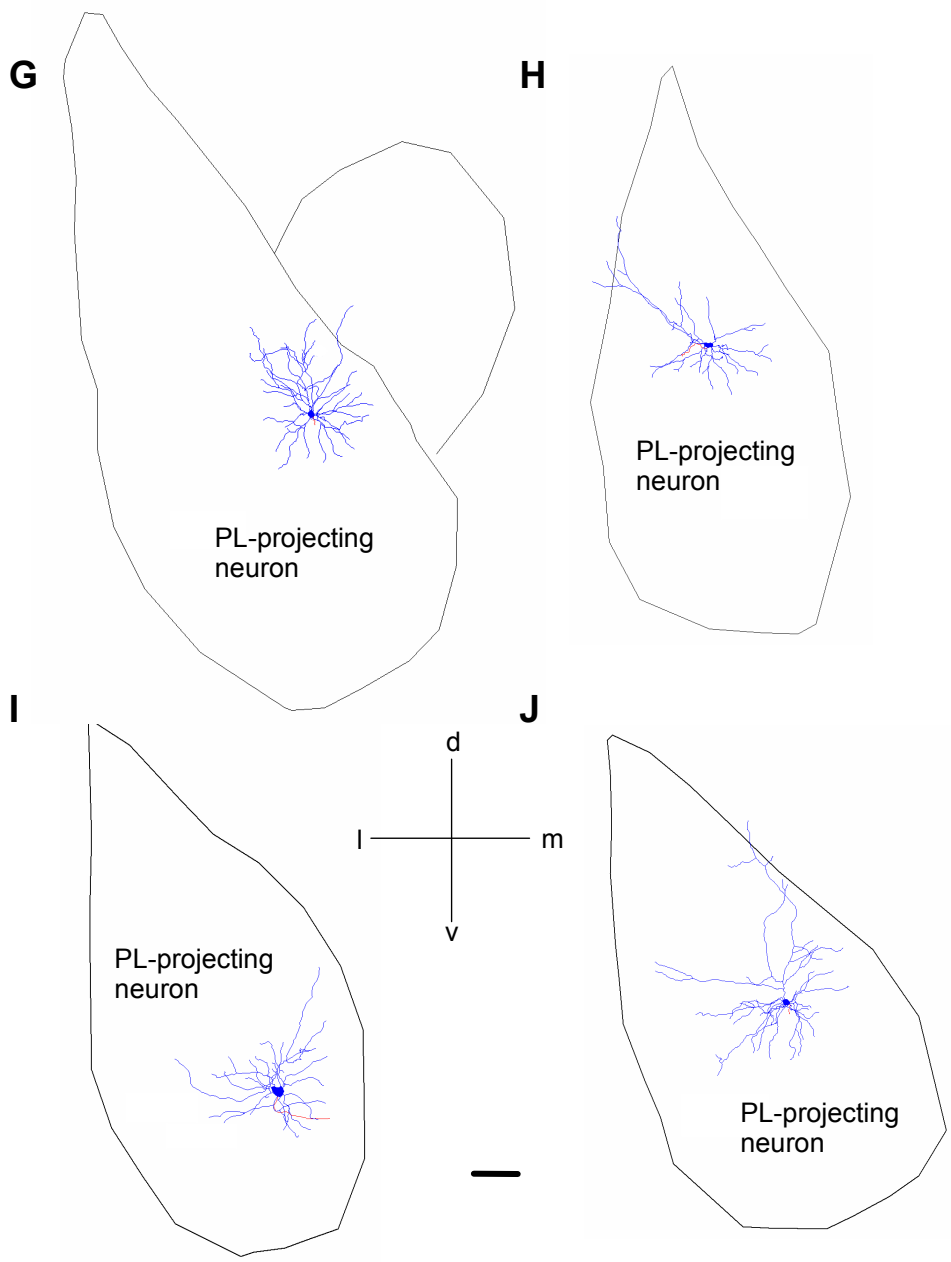


Figure 18: *Anatomical reconstructions of PL- and IL-projecting BA neurons. Blue = Cellbodies and dendrites, red = axons, black = amygdala contour; d = dorsal, m = medial, v = ventral, l = lateral; scalebar = 100 µm. LA = lateral amygdala, BA = basal amygdala, CE = central amygdala.*

4.2.6 cFos-positive cells generate less output following extinction

Following extinction training, cFos was induced in a greater number of BA neurons compared to controls (Fig. 8B). As cFos expression can be triggered upon Ca^{2+} influx, it is likely that these cells have been activated by extinction training. However, it is not clear what the physiological correlates of cFos

expression are, e.g. if plasticity is triggered in neurons that express cFos. To assess this I took advantage of a cFos-GFP transgenic mouseline expressing a cFos-GFP fusion-protein regulated by the *cfos*-promotor (Barth et al., 2004). Analysis of colocalization of AB stainings for cFos and GFP revealed, as expected for a fusion protein, 100% overlap for GFP positive neurons expressing cFos. Furthermore, overlap for cFos positive neurons being also GFP fluorescent was 97% (Fig. 19A; unpublished data, Dominic Trojer). In acute slices prepared from cFos-GFP transgenic mice, GFP expressing neurons could be visually identified (Fig. 19B). I recorded from GFP positive (X-GFP+) and GFP negative (X-GFP-) neurons after extinction training. In control animals, only GFP negative (CS only-GFP-) neurons were recorded due to low number of GFP+ cells. In addition, these animals were injected with red RB into the mPFC. Included to this analysis were only neurons that were not RB-labeled. First, I examined the passive properties of the neurons (Table 5). R_{series}, V_{rest}, the membrane time-constant Tau, and the capacitance (Cap) of CS only-GFP-, X-GFP- and X-GFP+ displayed no difference (R_{series} n.s., V_{rest} n.s., Tau n.s., Cap n.s.; one-way ANOVA). However, V_{rest} shows a trend to be increased after extinction. The input resistance (R_{in}) was lower in neurons recorded in extinction conditions compared to those from control conditions ($p < 0.01$ one-way ANOVA; CS only-GFP- vs X-GFP- $p < 0.05$, CS only-GFP- vs X-GFP+ $p < 0.05$, X-GFP- vs X-GFP+ n.s. post hoc Tukey test).

Table 5: *BA neurons have a smaller R_{in} following extinction learning. V_{rest}, R_{series}, Tau and Cap are not altered in CS only-GFP- cells compared to X-GFP- and X-GFP+ cells. $^{\#}$ $p < 0.05$ compared to CS only-GFP-.*

	CS only-GFP - (n=10)	**X-GFP - (n=22)**	**X-GFP + (n=10)**
V_{rest} (mV)	**- 71** +/- 1.6	**- 67** +/- 1.1	**- 67** +/- 2.3
R_{series} (Mohm)	**14.5** +/- 0.8	**14.7** +/- 1	**14.9** +/- 1.4
R_{in} (MOhm)	**211** +/- 24	**152** +/- 11$^{\#}$	**132** +/- 12$^{\#}$
Tau (ms)	**3.2** +/- 0.15	**3.3** +/- 0.17	**3.3** +/- 0.4
Cap (nF)	**254** +/- 17	**275** +/- 17	**238** +/- 17

The synaptic properties investigated by stimulating fibers from cortical and thalamic areas did not change across groups (Table 6; PPR_{ctx} n.s., PPR_{thal} n.s., one-way ANOVA). However, the PPR calculated from evoked events by stimulating the HC/mPFC pathway might be smaller in Fos-GFP+ cells compared

to both other groups, suggesting an increased probability of release in fibers from the mPFC and HC contacting these cells.

Table 6: *BA neurons share the same values for PPR on the different pathways after extinction training or cFos-GFP expression. Data for $PPR_{HC/mPFC}$ was not statistical analysed due to low n.*

	CS only-GFP -	X-GFP -	X-GFP +
PPR_{ctx}	**1.6** +/- 0.17 (n=6)	**1.82** +/- 0.23 (n=9)	**1.6** +/- 0.11 (n=3)
PPR_{thal}	**1.65** +/- 0.1 (n=4)	**1.61** +/- 0.08 (n=16)	**1.51** +/- 0.13 (n=6)
$PPR_{HC/mPFC}$	**1.85** +/- 0.22 (n=4)	**1.73** (n=2)	**1.2** +/- 0.05 (n=3)

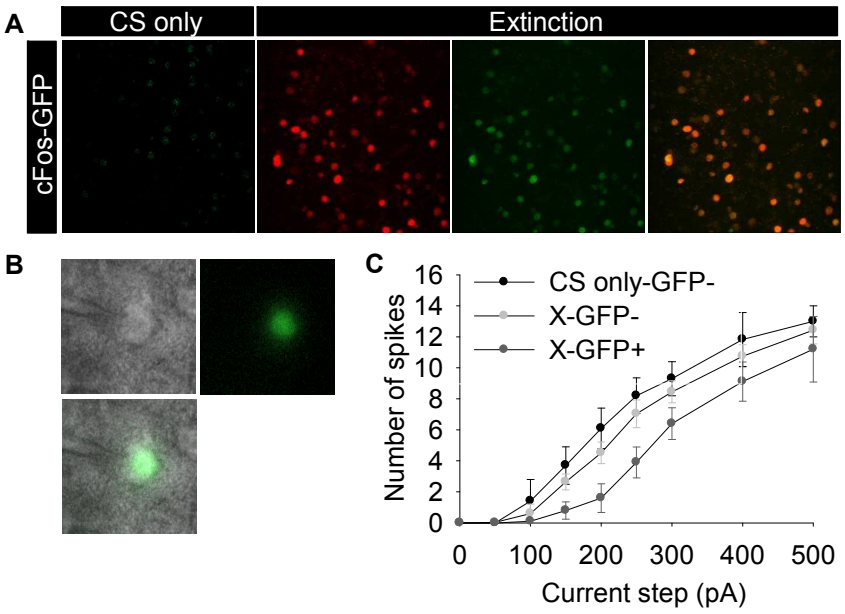


Figure 19: *cFos positive neurons after extinction are less excitable than cFos negative neurons in control and extinction conditions.* ***A:*** *cFos-GFP reporter mice express cFos and GFP following extinction training. Colocalization ~ 97%.* ***B:*** *Infrared DIC and fluorescent images of a living, cFos-GFP labeled BA neuron (exc = 488 nm, em = 525 nm and exc = 545 nm, em = 594 nm , respectively, magn = 40x).* ***C:*** *BA neurons expressing GFP are less excitable than GFP negative neurons. $p < 0.01$, two-way ANOVA.*

Overall, synaptic input to BA neurons was assessed by recording spontaneous EPSCs. All groups showed high variability and no difference was observed (Table 7; frequency n.s., amplitude n.s., rise-time n.s., decay-time n.s.). However, the frequency of events seemed to be almost doubled in X-GFP+ neurons compared to CS only-GFP- and X-GFP-. However, this was due to one outlier and might not reflect a real difference.

