Review

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New pharmacological strategies for augmenting extinction learning in anxiety disorders

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Abstract: Despite advances in the treatment of fear-, anxiety- and trauma-related disorders, a considerable proportion of patients shows only partial long-term therapeutic benefit with existing treatments. A promising option in improving therapy is speeding up and boosting the effect of exposure-based therapy (EBT) by pharmacological interventions. Here, we will discuss select examples of novel concepts in augmenting fear extinction, the central mechanisms of EBT. Based on accumulating knowledge from animal and human studies concerning the neurocircuitries and neurobiological mechanisms underlying successful fear extinction, diverse potential pharmacological targets have been identified to optimize the efficacy of fear extinction. We focus here on selected examples of these targets and present translational evidence for strengthening fear inhibitory learning by using L-DOPA and D-cycloserine. Furthermore, the potential of HDAC inhibitors and microRNAs (e. g. miR-144) as epigenetic targets, as well as neuropeptide S as a model substance with combined acute anxiolytic and extinction-facilitating properties are discussed. The presented mechanisms represent promising novel strategies that may be useful in the future for augmenting the efficacy and improving the acceptance of EBT in the treatment of anxiety disorders, although further work remains to be done in characterising the underlying modes of action and safety aspects.

Keywords: fear extinction; cognitive enhancer; L-DOPA; Neuropeptide S; D-cycloserine.

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Introduction

The lifetime prevalence of fear-, anxiety- or trauma-related disorders is 20–30%, constituting about 62 million people in Europe, who are diagnosed with one of these disorders (Wittchen et al., 2011; Bandelow and Michaelis, 2015). Characteristics of these disorders are the perception and occurrence of disproportionate fear and anxiety states, avoidance behaviour and stress reactions. These are often caused by certain key stimuli such as objects, situations and other extrinsic, but also intrinsic stimuli. Acquired behaviour patterns, like for example in the course of conditioning, also often play a role; the involved associations of conditioned stimuli (CS) and unconditioned stimuli (US) (see excursus 1) often occur subconsciously. The disease often starts early in life, making patients sometimes suffer for decades from their anxiety disorder. Many of those disorders are linked to a relatively high risk of relapse and various comorbidities like depression and addiction. Apart from their reduced quality of life and the burden of suffering both by the patients and their social environment, the economic costs for medical treatment, follow-up costs, in particular indirect costs for sick leave, early retirement, reduced productivity at work etc. are immense. However, the treated patients' labour participation and contribution to gross domestic product are about four times as much as costs for therapy (Chisholm et al., 2016).

Currently established therapies of anxiety disorders include and combine psychotherapeutic and pharmacological interventions. Therapies aim to attenuate the severity of symptoms and inhibit the acquired fear (see excursus 1). Ideally, patients are thereby being closely supervised by psychiatrists and psychotherapists. Medical long-term treatment mainly consists of antidepressants like selective serotonin- (SSRIs) and/or noradrenaline-reuptake inhibitors. These are capable of alleviating symptoms of fear and of adjusting impetus and motivation. During the acute phase of anxiety, anxiolytics like benzodiazepines (e. g. Lorazepam, Alprazolam) can temporarily be given in order to attenuate symptoms – long-term treatment is not recommended due to their addiction poten-

tial. Apart from medication there are different psychotherapeutic interventions, which are aimed at reinforcing, in anxiety patients often poorly established, fear inhibiting mechanisms as well as at learning to control fear symptoms more effectively. Exposure therapy is of particular importance for treatment by behaviour therapy. In such, the patient is repeatedly confronted in an *in vivo* setting or also in virtual reality, with stimuli and memories, which trigger fear or anxiety. The newly acquired learning experience, namely that the expected distress/unbearable situation does not occur or at least not in the expected dimension (expected contingency "prediction error") initiates new learning. The fear causing situation is re-assessed and ultimately anxiety symptoms and avoidance behaviour will be reduced (see excursus 1). Extinction as the central mechanism of exposure therapy involves active, new inhibitory associative learning while, especially during prolonged exposure, likewise non-associative habituation mechanisms are involved. The novel encoded memory traces for "CS-no US" (safety) suppress the behavioural characteristic of the still existing (fear)-memory traces. Implicit memory and, according to the extent of the therapist's cognitive instructions, also explicit memory is involved in this process.

Despite a high success rate, exposure therapy does not work for all patients. Up to 40% of the patients, who completed the therapy, do not show clinically relevant longterm treatment success (Bandelow and Michaelis, 2015). Even after completing an initially successful exposure therapy the relapse rate is relatively high, which can be attributed to the fact that the initial fear memory does not get erased by extinction. Thus, by certain stimuli/circumstances this initial fear memory trace can be reactivated and the fear reaction will be triggered again: i) by a prolonged period after completing the last exposure session (spontaneous recovery of the fear reaction), ii) by confrontation with the fear-triggering stimuli outside the "save" therapy environment (context-dependent renewal of the fear reaction) or iii) following confrontation with aversive stimuli like the original fear stimulus or general stress-laden events can revive the initial CS-US associations ("reinstatement"). Another potential drawback of therapy is the subjectively unbearable psychological burden of the exposure, which a considerable number of patients is anxious of ("fear of fear").

