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# Avènement de la peur conditionnée comme modèle animal du trouble de stress post-traumatique

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Avènement de la peur conditionnée comme modèle animal  
du trouble de stress post-traumatique.

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## RESUME

### Contexte

Le modèle animal de peur conditionnée (PC) a été développé dans les années 1910-1920. Il est fréquemment employé comme modèle animal du trouble de stress post-traumatique (TSPT). Cependant, le TSPT a été intégré à la nosographie psychiatrique en 1980 (DSM-III), et le modèle de peur conditionnée présente des limites de validité. Comment la PC est-elle devenue un modèle animal dominant pour l'étude du TSPT? L'objectif de ce travail était de comprendre l'évolution des liens conceptuels entre peur conditionnée et TSPT de 1980, année de la reconnaissance du TSPT, à la période actuelle (2019).

### Méthode

Une méthodologie qualitative (revue de la littérature) et quantitative (fouille de textes) issue des domaines de l'histoire et de la philosophie des sciences.

### Résultats

La première convergence conceptuelle PC/TSPT a été théorique, dans les années 1980, basée sur une analogie conditionnement/psychotraumatisme et PC/TSPT. L'utilisation de la PC comme modèle animal de TSPT est intervenue 10 ans après la création du premier modèle explicite de TSPT (2003 et 1993, respectivement). La PC a ensuite rapidement évolué vers le statut de modèle dominant du TSPT, porté par les concepts de circuits neuronaux de la peur et de son « extinction ». Les liens conceptuels FC/TSPT ont résulté de l'interaction entre les théories de la mémoire par plasticité synaptique, de la PC, et de ses circuits neuronaux, dans un contexte d'un intérêt politique fort pour la recherche sur le TSPT. La rencontre entre domaines a elle-même résulté d'interactions entre cliniciens-scientifiques américains.

### Conclusion

La convergence de la géopolitique avec l'histoire des sciences et de la médecine par le biais de collaborations entre chercheurs a mené à l'avènement du modèle animal de TSPT par la PC. Les modèles de peur conditionnée ont pris des formes diverses. Le flou conceptuel entourant ces modèles, et leur assimilation à un modèle théorique unique, nécessitent d'être clarifiés.

# THE ADVENT OF FEAR CONDITIONING AS AN ANIMAL MODEL OF POST-TRAUMATIC STRESS-DISORDER

## **Background**

Fear conditioning (FC) is one of the dominant models of post-traumatic stress disorder (PTSD). While the first results of FC experiments were published in the 1920s, PTSD appeared as a diagnosis in 1980 (DSM-III). Models of PTSD based of FC have been criticized for lacking validity (e.g. being adaptive, not pathological). How did FC become a dominant model of PTSD? The aim of this thesis was to shed light on the way conceptual links between the constructs of PTSD and FC have emerged and evolved.

## **Methods**

Qualitative (reviews of the literature) and qualitative (text mining) analyses were carried out, using methods borrowed from academic fields of the history and philosophy of science.

## **Results**

The constructs of FC and PTSD were first linked by analogy (conditioning/trauma and conditioned fear/PTSD re-experience symptoms) shortly after the recognition of PTSD (1980s). Fear conditioning only started to be used as an animal model of PTSD in 2003, ten years after the creation of the first explicit model of PTSD. From there, FC has rapidly expanded to its current dominant position. Conceptual links mostly concern neural circuits of fear and extinction. We propose that the convergence of scientific theories of fear and extinction circuits, and synaptic plasticity, with PTSD care, within a political context of strong interest in PTSD, has stimulated research on FC to model PTSD. Convergence materialized with collaborations between clinician-scientists within centers of the Department of Veterans Affairs and medical academic centers, in the USA.

## **Conclusion**

Collision of medical, scientific and political history has resulted in FC becoming a dominant model of PTSD. Current uses of FC in modeling PTSD are heterogeneous, however, introducing conceptual vagueness and ambiguity. These issues should be clarified in the future.

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## Avant-propos

Les modèles animaux sont employés dans l'ensemble des domaines de la Médecine, afin d'éclairer la compréhension des maladies et de développer des thérapeutiques nouvelles. Cette démarche de médecine expérimentale est en outre appliquée aux pathologies psychiatriques. La question de la validité des modèles animaux, et par conséquent de leur utilité, est exacerbée en Psychiatrie expérimentale. De toute évidence, modéliser chez l'animal des pathologies liées à l'activité mentale est éminemment problématique. La mesure du comportement animal offre néanmoins une possibilité de lien entre l'animal et la clinique.

Le trouble de stress post-traumatique (TSPT) survient après l'exposition directe ou indirecte d'un individu à un événement violent, tel une menace physique de mort ou une agression sexuelle, constituant un traumatisme psychologique. Le TSPT associe de manière prolongée des symptômes intrusifs (ex : cauchemars, reviviscences), un évitement persistant des stimuli associés à l'événement, une altération négative des cognitions et de l'humeur (ex : culpabilité, tristesse), et une altération de la réactivité (ex : irritabilité, sursauts). La faible efficacité des traitements du TSPT, en particulier médicamenteux, encourage la recherche biologique visant à comprendre les déterminants physiopathologiques de ce trouble.

Le comportement animal de peur conditionnée est fréquemment invoqué comme modèle de choix pour étudier le TSPT. Durant le conditionnement, un animal est soumis de manière répétée à un stimulus neutre, couplé dans le temps à un stimulus aversif (ex : un choc électrique modéré). Lors de la présentation ultérieure du stimulus neutre, l'animal présente des comportements défensifs de peur, dit « conditionnés ». Ces derniers sont facilement identifiables et quantifiables et sont interprétés comme l'expression mnésique de l'association conditionnée stimulus aversif/stimulus neutre.

L'expression pérenne et résistante des symptômes du TSPT présente des similitudes avec la peur conditionnée chez l'animal. Toutefois, l'apprentissage associatif de la prédiction d'un événement aversif est adaptatif. Il pourrait avoir contribué à la survie des espèces animales capables d'exprimer une peur apprise. Le TSPT *a contrario*, représente un état de dérégulation pathologique des émotions. Le modèle de peur conditionnée est également utilisé pour modéliser d'autres pathologies, telles que la phobie spécifique ou le trouble obsessionnel-compulsif. La peur conditionnée est aussi employée largement pour étudier d'un point de vue neurobiologique la mémoire et/ou les émotions. De plus, le modèle de peur conditionnée a été développé pour ce dernier usage dans les années 1910-1920, plus d'un demi-siècle avant la reconnaissance du TSPT en 1980.

De quelle manière la peur conditionnée est-elle devenue un modèle animal dominant pour l'étude du TSPT malgré ces inconvénients et ce hiatus historique? En d'autres termes, l'objectif de ce travail de thèse était de comprendre l'évolution des liens conceptuels entre peur conditionnée et TSPT entre 1980, année de la reconnaissance du TSPT, et la période actuelle.

En appliquant une méthodologie qualitative et quantitative issue des domaines de l'histoire et de la philosophie des sciences, l'ambition de cette initiative était de fournir une clarification des concepts encadrant le domaine d'étude biologique du TSPT utilisant le modèle animal peur conditionnée, et des éléments de réflexion sur le contexte global de ces travaux.

La confrontation à ces problématiques lors de la pratique combinée de la recherche en Neurosciences et de l'exercice clinique en Psychiatrie est à l'origine de ce questionnement et de ce travail de thèse.

Le texte est présenté en langue anglaise, en préparation d'une soumission pour publication dans une revue internationale à comité de lecture. Un argumentaire complet est exposé dans le présent manuscrit afin de permettre une discussion basée sur l'ensemble des analyses réalisées. La préparation d'un article pour publication nécessitera de présenter ces travaux sous une forme synthétique.



## List of abbreviations

ACTH: adrenocorticotrophic hormone

BOLD: Blood-oxygen-level dependent (signal)

CS: conditioned (sensory) stimulus

DSM: Diagnostic and Statistical Manual of Mental disorders

EMDR: Eye Movement Desensitization and Reprocessing

FC: fear conditioning

HPA: hypothalamic-pituitary-adrenal (axis)

IMO: immobilization stress

IS: inescapable shock

MRI: magnetic resonance imaging; fMRI = functional MRI

NMDA-R: N-Methyl-D-Aspartate type glutamate receptor

PFC: prefrontal cortex

PTSD: post-traumatic stress disorder

RDoC: Research Domain Criteria

SPS : single prolonged stress (single episode of prolonged exposure to stressors)

TDS: time-dependent sensitization

UCR: unconditioned response

UCS: unconditioned (sensory) stimulus

# I- Introduction

## Post-traumatic stress disorder

### Clinical descriptions

Post-traumatic disorder (PTSD) manifests itself by various stress-related reactions (stress disorder), resulting in significant impairment, after intense psychological distress (post-trauma), including man-made disaster or accident (e.g. car accident), war, sexual assault, or natural disaster (e.g. earthquake). It was first defined in the third edition of the Diagnostic and Statistical Manual of Mental disorders (DSM-III; 1980; (1)). The fifth edition (DSM-5 (2)), the current reference in international psychiatry, defines PTSD it as follows:

- A. *Exposure to actual or threatened death, serious injury, or sexual violence* in one (or more) of the following ways: 1-directly experiencing the traumatic event(s), 2-witnessing, in person, the event(s) as it occurred to others, 3-learning that the traumatic event(s) occurred to a close family member or close friend, or experiencing repeated or extreme exposure to aversive details of the traumatic event(s) [related to profession].
- B. *Intrusion symptoms* (Presence of one (or more) of the following) associated with the traumatic event(s), beginning after the traumatic event(s) occurred: 1-recurrent, involuntary, and intrusive distressing memories of the traumatic event(s), 2-recurrent distressing dreams in which the content and/or affect of the dream are related to the traumatic event(s), 3-dissociative reactions (e.g., flashbacks) in which the individual feels or acts as if the traumatic event(s) were recurring, 4-intense or prolonged psychological distress at exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event(s), 5-marked physiological reactions to internal or external cues that symbolize or resemble an aspect of the traumatic event(s).
- C. *Persistent avoidance of stimuli associated with the traumatic event(s)*, beginning after the traumatic event(s) occurred, as evidenced by one or both of the following: 1-avoidance of or efforts to avoid distressing memories, thoughts, or feelings about or closely associated with the traumatic event(s) or, 2-avoidance of or efforts to avoid external reminders that arouse distressing memories, thoughts, or feelings about or closely associated with the traumatic event(s).
- D. *Negative alterations in cognitions and mood associated with the traumatic event(s)*, beginning or worsening after the traumatic event(s) occurred, as evidenced by two (or more) of the

following: 1-inability to remember an important aspect of the traumatic event(s), 2-persistent and exaggerated negative beliefs or expectations about oneself, others, or the world, 3-persistent, distorted cognitions about the cause or consequences of the traumatic event(s) that lead the individual to blame himself/herself or others, 4-persistent negative emotional state, 5-markedly diminished interest or participation in significant activities, 6-feelings of detachment or estrangement from others, 7-persistent inability to experience positive emotions.

- E. *Marked alterations in arousal and reactivity associated with the traumatic event(s)*, beginning or worsening after the traumatic event(s) occurred, as evidenced by two (or more) of the following: 1-irritable behavior and angry outbursts, typically expressed as verbal or physical aggression toward people or objects, 2-reckless or self-destructive behavior, 3-hypervigilance, 4-exaggerated startle response, 5-problems with concentration, 6-sleep disturbance.
- F. Duration of the disturbance (Criteria B, C, D and E) is more than 1 month. (NB: symptoms of duration 3 days to 1 month are classified as acute stress disorder, DSM-5(2)).
- G. The disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.
- H. The disturbance is not attributable to the physiological effects of a substance or another medical condition.

DSM-5 specifies PTSD: with dissociative symptoms and with delayed expression (if the full diagnostic criteria are not met until at least 6 months after the event).

Clinical entities comparable to PTSD have been recognized under various names following historical events: railway spine from the Industrial revolution, soldier's heart from the American Civil War, shell shock and war/traumatic neurosis from the First World War, combat fatigue and traumatic neurosis of war from the Second World War. Vietnam veterans and their advocates obtained the recognition of war-related psychological distress ("post-Vietnam syndrome"), and PTSD was integrated into the American psychiatric nomenclature as a formal diagnostic entity with publication of DSM-III in 1980 (3,4).

Most articles reviewed in the present work were published prior to the publication of DSM-5. Definitions of PTSD in DSM editions III through IV-R (DSM-III: 1980, DSM-III-R: 1987; DSM-IV: 1994; DSM-IV-R: 2000) recognized only three symptom clusters. These were classified starting from DSM-III-R as re-experience (criteria B- equivalent of intrusion symptoms), avoidance/numbing (C), and hyperarousal (D). The fifth edition of DSM-5 has divided group C symptoms into C and D, and moved PTSD from anxiety-disorders to trauma- and stressor-related disorder (4).

## Epidemiology and treatment

Lifetime prevalence has been estimated to 5-10% in the general population (5), a figure similar to military cohorts (1-10%; (6,7)). Introduction of DSM-5 criteria has not been accompanied by major changes in lifetime prevalence estimates (8.3% with DSM-5 vs. 9.8% with DSM-IV criteria, (8)). In this 2013 study, 12-month prevalence was 4.7% (8).

Although 50-90% of the general population experience traumatic experience (5,8), only about 10-30% go on to develop full-blown PTSD (5,9–11), reflecting a degree of variability among subjects and traumatic experiences. This variability is linked to trauma type (the most severe being rape and war, around 40% to develop PTSD; (5)) and intensity (11), and individual vulnerability/susceptibility (5).

The time course of PTSD is limited to temporary expression in the majority of cases, but can last beyond 6 months in 25-40% of PTSD cases (9,12). Moreover, PTSD manifestation can be delayed (see definition), in 10% of cases, as suggested by a study on military population .

International guidelines recommend psychological and pharmacological treatments for long-term management of PTSD. Recommended psychological treatments are primarily trauma-focused, including Eye Movement Desensitization and Reprocessing (EMDR), and exposure-based (extinction) therapy (13–16).

In EMDR, Patients attend to the traumatic memory while their attention is also engaged by a bilateral physical stimulation (eye movements, taps or tones). The patient is guided into a receptive state in order to reprocess traumatic memories to resolution.

