Andreas Lüthi

THE CLOËTTA PRIZE 2016
IS AWARDED TO

PROFESSOR

ANDREAS LÜTHI

BORN IN 1968 IN BASEL, SWITZERLAND
SENIOR GROUP LEADER AT THE FRIEDRICH MIESCHER INSTITUTE
FOR BIOMEDICAL RESEARCH AND
TITULAR PROFESSOR AT THE UNIVERSITY OF BASEL

FOR HIS OUTSTANDING FINDINGS ABOUT UNDERSTANDING
LEARNING PROCESSES IN THE BRAIN

Basel, 4th November 2016

IN THE NAME OF THE FOUNDATION BOARD:

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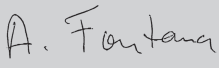

MEMBER

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BIOGRAPHY

Name:	Lüthi, Andreas
Date of Birth:	17th November 1968
Place of Birth:	Basel, Switzerland
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Education

1988–1992 M.Sc. in Biology	University of Basel, Switzerland
1993–1996 Ph.D. in Neurobiology	University of Basel, Switzerland

Employment

1996–1998	Postdoctoral Fellow	Department of Anatomy, University of Bristol, UK
1998–2000	Postdoctoral Fellow	Brain Research Institute, University of Zurich, Switzerland
2000–2003	Assistant Professor	Department of Pharmacology/Neurobiology, Biozentrum, University of Basel, Switzerland
2003–2004	Junior Group Leader	Friedrich Miescher Institute, Basel, Switzerland
2004–	Group Leader	Friedrich Miescher Institute, Basel; Titular Professor, University of Basel, Switzerland

Andreas Lüthi has since 2003 been Group Leader at the Friedrich Miescher Institute for Biomedical Research in Basel and since 2006 Titular Professor at the University of Basel. Born in 1968 in Basel, he studied Biology in Basel and received his PhD in Neuroscience in 1996 working on the synaptic mechanisms of hippocampal long-term potentiation. After postdoctoral fellowships with Graham L. Collingridge at the University of Bristol, UK and Beat H. Gähwiler at the University of Zurich, Switzerland, he established his own group as a Junior PI, first at the University of Basel and then at the Friedrich Miescher Institute for Biomedical Research, where he currently is a Senior Group Leader. Andreas Lüthi received several awards including the Pfizer Research Prize 2006 (with Y. Humeau), the Betty and David Koetser Award for Brain Research in 2009 and most recently an ERC advanced grant. He is a member of EMBO and of the National Research Council of the Swiss National Science Foundation. His research is focusing on the cellular and circuit mechanisms underlying associative learning using a multi-disciplinary approach to study classical conditioning paradigms in mice.

Honors and Fellowships

Swiss National Science Foundation/ EU Fellowship – 1996; EMBO Long-Term Fellowship – 1996; Borderline Personality Disorder Research Foundation, Junior Investigator Award – 2003; Swiss National Science Foundation, Professorship – 2003; Titular Professor, University of Basel – 2006; Pfizer Research Prize, Neuroscience – 2006; NARSAD Independent Investigator Award – 2008; Betty and David Koetser Award for Brain Research – 2009; Annual Meeting of the American Society for Neuroscience, Special Lecture – 2011; EMBO Member – 2012; ERC Advanced Grant – 2015; FENS Meeting Copenhagen, Plenary Lecture – 2016; Cloëtta Prize – 2016.

SELECTED PUBLICATIONS

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THE NEURONAL CIRCUITRY OF FEAR AND ANXIETY

Andreas Lüthi

Summary

Mood and anxiety disorders are the greatest societal burdens in terms of impairment and disability. However, the neurobiological mechanisms underlying the etiology and pathophysiology of mental disorders is poorly understood. It emerges that maladaptive neuronal plasticity caused by environmental and genetic factors can give rise to pathological neuronal circuit function, and that this process is key for the progression and manifestation of mental diseases.

To investigate the basic underlying circuit mechanisms, we are using classical Pavlovian fear conditioning (FC), a simple and robust form of learning, in which an animal learns to associate an initially neutral stimulus with an aversive outcome. A large number of studies in animals and humans have identified the amygdala as a key structure embedded in a brain-wide neuronal network mediating fear conditioning. Using a multidisciplinary approach in mice, we investigate the anatomical and functional logic of amygdala circuits, and their interactions with other brain areas during the formation, expression and extinction of learned fear in animal models for physiological and pathological fear and anxiety.