Various, in recent years newly established approaches are meant to improve the efficacy of exposure therapy and minimise therapy-related stress. The current focus of research is the significant facilitation of extinction learning during therapy sessions (extinction training) and the consolidation of extinction memory. Non-pharmacological optimisation approaches vary from adapting the "dosage" of exposure, i.e. modifying frequency, duration, intensity as well as varied and prolonged exposure sessions, restricting safety cues,... (Pittig et al., 2016) to exploiting consolidation mechanisms during sleep. It is still hoped that a combination of currently used anxioytic drugs will produce a synergistic (over-additive) therapeutic effect and at the same time will be able to attenuate the psychological stress of the exposure as such, however, this did not prove successful as yet (Hofmann et al., 2009; Otto et al., 2010). Thus, alternative pharmacological approaches aim at substances like neuroenhancers ("cognitive enhancers"), which help to initiate the new learning process and support formation of a fear-inhibiting extinction memory by theoretically modulating mechanisms like alertness, motivation, encoding but mainly by consolidating the extinction memory into a long term memory. This occurs via interaction of signal cascades in brain regions and circuits, which are important for extinction processes (excursus 2). Although experiencing fear during exposure might be an important element for the therapeutic learning effect, it is pursued to develop substances with acute anxiety reducing effects but without memory-impairing sedation. Currently no drug, approved for anxiety therapy, complies with those criteria [for review see (Singewald et al., 2015)].

We now present a selection of drugs and model substances, which have been developed based on growing animal and human data on extinction (Milad and Quirk, 2012), its underlying neuronal circuits and mechanisms (excursus 2). Apart from these selected examples, there are a number of other, here not further mentioned substances achieving similar effects via different pharmacological targets, such as glucocorticoids, yohimbine, neuropeptides like neuropeptide Y, opioids and oxytocin, growth factors like BDNF and fibroblast growth factor-2, cannabinoids, mGLUR7 receptors [for review see (Graham et al., 2011; Singewald et al., 2015)]. These drugs have been shown to promote mechanisms underlying the acquisition and/or consolidation (excursus 1) of extinction and some of them are already tested in clinical trials. In collaboration with others our research group has contributed to the development and/or further characterization of the following substances which will be discussed in more detail: L-DOPA and D-cycloserine (DCS) are examples of drugs approved for other indications and have been used in experimental studies in humans, while the other examples of substances discussed here are at very early stages of development. These are HDAC inhibitors and microRNAs as examples of epigenetic targets and neuropeptide S (NPS) as a rare example of an acute anxiolytic as well as

extinction-promoting model substance. Since a single administration of the substance occurs shortly before and/or after the exposure session, fewer side effects and better compliance of the patients are expected compared to chronic drug administration.

D-cycloserine: a translational success story

Based on animal studies, which revealed that activation of N-methyl-D-aspartate (NMDA) receptors is significantly involved in fear-extinction learning processes, the extinction facilitating effect of the NMDA partial agonist DCS was first reported in 2002 [for review see (Otto et al., 2016)]. DCS binds directly to glycine-sensitive NMDA-receptors in extinction-related brain areas like the amygdala (excursus 2) and promotes a fundamental molecular mechanism in memory formation, long-term potentiation (LTP). Other preclinical studies in different species confirmed the positive effect of DCS on augmentation of extinction learning and particularly consolidation of those memory contents [e. g. (Sartori et al., 2016); for review see (Singewald et al., 2015)]. Since DCS has been approved as a tuberculosis drug for many years, it took only two years until first positive results were published by K. Ressler and colleagues, reporting on a combination of DCS and exposure therapy in patients with fear of heights, which were followed by studies on patients with specific phobias, panic disorders, and posttraumatic disorders. Those first studies demonstrated that exposure therapy for certain anxiety disorders significantly benefits from DCS. This, however was followed by contradictory results so that several meta-analyses were unable to validate a clear result regarding the extinction augmenting effect of DCS [for review see (Otto et al., 2016)]. This could be due to the heterogeneity of the studied disorders and the participants' co-medications (e. g. SSRIs) as well as the fact of possible additional actions of DCS which have not received sufficient attention. Meanwhile it is known that during exposure sessions, in which no fear reduction takes place ("within session habituation"), augmentation of reconsolidation of the patient's fear memory is possible [for review see (Otto et al., 2016)]. Thus, the current recommendation is to administer DCS directly after "successful" exposure sessions in a controlled, stress-free environment. If DCS is given in a single to a few low doses in a restricted time period, it seems indeed able to increase the therapeutic effect of exposure sessions [for review see (Otto et al., 2016)]. Numerous currently registered studies involving different patient groups (see https://clinicaltrials.gov/) are supposed to clarify this further.