In exposure-based therapy, patients are repeatedly submitted to traumatic memories in a safe setting, in order to reduce the level of distress induced by the memories. Exposure therapy can be done with imaginal exposure (or narrative writing) or *in vivo*. It is similar to fear extinction (see below).

Recommended medication are mainly long-term (> 12 months) treatments with antidepressants Selective Serotonin Reuptake Inhibitors (SSRIs) fluoxetine, paroxetine and sertraline, or serotonin-noradrenaline reuptake inhibitor venlafaxine (13,14,17,16,15). Association of medication with psychological treatment is not recommended for first-line use, but may be used for augmentation, e.g. paroxetine may enhance exposure-based therapy (17).

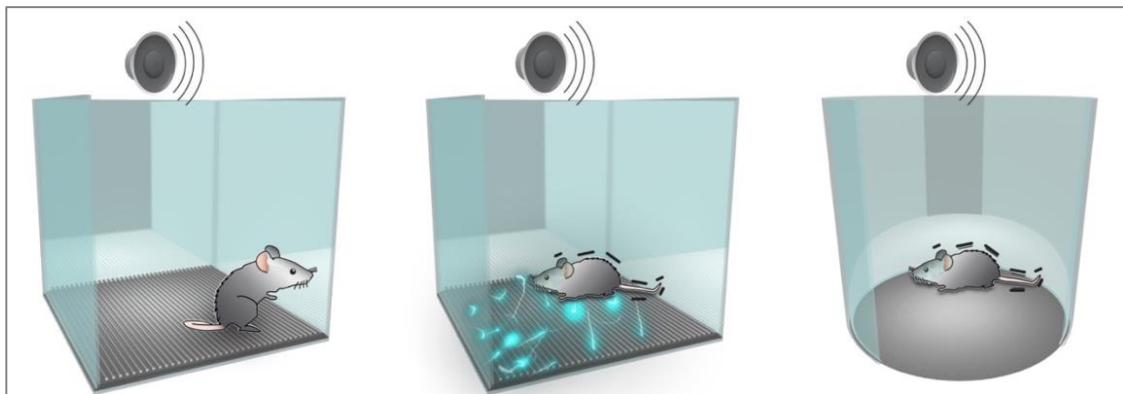
Standard of care is considered insufficiently efficacious. In particular, long-term treatment with antidepressants bring small beneficial effects (14). High prevalence and comorbidity rates (between 30-50% for depression or substance use disorders (5,18), and considerable impairment of daily functioning in PTSD set the need for better therapeutic interventions, stimulating research in the field of PTSD neurobiology.

## Neurobiological accounts of post-traumatic stress disorder

### The fear conditioning paradigm

The fear conditioning heuristic occupies a critical place in neurobiological accounts of PTSD. In classical fear conditioning, animals (typically rodents), or Human subjects, learn to assign a negative valence to an initially neutral (conditional) stimulus (e.g. a tone) after its repeated pairing with an aversive (unconditioned) stimulus (e.g. an electrical footshock; UCS), that evokes unconditioned defensive responses (UCR). Following this training, the conditioned stimulus (CS) is sufficient to elicit by itself a measurable defensive (“fear”) response, characterized by increased blood pressure and heart rate, potentiated startle, and freezing in rodents ((19,20); Figure 1).

The initial observation of conditioned fear responses is attributed to Pavlov after his experiments with dogs, hence the synonymous use of “pavlovian” for classical fear conditioning. Pavlov’s accounts of conditioning experiments were published in English language in 1927. Fear conditioning performed on an infant by pairing presentations of a rat with aversive loud sound (striking a hammer upon steel bar, the “little Albert experiment) was reported by Watson and Rayner in 1920 (21).



**Figure 1: Schematic representation of cued fear conditioning.** A mouse is habituated to the environment (square box) and the conditional stimulus, (a sound, loud speaker; Left). After habituation, electrical shocks are presented paired with the sound (Center). Later presentation of the conditioned sound in a novel environment (circular arena) elicits defensive behavior, here freezing (Right). SciLight©.

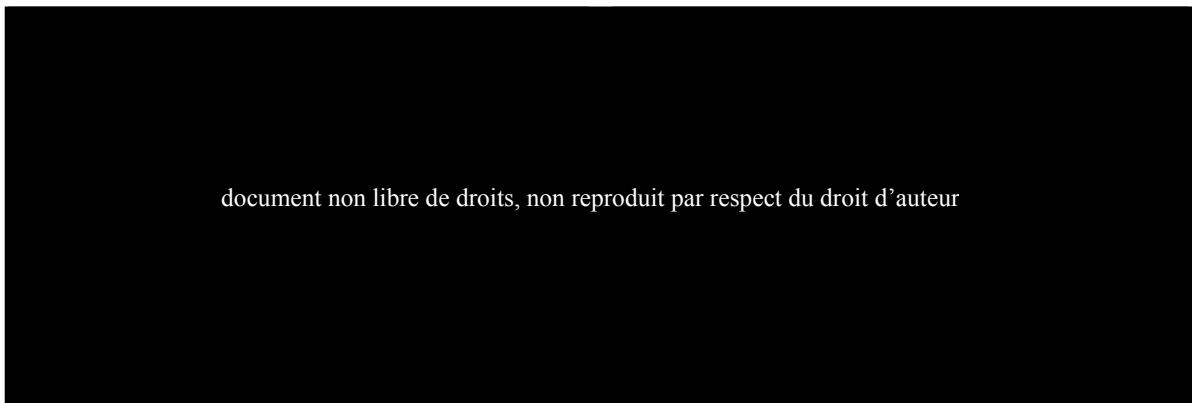
Conditioned fear responses have two variants, cued and contextual, depending on the nature of salient conditioned stimuli. Both can be tested in the same experiment by presenting either the cue or the context (22). More often, cued fear conditioning utilizes discrete CS (cues), tightly paired in time with UCS, so that conditioned responses to the cue is acquired (Figure 1). In contextual fear conditioning, UCS is given in the absence of discrete cue (or the temporal association between cue and shock is loose), so that fear of the environment (context) is learnt (e.g. Kaouane *et al.*, 2012; (23)).

After repeated presentations of conditioned stimuli in isolation, fear responses decrease in magnitude, a phenomenon referred to as “fear extinction”. Fear extinction is thought to represent an alternative form of learning rather than the erasure of the fear memory (24).

### Neurobiology of fear conditioning and extinction

Studies in rodents have shed light on some mechanisms of fear conditioning and extinction at the neurobiological level, from brain regions to molecular mechanisms (25). Because fear conditioning is readily implemented, standardized and therefore reproducible across space and time, it has become one of the leading learning models in modern neuroscience (20).

Fear conditioning and extinction implicate brain regions located in inner temporal lobe (amygdala and hippocampus), and the medial prefrontal cortex (mPFC). The main theory of fear conditioning neurobiology posits that the convergence of synaptic inputs to the basolateral complex of the amygdala from US and CS-mediating pathways (cued: thalamic; contextual: hippocampal) lead to the potentiation of the latter. As a result, subsequent activation of CS-mediating pathway alone is sufficient to provide suprathreshold activation of neurons in the basolateral amygdala, which in turn via the central nucleus of the amygdala activate brainstem effectors of the defense response (20) (Figure 2).



**Figure 2: Neurobiological theory of conditioned cued (Left) and contextual (Right) fear expression.** White disks represent the amygdala (nuclei= LA: lateral, B: basal, AB: accessory, CE: central). Modified from Ledoux, 2000 (20).

Prefrontal cortex and amygdala interact tightly during the various stages of fear and extinction learning and expression. It is thought from work in rodents that activation of the dorsal (prelimbic) mPFC sustains amygdala activity and fear expression, while activation of the ventral (infralimbic) part of mPFC is necessary for extinction learning and expression, dampening amygdalar activity (26).

Functional brain imaging studies performed along with fear conditioning and extinction in Human volunteers have revealed the implication of homologous structures (27).

## Clinical research

Clinical research on PTSD concerns mainly two biological systems. This section is based on two review articles, by Pitman and colleagues (28) and Ressler and colleagues (29), leading authors in the field.

- *The stress system*

Imbalanced stress systems have been found in PTSD that may recapitulate symptom of hyper-reactivity/hyperarousal. In PTSD, the negative feedback of cortisol on HPA axis is increased. This may account for normal to low basal levels of cortisol and has been proposed to play a pathogenic role in deficits of stress response termination. The sympathetic nervous system is hyperactive, spontaneously and with exaggerated evoked responses, leading to increased peripheral noradrenaline levels (28).

- *The fear system*

Structural and functional brain imaging in PTSD has supported the theory of a dysfunctional fear circuit. Findings implicate the amygdala, the hippocampus and the PFC, a network that is thought to underly fear conditioning and extinction learning and expression (this chapter). Structural imaging consistently found reduced hippocampal volume, which was proposed to explain impaired memories of the trauma (group D criteria, DSM-5).

Functional Resonance Magnetic Imaging (fMRI) evidenced in response to trauma reminders or fear conditioning in PTSD patients: 1) overactivation of the amygdala, 2) hyper-activity of the dorsal anterior cingulate cortex (equivalent to rodent prelimbic cortex), 3) decreased reactivity in ventromedial prefrontal cortex (equivalent to infralimbic cortex). These findings have been interpreted as an hyper-reactive fear circuit, with reduced inhibitory control of the PFC on the amygdala, relevant to symptom groups B and C (28,29).

The search for biomarkers of PTSD phenotype and vulnerability is also an intense subject of investigation, with the search for (epi)genetic correlates of the condition.

Current narratives of PTSD neurobiology (1) come from research in animal models and Human. In order to extract relevant knowledge from animal research, it is critical to analyze comprehensively the experimental protocols and the concepts they model.

## Modeling post-traumatic stress disorder

### Animal models in psychiatry

Animal models are used for different purposes in experimental psychiatry: to study the neurobiological substrates of psychiatric disorders, to test hypotheses regarding the natural course of a disorder or its risk factors, or to design potential therapeutic or diagnostic strategies (drugs, behavioral treatment, neuromodulatory devices, biology, neuroimaging), aiming ultimately at improving care. Indeed, they offer prospective and controllable conditions, a high level of reproducibility, access to invasive measurements of biological functions, and the ability to perform “preclinical” treatment trials faster.

Arguably, it is extremely challenging to model psychiatric conditions in animals. For instance, subjective dimensions, which might be the most important of all symptoms cannot be measured in animals, as their recognition relies on verbal reports. Despite significant limitations specific to mental disorders, experimental psychiatry has considerably developed during the last decades. It is widely thought that mental disorders stem in maladaptive functioning of the nervous system and that Human and non-human mammals share largely similar nervous systems. According to this biological view, animal models are useful to study mental disorders.

An animal model of a disease should ideally recapitulate all observable aspects of the clinical condition. A battery of criteria are employed to assess the usefulness (or “validity”) of animal models employed for scientific studies. Validity criteria are commonly clustered into three categories: construct validity, face validity and predictive validity (first proposed by Willner, 1984 (30)). It should be noted that validity criteria are polysemic. The three validity dimensions and the limitations of studying mental disorders in animals have been summarized in a seminal review by Nestler and Hyman (31). In this manuscript the definition given by Nestler and Hyman (31) are used as a reference. A refined classification has also been proposed that distinguishes criteria sub-categories (32) (Figure 3). This classification is helpful to clarify and/or refine conceptual boundaries between validity clusters and is therefore mentioned below in each relevant section.

*Construct validity* is used to assess the relevance of the way an animal model is *generated*. For a model to attain construct validity, it should recreate in an animal the factors that underlie the etiopathogenesis of the Human disorder. These factors comprise environmental and biological features, including genetics and epigenetics (31). Construct validity can be divided into homological and pathogenic validity (32).

- Homological (species and strain) validity refers to the choice of species and strain, with respect to vulnerability to developing abnormal phenotypes. For instance, the Lewis rat strain is more likely to display anxiety-like behavior than other common strains (33).



- Pathogenic validity contains ontopathogenic (or etiopathogenic) validity, experimental conditions that mimic environmental factors, and triggering validity, i.e. the factors that precipitate transition between individual susceptibility and diseased state. Ethological validity is in part implicitly contained in the concept of triggering validity. For instance, exposure to a cat can trigger anxiety-like symptoms in a mouse (see chapter 3).

*Face validity* indicates that a model recapitulates important measurable features of a human disease. This includes directly observable parameters such as behavior, but also biological variables that can be measured with a vast array of techniques.

According to Belzung and Lemoine, face validity encompasses ethological and mechanistic validity, that is, animal models should display behaviors that pertain to the human condition in the animal's behavioral repertoire and neurobiological or cognitive mechanisms should be similar in the model, respectively (32).

Face validity is probably the most widely accepted criterion, as resemblance between human and animal behavior can be easily conceived and readily measured in experiments.

*Predictive validity* signifies that the model organism displays biological changes (behavioral, biomarkers) to a treatment in a way that predicts the effects of this treatment in humans, according to the standard classification (31). Belzung and Lemoine used a logical definition and distinguished induction and remission validities. "The predictive validity of an animal model is the similarity of the relation between, on the one hand, the triggering factors and the occurrence of the disease and, on the other hand, between the therapeutic agent and the disease" (32). Induction validity is formally correct as a subtype of prediction validity but overlaps with triggering validity. It will not be used in this work. Remission validity is equivalent to the classical definition of predictive validity.

Developing an animal model on the sole basis of its predictive (or "remission") validity is fundamentally problematic. Indeed, responding to a drug that has demonstrated beneficial effects in clinical trials and modifies a biological parameter of the model does not guarantee that the model will respond in the same direction to a new molecule with different properties. Neslter and Hyman cite the famous example of the forced swim test as a model for depression. Rodents respond to acute doses of Selective Serotonin Reuptake Inhibitors by increasing their swimming time before stopping (stopping being considered as equivalent to despair). This has prompted the use of forced swim test to develop novel antidepressants, without any success. As the authors note, this is an "enormous anthropomorphic leap" and "the forced swim test [is] not [a] model of depression at all" (31). In conclusion, predictive validity may be useful to support a model with good construct and/or face validity but is not relevant when used alone.