Table 7: *BA neurons do not differ in terms of frequency, amplitude, rise- and decay-time of spontaneous activity following extinction training or cFos-GFP expression. Frequency is shown as number of events in 5 min.*

	CS only-GFP - (n=5)	X-GFP - (n=15)	X-GFP + (n=6)
Frequency	**1110** +/- 185	**1442** +/- 328	**2424** +/- 801
Amplitude (pA)	**13.9** +/- 0.5	**14.7** +/- 0.5	**14** +/- 1
Rise-time (ms)	**2.2** +/- 0.03	**2.1** +/- 0.05	**1.96** +/- 0.05
Decay-time (ms)	**4.1** +/- 0.1	**4.3** +/- 0.2	**3.8** +/- 0.5

Next, I explored if excitability and spiking properties of BA neurons expressing cFos-GFP following extinction learning were altered. While different types of AHP and spike half-width were not changed by extinction training and cFos expression, the spike threshold and spike amplitude seemed to be lowered (Table 8). This was not statistical significant (spike threshold p = n.s., spike amplitude p = n.s. one-way ANOVA). In stark contrast the excitability of neurons that express cFos was reduced following extinction training, particularly at smaller current steps (Fig. 19C, Table 8; $p < 0.001$ two-way anova with X-GFP- vs X-GFP+ $p < 0.01$ in a post hoc Tukey test). This was surprising as we hypothesized that the output of cFos expressing neurons might be potentiated by extinction learning. However, this result is in line with the low input resistance of X-GFP+ neurons (Table 5). It might be possible that these cells undergo homeostatic plasticity after receiving strong input during extinction learning. Moreover, this does not exclude that specific synapses were potentiated and input from these connections have a high impact in cells that are less excitable in general. This is further supported by a possible increase in release probability at synaptic input from HC and mPFC (Table 6).

Table 8: *Firing and AP properties of BA neurons. The excitability of GFP positive neurons (X-GFP+) is decreased following extinction learning. Other intrinsic properties are not changed following extinction training and cFos-GFP expression. Excitability = number of spikes at 250pA current injection. ** p < 0.01*

	CS only-GFP - (n=10)	X-GFP - (n=31)	X-GFP + (n=10)
Excitability	**8.2** +/- 1.2	**7** +/- 0.9	**3.9** +/- 1 **
Spike-threshold (mV)	**- 43.7** +/- 0.9	**- 41** +/- 0.8	**- 39.5** +/- 1
Spike-amplitude (pA)	**86** +/- 0.9	**82** +/- 1	**81** +/- 1
Spike half-width (ms)	**1.45** +/- 0.03	**1.56** +/- 0.03	**1.49** +/- 0.04
AHP_{fast} (mV)	**- 3.4** +/- 0.7	**- 2.2** +/- 0.5	**- 3.4** +/- 0.7
AHP_{medium} (mV)	**3.5** +/- 0.7	**3.8** +/- 0.6	**2.5** +/- 0.9
AHP_{slow} (mV)	**- 0.7** +/- 0.2	**- 0.74** +/- 0.1	**- 0.35** +/- 0.2

4.2.7 Physiological properties of PL- and IL-projecting BA neurons expressing cFos

As I found that PL/IL-projecting and cFos-GFP expressing BA neurons exhibited specific properties that were different from each other, I wanted to know if anatomically defined BA neurons identified to express cFos by GFP fluorescence possess similar properties. I showed before that neurons projecting to the PL did not exhibit any changes in their physiological properties after extinction training. In contrast, IL-projecting neurons exhibited a decreased AHP_{fast} and an increased spike half-width. BA neurons expressing GFP following extinction training were less excitable and might have had a lower R_{in}, but exhibited no change in AHP_{fast} or spike half-width. Consequentially, we asked how BA neurons behaved that were identified to project either to PL or to IL and express cFos (Fig. 20A).

Although this dataset is preliminary I can present considerable trends. The R_{in} was low in PL-GFP+X, while V_{rest} seemed not to be changed in PL-GFP+X cells compared to PL-CS only and PL-X (Table 9, R_{in} p = 0.07, V_{rest} p = n.s., one-way ANOVA). The membrane time constant Tau and the capacitance showed a slight trend being decreased in PL-GFP+X neurons (Table 9, Tau p = n.s., Cap p = n.s., one-way ANOVA).

Table 9: *Passive properties of PL-projecting BA neurons in control conditions and following extinction. After extinction cFos expressing PL-projecting neurons could be identified by GFP fluorescence (PL-GFP+X).* $^{\#\#}$ *p < 0.01 compared to PL-CS only, * p < 0.05 compared to PL-X.*

	PL-CS only (n=16)	**PL-X (n=27)**	**PL-GFP+ X (n=3)**
V_{rest} (mV)	**- 71** +/- 1.6	**- 69** +/- 1	**- 68** +/- 3.4
R_{series} (Mohm)	**14.1** +/- 0.5	**15.1** +/- 0.5	**13.3** +/- 0.4
R_{in} (MOhm)	**186** +/- 18	**194** +/- 13	**95** +/- 4
Tau (ms)	**3.2** +/- 0.1	**3.4** +/- 0.2	**2.6** +/- 0.2$^{\#\#}$ *
Cap (nF)	**263** +/- 14	**267** +/- 13	**232** +/- 14

In IL-GFP+X neurons the R_{in} and Tau were lower compared to the other cell groups (Table 10, R_{in} p = n.s., Tau p < 0.01, one-way ANOVA, IL-CS only vs. IL-GFP+X p < 0.01, IL-X vs. IL-GFP+X p < 0.05, IL-CS only vs. IL-X p = n.s., post hoc Tukey test). V_{rest} and Cap were not changed (Table 10, V_{rest} p = n.s., Tau p = n.s., one-way ANOVA). Note, that the recording quality for IL-GFP+X as indicated by a high R_{series} is worse than those of the other groups.

Table 10: *Passive properties of IL-projecting BA neurons in control conditions and following extinction. After extinction cFos expressing IL-projecting neurons could be identified by GFP fluorescence (IL-GFP+X).* $^{###}$*p < 0.001 compared to IL-CS only, * p < 0.05 compared to IL-X.*

	IL-CS only (n=26)	IL-X (n=10)	IL-GFP+ X (n=4)
V_{rest} (mV)	**- 69** +/- 2	**- 68** +/- 1.4	**- 68** +/- 2
R_{series} (Mohm)	**14.6** +/- 0.3	**15.7** +/- 0.6	**22.2** +/- 4.2$^{###}$ *
R_{in} (MOhm)	**175** +/- 21	**167** +/- 13	**134** +/- 28
Tau (ms)	**3.2** +/- 0.1	**3.5** +/- 0.2	**4.6** +/- 0.6
Cap (nF)	**258** +/- 13	**252** +/- 19	**267** +/- 7

Excitability was not altered in PL-projecting neurons expressing cFos-GFP following extinction training compared to GFP negative PL-projecting neurons (Table 11, Fig. 20B, p = n.s., one-way ANOVA). This is in contrast to anatomically unidentified neurons expressing cFos-GFP after extinction, which exhibited a decreased excitability (Table 8). The spike amplitude was smaller in PL-GFP+X neurons than in PL-CS only and PL-X (Table 11, $p < 0.05$, one-way ANOVA; IL-CS only vs. IL-X p = n.s., IL-CS only vs IL-GFP+ X $p < 0.05$, IL-X vs. IL-GFP+ X $p < 0.05$, post hoc Tukey test). Other intrinsic properties were unchanged (Table 11, spike threshold p = n.s., spike half-width p = n.s., AHP_{fast} p = n.s., AHP_{medium} p = n.s., AHP_{slow} p = n.s., one-way ANOVA).

Table 11: *Intrinsic properties of PL-projecting BA neurons.* $^{#}$ *p < 0.05 compared to PL-CS only, * p < 0.05 compared to PL-X.*

	PL-CS only (n=16)	PL-X (n=27)	PL-GFP+ X (n=5)
Excitability	**6.9** +/- 1.6	**7** +/- 0.9	**6** +/- 1.4
Spike-threshold (mV)	**-42** +/- 1	**-43** +/- 0.7	**-42.7** +/- 1.7
Spike-amplitude (pA)	**86** +/- 0.8	**86** +/- 0.8	**80** +/- 3.4$^{#}$ *
Spike half-width (ms)	**1.45** +/- 0.03	**1.51** +/- 0.03	**1.59** +/- 0.09
AHP_{fast} (mV)	**- 2.8** +/- 0.7	**- 2.8** +/- 0.6	**- 1.6** +/- 0.6
AHP_{medium} (mV)	**3.3** +/- 0.5	**2.9** +/- 0.3	**2.6** +/- 1.1
AHP_{slow} (mV)	**- 0.6** +/- 0.2	**- 1** +/- 0.1	**- 0.77** +/- 0.23

Excitability of IL-GFP+X neurons seemed to be lower compared to IL-CS only and IL-X (Fig. 20C, Table 12, p = n.s., two-way ANOVA). Additionally the spike amplitude was decreased in IL-GFP+X neurons compared to IL-CS only (Table 12, $p < 0.05$, one-way ANOVA, IL-CS only vs. IL-X p = n.s., IL-X vs. IL-GFP+X p = n.s., IL-CS only vs. IL-GFP+X $p < 0.05$, post hoc Tukey test). The spike half-width of IL-projecting neurons was increased following extinction, also of those that express cFos-GFP (Table 12, $p < 0.001$, one-way ANOVA, IL-CS only vs. IL-

X p < 0.001, IL-CS only vs. IL-GFP+X p < 0.05, IL-X vs. IL-GFP+X p = n.s.). Similarly, the AHP_{fast} was decreased in IL-projecting neurons after extinction independent of cFos-GFP expression (Table 12, p < 0.01 one-way ANOVA, IL-CS only vs. IL-X p < 0.01, IL-CS only vs. IL-GFP+X p = n.s., IL-X vs. IL-GFP+X p = n.s., post hoc Tukey test). The spike threshold, AHP_{medium} and AHP_{fast} were unchanged in IL-GFP+X cells (Table 12, spike threshold p = n.s., AHP_{medium} p = n.s., AHP_{slow} p = n.s., one-way ANOVA)

Table 12: *Intrinsic properties of IL-projecting BA neurons. . $^{###}$ p < 0.001, $^{##}$ p < 0.01, $^{#}$ p < 0.05 compared to IL-CS only.*

	IL-CS only (n=26)	**IL-X (n=10)**	**IL-GFP+ X (n=4)**
Excitability	**6** +/- 0.8	**6.5** +/- 0.9	**4** +/- 1.7
Spike-threshold (mV)	**-41** +/- 0.6	**-40** +/- 0.8	**-39** +/- 1.1
Spike-amplitude (pA)	**86** +/- 0.7	**84** +/- 1.4	**79** +/- 2.3 $^{#}$
Spike half-width (ms)	**1.4** +/- 0.04	**1.7** +/- 0.2 $^{###}$	**1.62** +/- 0.08 $^{#}$
AHP_{fast} (mV)	**-5.1** +/- 0.7	**-1.5** +/- 1.2$^{##}$	**- 2** +/- 2.3
AHP_{medium} (mV)	**2.3** +/- 0.6	**3.9** +/- 0.7	**2.7** +/- 1.2
AHP_{slow} (mV)	**-0.7** +/- 0.1	**0.9** +/- 0.3	**- 1.5** +/- 0.6