L-DOPA

The neurotransmitter dopamine belongs to the group of catecholamines and is abundant in the brain. Dopaminergic projections, which are potentially important for extinction processes, arise from the ventral tegmentum to the medial prefrontal cortex (mPFC), hippocampus and to the amygdala, which hold key functions in the processing of fear extinction (see excursus 2), as well as to the nucleus accumbens [for review see (Abraham et al., 2014)]. During and after a successful extinction session dopamine release in the mPFC is increased [for review and original literature see (Singewald et al., 2015)] and key markers of dopaminergic signaling pathways are upregulated in the amygdala (Whittle et al., 2016). In a translational study in collaboration with the groups of R. Kalisch and H.C. Pape we could show that administration of L-DOPA, a bioprecursor of dopamine, is able to augment extinction memory in extinction-competent mice and humans and importantly reduces relapse rates of fear reactions in both species (Haaker et al., 2013). Further, we were recently able to demonstrate an extinction-triggering effect of a single dose of L-DOPA in a clinically relevant mouse model of deficient extinction [(Whittle et al., 2016), see excursus 1)]. It remains to be shown whether these promising results can be transferred to clinical trials with anxiety patients. Since L-DOPA is already approved for the treatment of Morbus Parkinson, its single dose administration either before or after a limited number of exposure sessions is assumed to be safe, which expedites approval of clinical studies. This is substantiated by the previously described, improved and long-lasting effect of exposure therapy in a small group of therapy-resistant patients with post-traumatic stress disorder following a combination with MDMA (3,4-methylenedioxy-N-methylamphetamine; "ecstasy"), which activates dopaminergic (apart from serotonergic and other neurotransmitter) signaling pathways [(for review and original references see (Singewald et al., 2015)].

Mechanisms, by which L-DOPA improves extinction remain to be elucidated, but likely operate via improving consolidation of the new extinction learning process and modulating fear expression [for review see (Abraham et al., 2014; Singewald et al., 2015)]. Possibly promotion of dopaminergic signaling pathways also has an effect on motivation and contingency expectancy during the process of extinction learning [(Andre and Manahan-Vaughan, 2015;

Lissek et al., 2015); for review see (Abraham et al., 2014)]. Apart from potentially involved brain areas (see excursus 2) it remains to be shown which dopamine receptor(s) mediate the extinction augmenting effect of L-DOPA. Dopamine activates five metabotropic receptors, mainly coupled to either stimulating G_S or G_α proteins (D1-like receptors) or to an inhibiting $G_{i/0}$ protein (D2-like receptors), yet, also acting via other signaling pathways [for review see (Abraham et al., 2014)]. D1- and D2-like receptors control neuronal activity in the amygdala and in the mPFC in a finely tuned interaction (see excursus 2). In fact, activation of D1 receptors in the mPFC was shown to improve consolidation of fear extinction, possibly via interaction with excitatory NMDA receptors and LTP-dependent mechanisms [for review see (Abraham et al., 2014)]. However, due to the moderate selectivity of available ligands, a D5-mediated component cannot completely be ruled out [for review see (Abraham et al., 2014; Singewald et al., 2015)]. Avoiding the selectivity issue by using a conventional genetically modified mouse line, latest data suggest that D1-receptors are less involved in certain forms of association learning than assumed so far (Abraham et al., 2016). Thus, it seems important to consider the role of other dopamine receptors in fear extinction, especially because dopamine binds with a higher affinity to those than to D1-receptors.

Neuropeptide S

Another therapeutic problem worth improving is that about 30% of patients refuse participating in exposure therapy due to their anticipated (enormous) fear-/stress reaction during confrontation with the fear-triggering stimulus. Administration of acute anxiety-reducing substances like benzodiazepines prior to exposure therapy might have a beneficial effect in this respect. However, while benzodiazepines attenuate the arising anxiety, and therefore often allowed approaching exposure, they interfere with extinction processes e. g. via their sedative effect and can evoke an "interoceptive state", which also triggers state-dependent learning. On the whole, this rather diminished the efficiency of exposure therapy [for review see (Otto et al., 2010)]. Similarly, due to their absent anxiolytic effect, psychostimulants like amphetamines, which raise vigilance and attention, are not suitable for this purpose. Hence, there is a need for substances, which hold both anxiety-reducing and learning process-promoting properties.