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**Figure 3: Validity criteria for animal models of mental disorders** as conceived by Willner (similar to Nestler and Hyman: brown circles) and by Belzung and Lemoine: colored boxes. Reproduced from Belzung and Lemoine, 2011 (32).

### Stressor-based models of PTSD

The main animal models of PTSD are based on the application of stressors of different types, intensities and durations. Intense stressors are presented here, based on recent reviews of the literature (33–36). Common experimental paradigms in rodents can be divided in categories depending on the nature of stressors (33,35) and include:

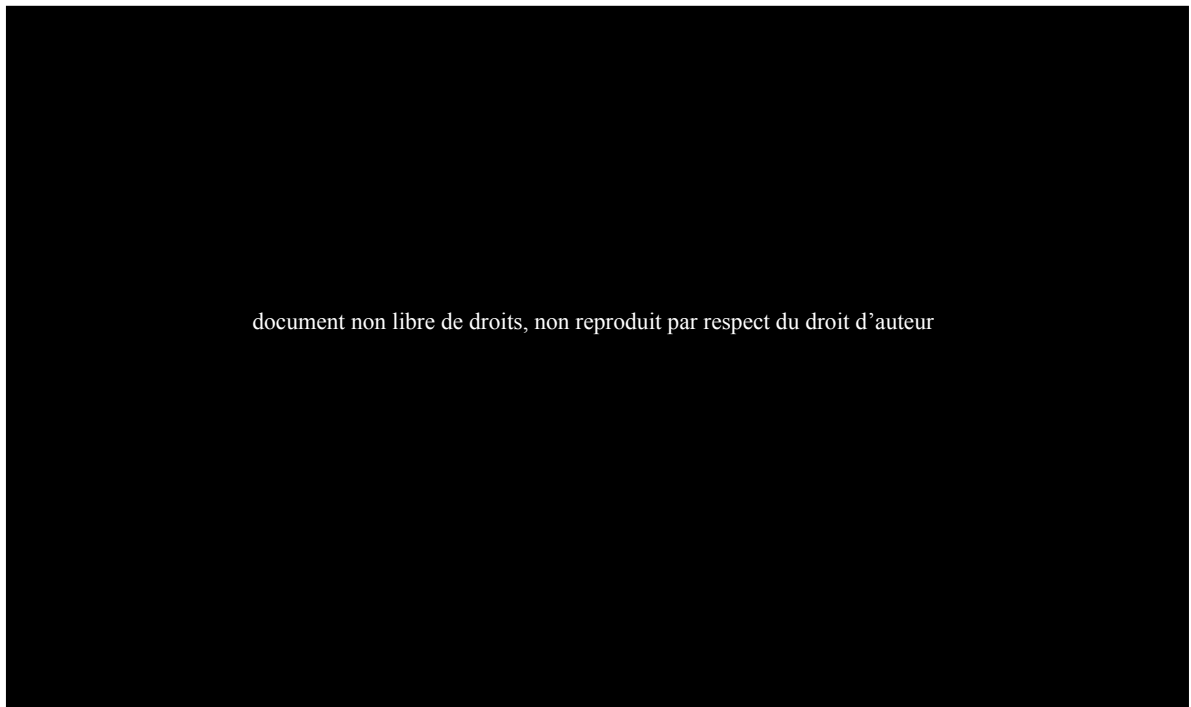
- Physical stressors: inescapable shock stress, single prolonged stress, immobilization stress, underwater trauma, unpredictable variable stress,
- Psychological stressors: predator stress (direct exposure or predator scent stress), and,
- Social stressors: social defeat stress, early life stress (e.g. maternal separation).

Declarers *et al.*, 2018, have “reviewed over 600 articles to examine the ability of current rodent models to probe biological phenotypes of PTSD”, that is, assess their face validity. This section largely uses conclusions of their comprehensive review of the literature. They defined relevant models as responding to the following face validity criteria: “1) focus on outcome variables that endure long after the trauma and/or stress has ended, 2) measure more than one behavioral outcome variable for reliability

and/or robustness, 3) have replicable effects across more than one laboratory, and 4) present an unpredictable, inescapable severe stressor (e.g., vary stressor intensity, duration) to avoid habituation and mimic life-threatening aspects of trauma associated with PTSD”. They retained inescapable footshocks, predator stress, single prolonged stress, immobilization stress, unpredictable variable stress, and social defeat stress (36).

Adding the construct validity criterion of high intensity and short-duration stressor (34), we focus here on stress models that are most specific to PTSD and most frequently applied: inescapable shock stress, predator stress, single prolonged stress and immobilization stress (Figure 4).

Measures of their validity are mainly behavioral, with anxiety and conditioned fear tests, biological with fear circuits and HPA axis dysfunction markers (face validity), individual susceptibility (construct validity) and pharmacological, with application of selective serotonin reuptake inhibitors (predictive validity).



(A) Inescapable shocks, (B) predator stress (unprotected and protected exposure, and predator scent), (C) single prolonged stress (SPS), (D) immobilization (IMO). Reproduced from Deslauriers *et al.*, 2018 (36).

- Inescapable shock : in this paradigm, brief electrical shocks are delivered to the tail or paws, typically within a single session, in a closed (inescapable) environment.

This stressor has been found to trigger enduring (several weeks) PTSD-like phenotypes with hyperarousal, sleep disturbances, contextual memory deficits, generalized avoidance, and depression the latter two sensitive to long-term SSRI treatment). Response variability exists in

this behavioral paradigm, reflecting individual vulnerability. Inescapable shocks also induce neuronal activity in the PFC and amygdala, and a decrease of hippocampal volume.

The main limitations of this model are its low ethological validity in induction, and the variability of protocols across laboratories (36).

- Predator stress : this paradigm consists in a single exposure of rodents to species-relevant predators (predator stress; either unprotected or protected with a physical barrier) or to their odor (predator scent stress).

Predator stress is ethologically relevant. It has been found to induce long-lasting (several months) behavioral and physiological abnormalities, including general avoidance, exaggerated fear response, and hyperarousal. Fear of trauma-related cues can be assessed by placing rodents back to predator-exposure context or presenting , in a different context, cues initially associated with predator exposure. Animals can be classified as susceptible versus resilient. Predator stress recapitulates some biological correlates of PTSD, including smaller hippocampus, increased amygdala activity, and enhanced negative feedback of the HPA axis.

Main limitations of predator stress are the heterogeneity in laboratory-specific procedures, including intensity ranging from severe (direct contact) to relatively mild (scent exposure), and the dependence of some paradigms on secondary stressors (e.g. social instability) (36).

- Single Prolonged Stress : in SPS, three severe stressors are presented to rodents in succession: a 2-hour restraint stress, followed by forced swimming for 20 minutes in water, and finally, after a 15 minute-recovery period, by exposure to ether vapors until loss of consciousness.

It is a standardized procedure that reliably produces PTSD-like phenotypes: avoidance, deficits in fear conditioning applied after SPS, in increased learning and reduced extinction (reversed by SSRI), hyperarousal, and negative feedback on the HPA axis.

Limitation of published SPS-based experiments include the lack of data for trauma-specific fear expression/avoidance and limited evidence for individual variability (36).

- Immobilization stress : in single immobilization/restraint stress (IMO), subjects are attached to a board or placed in a plastic tube for 30-120 minutes.

Immobilization stress induces deficits in subsequent fear extinction, increased activity in amygdala, microstructural alterations in hippocampus and decreased functional connectivity between amygdala and hippocampus. Measurements of HPA axis function after IMO resemble the PTSD phenotype (low corticosterone, increased negative feedback).

Limitations of IMO are mainly limited data on PTSD-relevant phenotypes, little evidence for long-term modifications, and limited work on individual vulnerability (36).

## Fear conditioning as a model of PTSD

In addition to stressor-based models, classical fear conditioning is used in combination with these stressors, or alone, to model PTSD. Deficits in conditioned fear extinction are particularly used to mimic PTSD-like phenotype. This section draws from theoretical reviews written on the subject (by Yehuda and Ledoux, 2007; Johnson *et al.*, 2012; Mahan *et al.*, 2012; Pitman *et al.*, 2012 (28,37–39))

*Combined with other stress paradigms*, classical fear conditioning is used either as *de novo* conditioning or as stress-enhanced conditioning (35).

- *De novo* conditioning is used to assess face validity of other models, typically the way stressors produce increased fear reactions, by enhanced fear learning or deficits in fear extinction (36). Deficits in extinction of *de novo* conditioned fear has also been reported in human subjects with PTSD (39).
- Stress-enhanced fear learning is similar, except that conceptually, the trigger of abnormal biological phenotypes is the conditioning procedure itself.

*Classical fear conditioning applied alone* is also regarded as a valuable tool to study PTSD, with regards to its construct, face and predictive validity.

- Construct validity: fear conditioning is considered valid in construction if the conditioning stress is considered as modeling trauma: “Pavlovian fear conditioning can be said to have a degree of etiological validity for the study of PTSD because the initial formation of memories that later develop to become PTSD involve the same process of Pavlovian fear”(38). Individual variability among animals of an experimental group or across genetic strains is also thought to carry validity in the sense of varying individual susceptibility to developing PTSD (28,38,39).
- Face validity is established mainly on the basis of neural circuits and extinction deficits. Animals showing elevated fear after classical fear conditioning and extinction are viewed as similar to PTSD subjects showing extinction deficits after *de novo* procedures. Implication of similar neural circuits in PTSD and fear conditioning/extinction (with interspecies conservation) is another argument used to support the fear conditioning model of PTSD (amygdala hyperactivation, PFC hypoactivation, hippocampal dysfunction) (28,38,39).
- Predictive validity: although few studies have been published on the subject, there is some evidence that prolonged SSRI application may facilitate fear extinction (40–42).

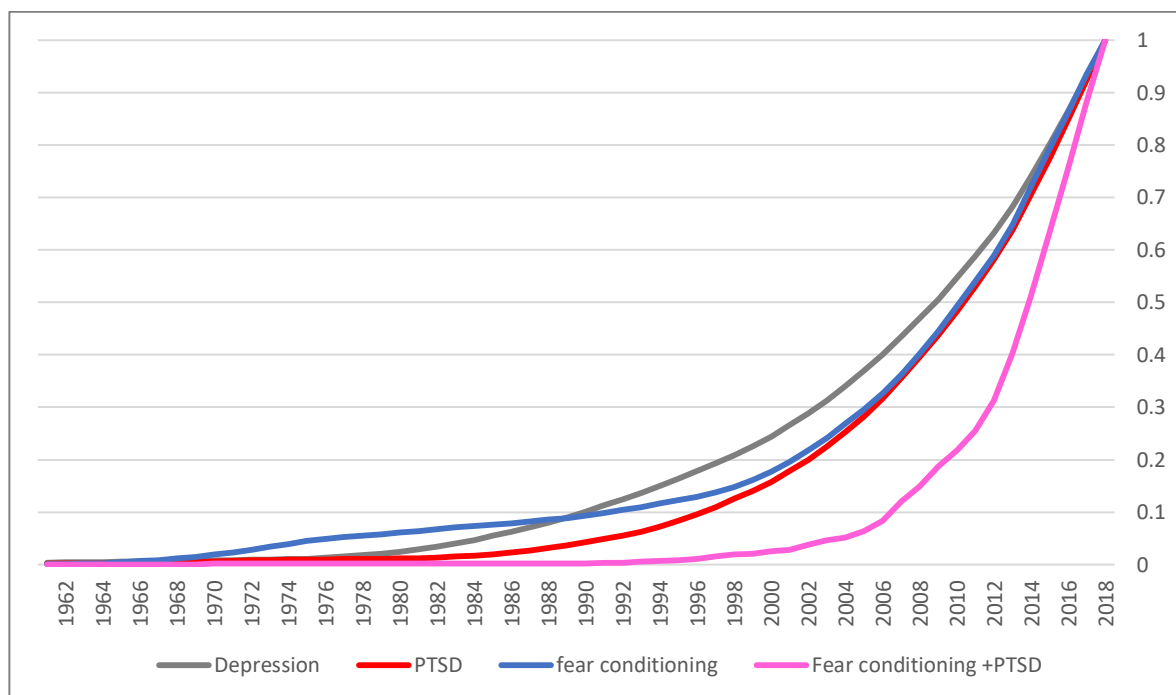
## Position of the problem and aims

### Position of the problem

Despite frequent use of fear conditioning to model PTSD, we have seen above that it is applied in many different forms, raising the question of *what* it is modeling. It has also been criticized on validity grounds. Some are: its low ethological validity, its reductionism (43), and its potential adaptive rather pathological nature (44), resulting from low aversive intensity, as opposed to life-threatening trauma (45).

Fear conditioning was not designed to study pathophysiology of mental disorders. It is mostly used to study fundamental neurobiology of memory, emotions or a combination of both (emotional memory;(20,45)). In disease and in addition to PTSD, it is also used to model other anxiety-related disorders, including specific phobia and obsessive-compulsive disorder (34,46).

It is intriguing that fear conditioning had been used for more than 50 years (47) when PTSD was recognized as a mental disorder (1980). Moreover, a cumulative distribution of publications in containing “fear conditioning”, “PTSD”, “fear conditioning” + “PTSD” and “depression” (Pubmed search, Figure 5), suggests that fear conditioning and PTSD have become a joint area of investigation with a delay of more than a decade after the development of research on PTSD (1980).



**Figure 5: cumulative plot of publication number** on the topics of depression, PTSD, fear conditioning, and the combination of fear conditioning and PTSD.

## Questions

This double temporal mismatch, the diverse use of fear conditioning to model PTSD and criticism formulated against the validity of fear conditioning to model PTSD raised the question of how fear conditioning has become a model of PTSD. In other words, how have PTSD and fear conditioning constructs intersected and what concepts have supported the use of fear conditioning to model PTSD? How have these concepts evolved to their current forms? Moreover, when, why, where and with whom has this conceptual convergence developed? What are the theoretical implications of this evolution?

## Aims

In order to understand how conceptual links between the constructs of PTSD and fear conditioning have emerged and evolved, and provide answers to the when, why, where and who questions, we have taken a qualitative and quantitative historical approach. A better understanding of the history of conceptual links between PTSD and fear conditioning should place current research on the neurobiology of PTSD in a clearer conceptual framework. Ultimately, the aim of this work was to advance our understanding of the fear conditioning model of PTSD, beyond criticism of its validity. The hope is that a better grasp of conceptual history will help select relevant information in the scientific literature, and refine and create experiments to study PTSD.