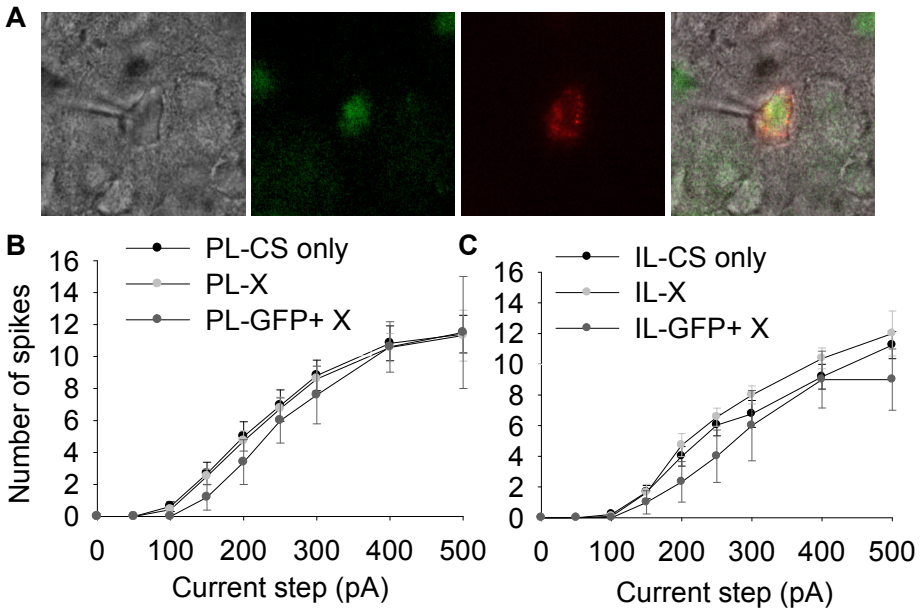


Figure 20: A: *Infrared DIC and fluorescent images of a living, cFos-GFP and retrobeads labeled BA neuron (exc = 488 nm, em = 525 nm and exc = 545 nm, em = 594 nm , respectively, magn = 40x).* ***B:*** *PL projecting neurons expressing GFP are as excitable as PL projecting neurons, whereas IL projecting neurons expressing GFP might by less excitable than IL projecting neurons.*

In summary, the R_{in} is low in PL-GFP+X and in IL-GFP+X as well as in anatomically unidentified GFP positive neurons (Table 5, 9, 10) suggesting a low

R_{in} as a consequence of cFos expression, independent of the cell-type that expresses cFos. Similarly, the spike amplitude seemed to be decreased in all cFos-GFP positive neurons. Interestingly, IL-GFP+X neurons showed, like anatomically unidentified X-GFP+ neurons, a decreased excitability, which could not be found in IL-X and PL-GFP+X neurons. Moreover, the AHP_{fast} and the spike half-width were modulated in IL-projecting neurons following extinction independent of cFos-GFP expression. This indicates that some some properties change subtype specific independent of cFos expression.

4.3 Behavioral relevance of IL- and PL-projecting BA neurons

4.3.1 Reversible asymmetric inactivation of IL and BA

To address the behavioral relevance of functional connections between the amygdala and the PL/IL in behaving animals I am currently performing experiments aimed at silencing the amygdala and the PL/IL in contralateral hemispheres by local iontophoretic application of the $GABA_A$-receptor agonist muscimol (Fig. 21A). These experiments provide a means to explore the role of connections between these two brain areas while leaving both structures unilaterally intact, but with no intact connectivity between (Garcia *et al*., 1999).

Because immunohistochemical and electrophysiological data pointed to a modulation of IL-projecting BA neurons following extinction training, I inactivated the IL and BA before the second extinction training session and compared this with inactivation of the PL and the BA (Fig. 21). Preliminary data suggest that mice with asymmetric inactivation of the IL or the PL and the BA show a different effect on fear behavior (Fig. 21A). Mice with an asymmetric IL-BA inactivation showed high freezing levels. In contrast, an animal with an asymmetric inactivation of PL and BA exhibited no effect or a decrease in fear behavior (more experiments need to be performed).

To test if this was specific for CS-presentation, we measured freezing throughout the second extinction session also in between CS presentation. We found that inactivation of IL and BA led to freezing behavior in between CS presentation

(Fig. 21B). This suggests that disconnecting the IL and the BA leads to a general effect on fear behavior, rather than a specific impact on extinction learning. Furthermore this may support the idea that the IL is implicated in inhibiting amygdala output, whereas the PL is necessary for learning/expression of high fear states. However, asymmetric inactivations affect reciprocal projections between PL/IL and BA. Therefore, we do not know if these effects are mediated by pathways to or from the BA or a combination.

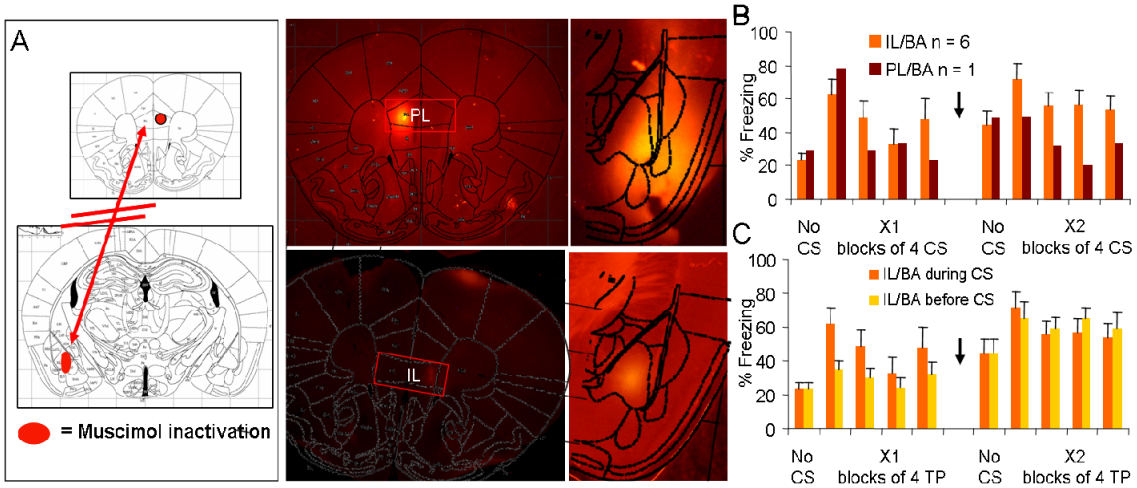


Figure 21: *Asymmetric inactivation of the PL/IL and the BA.* ***A:*** *Schematic drawing to illustrate the functional disconnection of IL and BA (left picture). Examples for successful muscimol application to PL/BA (upper panel) and IL/BA (lower panel).* ***B:*** *Following muscimol injection (↓) to IL/BA, mice showed increased baseline freezing and no extinction. One mouse with muscimol injection to PL/BA showed extinction.* ***C:*** *Mice injected to IL/BA display generalized freezing behavior to the context. TP=time point.*

4.3.2 A molecular approach to reversibly silence synaptic transmission: Molecules for inactivation of synaptic transmission (MISTs)

To explore the impact of BA projections to the PL and the IL I aimed to specifically and reversibly silence this pathway in freely moving mice. One possibility to silence specific groups of cells is to use a technique known as “Molecules for Inactivation of Synaptic Transmission” (MISTs) (Karpova et al., 2005). MISTs consist of presynaptic proteins, necessary for neurotransmitter release, which are genetically modulated allowing for reversible inactivation and can be expressed in subsets of cells or brain areas. Inactivation of presynaptic release is triggered by chemical induction of dimerization of a modified protein.

One of the MISTs developed is a modified small vesicle associated membrane protein 2 (Vamp2), also called Synaptobrevin. Vamp2 is fused to the FK506 binding protein (FKBP). This fusion protein can be triggered to homodimerize by applying the small molecule FK506. In neurons overexpressing Vamp2 fused to FKBP (VampFv2) presynaptic release was shown to be silenced upon application of the dimerizer FK506. Over-expression of VampFv2 in BA neurons and targeted injection of FK506 to the PL or the IL leads to a specific silencing of the respective pathway, by inactivation of presynaptic release in the IL only in axons originating from the BA.

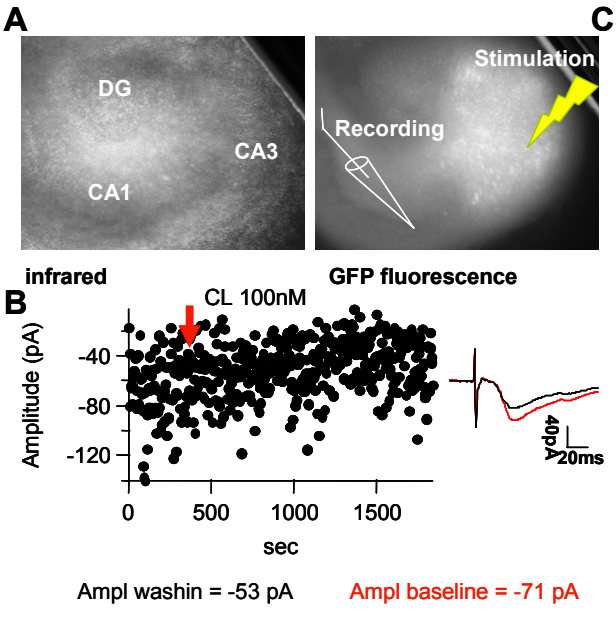
To over-express the Vamp MIST in amygdalar neurons I cloned the VampFv2-construct into an adeno-associated virus (AAV) vector. Pilot experiments revealed that the AAV2 serotype infected BLA neurons reliably (data not shown). To drive expression of VampFv2 two different promotors were chosen: The chicken β-actin promoter (CAG) is very strong and ubiquitous leading to high expression-levels in all cell-types, whereas the Syn-promotor is specific to neurons. Both generated constructs AAV-CAG-VampFv2 and AAV-Syn-VampFv2 also expressed eGFP.

To characterize both constructs, I infected the CA3 region in hippocampal slice cultures (generously provided by the laboratory of Thomas Oertner) with either AAV-CAG-VampFv2 or AAV-Syn-VampFv2. Infected cells were visualized by GFP fluorescence imaging (Fig. 22A). Axons from neurons within CA3 contact pyramidal neurons in CA1. Therefore, cells recorded in CA1, were stimulated by electric pulses in CA3 to assess changes in synaptic transmission upon bath-application of the dimerizer FK506 (Fig. 22A).

Treatment of slice cultures with the dimerizer (100 nM) only led to slight changes in the amplitude of evoked EPSCs in CA1 neurons (Fig. 22B). In total, wash in of FK506 reduced evoked EPSCs by only ~ 30%, independent of the recording temperature (RT and 36°C) (Fig. 22B, C). This was the same for both constructs. In some cells, a marked reduction in spontaneous activity was observed (data not shown). The low efficacy of the dimerizer might be a stimulation artifact, as it is possible that I stimulated a higher proportion of non-infected neurons and thereby

underestimated the silencing ability of the drug. To explore effects of FK506 application on VampFv2 expressing neurons in detail, Ingrid Ehrlich recorded from connected pairs of neurons in CA3, where one or both CA3 neurons were identified to express GFP. Also, in these conditions synaptic transmission was only impaired by ~ 30% (Fig. 22C, lower rows).

In conclusion, with the tools I developed, we could achieve a moderate presynaptic silencing in slice cultures. In vivo, a 30% reduction in synaptic transmission would likely not be enough to efficiently silence the BA-PL/IL pathway, particularly taking into account that most likely not all BA neurons would be infected by the AAV. For this reason, we decided not to explore this approach any further. It remains to be tested whether *in vivo* conditions are different from those in slice cultures, leaving the possibility that the VampFv2 system could be working better *in vivo*.