Some studies suggest that the 20 amino acids NPS might fulfil these requirements of a model substance. Expression of NPS in the brain is limited compared to other neuromodulators, however, the NPS receptor is expressed in rodent brain areas, which are relevant for extinction (see excursus 2). Animal studies revealed the specific pharmacological effects of NPS: anxiolytic and actiivty promoting and on top of that extinction memory augmenting [(Jungling et al., 2008; Slattery et al., 2015), for review see (Reinscheid et al., 2005)]. Moreover, NPS has been shown to elicit a very early anxiolytic/fear inhibiting effect and to initiate extinction learning in severely extinction-deficient rodents (Sartori et al., 2016). In the rodent brain the extinction augmenting, fear inhibiting effects of NPS likely result in attenuated activity of fear-output neurons in the centromedial amygdala, supposedly by stimulating highly specific inhibitory interneurons, the so-called intercalated cells [ITCs, see excursus 2; for review see (Pape and Wotjak, 2013)]. In addition, NPS seems capable of modulating other extinction-relevant neurotransmitter systems, including dopamine in the mPFC and hippocampus. At the moment it is not clear whether and how these rodent data can be translated into humans in particular due to lacking information on the exact distribution of the NPS system in the human brain. Even though polymorphisms in the NPS receptor, which alter its function, have been associated with panic disorders (Domschke et al., 2011), reported species-specific differences should be considered in its further development as potential pharmacological target (Adori et al., 2015).

Despite promising pre-clinical results, concomitant therapy with NPS and exposure therapy is unlikely in the near future, mainly because there is no blood brain barrier-penetrant NPS receptor agonist or biased agonist with a potentially even improved side effect profile (Clark et al., 2017) at present. While efforts are high to develop such non-peptide NPS receptor agonists, administration of NPS via the nasal mucosa might be a non-invasive alternative, which is already utilised for other neuropeptides like e. g. oxytocin. This approach is supported by preliminary data from mice which show that only 30 minutes after intranasal administration low concentrations of NPS can be found in the brain, where it binds to NPS receptors and mediates an anxiolytic effect (Ionescu et al., 2012; Lukas and Neumann, 2012).

Epigenetic mechanisms

Formation of extinction memories involves interaction of focused signal transduction, gene expression and translation of certain proteins, which are important for learning

and memory-associated mechanisms including synaptic plasticity. Gene activity and gene expression is regulated via various epigenetic mechanisms. During extinction learning, regulatory mechanisms, e. g. via microRNAs miR128b (Lin et al., 2011) or miR144 [(Murphy et al., 2017); for review see (Singewald et al., 2015)] and also modifications of DNA and DNA-associated proteins, the histones, play a role. It seems that the degree of histone acetylation is of particular importance for extinction processes. Histone acetyltransferases transfer acetyl residues to the amino acids lysine at the N-terminal end of histone proteins and promote gene expression by the thereby associated chromatin disaggregation. Histone deacetylases (HDAC) on the other hand remove acetyl groups from lysine and thus inhibit gene transcription. Fine tuning of acetylation and deacetylation of histones during and after extinction training determines whether and to what extent gene expression and downstream processes, crucial for development of a steady extinction memory, occur. Although at present our knowledge of involved substrates is still limited, it seems that increased acetylation in the promotor region of the growth factor BDNF (brain-derived neurotrophic factor) as well as certain NMDA receptor subunits (e. g. grin2b), neuroplasticity-associated immediate early genes including c-fos and arc and, as mentioned above, dopaminergic genes (Whittle et al., 2016) play a crucial role in consolidation of extinction [for review see (Singewald et al., 2015)].

Histone acetylation can be pharmacologically increased by HDAC-inhibitors. Administration of various HDAC inhibitors, concomitant to extinction, was studied in animal models. Trichostatin A, sodium butyrate, Entinostat (MS-275), Vorinostat (SAHA), valproate (VPA) and Cl-944 all exhibit fear extinction-promoting properties [for review see (Singewald et al., 2015)]. Cl-944, MS-275 and SAHA were able to compensate extinction deficits in various animal models, which suggests clinical potential of those substances. For example, it was shown that MS-275 improved fear extinction in extinction-deficient mice, which was accompanied by increased histone H4 acetylation in extinction-related brain regions (Whittle et al., 2016). To date, tested substances are unspecific HDAC inhibitors, which inhibit several HDAC isoforms with a preference for those of class 1 including HDAC1, HDAC2, HDAC3 and HDAC8. Studies in genetically modified mice revealed that gene silencing of HDAC2 but not HDAC1 in the forebrain has a beneficial effect on fear extinction [for original literature and review see (Singewald et al., 2015)]. Specific HDAC inhibitors are currently under development in order to reduce potential side effects and also to increase efficacy.

Concept of dual pharmacotherapy concomitant to exposure therapy

In extinction-deficient individuals including anxiety patients (see excursus 1), administration of a single drug concomitant to fear extinction does often not suffice to support the extinction memory augmenting mechanism in an extent that temporal, spatial or stress-dependent fear relapses can be prevented [for review see (Singewald et al., 2015)]. Moreover, at present, no drug, which is able to pass the blood brain barrier, has combined memory promoting, acute anxiolytic, as well as non-sedating properties. Using extinction-deficient mice, our group was able to show for the first time that only the administration of NPS before and DCS after successful extinction training, but not administration of NPS alone results in formation of a robust extinction memory, which withstands various types of fear relapses (Sartori et al., 2016). According to this dual pharmacotherapeutic concept it was shown that fear relapses in extinction-deficient mice can also be reduced by combined administration of L-DOPA and the HDAC-inhibitor MS-275, concomitant to extinction training (Whittle et al., 2016).