Our research shows that functionally, anatomically and genetically defined types of amygdala neurons are precisely connected both within local and within larger-scale neuronal networks, and that they selectively contribute to specific aspects of fear learning, expression and extinction of conditioned fear responses. Understanding the neuronal circuitry and the plasticity mechanisms underlying these processes will be fundamental not only for an understanding of memory processes in the brain in general, but also to inform new therapeutic strategies for psychiatric disorders involving dysregulated emotional responses associated with amygdala hyper- or hyposensitivity such as anxiety disorders or major depression.

Introduction

Fear and anxiety are emotional states that are crucial for eliciting appropriate defensive reactions in order to avoid harm and to ensure survival in threatening situations. However, in humans, excessive fear or chronic anxiety are core symptoms associated with severe mental diseases. The World Health Organization (WHO) has calculated that mental disorders are the greatest societal burdens in terms of impairment and disability (Murray et al., 1996). In particular, anxiety disorders and major depression are the most costly diseases among people in the middle years of life. These diseases thus represent one of the greatest preventive and therapeutic challenges in medicine. To meet these challenges, there is an urgent need for a better understanding of the underlying neurobiology.

Over the past decades, research on humans and on animal models has revealed that the neurobiological basis of anxiety disorders, and other mental diseases such as major depression or addiction, is not primarily based on neuronal degeneration and death, but rather on maladaptive functional and structural changes in the underlying neuronal circuits, e.g. a form of pathological learning (Lüthi & Lüscher, 2015). Most of what we know about the basic neurobiological mechanisms governing physiological and pathological forms of learning in the context of fear and anxiety originates from studies on classical fear conditioning (FC), a simple and robust form of associative learning, in which a human subject or an animal learns that an initially neutral sensory stimulus, the so-called conditioned stimulus (CS), predicts an unpleasant or aversive stimulus, the unconditioned stimulus (US).

Over the past decades, a large number of studies in animals and humans have identified the amygdala, a complex of multiple subcortical nuclei located in the temporal lobe, as a key structure embedded in a brain-wide neuronal network mediating fear conditioning (LeDoux, 2000; Davis, 2000; Fanselow and Poulos, 2005). Information about the CS and the US converge in the lateral nucleus of the amygdala (LA), which together with the basal (BA) and basomedial (BMA) nuclei, comprises the basolateral complex (BLA). Information then generally flows from the LA to the BA, which is strongly interconnected with other forebrain areas, including the medial prefrontal cortex (mPFC), the hippocampus (HC) and the

nucleus accumbens (NAc). The central nucleus of the amygdala (CEA) integrates information from the BLA and cortex to generate a complex fear response via its projections to downstream targets in the brainstem and hypothalamus. At the mechanistic level, an essential first step during the formation of CS-US associations involves activity-dependent synaptic plasticity. There is strong evidence supporting a role for NMDA receptor-dependent long-term potentiation (LTP) at glutamatergic sensory afferents to LA principal neurons (PNs)(LeDoux, 2000; Paré and Pape, 2010).

However, in contrast to the well-understood contributions of different brain areas to Pavlovian fear conditioning, and detailed knowledge on the basic mechanisms of synaptic plasticity in these brain areas, there is a big gap in our understanding of how these events relate to processing at the level of defined neuronal circuits during learning and memory.

A key feature of neuronal circuits is that they are composed of a multitude of excitatory and inhibitory neuron types. While the most of insights into the mechanisms and consequences of classical fear conditioning have been obtained from excitatory projection neurons, much less is known about the contribution of inhibitory interneurons to the processing of sensory information and to plasticity in amygdala circuits.

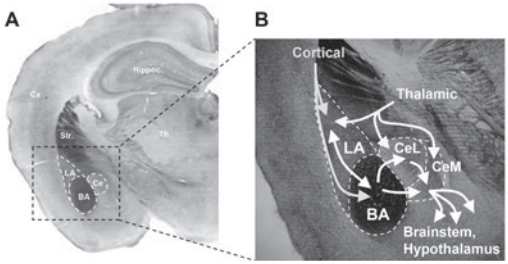

Fig. 1: Basic neuronal circuitry of the amygdala. A. *Coronal slice of a mouse brain stained for acetylcholinesterase illustrating location of the amygdala.* ***B.*** *The neural circuitry underlying classical auditory fear conditioning. Acoustic and somatosensory stimuli reach the lateral nucleus (LA) via thalamic and cortical afferents. Thalamic afferents also project to the central nucleus (CeL, CeM). By projections to brainstem and hypothalamus, the CeM initiates behavioral responses. Abbr.: LA: lateral amygdala; BA: basal amygdala; CeL: central lateral amygdala; CeM: central medial amygdala; Cx: Cortex; Str: Striatum.*