C

AAV-CAG-VampFv2	Evoked Ampl
extracellular stimulation	
cell1	30% ↓
cell2	30% ↓
AAV-Syn-VampFv2	
extracellular stimulation	
cell1	30% ↓
cell2	30% ↓
cell3	0 ---
cell4	0 ---
cell5	60% ↓
pairs	
pair1	30% ↓
pair2	30% ↓

Figure 22: *Molecules for inactivation of synaptic transmission (MISTs) in hippocampal slice cultures.* ***A:*** *Hippocampal slice culture infected with AAV-Syn-VampFV2. Pictures taken with a 5x objective in infrared (left) and GFP fluorescent settings (right).Scheme of recording and stimulation site (right).* ***B:*** *Representative recording of a CA1 neuron. Events triggered by stimulation of infected CA3 fibers. CL = crosslinker* ***C:*** *Table to summarize effects of CL wash-in on recorded cells*

5 Discussion

To examine the role of amygdala projection neurons in the expression and extinction of conditioned fear responses, I used activity-mapping by expression analysis of IEGs. I found that the two IEGs cFos and Zif268 were differentially expressed in the amygdala. While cFos was primarily found in the BA, Zif268 was mainly expressed in the LA. However, both were induced in response to fear conditioning and extinction. A combination of *in vivo* retrograde tracing techniques and analysis of expression of the activity-dependent IEGs cFos and Zif268 showed that amygdala neurons projecting to the mPFC or to the HC exhibited differential cFos and Zif268 expression in fear conditioned animals compared with non-conditioned control animals and with animals subjected to extinction. In particular, extinction resulted in a selective induction of cFos in mPFC- over HC-projecting neurons. Two subdivisions of the mPFC, the prelimbic (PL) and the infralimbic (IL) cortex are assumed to be differentially involved in fear conditioning and extinction. A more detailed analysis revealed that neurons projecting to the IL, but not those projecting to the PL, accounted for the specific increase in cFos expression following extinction. To investigate the physiological correlates of fear extinction in anatomically defined subpopulations of amygdala projection neurons I used an electrophysiological ex vivo approach. In these experiments, I recorded from identified BA neurons projecting to PL or IL in slices obtained from mice subjected to extinction. Extinction differentially affected intrinsic properties of PL- and IL-projecting cells. While there was no change in PL-projecting neurons, IL-projecting BA cells showed a learning-related increase in spike half-width and a concomitant decrease in the fast after-hyperpolarization (AHP_{fast}). In control animals, spike half-width and AHP_{fast} were influenced by the activation of VDPCs and BK-channels. Preliminary results indicated that after extinction training, only VDPCs contribute to the AHP_{fast} in IL-projecitng cells. This suggests a specific modulation of BK-channels in IL-projecting neurons following extinction learning. Moreover, our findings indicate that a change in the balance of activity between IL- and PL-projecting BA neurons may be involved in the extinction of conditioned fear.

5.1 BLA neuron subpopulations

I demonstrated that neuronal subpopulations within the amygdala were differentially activated following fear conditioning and extinction as suggested by expression analysis of the two IEGs cFos and Zif268. These IEGs were differentially activated by behavioral fear learning paradigms. While cFos positive neurons were predominately found in the BA after FC the majority of Zif268 expressing cells was found in the LA and was equally increased by FC and extinction. This suggests that distinct cell-populations within the BLA were differentially activated during fear learning and extinction. Overall, my data are in line with the notion that various IEGs can be induced by different stimuli (Alberini, 2009).

Double-injections of red and green RB to the mPFC and the HC revealed that the corresponding projection neurons in the BA are two separate populations. Even though it was known that the BA projects to both brain areas, it was not clear if these cell populations overlap (Pitkanen et al., 2000a). In contrast, in the vHC single neurons project simultaneously to the mPFC and the amygdala (Ishikawa and Nakamura, 2006).

5.2 Anatomically identified neurons are differentially activated following fear conditioning and extinction

IGE expression was specifically and differentially induced in anatomically identified subpopulations of BA neurons projecting either to the mPFC or the HC. While FC and fear consolidation induced cFos expression in mPFC- and HC-projecting neurons, extinction learning induced cFos only in mPFC-projecting neurons. In keeping, work from our laboratory showed that distinct neuronal subpopulations in the BA that are activated by fear conditioning (“fear neurons”) and extinction (“extinction neurons”) project to the mPFC (Herry et al., 2008). Furthermore, Zif268 expression in mPFC-projecting BA neurons was specifically induced following fear consolidation. This is in line with the postulated role of Zif268 expression in memory consolidation.It has been suggested that Zif268 induced by plasticity producing stimuli rather than by cellular activity alone

(Malkani et al., 2004; Alberini, 2009). Interestingly, no increase in Zif268-expression was found in HC-projecting neurons, suggesting that fear conditioning and extinction induced Zif268 expression in anatomically unidentified subpopulations of amygdala neurons. It is surprising that, in HC-projecting neurons, cFos was only moderately induced, and Zif268 not at all (Maren and Holt, 2004). However, while it has been shown that extinction related activity in the LA is driven by dHC input (Maren and Hobin, 2007), less is known about the BLA to HC pathway. It is possible that information about the riskiness of a context is directly assessed by sensory-evoked activity in the HC as inactivation of the HC can abolish FC (Maren, 1999; Corcoran and Maren, 2001). Additionally, the emotional value of a context could also be conveyed to the HC via the mPFC, as both amygdala and HC are reciprocally connected to the mPFC (Ishikawa and Nakamura, 2003).

“Fear neurons” and “extinction neurons” project to the mPFC, which consists of different subareas that are thought to be differentially implicated in fear conditioning and extinction (Vidal-Gonzalez et al., 2006). The PL is implicated in fear conditioning and the IL in extinction learning. In keeping, I found that BA neurons projecting to the PL expressed high cFos levels in high fear paradigms, whereas IL-projecting BA neurons are induced to express increased cFos levels following extinction (see Fig. 12). This indicates that not only IL and PL, but also BA neurons projecting to these areas are differentially activated by fear learning and extinction and might represent fear and extinction neurons, respectively. Interestingly, preliminary data showed that Zif268 expression is elevated particularly in PL- over IL-projecting neurons following fear consolidation and extinction, indicating again differential stimuli to induce cFos and Zif268 expression.

5.3 Physiological correlates of fear conditioning and extinction in PL- and IL-projecting BA neurons

When investigating electrophysiological correlates of fear learning, I found that BA neurons projecting to the PL did not change physiological properties following

extinction training, whereas IL-projecting BA neurons displayed a decreased AHP_{fast} and an increased spike half-width. How these two parameters may influence BA output to the IL, and perhaps IL activity is discussed below. My findings support the notion of the IL being implicated in extinction learning.

In the HC, the AHP_{fast} and the spike half-width are determined primarily through BK-channels (Poolos and Johnston, 1999). In contrast, in the LA, blockade of BK-channels increases spike half-width, but contributes little to the AHP_{fast}, which is mediated by VGPCs (Faber and Sah, 2002). Here, I show that in IL-projecting BA neurons both channel-types contributed to the AHP_{fast} and spike half-width. Interestingly, the AHP_{fast} has been shown before to be modulated by learning paradigms in rodents. Decreases in the AHP_{fast} were found following trace eyeblink conditioning in the HC (Matthews et al., 2008) and after fear extinction in the mPFC (Santini et al., 2008). This suggests an important role of BK-channels in learning. In keeping, my preliminary pharmacological data suggest a functional down-regulation of BK-channels after fear extinction learning. However, intra-hippocampal blockade of BK-channels does not fascilitate but block trace eyeblink conditioning (Matthews and Disterhoft, 2009). This is surprising, because a block of BK-channels should have mimicked the functional downregulation of BK-channels and the concomitant decrease in the AHP_{fast} following learning and therefore rather fascilitate learning. This is probably due to block of presynaptically expressed BK-channels by increasing release probability in an unspecific manner.

In the HC, the decrease in AHP_{fast} is accompanied by an increase in intrinsic cellular excitability (Matthews et al., 2008). Furthermore, trace eyeblink conditioning also decreases the AHP_{slow} which can account for an increase in excitability (Matthews et al., 2009). In contrast, I did not find changes in cellular excitability accompanying the changes in AHP_{fast} and spike half width. In the LA, it has been reported that fear extinction leads to no changes in excitability of neurons compared to naïve animals, but does increase the propensity of bursting of neurons which is accompanied by changes in AHP (Santini et al., 2008). Overall, this literature is still controversial. For example, Gu *et al.* found that blockade of BK-channels in hippocampal CA1 neurons decreases burstiness (Gu

et al., 2007). I did not find modulated burst behavior of IL-projecting BA neurons with decreased AHP_{fast}, but can not completely exclude a change in another subtle parameter of intrinsic cellular excitability. A possibility to be tested would be a change in EPSP-spike coupling.

Usually, APs are accompanied by a rise in cytosolic Ca^{2+}. The resulting Ca^{2+} influx during APs occurs largely during AP repolarisation (Llinas et al., 1981). Therefore, the spike duration is a key determinant of the amount of Ca^{2+} influx. It has been shown at presynaptic terminals that an increase in Ca^{2+} leads to increased neurotransmitter release (Augustine, 1990; Sabatini and Regehr, 1997; Geiger and Jonas, 2000). The increase in spike half-width in IL-projecting neurons following extinction could lead to increased presynaptic release in the IL and therefore to a strengthened BLA-IL pathway. However, I was only able to investigate properties of ion-channels at the soma of neurons. To further investigate a potential effect of an increased spike half-width on synaptic release in the IL we need to study these properties at the axonal level. Importantly, “extinction cells” show, like IL-projecting cells, an increase in spike half-width indicated by a broadened wave-form (Cyril Herry, unpublished data,). This suggests that IL-projecting BA neurons could be “extinction cells”. However, the spike half width increase in “extinction cells” happened already following fear conditioning and remained increased during extinction training. If this holds true for IL-projecting neurons recorded ex vivo remains to be tested.

Surprisingly, I found no change in synaptic input onto IL-projecting BA neurons after extinction training, even though the increase in the number of cFos expressing cells suggested activation of these cells. However, activation of a pathway does not necessarily result in plasticity of the activated synapses. Furthermore, I can not exclude that some of the inputs were altered, as indicated by trends for an increased and decreased PPR at the thalamic and HC/mPFC pathway, respectively. To dissect input-pathways more specifically, an approach using channelrhodopsin (ChR) would give more detailed information (Zhang et al., 2007). This could be achieved by expressing ChR in a brain-area specific manner, which would allow for long-range fibers in the amygdala to be selectively activated with light.

The fact that extinction training resulted in specific modulations of IL-projecting BA neurons over PL-projecting neurons suggests a change in the activity balance between these two anatomically identified BA neurons caused by extinction training. Whether these changes are also necessary remains to be tested. Interestingly, my anatomical reconstructions revealed that some IL-projecting neurons projected to the CEl, which was not found for PL-projecting neurons. The CE, consisting mainly of inhibitory neurons, is the “output station” of the amygdala and activity in CEm leads to high fear responses (Goosens and Maren, 2003; Wilensky et al., 2006). The CEm is downstream of the CEl and it was shown that CEl neurons can inhibit CEm neurons (Huber et al., 2005). Thus, IL-projecting neurons could locally excite CEl neurons during extinction and thereby inhibiting CEm output and the fear response.