Summary and outlook

In this article we review a selection of potential pharmacological strategies for strengthening extinction processes in the course of exposure sessions. These strategies aim at improved therapeutic success in regard to efficacy, clinical long-term success and acceptance of exposure therapy. Based on the advanced knowledge on extinction-related circuits and biological processes gained from animal and human work [see excursus 2; for review see (Milad and Quirk, 2012)], a rational administration of certain substances has been tested in validated animal models as well as even in volunteers and suitable patient groups (see excursus 3). Apart from targeting glutamatergic and dopaminergic signalling with DCS and L-DOPA, respectively, the results allowed to evolve novel (e. g. NPS), and a number of other pharmacological target candidates, which were not discussed in detail in this article (e. g. glucocorticoids, yohimbine, neuropeptides like neuropeptide Y, opioids and oxytocin, growth factors like BDNF and fibroblast growth factor-2, cannabinoids, mGLUR7 receptors), but also non-pharmacological strategies like deep brain stimulation in the nucleus accumbens [for review see (Graham et al., 2011; Singewald et al., 2015)].

In recent years there was increased awareness of the systematic administration of such potentially memory promoting substances tightly timed with extinction- and exposure training. While many of the mentioned substances are at a very early stage of investigation, the development of DCS is exemplary for successful, translational research from bench to bedside, which hopefully will be reproduced by more substances like e. g. L-DOPA in the near future (excursus 1–3). Based on animal and clinical research fear and fear extinction/exposure-based therapy supported by pharmacotherapy was further optimised in order to avoid off-target effects like the potential facilitation of reconsolidation of the initial fear memory traces. The concept of a dual pharmacotherapeutic approach opens up new possibilities to combine anxiety reducing and extinction memory facilitating substances. This might also be a potential way of reducing the patients' psychological stress before and during exposure therapy.

There are current studies on the molecular mechanisms, as well as the interplay of certain brain areas and circuits, which underlie extinction consolidation involving neuroenhancers, epigenetic modulators and other learning and memory modulating approaches. Studies in rodents need to be confirmed [see e. g. (Milad and Quirk, 2012)] and, if necessary, adapted in humans. Results, which have been and will be obtained, should contribute to identify effective pharmacological targets in order to strengthen psychotherapies and thus to provide optimised and permanent therapy of trauma- and anxiety-related disorders.

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Excursus 1: Modulation of extinction as an experimental approach of exposure therapy

Extinction of conditioned fear as the central mechanism of exposure therapy is based on various theoretical approaches, which have been significantly advanced by new data from the last decades. Apart from the experimental approach (the actual extinction training) and reduction of the conditioned reaction as such, extinction also describes a neuronal learning process, which forms the base of active re-learning and formation of a new inhibitory memory. **A.** Fear learning occurs via (Pavlovian) conditioning and represents a learning model important in many anxiety disorders. Thereby, a previously neutral, conditioned stimulus (CS; e. g. creaky car tyres in the traffic, in experimental setups e. g. sound, light or a computer image) is associated with aversive unconditioned stimuli [US; e. g. car accident, in experimental setups e. g. a mild electric shock to the hand, finger (humans) or foot/paw (animal)]. Circuits in the amygdala and other brain regions allow experience-dependent acquisition and storage of fear memory traces. Via activation of these memory traces the CS ultimately triggers an anticipatory fear reaction independent from the US. In patients suffering from anxiety disorders, this reaction is disproportional, which is partly based on dysregulated corticolimbic circuits of the fear system in the brain

During extinction training the CS is repeatedly presented without US in a usually different context. A new, inhibitory association "CS-no US" ("acquisition of extinction memory") is formed and consolidated. This now exists in parallel to the initial excitatory "CS-US" association and has an inhibitory effect. Fear symptoms are reduced and remain low in the long-term (see healthy/blue line) which can be used as a critical index for successful consolidation of the extinction acquisition. In patients with anxiety- or trauma-associated disorders these inhibitory learning mechanisms are often dysfunctional as relapse of the fear reaction (mildly deficient; yellow curve) occurs after initially successful extinction training or when even no initial extinction learning is present (severely deficient; red curve). **B.** Mechanisms of extinction learning can be pharmacologically augmented. In this context we introduce two pharmacological concepts: 1. Administration of *one* memory augmenting drug (neuroenhancer) for mildly deficient extinction with initial extinction learning, ideally following successful extinction training (see text). 2. Since the here presented selected examples DCS and HDAC-Is were not effective for severely deficient extinction when given before training (Hefner et al., 2008; Sartori et al., 2016), a combination of substances may be helpful with one given prior to the training, initiating extinction learning and allowing gradual fear reduction (extinction initiator), and a second given after the training, augmenting consolidation mechanisms (neuroenhancer). Abbreviations: DCS: D-cycloserine; HDAC-I: histone deacetylase inhibitor; NPS: neuropeptide S.