Neuronal mechanisms of fear learning: a role for inhibitory interneurons

The BLA is a cortex-like structure that contains glutamatergic principal (projection) neurons and local circuit GABAergic interneurons. Principal neurons account for the majority of the BLA neuronal population, their morphological features resemble cortical pyramidal neurons (Sah et al., 2003). As in the neocortex, a broad diversity of local GABAergic interneurons has been identified in the BLA. They can be subdivided based on molecular markers, electrophysiological properties, morphology and postsynaptic innervation pattern (McDonald & Mascagni, 2001). Interneurons constitute only about 20 % of the neuronal population, but tightly control pyramidal cell activity by releasing GABA onto principal neurons in a temporally and spatially distinct manner, thereby preventing neurons from firing to irrelevant stimuli (Klausberger & Somogyi, 2008; Ehrlich et al., 2009; Bienvenu et al., 2012). However, how each individual subtype of BLA interneurons contributes to sensory processing and fear learning is just beginning to be elucidated.

In our early work on the mechanisms of synaptic plasticity in the LA, we have addressed the impact of inhibitory transmission on the induction and specificity of LTP at thalamic and cortical inputs onto LA projection neurons. We could show that the synapses made by thalamic and cortical afferents are morphologically and functionally very different, even though they contact the same postsynaptic dendrites (Humeau et al., 2003, 2005). In particular, we found that thalamic and cortical afferent synapses exhibit input specific LTP mediated by distinct mechanisms. Whereas LTP at cortical afferents is mediated by presynaptic mechanisms involving the activation of presynaptic NMDA receptors (Humeau et al., 2003), the cAMP/PKA pathway, and the presynaptic active zone protein RIM1α (Fourcaudot et al., 2006), LTP at thalamic afferents is induced postsynaptically and requires activation of R-type voltage-dependent Ca^{2+} channels (R-VDCCs)(Humeau et al., 2005). Importantly, these pre- and postsynaptic forms of LTP at thalamic and cortical afferents to the LA are tightly controlled by local inhibition. Neuromodulators that are released in the amygdala upon stress gate induction of plasticity by transiently suppressing pre- or postsynaptic inhibition (Bissière et al., 2003; Lorétan et al., 2004; Shaban et al., 2006). Moreover, presynaptic inhibition in par-

ticular appears to play a major role to prevent generalization of amygdala LTP and conditioned fear, one of the hallmarks of anxiety disorders.

More recently, we have used a multidisciplinary analysis to identify key circuit elements underlying fear learning *in vivo.* We have obtained converging evidence that dis-inhibitory mechanisms play an important role in fear learning in multiple brain areas including the BLA and auditory cortex (Letzkus et al., 2011; Wolff et al., 2014).

Using a combination of *in vivo* single unit recordings and optogenetic manipulations, we found that in the BLA, parvalbumin (PV) and somatostatin (SOM) expressing interneurons bidirectionally control the acquisition of fear conditioning through two distinct disinhibitory mechanisms (Wolff et al., 2014). During the auditory CS, PV^+ interneurons are excited and indirectly disinhibit the dendrites of BLA principal neurons via SOM^+ interneurons, thereby enhancing auditory responses and promoting CS-US associations. During the aversive US, however, both PV^+ and SOM^+ interneurons are inhibited, which boosts postsynaptic US responses and gates plasticity and learning. These findings demonstrate that associative learning is dynamically regulated by the stimulus-specific activation of distinct disinhibitory microcircuits through precise interactions between different subtypes of local interneurons.