Chapter 2 (1980-1993) provides a systematic analysis of the way fear conditioning and PTSD intersected (1980-1993), and of the scientific framework in which this intersection was embedded. It traces the original link between fear conditioning and PTSD back to a theoretical analogy made in the 1980s between conditioning and trauma, and conditioned fear and reexperiencing. Other aspects of PTSD were proposed to be explained by sensitization processes.

Chapter 3 (1993-2005) systematically reports on the birth of the main animal models of PTSD (1993-2005). Its main finding is that sensitization models of PTSD were initially dominant, and designed after the theoretical work analyzed in chapter 2. Moreover, we have found that fear conditioning has not been used experimentally to model PTSD before 2003-2005.

Chapter 4 (2005-present) exposes a detailed analysis of the evolution of links between PTSD models and fear conditioning, to their current position. This work shows that although fear conditioning has been criticized as an animal model of PTSD and dedicated PTSD models have first been designed without conditioning, fear conditioning has gradually been included to model PTSD. This integration has been in the form of in various experimental paradigms. We propose that heterogeneous uses of fear conditioning to model PTSD have led to conceptual vagueness and ambiguity.

Chapter 5 takes a complementary quantitative approach to examine the evolution of the field. With the application of text mining (network analysis) on entire corpuses of literature, it provides an unbiased representation of the history of the field. The results confirm the main qualitative results of chapters 2-4, namely, they identify the links between pivotal publications, and the gradual aggregation of fear conditioning and PTSD, to become central in the field, on the basis of heterogeneous concepts.

Chapter 6 proposes tentative contextual explanations of the results presented in previous chapters. It suggests that development of fear conditioning models of PTSD developed as a result of interactions among of a small groups of individuals, with their scientific backgrounds and beliefs, in two academic medical centers of the USA, at a time when interest converged on PTSD and studying emotions with fear conditioning.

The last Chapter of this thesis discusses the findings, the methodology and the limitations of this work, as well as its contribution to the field. We also provide propositions to optimize conceptual translation of preclinical research in the field of PTSD.

This thesis represents working material for publication in a more synthetic format in a specialized philosophy of science journal. Although the present manuscript is somewhat beyond publication format, it was necessary to present in-depth analyses of the literature in order to allow conceptual clarifications. We argue that superficial conceptualization is a major problems in the field, hindering progress, and that a careful analysis is warranted.



## II- Early days: linking fear conditioning to PTSD by analogy (1980-1993)

*“The patient we described above pathetically likened himself to the dogs employed in canine combat units, whose training made them too vicious to be useful for anything else. Although we deplore the analogy, we note that our patient has offered an animal model of his own condition worth considering.”*  
Pitman, Orr and Shalev. 1993

First, we set out to identify the origins of the links between PTSD and fear conditioning and, in turn, what other conceptual links were implicated (referring to the when and what questions).

We systematically searched and analyzed the literature between years 1980-1993 for articles mentioning fear conditioning and PTSD. These dates were chosen as boundaries because:

- PTSD was recognized as a distinct mental disorder in 1980 (DSM-III).
- Results presented above suggest that scientific interest in fear conditioning to study PTSD started during the 1980s and early 1990s before animal models were developed.
- Animal models specifically designed to model PTSD were described in articles published from 1993.

It is noteworthy that academic publications reviewed in this section are contemporary of DSM-III editions (DSM-III: 1980-1987; DSMIII-R: 1987-1994).

Index words were “fear conditioning” and “PTSD” or “posttraumatic”. Four search engines were used: Google Scholar, Pubmed, Scopus, and ISI Web of knowledge, because articles published in 1980s-1990s were not exhaustively retrieved by standard Pubmed searches. Only articles explicitly referring to both concepts in the main text were included for analysis. Selected articles were screened for relevant references, and the latter were subsequently also included in the analysis. A total of 17 papers were included. The results of this systematic search are summarized in Table 1.

All articles were studied extensively to delineate conceptual trends that articulated the links between fear conditioning and PTSD during the period of interest.

The literature review indicates that the first movement of conceptual convergence between PTSD and fear conditioning has emerged from the analogy made between reexperiencing traumatic experience in PTSD on the one hand, and the behavioral paradigm of fear conditioning on the other hand. In this theory, traumatic events would act as aversive unconditioned stimuli (US), evoking a strong emotional unconditioned response (UCR). Contextual elements of the trauma would act as conditioned stimuli (CS). Subsequent exposure to reminders would evoke a pathological conditioned response, similar to reexperiencing symptoms of PTSD. The DSM-III and III-R criteria that pertain here are the following:

- *Trauma* (conceptualized as unconditioned stimulus evoking unconditioned responses): criterion A: DSM-III: “recognizable stressor that would evoke significant symptoms of distress in almost everyone and DSM-III-R : an event that is outside the range of usual human experience and that would be markedly distressing to almost anyone, involving serious threat to life or physical integrity”.
- *Re-experiencing* (conceptualized as conditioned responses): DSM-III-(R) criteria B: “intense psychological distress to reminders”.

### Theoretical accounts of fear conditioning to model PTSD

The original systematic bibliographic search (1980-1993) retrieved theoretical articles that examined psychological and neurobiological mechanisms underlying PTSD induction and expression. Here, we briefly summarize those studies and highlight relationships with fear conditioning. In virtually all of these studies, fear conditioning was discussed in the frame of PTSD traumatization and reexperiencing. Some authors directly compared conditioned fear and PTSD reexperiencing, but the majority derived modified theories from this original proposal.

#### Direct analogy between fear conditioning and PTSD

Kolb and Multalipassi were the first to liken delayed PTSD to a conditioned emotional response, in 1982 (48). Kolb was a Veteran Affairs physician, in charge of Vietnam Veterans, who also had acquired clinical experience from WW2. They compared PTSD reexperiencing to a “conditioned emotional response”.

Brett and Ostroff (1985), from a center treating Vietnam Veterans center and Yale university, reviewed psychological and biological theories of PTSD and referred to Kolb and Multalipassi for the conditioning theory (49).

Keane *et al.* (1985), another psychiatric team of a Veterans Affairs, conceptualized PTSD as resulting from “classical conditioning” (Pavlov’s experiment: the pairing of a bell sound with an electrical footshock to the extremities). In their view, a strong aversive stimulus is necessary to induce PTSD (50).

Pitman (1989), then a physician in a Veterans Affairs clinic, directly compared intrusion symptoms in response to trauma with conditioned responses to aversive conditioned stimuli (51), citing Kolb and Multalipassi (48).

In 1992, Shalev (then a physician in Jerusalem, Israel) and Pitman similarly made an explicit analogy between responses induced by trauma reminders in PTSD and fear conditioning: “combat veterans with PTSD respond with excessive arousal to sensory stimuli resembling the original traumatic event (e.g., sounds of machine gun fire) even years after the event's occurrence. This "conditioned emotional response" (Kolb 1987 (52)) bears an analogy to the conditioned fear responses in rodents” (53).

Shalev and Rogel-Fuchs (54) also referred to the “learned conditioning hypothesis, referring to Kolb (48) for the first formulation of the theory. In addition, they extended this theory to mental representations (see below).

Surprisingly, in a subsequent paper, the same group of physician-scientists claimed that “a problem faced by many of [PTSD sufferers] is that their emotional responses are not conditional enough” (55) (see below, “beyond reexperiencing”).

### Modified analogies

Van der Kolk, Krystal and colleagues (56–58), also physicians concerned with the clinical management of Vietnam Veterans and academic activities at Yale and Harvard Universities, started their essays by describing theories of PTSD that can be derived from Freud’s work or Pavlov’s experiments using aversive conditioning, citing Kolb and Mualipassi (48). They provided a clinical illustration of the phenomenon in “the sound of a passing helicopter can re-elicite the experience of combat as many as 15 years after the original association was forged” (57). They objected to the fear conditioning theory that “clinical experience suggests that the increased autonomic arousal is rather non-specific, and may be seen in response to a large variety of stimuli” (57). They favored a different behavioral paradigm to explain and model PTSD: *uncontrollable* stimulation (inescapable electrical shock) as an experimental alternative to Pavlovian fear conditioning. To support this choice, they established indirect links between inescapable shock and PTSD by listing neurochemical modifications induced by inescapable shock and the roles these neurotransmitters were thought to play in controlling behavior, and finally by linking those behaviors potentially affected by neurotransmitter defects to clinical features of PTSD. Although they meant to distinguish their model from fear conditioning, they referred to conditioning in sections describing the inescapable shock model, in the form of *conditioned* neurochemical responses (e.g. endorphin release).

Intriguingly, Kosten and Krystal (59) went back to including fear conditioning: “the conditioned emotional response model provides a mechanism for maintaining the central noradrenergic hyperactivity that seems to characterize PTSD”. They clarified the relative contributions of the fear conditioning and the inescapable shock models, that is, hyperactivity and “alarm” responses and “negative symptoms” (constricted affects, numbing, passivity etc.), respectively. Interestingly, they proposed that learned helplessness resulting from inescapable shocks and fear conditioning may interact,

to explain heroin craving induced by trauma reminder (conditioned endorphin release) in the context of drug abuse promoted by learned helplessness (59). This interaction is put forth again in a subsequent paper by Krystal *et al.* (1989; (60)).

In Krystal *et al.*, 1989, the authors called the transient symptoms of PTSD “specific learned responses to specific environment stimuli” and borrowed from Michael Davis a modified model of fear conditioning, the fear-enhanced acoustic startle response (60). In this paradigm, animals are tested for startle reflexes in response to loud sounds. If an aversive stimulus (typically an electrical shock) is paired to a neutral stimulus (e.g. a light), subsequent presentation of the conditioned stimulus with the sound increases the startle reflex. Increased startle is therefore a form of conditioned fear response. Krystal and colleagues linked associative, conditioned learning and general alarm responses in two ways: 1) biological: the locus coeruleus had been implicated in the two processes and synaptic plasticity in *Aplysia* could account for associative and non-associative learning, and 2) behavioral: exposure of animals to reminders of the context in which inescapable shocks were given may induce, on top of general stress responses, contextual defensive behavior (that they again explain with noradrenergic pathways). The same group, Charney, Davis and Krystal *et al.*, proposed that “the primary symptoms of PTSD -the persistent reexperiencing of the traumatic event, avoidance of stimuli associated with the trauma, and the symptoms of increased arousal- are related to the neural mechanisms involved in fear conditioning [in the fear-enhanced startle form, our note], experimental extinction and behavioral sensitization” (61).

A team of psychologists, led by Edna Foa, took a different approach to explain PTSD etiology (62,63). “ From a behavioral point of view, it is tempting to view PTSD as a prototype for etiology and symptomatology of phobia. Indeed, there is a recognizable traumatic stimulus, following which an individual shows fear reactions when confronted with situations associated with or similar to the original trauma. This is exactly what "Little Albert" in Watson and Rayner's experiment (1920) demonstrated: two-year-old Albert was confronted with a sudden loud noise while playing with a white rat and consequently displayed fear and avoidance when confronted with the rat and similar objects. Is PTSD, then, a prototype of phobia? This appears not to be the case”. They cited Keane’s work (50,64) and criticized the absence of theoretical explanations for increased nightmares, a symptom of great importance in PTSD according to these authors. They proposed a cognitive model whereby sensory experience factors such as unpredictability and uncontrollability are critical in evoking trauma. They also suggested that language and meaning be added when trying to understand PTSD. They compared this unpredictability and uncontrollability to the animal model of inescapable, unpredictable shock, inducing an alternation of explosive and inhibited behaviors, and took the example of rape victims who are more likely to develop PTSD if their attack is unpredictable, occurring in a familiar place and/or by a familiar person.

They added a putatively human-specific dimension, in that the meaning of the trauma, interpreted within the frame of individual beliefs, determines traumatic strength. The greater the difference between the expectation and the action, the stronger the traumatization. They illustrated this by the theoretical case of a woman who would be the victim of a rape in her own bed. Language and meaning could also modify the affective content of the initial experience. For instance, if a rape victim learns that her assailant killed his next victim, this may trigger PTSD.

Despite the criticism of the fear conditioning model, Foa *et al.*, concluded by stating: “traditional [US-CS] response learning theories, we proposed, can *adequately* account for the acquisition and maintenance of fear and avoidance of previously neutral situations following a traumatic event” (62). The concepts of unpredictability, uncontrollability and meaning would only come to complement fear conditioning in order to account for induction of the full-blown clinical picture of PTSD.

Quite similar to Foa and colleagues, Shalev and Rogel-Fuchs extended the conditioning theory of PTSD to mental representations of the trauma (54). In their argumentation, mental representations could be analogous to conditioned stimulus, evoking conditioned responses in the absence of external reminders of the trauma. They proposed that such system would be equivalent to Pavlov’s “secondary system of representations” -a mental network of recollections, verbal representations and images which, in humans, plays a role of a “second reality” (54). Mental imagery was also central to Brett and Ostroff’s theoretical account of PTSD (49).

Of note, another team of psychologists interested in anxiety disorders (Davey *et al.*, 1993) also used the fear conditioning analogy for PTSD (among other anxiety disorders such as phobia) and the verbal affective content of the traumatic event to explain the secondary enhancement of conditioned fear. They used the example of a bank employee who would be fine after a robbery, but would go into developing PTSD several days later after being told that the robber had previously killed a man. They explained this phenomenon by the mental reevaluation of unconditioned stimuli, that they coined “unconditioned stimulus inflation” (65).

Whether modified or direct, early work has implanted theoretical analogies of conditioning/trauma and conditioned responses/reexperiencing. The importance of PTSD clinical features used to establish the analogies have contributed to the strength of the conditioning model. Moreover, physiological experiments in patients with PTSD has lent support to the concept of conditioned fear responses in this disorder.