Excursus 1.

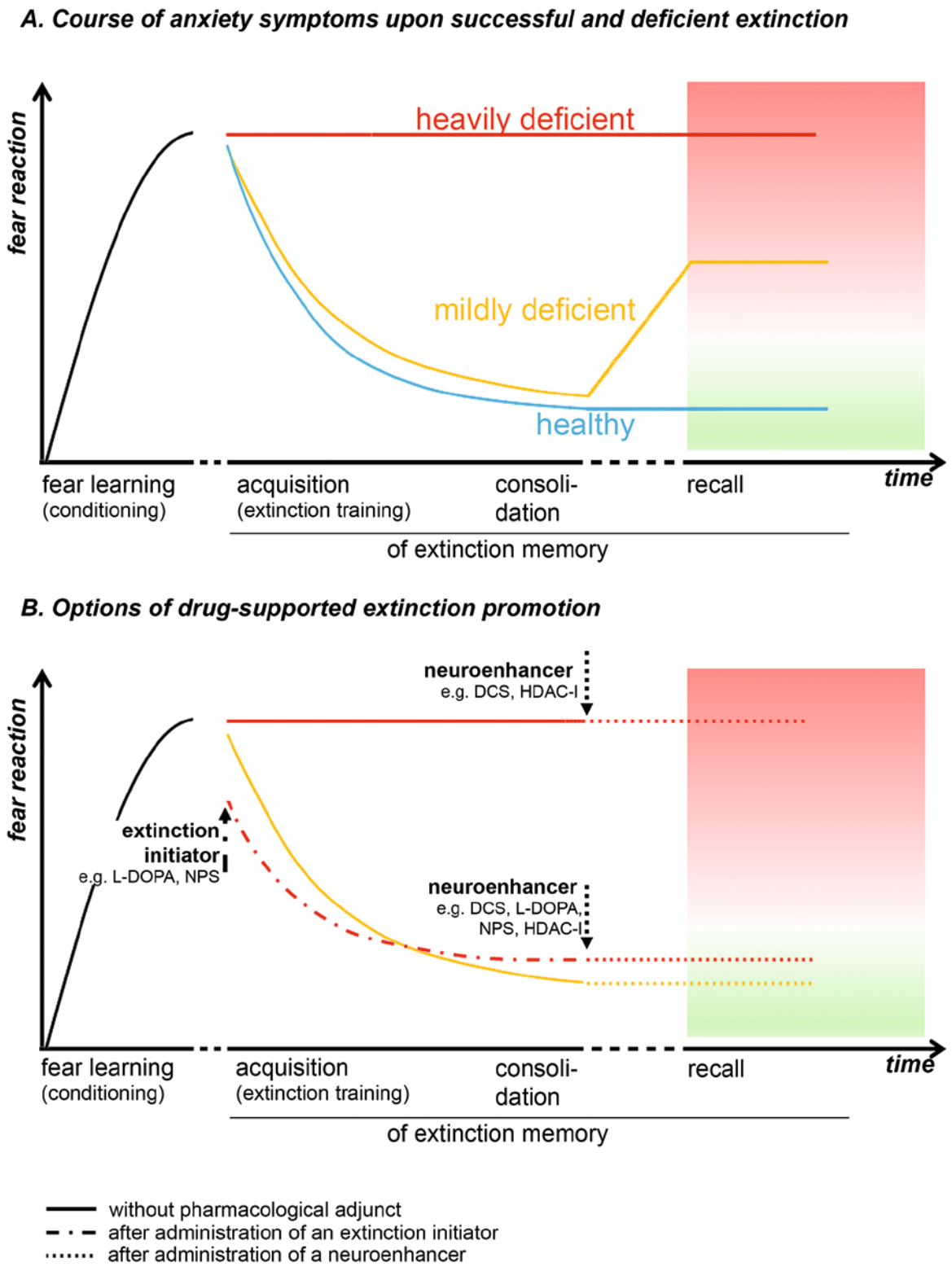

Fig. 1: Excursus 1: Modulation of extinction as an experimental approach of exposure therapy

Excursus 2: Central circuits of fear extinction and pharmacological targets for extinction augmentation