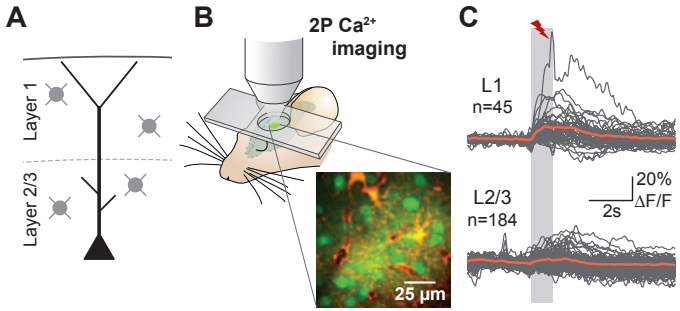

Fig. 2: Somatosensory responses in auditory cortex L1 interneurons A: *Cytoarchitecture of upper layers of auditory cortex (interneurons grey, pyramidal neuron black).* ***B:*** *2-photon calcium imaging in anesthetized mice using the membrane permeant dye OGB-1 AM (green). Glial cells were counterstained with sulforhodamine 101 (red).* ***C:*** *Responses in L1 and L2/3 to hindpaw stimulation in single neurons. Layer 1 interneurons display much stronger activation than layer 2/3 neurons.*

Disinhibition emerges as a general mechanism of aversive learning that is not limited to the BLA. Recordings in auditory cortex revealed that US stimulation elicits strong, time-locked firing in layer 1 interneurons. This response was mediated by rapid acetylcholine release from basal forebrain afferents activating nicotinic receptors on these interneurons. Firing of layer 1 interneurons in turn caused an inhibition of fast-spiking PV+ basket cells in layer 2/3 leading to disinhibition of layer2/3 pyramidal neurons and to a marked increase in the neuronal responses to concomitantly presented auditory CSs (Letzkus et al., 2011). Optogenetic interference with these processes led to a strong deficit in fear learning, indicating that disinhibition in auditory cortex is required for fear learning possibly by gating plasticity at afferent synapses or in downstream areas such as the BLA.

Circuit mechanisms underlying the expression and selection of fear behavior

In addition to acquisition, disinhibition also plays an important role in fear memory expression. One example is the control of fear expression by the central amygdala, a striatum-like nucleus composed mainly of GABAergic neurons. It can be subdivided into a lateral (CEl) and medial (CEm) part (McDonald, 1982), the latter being one of the main output pathways of the amygdala to downstream areas involved in fear responses (Ehrlich et al., 2009). Neurons in CEl project to CEm (Ehrlich et al., 2009), suggesting that amygdala output is under tight inhibitory control from CEl. In line with this scenario, we found that pharmacological inactivation of CEl causes unconditioned freezing (Ciocchi et al., 2010), as did pharmacogenetic inhibition of a population of CEl neurons expressing PKC delta (Haubensak et al., 2010). Conversely, optogenetic activation of CEm neurons causes unconditioned freezing (Ciocchi et al. 2010). While the central amygdala was previously thought to be mainly involved in the behavioral expression of fear memory residing in the BLA, several lines of evidence suggest that the central amygdala is also a critical site for plastic changes during fear learning (Ciocchi et al., 2010). First, activity in CEl was required for fear memory acquisition, whereas memory expression was selectively impaired by CEm inactivation. Moreover, fear conditioning caused plastic changes in CS respons-

es of different central amygdala neurons. In the CEl, one population of neurons acquired an excitatory response (CEl_{on} neurons), whereas an equal number of cells displayed inhibitory responses to the conditioned tone (CEl_{off} neurons) **(Fig. 3)**. CEl_{off} neurons correspond to $PKC\delta^+$ positive cells (Haubensak et al., 2010), and their inhibitory response to the conditioned tone likely mediates dis-inhibition of CEm output neurons, which in turn elicits conditioned freezing.

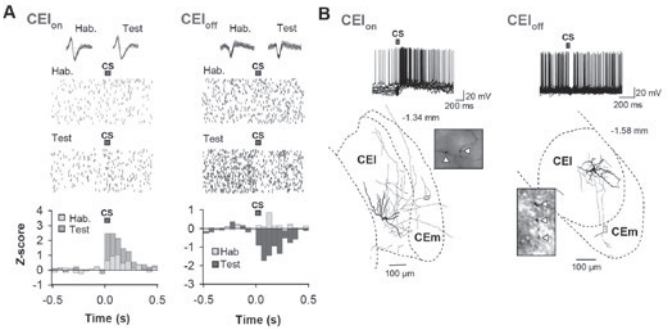

Fig. 3. Fear conditioning induces cell type specific plasticity in CEl circuits. A: *Extracellular single unit recordings in the central amygdala of behaving mice reveals two distinct sub-populations of CEl units showing an increase (CEl_{on} neurons) or a decrease (CEl_{off} neurons) in CS-evoked firing after fear conditioning.* ***B:*** *Intracellular recordings from CEl on and CEl_{off} neurons in anaesthetized mice. Anatomical reconstructions of recorded neurons demonstrates that both CEl_{on} and CEl_{off} neurons send axon collaterals to CEm, the output nucleus of the amygdala. Modified from Ciocchi et al., 2010.*