5.4 cFos-GFP expressing neurons in the BA are less excitable

In a parallel approach I was able to record from cFos-GFP reporter mice ex vivo following behavioral training. This revealed that anatomically unidentified neurons expressing cFos-GFP after fear extinction were less excitable than neurons negative for cFos-GFP. As these cells express cFos probably as a direct response to activation this might be due to homeostatic scaling. Homeostasis is thought to stabilize the activity of neurons and thereby maintains the stability of neuronal networks (Turrigiano, 2007). It was shown that this can involve activity-dependent regulation of intrinsic neuronal firing properties (Marder and Prinz, 2003; Zhang and Linden, 2003), as well as synaptic scaling (Turrigiano, 2007). Possibly, cFos is expressed in strongly activated brain areas in a certain percentage of cells to keep the network balanced. However, recordings in the barrel cortex from cFos positive and cFos negative neurons following single-whisker stimulation revealed no such difference (Barth et al., 2004). The difference to our protocol is that their stimulation was ongoing for 24 – 48 h. As cFos-GFP is a transient protein, which is gone ~ 4 h after expression, the authors might have recorded from neurons with a recent history of cFos expression and thereby missed possible physiological consequences.

The decrease in excitability in cFos-GFP+ neurons might be a direct consequence of the lower R_{in} in these cells, compared to CS only-GFP- and X-GFP-. The lower R_{in} will decrease the depolarizytion associated with a given (synaptic) current as given by Ohm's law. Thus, with constant spike threshold, the number of spikes will be reduced. However, when looking at anatomically defined subpopulations, the R_{in} was also low in cFos-GFP expressing neurons identified to project to PL and IL, but only IL-projecting cells exhibited a decrease in excitability. Interestingly, the physiological changes of the AHP and spike-half width found in IL-projecting neurons after extinction training were cell-type specific and occurred independent of cFos-GFP induction. However, the numbers of recordings in cFos-GFP expressing anatomically defined neurons are very low and more experiments are required to clarify the consequences of cFos expression in these cells.

5.5 Summary

PL- and IL-projecting BA neurons were differentially activated and modulated upon fear extinction in mice and might represent previously idetified "fear and extinction neurons" (Herry et al., 2008). This was indicated by analysis of the IEG cFos, which was expressed in a higher proportion of IL- vs. PL-projecting neurons after extinction. This probably reflects increased activation of these cells. In contrast, fear conditioning induced increased cFos expression specifically in PL-projecting neurons. Despite using cFos expression as activity-mapping, I found that neurons in the BA identified to express cFos were less excitable. This might reflect previous strong activation and subsequent homeostatic scaling of these cells. Further indication that IL-projecting neurons were "extinction cells" came from electrophysiological recordings in anatomically identified neurons from acute slices. Only IL-projecting neurons showed extinction learning related changes of physiological properties. They displayed a decreased AHP_{fast} and an increased spike half-width. A decrease in AHP_{fast} has been shown before to be regulated in a learning-dependent manner (Matthews et al., 2008). Importantly, also "extinction cells" showed an increase in the spike half-width after extinction compared to naïve conditions (Cyril Herry, unpublished data).

6 Outlook

Overall, based on my findings more questions can be addressed. The first one is the behavioral impact of projections from the BA to the PL and the IL. To address this, I tested two different approaches, asymmetric inactivation and silencing using MISTs. Both approaches appeared to be not suitable. What might be alternative approaches to assess the behavioral relevance of BA projections to the PL/IL? One possibility is the use of optogenetics (Zhang et al., 2007). This approach involves infection of neurons with retrogradely transported herpes simplex-virus (HSV) driving the expression of light-gated channels. Two different channels are available so far, channelrhodopsin (ChR) which depolarizes cells upon illumination with blue light, and halorhodopsin (NpHR) which hyperpolarizes and silences cells as a response to yellow light. After injection with HSV, amygdala neurons projecting to the mPFC express ChR and NpHR. As it is possible to illuminate deep brain structures with optical fibres (Adamantidis et al., 2007), targeted photostimulation to the BA could specifically excite (blue light) or silence (yellow light) PL/IL-projecting neurons in the behaving animal. This would allow for testing the impact of activity changes in PL- and IL-projecting BA neurons for the expression and extinction of conditioned fear.

A second possibility would be to ablate subpopulations of BA neurons that project to the mPFC and the IL and PL. For this, we could use a mouse-model for conditional ablation of diphtheria toxin receptor (DTR) expressing cells by application of diphtheria toxin (DT) (Han et al., 2009). Neurons in mice carrying a floxed STOP-cassette before the DTR coding sequence can be made vulnerable to DT by expression of the Cre-recombinase. Expression of Cre-recombinase via HSV can be achieved in mPFC-projecting BA neurons by a targeted injection of the HSV to mPFC subdivisions. Targeted injection of DT to the BA will thus only ablate those cells expressing the DTR. If this approach works remains to be tested.

A second big question is the link between the physiological changes I found in IL-projecting BA neurons and the impact on IL activity. Two different experiments are planned to explore the possibility that the increased spike half width of IL-

projecting neurons in the BA leads to increased release probability at their axon terminals in the IL following extinction training. First, we will assess if the changes in AHP_{fast} and spike half-width are not only somatic but also axonal. Therefore, we plan to perform simultaneous somatic and axonal recordings in control conditions and following extinction training. Secondly, we plan to directly study if Ca^{2+} influx is increased within the IL in terminals originating from the amygdala after fear extinction, thus providing direct indication for increased release probability. Therefore, we plan to identify terminals in the IL originating from the amygdala by application of a GFP-expressing virus to the amygdala. In an ex vivo approach we will test, using Ca^{2+} indicators in acute slices, the Ca^{2+} response to minimal stimulation in GFP-expressing terminals.

The third central question is to explore whether PL- and IL-projecting neurons are correlated with the reported “fear and extinction neurons” (Herry et al, 2008). To address this, we need to record from PL- and IL-projecting neurons in an ex vivo approach directly following fear conditioning. This will provide information whether PL-projecting neurons change physiological properties with fear learning. Furthermore, this will tell us whether physiological parameters in IL-projecting cells like the AHP_{fast} and spike half-width already change directly following fear conditioning, like they do in “extinction cells” recorded in vivo. These data could at least provide strong correlative evidence for a connection between fear and extinction neurons and PL-and IL-projecting cells, respectively.

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8 Curriculum Vitae

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2002-2004 **Graduate studies in Biology** (Diploma mark 1.1/Top 5%)
Philipps-University (Marburg, Germany)
Majors: Biochemistry, Genetics, Microbiology

1999-2002 **Undergraduate studies in Biology** (intermediate degree 2.0) Technical University Darmstadt (Darmstadt, Germany)

Scientific experience

2004-present **Friedrich-Miescher-Institute** (Basel, Switzerland)
Ph.D. candidate; Laboratory of Neurobiology, Prof. A. Lüthi.
- Topic: Dissecting defined cell populations within the basolateral amygdala, which are implicated in learning and memory of conditioned fear.
- Methods: immunohistochemical stainings, behavioral tests, mouse brain surgeries, viral gene transfer (in vivo) and whole-cell patch clamp in brain slices.

01/2009 **PENS-Hertie winter school** (Obergurgl, Austria)
Structure and Function of Neural Circuits. Organizers: Bert Sakmann (Martinsried, Germany) and Michael Häusser (London, UK)

03/2005	**Friedrich-Loeffler-Institute** (Greifswald, Germany) Visiting scientist; Laboratory of Molecular Biology of Herpesvirus, Prof. Thomas C. Mettenleiter (Head of Institute) - Training in cell culture and virus production.
2004	**Philipps-Uninversity** (Marburg, Germany) Diploma thesis; Laboratory of Biochemistry, Prof. M.A. Marahiel. - Topic: Cyclases from nonribosomal peptidesynthtases - Methods: Cloning of various constructs and protein purification using Fast Protein Liquid Chromatography (FPLC), enzyme activity assays, High Pressure Liquid Chromatography (HPLC), Matrix-Assisted, Laser Desorption/Ionisation-Time Of Flight (MALDI-TOF), chemical synthesis of small Peptides.
2003	**Philipps-Uninversity** (Marburg, Germany) Research assistant; Laboratory Biochemistry, Prof. M.A. Marahiel, Department Chemistry - Enzyme assays in a biochemical screen.
2003	**Max-Planck-Institute for terrestrial Microbiology** (Marburg, Germany) Intern, Laboratory Microbiology, Prof. R. Kahmann (Director and Head of Institute, Winner of Leibniz price) -UV-Mutation screen using the maize pathogen Ustilago maydis.
12/2001- 03/2002	**Chemisch-Analytisches-Labor** (Darmstadt, Germany) Student job; environmental chemical testing

Teaching experience

2004	Part time supervision of an intern
2007-present	Supervision of two master students

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American Association for the Advancement of Science (AAAS)
Society for Neuroscience (SfN)

Fellowship

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Publications and presentations at congresses

Publication:

Herry C., Ciocchi S., Senn V., Demmou L., Müller C., Lüthi A.; Switching on and off context-dependent fear memories by specific amygdala circuits. Nature 454, 600-606 (2008).

Presentations at congresses:

Talk:

5th Meeting on "Cellular and Molecular Neurobiology of Mental Disease", Giessbach, 2009.
Fear extinction induces cell-type specific neuronal plasticity in amygdala-prefrontal cortex pathways

Poster presentations:

Senn V., Herry C., Ehrlich I., Mueller C. and Lüthi A.; Identification of amygdala neuronal subpopulations by immediate early gene analysis. FMI Annual Meeting, Grindelwald (CH) 2008.

Senn V., Kucsera S., Herry C., Ehrlich I., Mueller C. and Lüthi A.; Identification of amygdala neuronal subpopulations by immediate early gene analysis. Conferences Jaques Monod, Roscoff (F) 2008

Senn V., Kucsera S., Herry C., Ehrlich I., Mueller C. and Lüthi A.; Identification of amygdala neuronal subpopulations by immediate early gene analysis. FENS meeting, Geneva (CH) 2008

Herry C., Ciocchi S., **Senn V.**, Müller C., Lüthi A.; Encoding of fear extinction in the basal amygdala. Society for Neuroscience, San Diego (USA) 2007

Senn V., Kucsera S., Herry C, Mueller C. and Lüthi A.; Extinction of conditioned fear induces cFos in defined subpopulations in amygdala neurons. FMI Annual Meeting, Grindelwald (CH) 2007

Senn V., Kucsera S., Herry C, Mueller C. and Lüthi A.; Dissecting defined cell populations in the basal amygdala: a combined anatomical and electrophysiological approach. FMI Annual Meeting, Murten (CH) 2006

Senn V., Herry C. and Lüthi A.; Dissecting neuronal circuits in the basal amygdala: a combined anatomical and electrophysiological approach. FMI Annual Meeting, Pontresina (CH) 2005

Diploma thesis:

Senn V.,„Untersuchungen zur Rolle der der Lidregion in der Substratspeziefitaet von Zyklasen aus Nichtribosomalen Peptidsynthetasen“ (Translation: Testing substrate specificity of the lid-region of cyclases in nonribosomal peptide synthetases). Diploma Thesis 2004

9 References

Abbott LF, Nelson SB (2000) Synaptic plasticity: taming the beast. Nat Neurosci 3 Suppl:1178-1183.

Abraham WC, Mason SE, Demmer J, Williams JM, Richardson CL, Tate WP, Lawlor PA, Dragunow M (1993) Correlations between immediate early gene induction and the persistence of long-term potentiation. Neuroscience 56:717-727.

Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, de Lecea L (2007) Neural substrates of awakening probed with optogenetic control of hypocretin neurons. Nature 450:420-424.

Aizenman CD, Linden DJ (2000) Rapid, synaptically driven increases in the intrinsic excitability of cerebellar deep nuclear neurons. Nat Neurosci 3:109-111.

Aizenman CD, Akerman CJ, Jensen KR, Cline HT (2003) Visually driven regulation of intrinsic neuronal excitability improves stimulus detection in vivo. Neuron 39:831-842.

Alberini CM (2005) Mechanisms of memory stabilization: are consolidation and reconsolidation similar or distinct processes? Trends Neurosci 28:51-56.

Alberini CM (2009) Transcription factors in long-term memory and synaptic plasticity. Physiol Rev 89:121-145.

Amorapanth P, LeDoux JE, Nader K (2000) Different lateral amygdala outputs mediate reactions and actions elicited by a fear-arousing stimulus. Nat Neurosci 3:74-79.

Anglada-Figueroa D, Quirk GJ (2005) Lesions of the basal amygdala block expression of conditioned fear but not extinction. J Neurosci 25:9680-9685.

Armano S, Rossi P, Taglietti V, D'Angelo E (2000) Long-term potentiation of intrinsic excitability at the mossy fiber-granule cell synapse of rat cerebellum. J Neurosci 20:5208-5216.

Augustine GJ (1990) Regulation of transmitter release at the squid giant synapse by presynaptic delayed rectifier potassium current. J Physiol 431:343-364.

Bacon SJ, Headlam AJ, Gabbott PL, Smith AD (1996) Amygdala input to medial prefrontal cortex (mPFC) in the rat: a light and electron microscope study. Brain Res 720:211-219.

Bading H, Ginty DD, Greenberg ME (1993) Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. Science 260:181-186.

Barth AL, Gerkin RC, Dean KL (2004) Alteration of neuronal firing properties after in vivo experience in a FosGFP transgenic mouse. J Neurosci 24:6466-6475.

Bauer EP, Schafe GE, LeDoux JE (2002) NMDA receptors and L-type voltage-gated calcium channels contribute to long-term potentiation and different components of fear memory formation in the lateral amygdala. J Neurosci 22:5239-5249.

Baumgartel K, Genoux D, Welzl H, Tweedie-Cullen RY, Koshibu K, Livingstone-Zatchej M, Mamie C, Mansuy IM (2008) Control of the establishment of aversive memory by calcineurin and Zif268. Nat Neurosci 11:572-578.

Berendse HW, Galis-de Graaf Y, Groenewegen HJ (1992) Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. J Comp Neurol 316:314-347.

Berretta S, Pantazopoulos H, Caldera M, Pantazopoulos P, Paré D (2005) Infralimbic cortex activation increases c-Fos expression in intercalated neurons of the amygdala. Neuroscience 132:943-953.

Bi G, Poo M (2001) Synaptic modification by correlated activity: Hebb's postulate revisited. Annu Rev Neurosci 24:139-166.

Blair HT, Schafe GE, Bauer EP, Rodrigues SM, LeDoux JE (2001) Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. Learn Mem 8:229-242.

Bouton ME, Nelson JB (1994) Context-specificity of target versus feature inhibition in a feature-negative discrimination. J Exp Psychol Anim Behav Process 20:51-65.

Bouton ME, Rosengard C, Achenbach GG, Peck CA, Brooks DC (1993) Effects of contextual conditioning and unconditional stimulus presentation on performance in appetitive conditioning. Q J Exp Psychol B 46:63-95.

Campeau S, Davis M (1995) Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. J Neurosci 15:2301-2311.

Carlsen J, Heimer L (1988) The basolateral amygdaloid complex as a cortical-like structure. Brain Res 441:377-380.

Coggeshall RE (2005) Fos, nociception and the dorsal horn. Prog Neurobiol 77:299-352.

Collins DR, Pare D (2000) Differential fear conditioning induces reciprocal changes in the sensory responses of lateral amygdala neurons to the CS(+) and CS(-). Learn Mem 7:97-103.

Condorelli DF, Dell'Albani P, Amico C, Lukasiuk K, Kaczmarek L, Giuffrida-Stella AM (1994) Glutamate receptor-driven activation of transcription factors in primary neuronal cultures. Neurochem Res 19:489-499.

Corcoran KA, Maren S (2001) Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. J Neurosci 21:1720-1726.

Corcoran KA, Quirk GJ (2007) Activity in prelimbic cortex is necessary for the expression of learned, but not innate, fears. J Neurosci 27:840-844.

Cousens G, Otto T (1998) Both pre- and posttraining excitotoxic lesions of the basolateral amygdala abolish the expression of olfactory and contextual fear conditioning. Behav Neurosci 112:1092-1103.

Damasio H, Grabowski T, Frank R, Galaburda AM, Damasio AR (1994) The return of Phineas Gage: clues about the brain from the skull of a famous patient. Science 264:1102-1105.

Daoudal G, Debanne D (2003) Long-term plasticity of intrinsic excitability: learning rules and mechanisms. Learn Mem 10:456-465.

Davidson RJ, Irwin W (1999) The functional neuroanatomy of emotion and affective style. Trends Cogn Sci 3:11-21.

Davis M (2000) The role of the amygdala in conditioned and unconditioned fear and anxiety. In: The Amygdala (Aggleton JP, ed), pp 213-288: Oxford University Press.

Delgado JM, Rosvold HE, Looney E (1956) Evoking conditioned fear by electrical stimulation of subcortical structures in the monkey brain. J Comp Physiol Psychol 49:373-380.

Desgranges B, Levy F, Ferreira G (2008) Anisomycin infusion in amygdala impairs consolidation of odor aversion memory. Brain Res 1236:166-175.

Disterhoft JF, Oh MM (2006) Learning, aging and intrinsic neuronal plasticity. Trends Neurosci 29:587-599.

Disterhoft JF, Coulter DA, Alkon DL (1986) Conditioning-specific membrane changes of rabbit hippocampal neurons measured in vitro. Proc Natl Acad Sci U S A 83:2733-2737.

Douglas RM, Dragunow M, Robertson HA (1988) High-frequency discharge of dentate granule cells, but not long-term potentiation, induces c-fos protein. Brain Res 464:259-262.

Doyere V, Schafe GE, Sigurdsson T, LeDoux JE (2003) Long-term potentiation in freely moving rats reveals asymmetries in thalamic and cortical inputs to the lateral amygdala. Eur J Neurosci 17:2703-2715.

Dragunow M, Robertson HA (1987) Generalized seizures induce c-fos protein(s) in mammalian neurons. Neurosci Lett 82:157-161.

Dragunow M, Peterson MR, Robertson HA (1987) Presence of c-fos-like immunoreactivity in the adult rat brain. Eur J Pharmacol 135:113-114.

Egorov AV, Hamam BN, Fransen E, Hasselmo ME, Alonso AA (2002) Graded persistent activity in entorhinal cortex neurons. Nature 420:173-178.

Faber ES, Sah P (2002) Physiological role of calcium-activated potassium currents in the rat lateral amygdala. J Neurosci 22:1618-1628.

Faber ES, Sah P (2003) Ca2+-activated K+ (BK) channel inactivation contributes to spike broadening during repetitive firing in the rat lateral amygdala. J Physiol 552:483-497.

Faber ES, Callister RJ, Sah P (2001) Morphological and electrophysiological properties of principal neurons in the rat lateral amygdala in vitro. J Neurophysiol 85:714-723.

Falls WA, Miserendino MJ, Davis M (1992) Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. J Neurosci 12:854-863.

Fanselow MS (2000) Contextual fear, gestalt memories, and the hippocampus. Behav Brain Res 110:73-81.

Fanselow MS, LeDoux JE (1999) Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. Neuron 23:229-232.

Fanselow MS, Poulos AM (2005) The neuroscience of mammalian associative learning. Annu Rev Psychol 56:207-234.

Fleischmann A, Hvalby O, Jensen V, Strekalova T, Zacher C, Layer LE, Kvello A, Reschke M, Spanagel R, Sprengel R, Wagner EF, Gass P (2003) Impaired long-term memory and NR2A-type NMDA receptor-dependent synaptic plasticity in mice lacking c-Fos in the CNS. J Neurosci 23:9116-9122.

Floresco SB, Tse MT (2007) Dopaminergic regulation of inhibitory and excitatory transmission in the basolateral amygdala-prefrontal cortical pathway. J Neurosci 27:2045-2057.

Fourcaudot E, Gambino F, Humeau Y, Casassus G, Shaban H, Poulain B, Lüthi A (2008) cAMP/PKA signaling and RIM1alpha mediate presynaptic LTP in the lateral amygdala. Proc Natl Acad Sci U S A 105:15130-15135.

Gass P, Herdegen T, Bravo R, Kiessling M (1992) Induction of immediate early gene encoded proteins in the rat hippocampus after bicuculline-induced seizures: differential expression of KROX-24, FOS and JUN proteins. Neuroscience 48:315-324.

Geiger JR, Jonas P (2000) Dynamic control of presynaptic Ca(2+) inflow by fast-inactivating K(+) channels in hippocampal mossy fiber boutons. Neuron 28:927-939.

Gewirtz JC, Falls WA, Davis M (1997) Normal conditioned inhibition and extinction of freezing and fear-potentiated startle following electrolytic lesions of medical prefrontal cortex in rats. Behav Neurosci 111:712-726.

Ghosh A, Ginty DD, Bading H, Greenberg ME (1994) Calcium regulation of gene expression in neuronal cells. J Neurobiol 25:294-303.

Gilmartin MR, McEchron MD (2005) Single neurons in the medial prefrontal cortex of the rat exhibit tonic and phasic coding during trace fear conditioning. Behav Neurosci 119:1496-1510.

Goosens KA, Maren S (2001) Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. Learn Mem 8:148-155.

Goosens KA, Maren S (2003) Pretraining NMDA receptor blockade in the basolateral complex, but not the central nucleus, of the amygdala prevents savings of conditional fear. Behav Neurosci 117:738-750.

Goosens KA, Maren S (2004) NMDA receptors are essential for the acquisition, but not expression, of conditional fear and associative spike firing in the lateral amygdala. Eur J Neurosci 20:537-548.

Goosens KA, Hobin JA, Maren S (2003) Auditory-evoked spike firing in the lateral amygdala and Pavlovian fear conditioning: mnemonic code or fear bias? Neuron 40:1013-1022.

Grissmer S, Nguyen AN, Aiyar J, Hanson DC, Mather RJ, Gutman GA, Karmilowicz MJ, Auperin DD, Chandy KG (1994) Pharmacological characterization of five cloned voltage-gated K+ channels, types Kv1.1, 1.2, 1.3, 1.5, and 3.1, stably expressed in mammalian cell lines. Mol Pharmacol 45:1227-1234.

Groenewegen HJ, Berendse HW, Wolters JG, Lohman AH (1990) The anatomical relationship of the prefrontal cortex with the striatopallidal system, the thalamus and the amygdala: evidence for a parallel organization. Prog Brain Res 85:95-116; discussion 116-118.

Gu N, Vervaeke K, Storm JF (2007) BK potassium channels facilitate high-frequency firing and cause early spike frequency adaptation in rat CA1 hippocampal pyramidal cells. J Physiol 580:859-882.

Guzowski JF, Timlin JA, Roysam B, McNaughton BL, Worley PF, Barnes CA (2005) Mapping behaviorally relevant neural circuits with immediate-early gene expression. Curr Opin Neurobiol 15:599-606.