According to the current state of knowledge, a context-dependent, inhibitory "CS-no US" memory trace is primarily formed by neuronal circuits, which involve the amygdala, medial prefrontal cortex (mPFC) and hippocampus (HPC) representing important elements of fear circuits [for review see (Pape and Wotjak, 2013)]. Although homologies between the animal and human brain cannot always be clearly drawn, the amygdala function in emotional processing is highly conserved across species and similar activity changes in parts of the cortex and amygdala are observed in humans and rodents during fear extinction [for review see (Milad and Quirk, 2012)]. The ventrally localised infralimbic region (IL) of the mPFC (*ventromedial prefrontal cortex, vmPFC in human brain*) is important for retrieving and possibly forming extinction memory and can activate excitatory extinction neurons (green symbols) in the basal amygdala (BA). Extinction neurons activate highly specialised, intercalated inhibitory interneurons, the so-called intercalated cells (ITCs), and/or inhibitory OFF-neurons in the central lateral amygdala (CeL). Both types of neurons project to the important output station of fear circuits, the central medial amygdala (CeM), contributing to the inhibition of fear symptoms. Extinction also occurs context-dependent. The HPC is linked with the IL as well as the BA and is crucial for encoding the context of fear learning and extinction. A dysregulation of extinction-related circuits has been observed in anxiety disorders (e. g. hypoactivity in the IL/vmPFC,), which leads to disinhibition of microcircuits in the amygdala. These are fairly well characterised in the rodent, but not in the human brain mainly due to limited resolution of current imaging techniques. Furthermore, the prelimbic part of the mPFC (PrL; *dorsal anterior cingulate cortex, dACC in human brain*), is thought to contribute to excessive neuronal activity in the amygdala, e. g. in its output region CeM, as shown in rodents [for review see (Tovote et al., 2015)].

Based primarily on animal studies and a few emerging human studies (see text), D-cycloserine (DCS), L-DOPA (dopamine), neuropeptide S (NPS), HDAC-inhibitors (HDAC-I) and microRNAs (like miR-128 and miR-144) seem to augment extinction-related mechanisms at various levels of those circuits [yellow boxes); for review see also (Singewald et al., 2015)], reduce fear symptoms and in the case of NPS exert anxiolytic effects [purple boxes; (Jungling et al., 2008; Dine et al., 2013)], respectively. Neurobiological mechanisms, which contribute to maintain the effects of extinction as a long-term memory, are partly decoded. For example, it has been revealed that protein synthesis is mediated via the transcription factor CREB in the amygdala and in the IL of the mPFC. CREB activity is regulated by various neurotransmitters including noradrenaline and dopamine [for details see review (Singewald et al., 2015)].

Excursus 2.

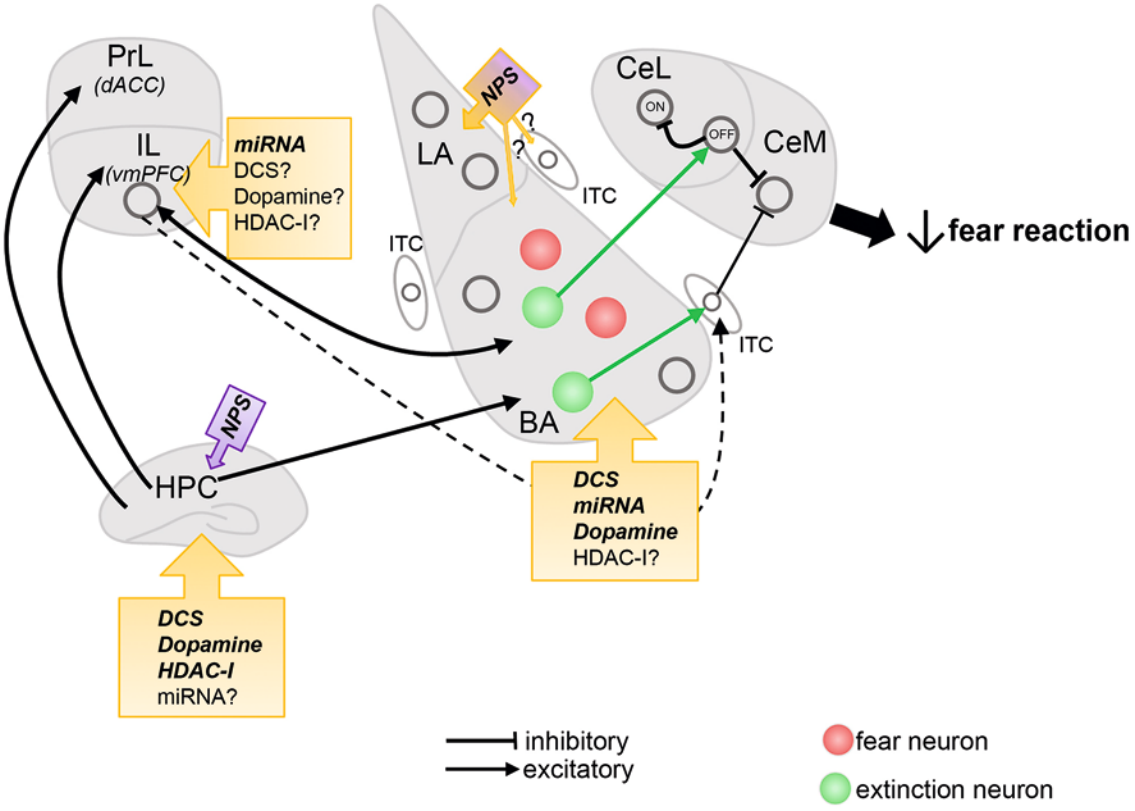

Fig. 2: Excursus 2: Central circuits of fear extinction and pharmacological targets for extinction augmentation