Authors	Year	Title	Reference
Kolb, Multalipassi	1982	The conditioned emotional response: a sub-class of the chronic and delayed post-traumatic stress disorder;	48. Kolb LC, Multalipassi LR. <i>Psychiatric Annals</i> . 1982;12:979–87.
Brett, Ostroff	1985	Imagery and posttraumatic stress disorder, an overview	49. Brett EA, Ostroff R. <i>American Journal of Psychiatry</i> . 1985 Apr;142(4):417–24.
Keane, Zimering, Kaddell	1985	A behavioral formulation of posttraumatic stress disorder in Vietnam veterans	50. Keane TM et al. <i>The Behavior Therapist</i> 1985;8(1):9-12
Van Der Kolk, Greenberg, Boyd, Krystal	1985	Inescapable Shock, Neurotransmitters, and Addiction to Trauma: Toward a Psychobiology of Post Traumatic Stress	56. van der Kolk B, Greenberg M, Boyd H, Krystal J. <i>Biological Psychiatry</i> . 1985 Mar;20(3):314–25.
Kolb	1987	A neuropsychological hypothesis explaining posttraumatic stress disorder	52. Kolb LC. <i>American Journal of Psychiatry</i> . 1987 Aug;144(8):989–9
Van der Kolk	1987	The drug treatment of post-traumatic stress disorder	57. van der Kolk BA. <i>Journal of Affective Disorders</i> . 1987 Sep;13(2):203–13.
Kosten, Krystal	1988	Biological mechanisms of posttraumatic stress disorder, relevance for substance abuse	59. Kosten T.R., Krystal J. (1988) In: Galanter M. et al. (eds) <i>Recent Developments in Alcoholism</i> . Springer, Boston, MA
Foa, Steketee, Rothbaum	1989	Behavioral/cognitive conceptualizations of post-traumatic stress disorder	62. Foa EB, Steketee G, Rothbaum BO. <i>Behavior Therapy</i> . 1989;20(2):155–76.
Pitman	1989	Post-traumatic stress disorder, hormones, and memory	51. Pitman RK.. <i>Biological Psychiatry</i> . 1989;26:221–3.
Krystal	1990	Animal Models for Posttraumatic Stress Disorder	78. Krystal JH. In: <i>Biological assessment and treatment of posttraumatic stress disorder</i> . Griller E.L., Spiegel D. <i>American Psychiatric Press Inc.</i> ; 1990. p. 3–26.
Foa, Zinbarg, Rothbaum	1992	Uncontrollability and unpredictability in post-traumatic stress disorder: an animal model	62. Foa EB, Zinbarg R, Rothbaum BO. <i>Psychological Bulletin</i> . 1992;112(2):218–38.
Shalev, Rogel-Fuchs, Pitman	1992	Conditioned fear and psychological trauma	53. Shalev AY, Rogel-Fuchs Y, Pitman RK.. <i>Biological Psychiatry</i> . 1992;31:863–5.
Charney, Deutch, Krystal, Southwick, Davis	1993	Psychobiologic mechanisms of posttraumatic stress disorder	61. Charney DS. <i>Archives of General Psychiatry</i> . 1993 Apr 1;50(4):294.
Davey, De Jong, Tallis	1993	UCS inflation in the aetiology of a variety of anxiety disorders: some case histories	65. Davey GCL, De Jong PJ, Tallis F. <i>Behaviour Research and Therapy</i> . 1993 Jun;31(5):495–8.
Pitman, Orr, Shalev	1993	Once bitten, twice shy: beyond the conditioning model of PTSD	55. Pitman RK, Orr SP, Shalev AY. <i>Biological Psychiatry</i> . 1993;33:145–6.
Shalev, Rogel-Fuchs	1993	Psychophysiology of the posttraumatic stress disorder: from sulfur fumes to behavioral genetics	54. Shalev AY, Rogel-Fuchs Y. <i>Psychosomatic Medicine</i> . 1993 Sep;55(5):413–23.
Yehuda, Antelman	1993	Criteria for rationally evaluating animal models of posttraumatic stress disorder	66. Yehuda R, Antelman SM. <i>Biological Psychiatry</i> . 1993;33:479–86.

**Table 1 results of the systematic search of articles dealing with PTSD and fear conditioning.** The position of the articles with regard to fear conditioning is represented by increasing shading with distance to the fear conditioning theory (and, conversely, proximity to sensitization theory).

## Experimental and theoretical support to the conditioning model

### A behavioral model analogous to central features of PTSD

The analogy between fear conditioning and PTSD rests on clinical features that make PTSD unique. If a behavioral paradigm can model the critical aspects of a mental disorder, it logically follows that this model will play a central role in experimentally studying that disorder. Trauma and reexperiencing have indeed led to PTSD recognition as a clinical entity, and are the core of the PTSD-conditioning analogy.

- 1- Traumatic experience. Posttraumatic stress disorder recognizes a direct, causal, link between a single aversive event and the installation of a pathological state. As such, it is the first and only DSM diagnosis to include an explicit induction factor. Yehuda and Antelman, and Pitman pointed this. “In the case of PTSD, there is a greater potential to accurately model the disorder because the major precipitating factors are known, i.e., PTSD occurs in response to severe and unusual stressful or traumatic situations)” (66). “[An] essential feature of PTSD is its cause, which by definition is an external event” (51). Keane added the phylogenic similarity to support the analogy “it is likely that different organisms acquire fear through similar mechanisms” (50).
- 2- Reexperiencing. It is the distinguishing clinical feature of PTSD; other symptoms are rather non-specific of this mental pathology and can be identified, for instance, in depression (67). Therefore, the analogy between fear conditioning and *reexperiencing* may have strongly supported the interest in this behavioral model. Keane, Pitman and their collaborators recognized the importance of reexperiencing elements: they are “benchmark symptoms for the diagnosis of PTSD” (50); “the intrusion symptoms are PTSD’s characteristic phenomenological feature” (51).

### Psychophysiological experiments with PTSD patients

To back the analogy between fear conditioning and reexperiencing of traumatic experience with experimental observations, many of the studies reviewed in this chapter (52–54,59–61) listed and discussed results from “psychophysiological” experiments performed with PTSD patients. These psychophysiological studies showed stress responses in Vietnam war veterans exposed to reminders of their traumatic experience. The experiments consisted in exposing subjects to trauma reminders while collecting a combination of physiological and/or biological parameters, namely skin resistance, heart rate, blood pressure and muscular activity (psychophysiological) and blood levels of adrenaline, noradrenaline (biological). All these parameters increase in response to stress, reflecting mainly sympathetic nervous system activation. Shalev extensively discussed these findings in the frame of fear condition to explain PTSD (54).

Reminders of trauma could be of two types:

- *external, sensory stimuli* (e.g. combat videos; (68), from the group of Keane)
- *mental imagery* of trauma-related factors triggered by exposing subjects to the recorded scripts of accounts of their own traumatic experiences (e.g Pitman *et al.*, 1987 (69)).

Some of these studies (e.g. Malloy *et al.*, 1983 (68)) were designed to provide experimental support for the diagnosis and evaluation of PTSD treatments.

Remarkably, the group of Kolb (70,71), as well as McFall and colleagues (72), and Boudewyns and colleagues (73), explicitly set out to provide empirical support to the conditioning theory of the “distinctive pattern of psychophysiological responses [in PTSD]” (71).

Although the paradigm of fear conditioning in animals had received theoretical and experimental support to model the induction and the reexperiencing symptoms of PTSD, researchers soon recognized the limitations of this paradigm in explaining some features of PTSD.

### Beyond reexperiencing: explaining other PTSD symptoms

Some limitations of the fear conditioning model were recognized. The explanatory power of fear conditioning is derived from trauma reminder-induced symptoms, but applied to the full reexperiencing spectrum. Moreover, authors of most articles reviewed above have acknowledged that although classical fear conditioning and conditioned responses are valid concepts to explain reexperiencing in PTSD, they are not sufficient to account for the full clinical picture. *Chronicity* of the disorder, observed in a number of patients, must be matched with durable behavioral alterations in experimental models. Similarly, symptoms of PTSD (DSM-III(R)) other than reexperiencing are not phasic but *permanent*, including avoidance/numbing and hyperarousal. Taking into account chronicity and other symptoms required complementary or replacement animal models, according to the authors.

### Chronicity

Classically conditioned fear can be readily reduced in the absence of aversive unconditioned stimuli, mainly, through the process of fear extinction (the gradual decrease of conditioned responses by unpaired CS presentations; see chapter 1). Authors noted that if PTSD was to be explained by fear conditioning, then learnt fear had to be insufficiently reduced by extinction in pathology. Indeed, PTSD can manifest itself for a lifetime despite repeated exposure to trauma-related cues in a safe environment. Insufficient extinction of conditioned fear in PTSD was conceptualized by two non-exclusive theories: poor extinction, and strong memory traces.



### *Maladapted extinction weakness*

Keane and colleagues proposed that resistance of conditioned fear results from insufficient extinction, due to incomplete exposure (actual or mental) of subjects to the full context of their trauma. This would be explained by individual avoidance, incomplete memories of trauma, and social pressure (prohibition of discussing traumatic experience in the US army during Vietnam war) (50).

Kolb postulated for the persistence of symptoms that “[it is] explainable as expressive of cortical synaptic change related to those processes which underlie sensitization, learning, and habituation”. Neuronal changes would be of synaptic nature, and *permanent*, suppressing in turn the cortical control of hindbrain centers, whose activation would mediate PTSD symptoms. To explain the irreversibility of reactions to trauma, he compared psychological trauma, rendering the individual constantly affected, to sound trauma inducing deafness (52).

Charney, Krystal and colleagues also hypothesized that “a failure in extinction in PTSD may relate to the persistence in recalling traumatic memories” (61).

Shalev and colleagues used the concept of deficient cortical control to explain the onset of PTSD -possibly delayed-, for instance when soldiers go home and loosen their psychological defenses (54). In other words, the traumatic memory would be permanently engraved but submitted to cortical control of varying strength. This is reminiscent of Kolb’s initial proposal (52).

### *Indelible memories*

Keane et al proposed by analogy with previous experiments in rodents that persistence of symptoms might result from the strength of US and complex CSs, composed of multiple sensory cues (50).

Kosten and Krystal hypothesized that symptomatology appearing in response to strong unconditioned stimuli would be remanent by implication of the locus coeruleus but they did not propose mechanistic explanations (59).

Pitman introduced the concept of “*superconditioning*”, whereby stress hormones released in response to traumatic event would strengthen memory consolidation, resulting in “overconsolidation”. Superconditioning would confer traumatic memories resistance to extinction. Each exposure to trauma reminder would lead to further consolidation of the memory, resulting in gradual increase of responses. This would account for delayed forms of the disorder, the author proposed (51).

Similarly, Shalev formulated that psychological trauma induced strong memories, enhanced by stress hormones such as noradrenaline (53). Endless repetitions of traumatic experiences in mental representations would continuously reinforce conditioning, even in the absence of external reinforcer. This self-maintained trend towards ever increasing fear thus resembles Pitman’s hypothesis.

Shalev, Charney and their groups (54,61) formulated the hypothesis that chronicity of PTSD symptoms results from the interaction between a deficient extinction and indelible subcortical memory traces of the trauma. This borrows from Ledoux's theory, according to which conditioned fear memory traces might be indelible. Ledoux and colleagues performed visual fear conditioning in water-deprived rats, where US was an electrical shock and CS a flash light. Conditioned response to flash light was the diminution of water intake. They showed that large lesions of the visual cortex did not impair conditioning but prevented extinction of conditioned fear. From this, they concluded that fear memories are mediated by subcortical structures and, more surprisingly, that they are indelible (74). According to Shalev and colleagues, these experiments "[offered] a potentially sobering neuroanatomical insight into PTSD. ... therapists work to strengthen their patients' control over underlying traumatically conditioned emotional response predilections that are unlikely to go away" (54).

Foa and colleagues took some distance from the conditioning model of PTSD, and attributed duration of PTSD symptoms to the enduring beliefs about their environment developed by trauma victims.

If conditioning to strong unconditioned stimuli and/or extinction deficit might theoretically account for chronicity of reexperiencing symptoms following an analogical reasoning, what about other symptoms?

#### Extended associative learning: avoidance and generalization

Keane (50) introduced Mowrer's two-factor learning theory (75) to explain avoidance, and high-order conditioning and generalization to explain diffuse anxiety. In two-factor learning, avoidance of fear-evoking stimuli is considered rewarding, by suppression of fear. Therefore, avoidance of thoughts or physical reminders of the trauma would tend to become chronic by such instrumental learning process of "negative reinforcement".

High-order conditioning consists in the formation of new memory links between reexperienced conditioned stimuli and neutral sensory cues, remotely from the traumatic experience. For instance, a soldier may come to fear and avoid his own bedroom by repetitive reexperiencing of the trauma in this context. Generalization is the triggering of fear by constitutive similarities between a neutral cue and a feature conditioned by the trauma, accounting for diffuse responses to sensory stimulation (50). As a result, higher-order conditioning and generalization may interact to produce enhanced fear reactions to a variety of stimuli that may not represent traumatic experiences.

Foa partly accepted the argumentation developed by Keane and colleagues, that she criticized on the ground of its incapacity to account for the full PTSD spectrum. She preferred a cognitive theory, including semantic and cognitive generalization (62,63): "[fear conditioning] can partly explain why

PTSD sufferers exhibit greater generalization of fear than simple phobics, under the assumption that PTSD traumas are more intense and the stimulus compound more complex”. “The notion of predictability is easily accommodated by traditional learning theories. However, they do not explain generalization across dissimilar situations. If a woman was raped at home at night, why does she fear jogging through the park at midday? S-S theories [higher-order conditioning, our note] account better than S-R theories [classical fear conditioning, our note] for generalization across dissimilar situations through higher order conditioning. However, in PTSD, the common denominator for the feared situations is the perceived threat, a concept that is more parsimoniously addressed via a semantic theory”. In this theory, evaluation of emotional contents by verbal reports and cognitive processing would prevail. Moreover, cognitive biasing would account for generalization. Foa based extensively her argumentation on the example of rape victims, and put forward the idea that erroneous evaluation of the environment, perceived as permanently uncontrollable and dangerous, underlies generalization and avoidance.

According to Charney, Krystal and colleagues (61), fear conditioning can result in avoidance of trauma-related cues and general numbing, but they provided no mechanistic explanation. “Fear conditioning may account for the common clinical observation in patients with PTSD that sensory and cognitive stimuli associated with or resembling the original trauma elicit symptoms, including anxiety, flashbacks, and hyperarousal; this results in the frequent reexperiencing of the traumatic event, a persistent avoidance of such stimuli, and a compensatory numbing of general responsiveness” (61).