Haghdoost-Yazdi H, Janahmadi M, Behzadi G (2008) Iberiotoxin-sensitive large conductance Ca2+ -dependent K+ (BK) channels regulate the spike configuration in the burst firing of cerebellar Purkinje neurons. Brain Res 1212:1-8.

Han JH, Kushner SA, Yiu AP, Cole CJ, Matynia A, Brown RA, Neve RL, Guzowski JF, Silva AJ, Josselyn SA (2007) Neuronal competition and selection during memory formation. Science 316:457-460.

Han JH, Kushner SA, Yiu AP, Hsiang HL, Buch T, Waisman A, Bontempi B, Neve RL, Frankland PW, Josselyn SA (2009) Selective erasure of a fear memory. Science 323:1492-1496.

Herrera-Morales W, Mar I, Serrano B, Bermudez-Rattoni F (2007) Activation of hippocampal postsynaptic muscarinic receptors is involved in long-term spatial memory formation. Eur J Neurosci 25:1581-1588.

Herrera DG, Robertson HA (1996) Activation of c-fos in the brain. Prog Neurobiol 50:83-107.

Herry C, Mons N (2004) Resistance to extinction is associated with impaired immediate early gene induction in medial prefrontal cortex and amygdala. Eur J Neurosci 20:781-790.

Herry C, Ciocchi S, Senn V, Demmou L, Muller C, Lüthi A (2008) Switching on and off fear by distinct neuronal circuits. Nature 454:600-606.

Hestrin S, Nicoll RA, Perkel DJ, Sah P (1990) Analysis of excitatory synaptic action in pyramidal cells using whole- cell recording from rat hippocampal slices. J Physiol (Lond) 422:203-225.

Hobin JA, Ji J, Maren S (2006) Ventral hippocampal muscimol disrupts context-specific fear memory retrieval after extinction in rats. Hippocampus 16:174-182.

Holson RR (1986) Mesial prefrontal cortical lesions and timidity in rats. I. Reactivity to aversive stimuli. Physiol Behav 37:221-230.

Hope BT (1998) Cocaine and the AP-1 transcription factor complex. Ann N Y Acad Sci 844:1-6.

Huang YY, Kandel ER (1998) Postsynaptic induction and PKA-dependent expression of LTP in the lateral amygdala. Neuron 21:169-178.

Huber D, Veinante P, Stoop R (2005) Vasopressin and oxytocin excite distinct neuronal populations in the central amygdala. Science 308:245-248.

Hughes P, Lawlor P, Dragunow M (1992) Basal expression of Fos, Fos-related, Jun, and Krox 24 proteins in rat hippocampus. Brain Res Mol Brain Res 13:355-357.

Humeau Y, Reisel D, Johnson AW, Borchardt T, Jensen V, Gebhardt C, Bosch V, Gass P, Bannerman DM, Good MA, Hvalby O, Sprengel R, Lüthi A (2007) A pathway-specific function for different AMPA receptor subunits in amygdala long-term potentiation and fear conditioning. J Neurosci 27:10947-10956.

Ishikawa A, Nakamura S (2003) Convergence and interaction of hippocampal and amygdalar projections within the prefrontal cortex in the rat. J Neurosci 23:9987-9995.

Ishikawa A, Nakamura S (2006) Ventral hippocampal neurons project axons simultaneously to the medial prefrontal cortex and amygdala in the rat. J Neurophysiol 96:2134-2138.

Ji J, Maren S (2007) Hippocampal involvement in contextual modulation of fear extinction. Hippocampus 17:749-758.

Jones MW, Errington ML, French PJ, Fine A, Bliss TV, Garel S, Charnay P, Bozon B, Laroche S, Davis S (2001) A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories. Nat Neurosci 4:289-296.

Kandel ER (2001) The molecular biology of memory storage: a dialogue between genes and synapses. Science 294:1030-1038.

Karpova AY, Tervo DG, Gray NW, Svoboda K (2005) Rapid and reversible chemical inactivation of synaptic transmission in genetically targeted neurons. Neuron 48:727-735.

Kemppainen S, Pitkanen A (2000) Distribution of parvalbumin, calretinin, and calbindin-D(28k) immunoreactivity in the rat amygdaloid complex and colocalization with gamma-aminobutyric acid. J Comp Neurol 426:441-467.

Kent JM, Rauch SL (2003) Neurocircuitry of anxiety disorders. Curr Psychiatry Rep 5:266-273.

Knapska E, Kaczmarek L (2004) A gene for neuronal plasticity in the mammalian brain: Zif268/Egr-1/NGFI-A/Krox-24/TIS8/ZENK? Prog Neurobiol 74:183-211.

Kogure K, Kato H (1993) Altered gene expression in cerebral ischemia. Stroke 24:2121-2127.

Krettek JE, Price JL (1978) Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. J Comp Neurol 178:225-254.

Lacroix L, Spinelli S, Heidbreder CA, Feldon J (2000) Differential role of the medial and lateral prefrontal cortices in fear and anxiety. Behav Neurosci 114:1119-1130.

Lamprecht R, Dudai Y (1996) Transient expression of c-Fos in rat amygdala during training is required for encoding conditioned taste aversion memory. Learn Mem 3:31-41.

Lancaster B, Nicoll RA (1987) Properties of two calcium-activated hyperpolarizations in rat hippocampal neurones. J Physiol 389:187-203.

LeDoux J (1998) Fear and the brain: where have we been, and where are we going? Biol Psychiatry 44:1229-1238.

LeDoux JE (1995) Emotion: clues from the brain. Annu Rev Psychol 46:209-235.

LeDoux JE (2000) Emotion circuits in the brain. Annu Rev Neurosci 23:155-184.

LeDoux JE, Farb C, Ruggiero DA (1990) Topographic organization of neurons in the acoustic thalamus that project to the amygdala. J Neurosci 10:1043-1054.

LeDoux JE, Farb CR, Milner TA (1991) Ultrastructure and synaptic associations of auditory thalamo-amygdala projections in the rat. Exp Brain Res 85:577-586.

LeDoux JE, Iwata J, Cicchetti P, Reis DJ (1988) Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. J Neurosci 8:2517-2529.

Likhtik E, Popa D, Apergis-Schoute J, Fidacaro GA, Paré D (2008) Amygdala intercalated neurons are required for expression of fear extinction. Nature 454:642-645.

Lin CH, Yeh SH, Leu TH, Chang WC, Wang ST, Gean PW (2003) Identification of calcineurin as a key signal in the extinction of fear memory. J Neurosci 23:1574-1579.

Llinas R, Steinberg IZ, Walton K (1981) Presynaptic calcium currents in squid giant synapse. Biophys J 33:289-321.

Lu KT, Walker DL, Davis M (2001) Mitogen-activated protein kinase cascade in the basolateral nucleus of amygdala is involved in extinction of fear-potentiated startle. J Neurosci 21:RC162.

Mahanty NK, Sah P (1998) Calcium-permeable AMPA receptors mediate long-term potentiation in interneurons in the amygdala. Nature 394:683-687.

Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. Neuron 44:5-21.

Malkani S, Rosen JB (2000a) Specific induction of early growth response gene 1 in the lateral nucleus of the amygdala following contextual fear conditioning in rats. Neuroscience 97:693-702.

Malkani S, Rosen JB (2000b) Differential expression of EGR-1 mRNA in the amygdala following diazepam in contextual fear conditioning. Brain Res 860:53-63.

Malkani S, Wallace KJ, Donley MP, Rosen JB (2004) An egr-1 (zif268) antisense oligodeoxynucleotide infused into the amygdala disrupts fear conditioning. Learn Mem 11:617-624.

Marder E, Prinz AA (2003) Current compensation in neuronal homeostasis. Neuron 37:2-4.

Maren S (1999) Neurotoxic or electrolytic lesions of the ventral subiculum produce deficits in the acquisition and expression of Pavlovian fear conditioning in rats. Behav Neurosci 113:283-290.

Maren S, Holt W (2000) The hippocampus and contextual memory retrieval in Pavlovian conditioning. Behav Brain Res 110:97-108.

Maren S, Holt WG (2004) Hippocampus and Pavlovian fear conditioning in rats: muscimol infusions into the ventral, but not dorsal, hippocampus impair the acquisition of conditional freezing to an auditory conditional stimulus. Behav Neurosci 118:97-110.

Maren S, Hobin JA (2007) Hippocampal regulation of context-dependent neuronal activity in the lateral amygdala. Learn Mem 14:318-324.

Martin SJ, Grimwood PD, Morris RG (2000) Synaptic plasticity and memory: an evaluation of the hypothesis. Annu Rev Neurosci 23:649-711.

Mascagni F, McDonald AJ (2003) Immunohistochemical characterization of cholecystokinin containing neurons in the rat basolateral amygdala. Brain Res 976:171-184.

Matthews EA, Disterhoft JF (2009) Blocking the BK channel impedes acquisition of trace eyeblink conditioning. Learn Mem 16:106-109.

Matthews EA, Linardakis JM, Disterhoft JF (2009) The fast and slow afterhyperpolarizations are differentially modulated in hippocampal neurons by aging and learning. J Neurosci 29:4750-4755.

Matthews EA, Weible AP, Shah S, Disterhoft JF (2008) The BK-mediated fAHP is modulated by learning a hippocampus-dependent task. Proc Natl Acad Sci U S A 105:15154-15159.

McDonald AJ (1991) Organization of amygdaloid projections to the prefrontal cortex and associated striatum in the rat. Neuroscience 44:1-14.

McDonald AJ (1992) Neuroanatomical labeling with biocytin: a review. Neuroreport 3:821-827.

McDonald AJ (1998) Cortical pathways to the mammalian amygdala. Prog Neurobiol 55:257-332.

McDonald AJ, Augustine JR (1993) Localization of GABA-like immunoreactivity in the monkey amygdala. Neuroscience 52:281-294.

McDonald AJ, Mascagni F (2001) Colocalization of calcium-binding proteins and GABA in neurons of the rat basolateral amygdala. Neuroscience 105:681-693.

McDonald AJ, Mascagni F, Guo L (1996) Projections of the medial and lateral prefrontal cortices to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat. Neuroscience 71:55-75.

McKernan MG, Shinnick-Gallagher P (1997) Fear conditioning induces a lasting potentiation of synaptic currents in vitro. Nature 390:607-611.

Medina JF, Repa JC, Mauk MD, LeDoux JE (2002) Parallels between cerebellum- and amygdala-dependent conditioning. Nat Rev Neurosci 3:122-131.

Milad MR, Quirk GJ (2002) Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 420:70-74.

Millan MJ (2003) The neurobiology and control of anxious states. Prog Neurobiol 70:83-244.

Millhouse OE, DeOlmos J (1983) Neuronal configurations in lateral and basolateral amygdala. Neuroscience 10:1269-1300.

Morgan JI, Cohen DR, Hempstead JL, Curran T (1987) Mapping patterns of c-fos expression in the central nervous system after seizure. Science 237:192-197.

Morgan MA, Romanski LM, LeDoux JE (1993) Extinction of emotional learning: contribution of medial prefrontal cortex. Neurosci Lett 163:109-113.

Murphy TH, Worley PF, Baraban JM (1991) L-type voltage-sensitive calcium channels mediate synaptic activation of immediate early genes. Neuron 7:625-635.

Nader K, Schafe GE, Le Doux JE (2000) Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature 406:722-726.

Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurones. Nature 307:462-465.

O'Keefe J, Conway DH (1978) Hippocampal place units in the freely moving rat: why they fire where they fire. Exp Brain Res 31:573-590.

Pare D, Smith Y (1993) The intercalated cell masses project to the central and medial nuclei of the amygdala in cats. Neuroscience 57:1077-1090.

Pare D, Gaudreau H (1996) Projection cells and interneurons of the lateral and basolateral amygdala: distinct firing patterns and differential relation to theta and delta rhythms in conscious cats. J Neurosci 16:3334-3350.

Pare D, Smith Y (1998) Intrinsic circuitry of the amygdaloid complex: common principles of organization in rats and cats. Trends Neurosci 21:240-241.

Pare D, Smith Y, Pare JF (1995) Intra-amygdaloid projections of the basolateral and basomedial nuclei in the cat: Phaseolus vulgaris-leucoagglutinin anterograde tracing at the light and electron microscopic level. Neuroscience 69:567-583.

Paré D, Quirk GJ, Ledoux JE (2004) New vistas on amygdala networks in conditioned fear. J Neurophysiol 92:1-9.

Pavlov (1927) Conditioned Reflexes
Oxford University Press:
Oxford, UK.

Pawlowski TL, Bellush LL, Wright AW, Walker JP, Colvin RA, Huentelman MJ (2009) Hippocampal gene expression changes during age-related cognitive decline. Brain Res 1256:101-110.

Pikkarainen M, Ronkko S, Savander V, Insausti R, Pitkanen A (1999) Projections from the lateral, basal, and accessory basal nuclei of the amygdala to the hippocampal formation in rat. J Comp Neurol 403:229-260.

Pitkanen A (2000) Connectivity of the rat amygdaloid complex. In: The amygdala: A functional analysis (Aggleton JP, ed), pp 31-115. Oxford, UK: Oxford University Press.

Pitkanen A, Savander V, LeDoux JE (1997) Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. Trends Neurosci 20:517-523.

Pitkanen A, Pikkarainen M, Nurminen N, Ylinen A (2000a) Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. Ann N Y Acad Sci 911:369-391.

Pitkanen A, Nissinen J, Lukasiuk K, Jutila L, Paljarvi L, Salmenpera T, Karkola K, Vapalahti M, Ylinen A (2000b) Association between the density of mossy fiber sprouting and seizure frequency in experimental and human temporal lobe epilepsy. Epilepsia 41 Suppl 6:S24-29.

Poolos NP, Johnston D (1999) Calcium-activated potassium conductances contribute to action potential repolarization at the soma but not the dendrites of hippocampal CA1 pyramidal neurons. J Neurosci 19:5205-5212.

Quirk GJ, Mueller D (2008) Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology 33:56-72.

Quirk GJ, Repa C, LeDoux JE (1995) Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. Neuron 15:1029-1039.

Quirk GJ, Armony JL, LeDoux JE (1997) Fear conditioning enhances different temporal components of tone-evoked spike trains in auditory cortex and lateral amygdala. Neuron 19:613-624.

Quirk GJ, Garcia R, Gonzalez-Lima F (2006) Prefrontal mechanisms in extinction of conditioned fear. Biol Psychiatry 60:337-343.

Quirk GJ, Russo GK, Barron JL, Lebron K (2000) The role of ventromedial prefrontal cortex in the recovery of extinguished fear. J Neurosci 20:6225-6231.

Reijmers LG, Perkins BL, Matsuo N, Mayford M (2007) Localization of a stable neural correlate of associative memory. Science 317:1230-1233.

Repa JC, Muller J, Apergis J, Desrochers TM, Zhou Y, LeDoux JE (2001) Two different lateral amygdala cell populations contribute to the initiation and storage of memory. Nat Neurosci 4:724-731.

Richardson DP, Byrnes ML, Brien JF, Reynolds JN, Dringenberg HC (2002) Impaired acquisition in the water maze and hippocampal long-term potentiation after chronic prenatal ethanol exposure in the guinea-pig. Eur J Neurosci 16:1593-1598.

Rodrigues SM, Schafe GE, LeDoux JE (2001) Intra-amygdala blockade of the NR2B subunit of the NMDA receptor disrupts the acquisition but not the expression of fear conditioning. J Neurosci 21:6889-6896.

Rogan MT, LeDoux JE (1995) LTP is accompanied by commensurate enhancement of auditory-evoked responses in a fear conditioning circuit. Neuron 15:127-136.

Rogan MT, Staubli UV, LeDoux JE (1997) Fear conditioning induces associative long-term potentiation in the amygdala [see comments] [published erratum appears in Nature 1998 Feb 19;391(6669):818]. Nature 390:604-607.

Romanski LM, LeDoux JE (1993) Information cascade from primary auditory cortex to the amygdala: corticocortical and corticoamygdaloid projections of temporal cortex in the rat. Cereb Cortex 3:515-532.

Rosen JB, Adamec RE, Thompson BL (2005) Expression of egr-1 (zif268) mRNA in select fear-related brain regions following exposure to a predator. Behav Brain Res 162:279-288.

Rosen JB, Hitchcock JM, Miserendino MJ, Falls WA, Campeau S, Davis M (1992) Lesions of the perirhinal cortex but not of the frontal, medial prefrontal, visual, or insular cortex block fear-potentiated startle using a visual conditioned stimulus. J Neurosci 12:4624-4633.

Rosenkranz JA, Moore H, Grace AA (2003) The prefrontal cortex regulates lateral amygdala neuronal plasticity and responses to previously conditioned stimuli. J Neurosci 23:11054-11064.

Rumpel S, LeDoux J, Zador A, Malinow R (2005) Postsynaptic receptor trafficking underlying a form of associative learning. Science 308:83-88.

Saar D, Grossman Y, Barkai E (1998) Reduced after-hyperpolarization in rat piriform cortex pyramidal neurons is associated with increased learning capability during operant conditioning. Eur J Neurosci 10:1518-1523.

Sabatini BL, Regehr WG (1997) Control of neurotransmitter release by presynaptic waveform at the granule cell to Purkinje cell synapse. J Neurosci 17:3425-3435.

Sah P, Westbrook RF, Luthi A (2008) Fear conditioning and long-term potentiation in the amygdala: what really is the connection? Ann N Y Acad Sci 1129:88-95.

Sah P, Faber ES, Lopez De Armentia M, Power J (2003) The amygdaloid complex: anatomy and physiology. Physiol Rev 83:803-834.

Samson RD, Paré D (2005) Activity-dependent synaptic plasticity in the central nucleus of the amygdala. J Neurosci 25:1847-1855.

Santini E, Quirk GJ, Porter JT (2008) Fear conditioning and extinction differentially modify the intrinsic excitability of infralimbic neurons. J Neurosci 28:4028-4036.

Savander V, Go CG, LeDoux JE, Pitkanen A (1995) Intrinsic connections of the rat amygdaloid complex: projections originating in the basal nucleus. J Comp Neurol 361:345-368.

Schafe GE, LeDoux JE (2000) Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. J Neurosci 20:RC96.

Schafe GE, Nader K, Blair HT, LeDoux JE (2001) Memory consolidation of Pavlovian fear conditioning: a cellular and molecular perspective. Trends Neurosci 24:540-546.

Schreurs BG, Tomsic D, Gusev PA, Alkon DL (1997) Dendritic excitability microzones and occluded long-term depression after classical conditioning of the rabbit's nictitating membrane response. J Neurophysiol 77:86-92.

Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry 20:11-21.

Sierra-Mercado D, Jr., Corcoran KA, Lebron-Milad K, Quirk GJ (2006) Inactivation of the ventromedial prefrontal cortex reduces expression of conditioned fear and impairs subsequent recall of extinction. Eur J Neurosci 24:1751-1758.

Sjostrom PJ, Nelson SB (2002) Spike timing, calcium signals and synaptic plasticity. Curr Opin Neurobiol 12:305-314.

Sourdet V, Debanne D (1999) The role of dendritic filtering in associative long-term synaptic plasticity. Learn Mem 6:422-447.

Thomas KL, Arroyo M, Everitt BJ (2003) Induction of the learning and plasticity-associated gene Zif268 following exposure to a discrete cocaine-associated stimulus. Eur J Neurosci 17:1964-1972.

Tsubokawa H, Offermanns S, Simon M, Kano M (2000) Calcium-dependent persistent facilitation of spike backpropagation in the CA1 pyramidal neurons. J Neurosci 20:4878-4884.

Tsvetkov E, Carlezon WA, Benes FM, Kandel ER, Bolshakov VY (2002) Fear conditioning occludes LTP-induced presynaptic enhancement of synaptic transmission in the cortical pathway to the lateral amygdala. Neuron 34:289-300.

Turner BH, Herkenham M (1991) Thalamoamygdaloid projections in the rat: a test of the amygdala's role in sensory processing. J Comp Neurol 313:295-325.

Turrigiano G (2007) Homeostatic signaling: the positive side of negative feedback. Curr Opin Neurobiol 17:318-324.

Tzounopoulos T, Stackman R (2003) Enhancing synaptic plasticity and memory: a role for small-conductance Ca(2+)-activated K+ channels. Neuroscientist 9:434-439.

Uylings HB, van Eden CG (1990) Qualitative and quantitative comparison of the prefrontal cortex in rat and in primates, including humans. Prog Brain Res 85:31-62.

Uys JD, Stein DJ, Daniels WM, Harvey BH (2003) Animal models of anxiety disorders. Curr Psychiatry Rep 5:274-281.

Van der Gucht E, Clerens S, Jacobs S, Arckens L (2005) Light-induced Fos expression in phosphate-activated glutaminase- and neurofilament protein-immunoreactive neurons in cat primary visual cortex. Brain Res 1035:60-66.

Veening JG, Swanson LW, Sawchenko PE (1984) The organization of projections from the central nucleus of the amygdala to brainstem sites involved in central autonomic regulation: a combined retrograde transport-immunohistochemical study. Brain Res 303:337-357.

Vidal-Gonzalez I, Vidal-Gonzalez B, Rauch SL, Quirk GJ (2006) Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. Learn Mem 13:728-733.

Weiskrantz L (1956) Behavioral changes associated with ablation of the amygdaloid complex in monkeys. J Comp Physiol Psychol 49:381-391.

Wilensky AE, Schafe GE, Kristensen MP, LeDoux JE (2006) Rethinking the fear circuit: the central nucleus of the amygdala is required for the acquisition, consolidation, and expression of Pavlovian fear conditioning. J Neurosci 26:12387-12396.

Wilson CJ, Sachdev RN (2004) Intracellular and juxtacellular staining with biocytin. Curr Protoc Neurosci Chapter 1:Unit 1 12.

Zangenehpour S, Chaudhuri A (2002) Differential induction and decay curves of c-fos and zif268 revealed through dual activity maps. Brain Res Mol Brain Res 109:221-225.

Zhang F, Aravanis AM, Adamantidis A, de Lecea L, Deisseroth K (2007) Circuit-breakers: optical technologies for probing neural signals and systems. Nat Rev Neurosci 8:577-581.

Zhang W, Linden DJ (2003) The other side of the engram: experience-driven changes in neuronal intrinsic excitability. Nat Rev Neurosci 4:885-900.

Zimmerman JM, Rabinak CA, McLachlan IG, Maren S (2007) The central nucleus of the amygdala is essential for acquiring and expressing conditional fear after overtraining. Learn Mem 14:634-644.