Excursus 3: Translational research for drug-supported augmentation of exposure therapies

The ideal development of concepts for improving therapy of anxiety disorders is illustrated schematically. There is a need for rational, multi-species basic and translational research in order to augment fear extinction learning as the central mechanisms of exposure-based therapy. The focus of this research is development of clinically approved drugs to maximise efficacy and acceptance of exposure therapy.

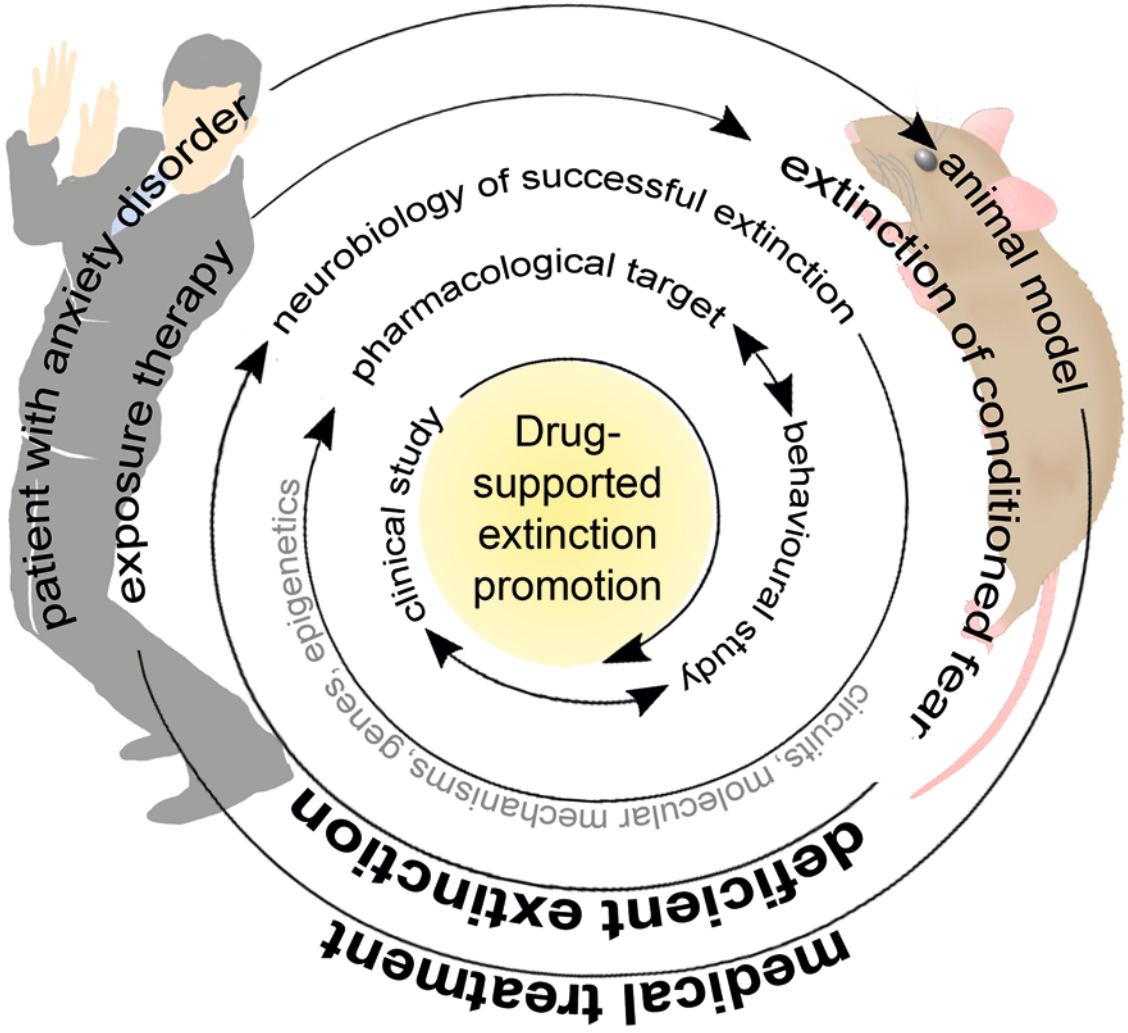

Fig. 3: Excursus 3: Translational research for drug-supported augmentation of exposure therapies

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