Arguably, despite generalization of fear and avoidance, a number of PTSD symptoms were difficult to accommodate with the fear conditioning paradigm. As Pitman put it: “an understanding of the role of heightened unconditional responses potentially informs aspects of PTSD that resist conditioning explanations, for example, DSM-III-R "symptoms of increased arousal," including irritability, hypervigilance, and exaggerated startle response” (55).

### Stimulus-induced reviviscence reduction

Importantly, using analogy with trauma reminder-evoked responses to conclude on mechanisms of re-experiencing in PTSD is a form of generalization. Conversely, taking trauma reminder-evoked symptoms as representative for all the re-experiencing cluster is a reduction. We coined this trend we have found in articles “stimulus-induced reviviscence reduction”. Foa *et al.* wrote : “Keane *et al.* (1985) attempted to explain flashbacks, nightmares, and intrusive images of the event via the high degree of generalization which renders avoidance of traumatic memories impossible. Although this may plausibly explain intrusive thoughts and perhaps flashbacks, it does not adequately account for nightmares” (62). Krystal or Kolb, on the contrary, thought daytime and nighttime symptoms were similar (52,57).

### Non-associative features: sensitization and kindling

Symptoms not readily explained by fear conditioning set a clear problem for theoreticians. Although some authors have used the notion of generalization and avoidance to explain diffuse symptomatology, including numbing, most have proposed other concepts to account for the full clinical picture of PTSD, advancing alternative and complementary proposals of animal modeling.

The main idea is that explaining permanent, non-associative symptoms required a theoretical model in which traumatization induces enduring changes in the individual, that can express themselves independently of trauma reminders. Two close concepts can summarize this: *sensitization* and *kindling*.

- Sensitization is the increase in response magnitude to stimuli following exposure to a stimulus. Here, traumatic experience would sensitize emotional responsivity of their victims.
- In kindling, repeated application of stimuli too small to produce overt effects can eventually have consequences. In 1969 Goddard first coined “kindling” (76) his experimental observation in rats that the repeated intermittent stimulation of the brain with an electrical current, initially insufficient to induce seizures, gradually induced seizures, sometimes after extended periods of stimulation (77).

Symptoms have been divided into positive and negative clusters: “the positive symptoms consist[ing] of hyperreactivity, explosive aggressive outbursts, startle responses, and intrusive recollections; the negative symptoms consist[ing] of constriction, social isolation, retreat from family obligations, occupational failure, anhedonia, and a global sense of estrangement” (56). This conceptual delineation is useful to understand theoretical considerations, and we will use it to recapitulate the scope of sensitization and kindling theories of PTSD induction and expression.

#### *Positive symptoms*

Van Der Kolk proposed that “kindling phenomena leading to lasting neurobiological and behavioral changes are produced by repeated traumatization, as in child abuse, or by one trauma followed by intrusive reexperiences. Kindling may also account for the frequency of neurological abnormalities in trauma victims, especially child victims of physical or sexual abuse” (57). This group of authors used the analogy of positive clinical responses to an anti-convulsant in PTSD (carbamazepine) to experimentally support the kindling theory of non-associative learning in PTSD (60,78).

The notion of psychiatric disorder induced by repeated trauma is noteworthy. Indeed, this phenomenon had been reported for war veterans, but was not recognized in DSM-III(-R). Kindling induced by reexperiencing, on the other hand, is reminiscent of Pitman’s *superconditioning* (51) and Shalev’s theory of traumatic memory consolidation by mental reexperiencing (53).

Pitman and colleagues, who dedicated an article to PTSD phenomena that cannot be accounted for by fear conditioning theories, proposed that emotional (fear) biasing could explain phenomena such as irritability, increased arousal or hypervigilance. They likened the installation of emotional bias to kindling, where kindling relates to electrical sensitization to seizure induction by repeated electrical stimulation, and emotional biasing relates to “temperamental alterations” by “experience [that] can kindle the brain”. “All of these [increased arousal, including irritability, hypervigilance, and exaggerated startle response] reflect consistent emotively biased fear” (55). Pitman compared sensitization with stress responses after predator stress exposure, with reference to Adamec’s work.

Early conceptualization of PTSD by Kolb also used sensitization (52). The author reviewed conditioning theories of Keane *et al.* (50), that he had “discussed and critiqued”. Kolb did not explicitly rule out fear conditioning paradigm, but proposed a theory where overwhelmingly strong stimuli would lead to neuronal changes in cortex, in analogy to synaptic plasticity described by Kandel (see above and chapter 6). This would result in the release of deep brain structures (temporal complex, brainstem) from cortical control, rendering the individual generally “hyper-reactive”. In this sense, without the author stating it, we can infer that he considered responses to arousing stimuli in PTSD could be non-associative: “the constant symptoms of PTSD are explainable as expressive of cortical synaptic change related to those processes which underlie sensitization, learning, and habituation” (52). Conceptually close, Pitman criticized Kolb for resting on analogies ignoring neurobiological knowledge (51).

According to Charney, Krystal, and colleagues, sensitization would explain the general increased arousal in PTSD. Importantly, a single stressor of *strong* intensity would be sufficient to sensitize the organism. In this schema, sensitization and fear conditioning would be co-existing mechanisms, mediated by largely non-overlapping brain mechanisms (61) (see below).

Foa and colleagues again proposed alternative theories (62,63). In line with arguments reviewed above, they used the concept of cognitive bias induced by semantically signifying, uncontrollable and unpredictable traumatic events to explain symptoms “Traditional S-R learning theories, ..., can adequately account for the acquisition and maintenance of fear and avoidance of previously neutral situations following a traumatic event. They can partly explain why PTSD sufferers exhibit greater generalization of fear than simple phobics, under the assumption that PTSD traumas are more intense and the stimulus compound more complex. However, intensity and complexity of the traumatic events do not, by themselves, explain the development of PTSD symptoms. The literature on experimental neurosis suggests that unpredictable and uncontrollable events produce responses that are highly reminiscent of post-traumatic stress disorder irrespective of intensity and complexity” (63).

### *Negative symptoms*

Similar to positive symptoms, sensitization was used to explain constant negative symptoms, including, numbing. This work is mostly the production of Krystal and colleagues. They proposed a model of uncontrollable shock: “the best available biological model for PTSD involves exposure of animals to inescapable shock (IS)” and that “behavioral sequelae of inescapable shock in animals closely parallel the negative symptoms of PTSD in humans ” (1).

In this procedure, animals are given aversive stimuli, electrical shocks (more or less intense), in a behavioral context they cannot get away from. For instance, the escape door of a behavioral arena the animal previously explored is closed before electrical shock is given. Inescapable shock results in behavioral inhibition and passivity, also known as “learned helplessness”. A series of papers by the same group defended the inescapable stress model of PTSD and explored mechanistic understanding it could give to PTSD at the neurobiological level. (57,59,60).

Although inescapable, uncontrollable aversive stimulation offers resemblance to traumatic events, Krystal and colleagues surprisingly did not stress this argument. In fact, they gave little conceptual explanation for this choice, except the behavioral similarity with negative symptoms of PTSD, leaving the reader to wonder why this model would then be the best to model PTSD. Foa, on the contrary, insisted on the meaning of trauma given by uncontrollability unpredictability, and expressed interest in this learned helplessness model, stating that it had not been tested to study PTSD (62,63). Interestingly, they used the term “experimental neurosis” that was introduced by Pavlov (47).

Tightly linked with sensitization and kindling is the concept of individual susceptibility, that is examined in the next section. Conceptual and animal models have been proposed to complement the fear conditioning paradigm, and account for the full picture DSM-III-R trauma, reexperiencing, avoidance/numbing and hyperarousal. In the next section, we will examine the implications and conceptual correlates of theorizing PTSD with animal models of fear conditioning and inescapable shock, that these authors recognized.

### Implications of modeling PTSD

Experimental and conceptual modeling has several implications and correlates in understanding PTSD. The promise of the fear conditioning-PTSD analogy was that because we could and started to understand fear conditioning in scientific terms, we might also gain insight into PTSD neurobiology to design scientifically-informed biological diagnosis tools, treatments and treatment outcome assessment.

Knowledge derived from behavioral paradigms, coupled to biological measurements and confronted with clinical observations in PTSD patients has led to theories taking into account the individual

susceptibility of developing PTSD, to neurobiological accounts of mechanisms underlying PTSD, and to therapeutic proposals.

#### Individual variability and diathesis

Individual susceptibility to develop mental disorders in reaction to intense psychological had long been recognized, as for instance in army corps during WW1 and WW2 (67). Comparing PTSD to animal models suggests that individual susceptibility might be biologically-mediated and perhaps, constitutive of the organism. Authors of papers reviewed in this chapter offered mechanistic hypotheses to explain different individual vulnerability to developing PTSD after trauma exposure, an extension of the diathetic theory of mental disorders.

Kean *et al.* proposed that “biochemical factors, [among others] possibly play a role in who does and who does not develop a chronic stress disorder following trauma”, and recognized a promising research avenue in this field (50). Similarly, Charney stressed that “vulnerability factors that result in the development of acute and chronic PTSD in traumatized individuals need to be identified” (61).

Kolb hypothesized that variability could be explained by the initial strength of cortical control over subcortical structures. For instance, he wrote “as for the reported greater resistance of military officers to developing posttraumatic stress disorder, we may presume that their neuronal network is numerically larger than that of nonofficers and, through education and diverse experiences, better evolved and integrated and therefore more flexible” (52). In the same article, he implies that vulnerability may also develop through sensitization, taking the examples of early life trauma and of soldiers who develop symptoms a first time on the battlefield and are sent back to combat, where they develop severe forms of PTSD (52). Van der Kolk and colleagues argued similarly that “massive trauma [may] precipitat[e] a vulnerability to respond with excessive autonomic reactivity” (56).

Interestingly, Shalev and colleagues noticed that some symptoms of a composite disorder such as PTSD might be conceptualized as acquired as a result of the exposure to stress, or as a precondition (or trait), such for example as non-habituating auditory startle responses. In fact, that precondition might reflect a vulnerability factor, he proposed, because non-habituating startle predisposes to strong conditioning (and by extension, perhaps to PTSD). In turn, he speculated that vulnerability traits might be genetically transmitted (54).

Krystal and colleagues focused on susceptibility factors to PTSD development that are paralleled in the inescapable shock model by increased behavioral effects. Susceptibility factors include developmental factors, e.g. maternal deprivation, and constitutive factors that are inherited biologically, because animals may be bred for susceptibility to inescapable shock. He further formulated hypotheses about which genes might mediate this experimental finding (Krystal *et al.*, 1989 (60); see below).

Pitman advanced that vulnerability may guide research on neuronal mechanisms of PTSD : “a worthy task for biological psychiatric research is to formulate and test models of the lasting transformations in emotional disposition that are found in PTSD. This endeavor may be aided by research into the phenomenon of "emotive biasing". Pitman cited Adamec’s 1991 article on “anxious” cats (79) that showed facilitated amygdala to ventro-medial hypothalamus transmission (55).

Embedded in the process of modeling mental disorders with experimental behavioral tasks in animal is the search for biological explanations to risk factors, symptoms and pathophysiology, to better understand, diagnose and treat. Main neurobiological concepts included in the 1980-1993 corpus are reviewed below.

### Neurobiological mechanisms

Articles described in this chapter have set PTSD into a neurobiological framework. They established a direct link between neurobiology and behavior, and, as a result, an indirect link between pathology and neurobiology. Interactions between environment on the one hand and organs (brain, autonomic nervous system and hormonal systems) on the other hand, would underlie PTSD induction (traumatization) and symptomatic expression.

Charney *et al.* reviewed in 1993 neurobiological mechanisms that had been proposed to underly PTSD ((61); see Table 1). It is obvious from their overview that they considered fear conditioning and sensitization as complementary behavioral paradigms, mediated by largely non-overlapping biological mechanisms.

#### *Autonomous nervous system and hormones: the stress system*

Non-associative symptoms of PTSD were mainly linked to biology of hormonal systems comprising the HPA axis, autonomic nervous system and central catecholamines and endorphins, a series of paper by Krystal and colleagues suggested (56,59–61). They examined the putative roles of cortisol, of central and circulating noradrenaline, and of endorphins.

They proposed that noradrenaline depletion, hyper-sensitivity to noradrenaline and conditioned endorphin release followed by endorphin withdrawal after reexperiencing would account for permanent emotional hyper-reactivity, autonomic arousal and baseline numbing (56,57,59,60). The potential contribution to PTSD of peripheral and central noradrenaline systems (chiefly the locus coeruleus) were extensively reviewed in these papers, as mediating permanent but also possibly associative manifestations of PTSD. Moreover, vulnerability to PTSD, was speculated to be determined in part by genetically-determined monoamine oxidase expression levels (the enzyme responsible for the degradation of catecholamines; (60)).



### *Brain regions: neurobiology of fear conditioning*

Two competing models co-existed to explain associative symptoms, analogous to classical fear conditioning. Krystal and colleagues proposed theories accounting for trauma reminder-evoked symptoms of PTSD by conditioned locus coeruleus activation. As such, central noradrenaline could play a role in associative, non-associative, negative and positive manifestations of PTSD (a “trauma center”, (60)). However, as in the case of the rejection and later adoption of the conditioning theory, what parts of PTSD symptoms are thus explained, and how, remained ambiguous. Of note, this speculation was not backed by experimental data, either. They also used the model of fear-enhanced startle reflex, that appeared to implicate the amygdala in Davis’ experiments. Again, the relative contributions of the two mechanisms remained unclarified.

Other articles (53,54,61) referred to the work of LeDoux and colleagues, who studied the neurobiology of classical auditory fear conditioning in rats. Ledoux and colleagues had proposed on the basis of brain region lesion and anatomical pathways tracing that connections from the thalamus to the lateral and subsequently medial amygdala constitute a "quick-and-dirty" pathway for the mediation of conditioned fear (80). Parallel to the thalamo-amygdala subcortical connection is a thalamo-cortico-amygdala pathway, which allows more precise, but slower elaboration and discrimination of sensory input” (53).