Generalization of conditioned fear responses can be desirable if expressed under the appropriate circumstances. Understanding these processes is critical not only for the identification of neuronal processes that specifically relate to associative fear learning, but also for gaining insights into the transition of emotional states from normal fear to pathological anxiety exhibited in affective disorders, PTSD or generalized anxiety. These disorders may be viewed as instances of overgeneralization. However, we know little about the neural mechanisms mediating appropriate generalization during fear learning.

$PKC\delta^+$ neurons not only gate acute fear responses through a dis-inhibitory mechanism, but also show increased spontaneous activity in animals exhibiting fear generalization. Fear generalization is often associated with

states of anxiety and has been described in PTSD patients. The neuronal mechanisms underlying such maladaptive behavioral changes are, however, poorly understood. We recently found that acute stress regulates the excitability of PKCδ^+ neurons through α5GABA$_A$ receptor-mediated extrasynaptic inhibition, thereby controlling anxiety behavior and fear generalization (Botta et al., 2015). Our findings demonstrate that the neuronal circuitries of fear and anxiety overlap at the level of defined populations of CEA neurons and indicate that persistent changes in cellular excitability within CEA circuitry underlies concerted changes in conditioned fear and unconditioned anxiety.

Our work over the past years mainly focused on amygdala circuit plasticity underlying learning and expression of conditioned freezing, one particular behavior that can be part of a conditioned fear response. In a recent project, we have addressed how the amygdala taps into downstream circuits in the midbrain, which drive specific behavioral aspects of conditioned fear including passive and active defensive behaviors (Tovote et al., 2016). Understanding the neuronal circuitry underlying the selection and regulation of distinct behavioral coping strategies will provide new insight into evolutionary conserved survival mechanisms. An imbalance between active and passive coping strategies is observed in highly prevalent psychiatric conditions.

The midbrain periaqueductal grey (PAG) has been implicated in generating active and passive defensive behaviors evoked by threatening situations, but the identity and function of forebrain inputs to the PAG, local microcircuits and outputs underlying specific defensive behaviors is not known. We used circuit-based optogenetic, in vivo and in vitro recording, and neuroanatomical tracing methods to characterize PAG circuits for specific defensive behaviors (Tovote et al., 2016). We identified an inhibitory pathway from the central nucleus of the amygdala (CEA) to the PAG that produces freezing by disinhibition of ventrolateral PAG excitatory outputs to pre-motor targets in the magnocellular nucleus of the medulla. In addition, we found that this freezing pathway functionally interacts with long-range and local circuits mediating flight. Our findings define the neuronal circuitry underlying important components of a conditioned fear response and indicate that PAG circuitry plays a key role in the selection and execution of appropriate behavioral defensive programs.

Mechanisms of fear extinction

While tremendous progress has been made in identifying the mechanisms underlying fear learning, much less is known about inhibition of conditioned fear, although this question is attracting increasing interest because of its clinical importance. Inhibition of conditioned fear can be obtained if the conditioned stimulus is repeatedly presented alone, a phenomenon called fear extinction. Behavioral studies in animals demonstrate that fear extinction is not simply the forgetting of previously learned fear, but rather an active learning process (Myers and Davis, 2006). Fear extinction requires exposure to the CS in the absence of the US as opposed to the simple forgetting of CS-induced fear behavior over time. Moreover, fear extinction is generally not permanent, that is the original CS-evoked fear behavior can spontaneously recover over time, or can be recovered by exposing animals to a novel context, or to simple US presentations (Myers and Davis, 2006).

The amygdala is thought to play a central role for the acquisition of fear extinction. Studies using the fear-potentiated startle paradigm demonstrate that local infusion of an NMDA receptor antagonist or a blocker of the ERK/MAPK pathway prevents extinction (Falls et al., 1992; Lu et al., 2003). However, because using the fear-potentiated startle paradigm it is not possible to discriminate between acquisition and retention, the precise role of the amygdala during fear extinction remained unclear. Recently, we and others have used classical fear conditioning to show that intra-amygdala application of NMDA receptor antagonists and ERK/MAPK pathway blockers interfere with the acquisition of fear extinction (Herry et al., 2006). Single unit studies in the LA demonstrate that fear extinction decreases CS-evoked unit activity (Quirk et al., 1997) in a context-specific manner (Hobin et al., 2003). However, there might be different neuronal subpopulations, one of which appears resistant to extinction training (Repa et al., 2001). Together, these experiments establish a strong case for synaptic plasticity in the amygdala during the initial phase of fear extinction.

The formation of a context-specific long-term memory for fear extinction is believed to involve a more distributed neuronal network comprising the medial prefrontal cortex (mPFC)(Quirk et al., 2006) and the hip-

pocampus (Bouton et al., 2006), both of which are strongly interconnected with the amygdala (Ottersen, 1982; Pitkanen et al., 2000). Although the neuronal circuitry underlying amygdala-mPFC and amygdala-hippocampus interactions are still poorly understood, it has been proposed that the role of the mPFC and the hippocampus in fear extinction is ultimately mediated by controlling amygdala activity (Quirk et al., 2006). In analogy to animal experiments, functional imaging studies in human are consistent with the concept that fear extinction is mediated by an amygdala-hippocampus-mPFC network (Phelps et al., 2004; Kalisch et al., 2006). Moreover, pathological amygdala hypersensitivity in patients with anxiety disorders has been repeatedly associated with poor prefrontal control (Phelps and LeDoux, 2005).

Recently, we identified two functionally distinct classes of neurons in the basal nucleus of the amygdala (BA), so-called fear and extinction cells (Herry et al., 2008). While fear neurons were not responsive to tone presentations in unconditioned animals, they showed increased firing rates when the tone was presented during and after fear conditioning (**Fig. 4**). Repeated tone presentations in turn, caused a loss of CS evoked firing. Contrary to fear neurons, extinction neurons became tone responsive only during extinction learning, when the CS was presented repeatedly without being paired with a foot shock (**Fig. 4**).

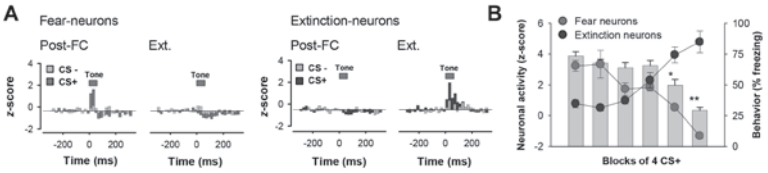

Fig. 4. Fear neurons and extinction neurons in basal amygdala. A: *Fear-neurons exhibited selective CS$^+$-responses after fear conditioning, which were fully reversed upon extinction. In contrast, CS$^+$-evoked firing of extinction-neurons was selectively increased after extinction.* ***B:*** *Averaged time courses of freezing behavior (grey bars) and neuronal activity (z-scores) of BA fear-neurons (red circles) and extinction neurons (blue circles) during extinction training. Modified from Herry et al., 2008.*

Fear and extinction cells are largely overlapping with projection neurons targeting the prelimbic (PL) or infralimbic (IL) subdivision of the medial prefrontal cortex, respectively. Based on our previous findings that the

BA contains functionally distinct neurons selectively activated by either fear conditioned or extinguished CSs (Herry et al., 2008), we recently used a combined anatomical and optogenetic approach to test the hypothesis whether BA fear and extinction neurons might differ with regard to their long-range projection targets (Senn et al., submitted). Using the immediate early gene cFOS as an activity marker, we found that BA neurons projecting to the PL were preferentially activated when the animal was in a state of high fear, whereas BA neurons projecting to the IL were active when animals exhibited low fear levels (i.e. after extinction; Fig. 4). Consistent with this finding, when recording from optogenetically identified PL- or IL-projecting BA neurons, we were able to show that this was also the case in awake behaving animals, and that optogenetic manipulations of the BA→PL and BA→IL pathways had opposite behavioral effects on the formation of long-term extinction memories (Fig. 4). Together, these findings indicate that extinction learning is driven by the switch of activity between two distinct neuronal pathways from the BA to separate subdivisions of the mPFC.