Related to the work of Ledoux is the concept of insufficient cortical control over subcortical structures in PTSD ((52,54), see above). “PTSD, a disorder in which a defect in cortical control over subcortical processes mediating aggression and arousal exists, might represent a human equivalent to Ledoux’s proposition” (that subcortical memories are indelible, our note). Cortex would provide verbal representations and mental associations explicit control over trauma (54).

The amygdala was also cited in Pitman hypothesis of a potential neurobiological basis for vulnerability to trauma as an overactive basal amygdala to ventromedial hypothalamus pathway (55).

### *Synaptic plasticity*

To explain the physical effects of environment on the brain accounting for persistent traces, authors theorized that conditioning and sensitization were linked to the process of synaptic plasticity.

Kolb introduced synaptic changes in “temporo-amygdaloid complex” (with reference to Kandel’s work on Aplysia, see chapter 6) to explain loss of cortical control underlying sensitization (52).

Kystal and colleagues also theoretically used the synaptic plasticity comparison, in the form of Long Term Potentiation by “the similarity of invertebrate and mammalian cellular models for producing stable alterations in neural and behavioral plasticity within the systems mediating alarm and memory”

(60). The implication of synaptic long-term potentiation in PTSD by fear conditioning was further proposed by Shalev (53), after it had received experimental support. Indeed, Davis and colleagues had shown that pharmacological blockade of NMDA-type glutamate receptors (often involved in long-term potentiation) in the basolateral amygdala prevents acquisition of the fear-potentiated startle response (81). Intriguingly, synaptic plasticity (as proposed in Charney *et al.*, 1993 (61), see above) and reinforcement of memories by stress hormones in the amygdala (McGaugh 1990), two phenomena possibly interconnected, provided theoretical links between associative and non-associative features of PTSD.

## Summary

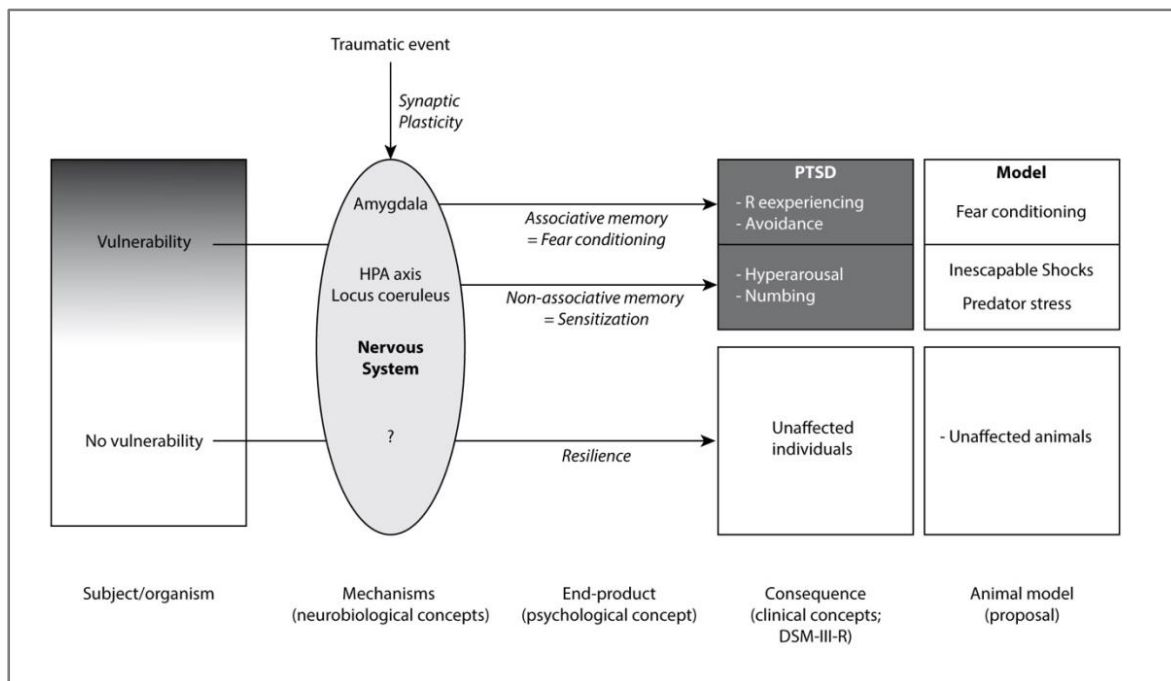
Systematically reviewing the literature containing both “PTSD” and fear conditioning” terms and related articles has suggested that in the first period following PTSD recognition, fear conditioning has been linked to PTSD by analogy (trauma/conditioning and reexperiencing/conditioned responses, linked by plasticity/memory processes). This analogy was strongly supported by implication of the specific clinical features of PTSD: trauma and reexperiencing, and by experimental parallels in PTSD patients. It was argued that fear conditioning could explain reexperiencing -except nightmares and spontaneous reexperiencing-, avoidance and a level of generalization. On the basis of animal work, conditioning was proposed to be enduring by virtue of indelible memory traces made in the amygdala and/or deficits of fear extinction, leading to chronic PTSD.

To account for clinical features beyond reexperiencing and avoidance, non-associative theories of sensitization and kindling, and corresponding animal models were proposed in addition to fear conditioning, completing the full clinical picture of PTSD (DSM-III-R).

Building on conceptual analogies, mechanistic neurobiological accounts of PTSD were formulated. Those included the implication of the stress and fear systems as loci of trauma-induced changes and effectors of the stress response. Synaptic plasticity was proposed to be the mechanism leading to long-term modifications of these fear and stress systems. It followed that behavioral paradigms that pre-existed PTSD recognition were proposed to model the disorder :

- Fear conditioning
- Inescapable (+/- uncontrollable) shock to fill the theoretical gaps of the fear conditioning paradigm.
- Combination of inescapable shocks and contextual fear conditioning (61)
- Sensitization with predator stress (Pitman (55) citing Adamec)

The results of this chapter are summarized in Figure 6.



**Figure 6: Summary of the conceptual models for PTSD identified in the 1980-1993 literature.**

Traumatic sensory stimulation leads in vulnerable organisms to enduring neurobiological modifications, through synaptic plasticity in fear (amygdala) and stress systems (HPA axis, Locus coeruleus). These changes are responsible for fear conditioning and sensitization, respectively, that in turn underly reexperiencing and avoidance, and hyperarousal and numbing. Animal models have been proposed accordingly.

Up to this point, mechanistic accounts of PTSD were theoretical, mostly derived by analogy from pre-existing animal models. These two model categories of conditioning and sensitization were complementary, but their limitations of being only associative and non-associative, respectively, have motivated researchers to build dedicated animal models of PTSD.

Yehuda and Antelman explicitly set specifications of animal models that would be valid to model and study PTSD (66). They noted to justify their work that “it is critical to differentiate between factors that can influence the manifestations or course of PTSD and those that are essential for its induction”, and that “The dangers inherent in the uncritical acceptance of a model based principally on a superficial face validity graphically point to the need for establishing indisputable criteria for evaluating the appropriateness of proposed animal models for PTSD”.

They proposed five criteria that “were derived by paring down PTSD phenomenology to its most basic components and identifying relevant counterparts for these clinical characteristics based on animal studies:

- 1- Even brief stressors should be capable of inducing biological and behavioral sequelae of PTSD;  
The stressor should
- 2- be capable of producing the PTSD-like sequelae in a dose-dependent manner;

- 3- produce biological alterations that persist over time or become more pronounced with the passage of time;
- 4- induce biobehavioral alterations that have the potential for bidirectional expression (e.g. arousal and numbing); and
- 5- Interindividual variability in response to stressor should be present either as a function of experience, genetics, or a combination of the two.”

The next chapter studies the development of experimental paradigms that explicitly claimed to model PTSD in animals, and their links with fear conditioning.

### III- Designing animal models for the study of PTSD (1993-2005)

*“Recently, PTSD model has become a somewhat fashionable term used in animal studies for almost every stress-induced behavioral alteration.” Siegmund and Wotjak, 2006.*

Proposals for animal models relevant to study the neurobiology of PTSD have emerged during the first decade following PTSD recognition (in DSM-III, 1980), on the basis of theoretical considerations. Namely, authors considered relevant to PTSD models encompassing associative (fear conditioning) and non-associative paradigms (sensitization with predator stress or inescapable electrical shocks). Behavioral paradigms dedicated to studying PTSD in rodents have been developed during the following decade (1993-2005).

This chapter aimed to trace the history of PTSD animal models, and to understand their original links with fear conditioning and earlier theories. The concepts used as theoretical support of the use of these models were scrutinized. Attention was given to identify references to articles reviewed in chapter 2, in these seminal papers, in order to understand if and how dedicated PTSD models were influenced by earlier theoretical considerations.

To find original studies that explicitly claimed the design of animal models of PTSD, we have searched and reviewed papers cited by review articles, in particular those of Berardi *et al.*, 2012, Siegmund and Wotjak 2006, and Deslauriers *et al.*, 2018 ((34,36,82), see chapter 1). Selected articles were screened to find references of potential predecessor PTSD models. If any, they were included in the analysis. When explicitly indicated by authors, articles from which their PTSD model was derived were reviewed and described. Analysis was restricted to the five dominant models of PTSD, namely fear conditioning, immobilization stress, inescapable shock, single prolonged stress, and predator stress ((36), see Chapter 1 “stressor-based models of PTSD”).

Results are presented in chronological order, analyzing articles proposing to model PTSD in rodents with trauma-like stressors: predator stress (1993), inescapable shock (1995-1996), single prolonged stress (1997), immobilization stress (2001), and fear conditioning (2003-2005). For each model, the seminal paper(s) is (are) first described (conceptual framework, procedure, results), the way the model was taken and/or modified from previous experiments are listed, then its relative position to 1980-1993 articles is examined, and finally its relationships with fear conditioning theories and paradigms are discussed.

The 1990s have witnessed the publication of animal models that are currently dominant in the field of PTSD: predator stress, inescapable shock, single prolonged stress, immobilization stress and (various forms of) classical fear conditioning. This review of the literature confirmed strong links

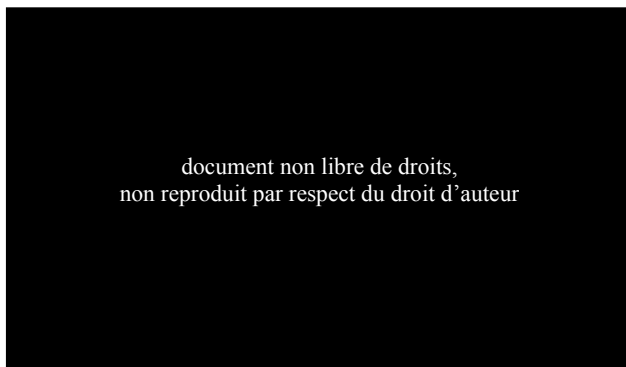
between creation of dedicated animal models of PTSD and theoretical proposals previously formulated. In particular, models based on sensitization theories were the only models during 10 years. Indeed, no experimental work on fear conditioning in rodents has been published to model PTSD before year 2003. From there, fear conditioning has been used *per se* or after sensitization procedures. Conceptual vagueness appeared regarding the use of fear conditioning to model PTSD.

### Predator stress (1993)

The use of predator stress was formalized for PTSD modeling by Adamec and Shallow, two researchers in experimental psychology, in 1993 (83). They explicitly claimed their procedure was a sensitization paradigm, the validity of which they articulated around “ecological” validity: “species-relevant, life-threatening inescapable stress”, with face validity in the form of increased anxiety for an extended period of time (83).

In their 1993 study, half of a hundred rats (unspecified strain) were exposed during 5 minutes to a real cat in a large enclosure (Figure 7). The remaining rats served as controls. Rats were later tested for exploratory behavior and activity, and risk assessment and anxiety-like behavior in a hole board and in an elevated plus-maze, respectively.

Rats exposed to cats displayed more anxiety-like behavior (fewer time and entries in open arms of the plus maze) and fewer risk assessment (approach of open arms) than controls. Anxiety levels remained unchanged for 3 weeks following cat exposure. The authors compared the duration of this effect with rats lifespans to support its relevance to mimic PTSD: “twenty-one rat days, then, is roughly equivalent to 18 human months. So the persistence of anxiety symptoms beyond 7 days may model chronic PTSD-related anxiety” (83). Adamec listed validity criteria for his model in 1997, which included “ecological” (ethological) validity, behavior (enhanced acoustic startle -a component of face validity), neurobiological mechanisms processes (dysregulation of the HPA axis -mechanistic face validity), and individual variability (similar to construct validity) (84).



**Figure 7 the arena that served for cat exposure in Adamec’s experiments**

Dimensions were 59 x 83 inches (150 x 211 cm)

After the cat was habituated to the enclosure for 5 minutes, rats were introduced from a shuttle box into the arena with a sliding platform, through the “prey door”.

Figure and legend adapted from Adamec *et al.*, 1980 (85).

The predator stress model of PTSD derives from previous work by the same group. Indeed, Adamec had published a number of studies using stress exposure for other purposes (e.g. developmental factors influencing predator aggressiveness (Adamec *et al.*, 1980 (85)), but adapted behavioral assays to

specifically test for enduring anxiety-like effects. Other authors had used cat stress before, as a naturalistic stress. For instance Hamilton and colleagues had assessed the effect of cat exposure on the immune response of mice to helminth infection (86).

The study of Adamec and Shallow (83) is strongly anchored in the context described in chapter 2. Indeed, they described in their introduction the two axes of PTSD modeling that had been proposed before (conditioning and sensitization), with explicit references to earlier work :

- “Very long-lasting classical conditioning of emotion first described in animals has provided one explanatory model for many features of PTSD” (references to Keane *et al.*, 1985, Kolb and Multalipassi 1982 (48,50)).

- “Kolb [(52)] has suggested sensitization may occur, which results in generalization of emotional responsiveness. The behavioral and physiological consequences of inescapable shock in rodents has also provided another model for the aftermath of intense and inescapable stress”. No reference is given for inescapable stress, while the importance of inescapability and uncontrollability for aversive situations to trigger PTSD had been discussed extensively by Foa, Krystal and their colleagues.

Though they recognized the influence of fear conditioning and inescapable shock models, Adamec and Shallow departed from these paradigms, stressing their limitations. According to Adamec and Shallow, these limitations were the partial explanatory power of fear conditioning (inability to explain general responsiveness; citing Pitman, 1993 (55)), and short-lived manifestations, respectively (citing only a study of WWII prisoners of war finding PTSD in 30% after 50 years (87), but no animal research showing effects of inescapable shock on behavior fading with time).