One possible mechanism that could underlie this switch in the balance of activity between distinct output pathways may involve local inhibitory circuits in the BA. We recently found that retrograde endocannabinoid signaling and CB1R-mediated regulation of inhibitory synaptic transmission of CCK-expressing interneurons onto BA PNs strongly depend on PN projection target (Vogel et al., 2016). It is conceivable that such cell-type specific short-term synaptic plasticity may transform uniform recruitment of CCK-lNs into asymmetric inhibitory input onto PL and IL projection-specific subpopulations of PNs during fear extinction. More generally, projection-specific shifts in the balance between inhibition and disinhibition may enhance contrast in activity between distinct amygdala output pathways thereby inducing rapid behavioral adaptations.

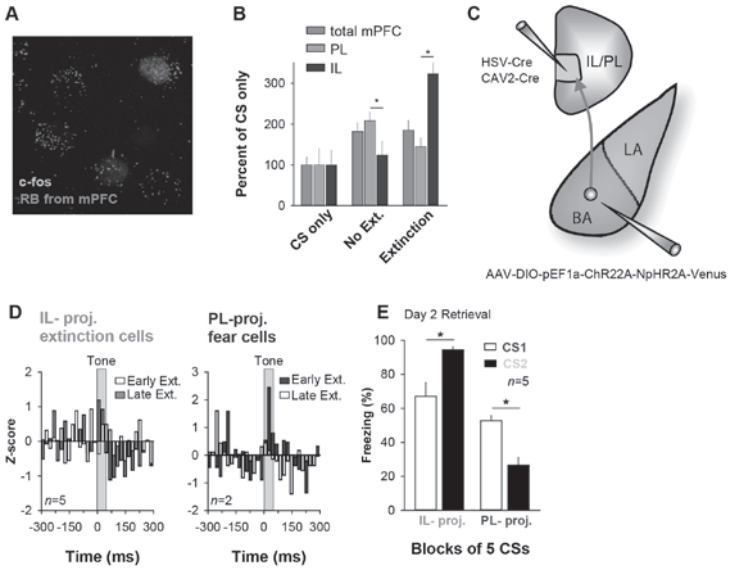

Fig. 5. Fear and extinction neurons target PL and IL subdivisions of mPFC. A: *Immediate early gene expression in anatomically defined neuronal subpopulations. Combining retrograde labeling (red fluorescent beads) with Fos immunostaining (green) reveals Fos expression in subsets of mPFC-projecting neurons.* ***B:*** *Neurons projecting to the infralimbic division (IL) of the mPFC exhibit extinction-specific changes in Fos expression, whereas neurons projecting to the prelimbic division (PL) show increased Fos expression 2 hrs after fear conditioning or upon CS exposure 2 days after consolidation.* ***D:*** *Intersectional viral strategy targeting specific subpopulations of BA output neurons.* ***D:*** *Optogenetically identified IL- or PL-projecting neurons specifically respond to extinguished or fear conditioned CSs.* ***E:*** *Optogenetic manipulation of IL-projecting or PL-projecting BA neuron activity oppositely affects long-term extinction memory acquisition.*

Conclusions and outlook

Together with research from other laboratories, our work over the past years revealed that functionally, anatomically and genetically defined types of amygdala neurons are precisely connected both within local and within larger-scale neuronal networks, and that they selectively contribute to specific aspects of fear learning and extinction. For instance, the activity of distinct sets of amygdala projection neurons can mediate rap-

id, switch-like changes in fear behavior. Moreover, we have identified specific subpopulations of inhibitory interneurons that gate and regulate circuit plasticity and behavior. Finally, we have started to address how the amygdala taps into downstream midbrain and brain stem circuits, which drive specific behavioral aspects of conditioned fear responses, including passive and active coping strategies.

During the last decade, systems neuroscience has witnessed a revolution in terms of novel technologies allowing for imaging and manipulating the activity of defined neuronal circuits *in vivo.* The advent of two-photon microscopy along with optogenetics and molecular genetic tools has allowed to address questions and causalities at a hitherto unprecedented level. These developments have had great impact also on our understanding of the neuronal circuit mechanisms underlying pathological fear and anxiety. Novel deep brain imaging approaches (Gosh et al., 2011) will even further accelerate progress in this field. One of the key challenges will be to integrate across different levels of analysis, from genetics and molecular mechanisms to cellular and synaptic physiology up to circuit function and behavior. Ultimately, this approach will enable us to selectively target and treat pathological circuit states underlying psychiatric conditions caused by maladaptive plasticity within fear and anxiety circuits.

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