It is noteworthy that the protocol of Adamec and Shallow (83) was designed in response to the paper of Pitman published the same year (55). Pitman proposed modeling non-conditional, emotional biasing aspects of PTSD (equivalent to sensitization) with predator stress (55). It should be noted that Pitman himself had based his argumentation on previous work by Adamec on emotional biasing/sensitization induced by unnatural stressors, including a benzodiazepine inverse-agonist. Similar to Pitman (55), Adamec and Shallow stressed the need for a PTSD model that would incorporate sensitized emotional responses. “Pitman *et al.* (1993) have suggested that animal models of sensitization to fear-provoking stimuli would better approximate the clinical situation if it could be shown that exogenous, more natural stimuli could provoke lasting changes in defensive responsiveness. The present study was designed to devise a situation that satisfied the Pitman *et al.* [Pitman *et al.* 1993 (55)] criterion.” (83). In fact, the two scientist collaborated, as indicated in the acknowledgement section of Adamec, 1997 (84).

Adamec and Shallow hypothesized that long-term sensitization results from changes in excitability of a temporo-limbic pathway, citing Kolb’s 1987 study, Kolb having proposed synaptic plasticity as a biological substrate ((52); see chapter 2).

Adamec and Shallow distinguished their behavioral procedure from conditioning and even highlighted that their findings on predator stress-induced anxiety excluded potential confounding effects of conditioning. They criticized an earlier study that used predator stress on the ground of studying behavior in the same environment in which the cat was presented (70), as it might have induced conditioned responses. They added that “a test for sensitization following trauma should be done in an environment different from the one in which the trauma was experienced”.

### Inescapable shock (1995-1996)

Inescapable -electrical- shock was revindicated to model PTSD for the first times by two groups: Servatius and colleagues in 1995 and Pynoos and colleagues in 1996 (88,89).

The first article was published by Servatius and Ottenweller, scientists in experimental psychology, and Natelson, neurologist of a Veterans Affairs medical center in New Jersey (88).

Servatius and colleagues used a task they referred to as “inescapable stress”. They submitted adult male Sprague-Dawley rats to 1 day-stress or 3 day-stress (they coined 1DS and 3DS, respectively), each session-day consisting in electrical shocks given to the tail of the animals (40 pulses, 3 seconds, 2 mA shocks of a duration of 200 ms, over 2 hours), while they were restrained in a tube (88).

Auditory startle was increased relatively to control rats in shocked animals at 4, 7, and 10 days post-stressor. This effect was transient, at day 7, in the 1DS group, and remanent at 7 and 10 days in the 3DS group. Basal plasma corticosterone was transiently increased at day 1 in both stressed groups (though to a lesser extent in 1DS), but did not differ from controls 4 days post-stressor (88).

They discussed and defended the validity of their model: “animal models (...) evaluated on two fronts: similarities in cause [i.e. construct validity, our note] and the degree of resemblance between abnormalities in the animal model and the objective signs of PTSD [referring to behavioral and physiological signs; equivalent of face validity; our note]”. Support for their model was argued around sensitization processes (explicitly named as such), on the basis of this article and previous work of the group using the same paradigm showing increased level of corticosterone, CRH and ACTH, or suppressed open field activity. With these modifications present in various combinations in their unique (1DS) and repeated stress paradigms (3DS), they argue that those represent best isolated trauma (giving the example of rape) or repeated trauma (example of combat), respectively (88).

This group of scientists had indeed used the same protocol to study the effects of repeated stress (3DS) on behavior and plasma cortisol levels (90) and of single (1DS) and repeated stress on plasma cholesterol (91) and had argued that these may be relevant to PTSD (90). However, their 1995 article explicitly claimed for the first time the design of an animal model of PTSD.



Servatius and colleagues referred to previous theoretical work, of which the article by Kolb has been reviewed above ((52), chapter 2), to describe permanent non-associative dimensions of PTSD, i.e. sensitization (increased startle, sympathetic hyperreactivity, behavioral hyperresponsivity): “PTSD can be characterized by persistent tonic sympathetic activation and sympathetic hyperreactivity to stimuli associated with the precipitating stressor” (88).

The second article was published in 1996 by Pynoos, MD, a psychiatrist and specialist of child trauma and his colleagues at UCLA (89).

Pynoos and colleagues applied to adult male C57BL/6 mice a single 10-second bout of electrical footshocks (2 mA; the “traumatic stress”), and imposed situational reminders of the trauma to “prevent desensitization, which might occur if the mouse entered the shock compartment and did not receive a shock; and, second, to develop an analogue that resembles a common posttraumatic human phenomenon in which traumatized individuals are often confronted with reminders of a traumatic event, but not, except for repeated trauma, with a replication of the traumatic moment(s)”. Reminder exposure consisted in placing animals in the chamber adjoining the shock arena. Four groups were tested: shock/no shock with or without situational reminders. Pynoos and colleagues examined the behavioral effects of this procedure 24 hours, 3 weeks and 6 weeks later. They called the protocol “Time Dependent Sensitization”, without the article containing the term “inescapable shock” (89).

Locomotor activity increased initially in shocked animals, but this effect went away by the third week of testing, a phenomenon they interpreted as transient hypervigilance and/or lack of habituation in the context of acute stress. Animals exposed to shocks displayed increased “abnormal behavior” (statistically-based deviation from the control), an effect that was enhanced by situational reminders. Thus, mice exposed to shocks and situational reminders showed bidirectional responses (more or less entries in open arms), behavior they likened to human fearfulness/anxiety. Exposure to situational reminders was associated with a progressive increase over time in the magnitude of the startle reflex, while other groups showed no change from baseline. The authors interpreted this as time-dependent heightened arousal (89).

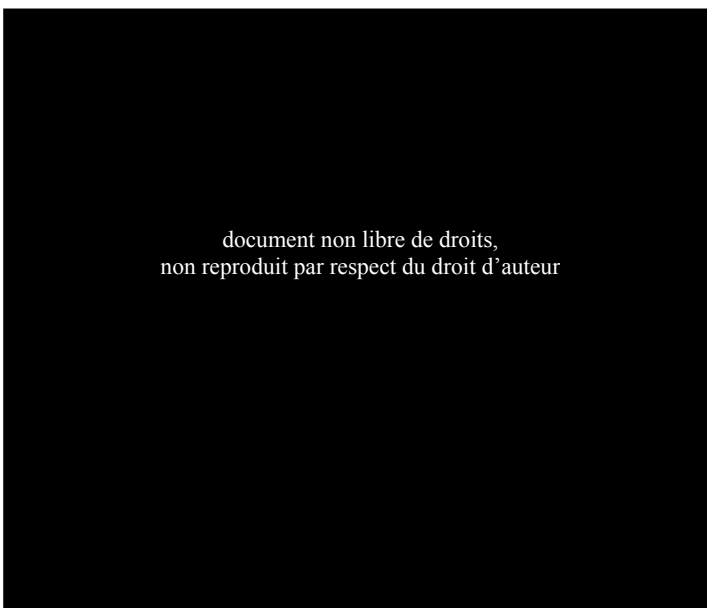
The validity of the paradigm was exclusively assessed with Yehuda and Antelman’s 1993 criteria (66), all of which they claim to fulfilled with their “Time Dependent Sensitization” protocol.

As for protocols from which that of Pynoos and colleagues derived, they refer to time dependent sensitization protocols used by Antelman and colleagues (e.g. Antelman *et al.*, 1991 (92)), but those used immobilization stress. In fact, it seems that unlike other groups, Pynoos and colleagues specifically designed the experiment to model PTSD.

Regarding conditioning, Pynoos *et al.* (89) distinguished the startle increase following shocks and situational reminders from fear-enhanced startle. They also made a surprising distinction between situational reminders, that could be conceptualized as a conditioned context, and conditioned stimuli: “we believe that this [Time Dependent Sensitization] model can provide new avenues for the investigation of PTSD. These include investigation of the effect of frequency and duration of exposure to reminders, the effects of different types of reminders (i.e., situational vs. reminders linked to the aversive stimulus by conditioning)”.

Interestingly, in their discussion, they reported additional observations, one of which is reminiscent of defensive behavior/freezing during situational reminder (“a state characterized by increased breathing and huddling near the back wall”; (89)). The authors did not exploit this observation as a resembling associative aspects of PTSD.

The inescapable stress paradigm -that has also been conceptualized as and called “learned helplessness”- can be traced back to work of Weiss and colleagues in 1970. These authors studied coping, and/or depression in response to repeated stress ((93); Figure 8). Servatius and colleagues defended the relevance of their model despite its resemblance with models of depression, in fact turning this as an advantage “both [PTSD and depression] appear to be characterized by dysfunction of the physiological stress-response systems.” (88). This pre-existing inescapable shock/learned helplessness model had been suggested to model PTSD by Krystal (56) and Foa (62) and their colleagues, and had been criticized by Yehuada and Antelman ((66), see previous chapter). Surprisingly none of these authors were cited by Servatius or Pynoos.



**Figure 8: Original figure illustrating the learned helplessness experiment of Weiss and colleagues; 1970 (93).**

Rats in the foreground could escape electrical shocks while rats in the middle (yoked) could not escape. The last rat served as no-shock control.

## Single prolonged stress (1997)

Single prolonged stress is one of the most commonly used PTSD model (see chapter 4). It was first introduced by Liberzon and colleagues in 1997, who proposed it might represent an animal model of PTSD-specific neuroendocrine abnormality (94,95). Liberzon, psychiatrist MD, was then in charge of a VA clinic in Michigan. The model is articulated around the construct validity of a single stressful event and the face (mechanistic) validity (our note) of enhanced negative cortisol feedback on HPA axis. Sensitization, increased reactivity, is the central concept of the study, which compared the enhancement of HPA feedback to other forms of sensitization: behavioral (e.g. aggressivity) or neurochemical (exaggerated catecholamine response). Liberzon and colleagues wrote “an animal model that would mimic HPA abnormalities found in PTSD and could reproduce sensitization of both facilitatory and inhibitory elements of stress systems would therefore constitute a good model for [PTSD]”.

The protocol of Liberzon *et al.* consisted in submitting adult male Sprague-Dawley rats to a “single session of prolonged stress” consisting in 2 hours of restraint in a Plexiglas apparatus, followed by forced swim for 20 minutes, and after a 15 minute-recovery period, by exposure to ether vapors until loss of consciousness. Adrenocorticotropin (ACTH) levels were measured during the initial restrain task, and intraperitoneal cortisol was given to assess HPA feedback. After a week of rest, animals were challenged to a restrain test (restress), in order to evaluate again the magnitude of HPA response and feedback strength in response to cortisol administration (Figure 9 from Liberzon *et al.*, 1997; (94)). Adrenocorticotropin hormone levels were similar at stress and restress, but ACTH levels were more reduced at day 7 in stress-restressed than in naive rats stressed for the first time. The authors concluded that SPS sensitizes the HPA feedback response and that SPS might constitute a valid model of PTSD, provided additional studies measuring of behavior and other biological parameters were performed. Remarkably, the SPS protocol described in 1997 is still used unchanged twenty years after. Interestingly, the apparent oxymora “single prolonged stress” is explained by the contraction of its original form “single session of prolonged stress”.

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### Figure 9 : Study diagram of Liberzon *et al.*, 1997.

Note the restress session includes restraint stress only. The sequence of the stressors, the length of exposure and the timing of the plasma samples during the single prolonged stress are shown. The timing of the blood samples during the restress session is similar to the single prolonged stress. (Figure and legend as in Liberzon *et al.*, 1997 (94)).

The SPS protocol was derived from previous work of the team. “In our earlier studies, we examined effects of stress on oxytocin receptors in hippocampus and used the stress-restress paradigm that included single prolonged stress followed by 1 week of rest and then a restress session.” (94). Indeed, they had used non-habituating stress (7 days of cold, restrain, forced swim and ether) to study the effect of stress on oxytocin binding in the hippocampus (96). However, no previous study of theirs was found using discrete stress-restress exposure prior to the 1997 article (94).

Regarding relationships with 1980-1993 literature, Liberzon and colleagues claimed inspiration from Yehuda and Antelman (66). Liberzon and colleagues criticized other models, in particular inescapable shock, for lacking validity with respect to this enhanced feedback, and here again, to support their position, they cite Yehuda and Antelman: “early animal models, like Inescapable Shock-Learned Helplessness models, while having good face validity, failed to reproduce the HPA axis changes characteristic of PTSD (for detailed discussion of this topic see Yehuda and Antelman (1993))”.

The group of Krystal is cited for the proposal that CRF abnormalities in the amygdala underlay PTSD symptomatology in Charney *et al.*, 1994 (97), an essay very similar to their previous review ((61); see chapter 2). Overall, references to earlier theoretical work were restricted or distorted to support their model. Charney and colleagues were credited for only one of their many PTSD theories without reference to alternatives (e.g. fear conditioning through the amygdala, sensitization of the catecholaminergic systems etc.) More intriguing is the fact that no reference to “stress-restress” paradigms was found in Antelman’s or Yehuda’s articles (66,98), while Liberzon and colleagues claimed they followed this link (94).

The SPS paradigm is considered as a sensitization method (see chapter 2 for definition, and Figure 6). Finally, fear conditioning is not mentioned in Liberzon *et al.*, 1997 (94).

### Immobilization stress (2001)

The immobilization stress paradigm (IMO) has been developed to study PTSD after year 2000, by two groups: Armario and colleagues, a group of researchers in experimental psychology, in Barcelona, Spain: (Marti *et al.*, 2001; (99)) and Shinba, physician-scientist in psychiatry and his colleagues in Tokyo, Japan (Shinba *et al.*, 2001; (100)).

Shinba and colleagues explicitly claimed they “used stress-induced locomotion reduction in the rat as an animal model of PTSD”, the stressor being IMO. The validity of the model was discussed by the sensitivity of the stress-induced behavioral responses to anxiolytic agents, their ability to decrease avoidance, and the increased mobility in forced swim test (citing three articles by Van Dijken (101–103)). However, the three articles used inescapable shock as a stressor, and Shinba *et al.* using these references represents a conceptual assimilation of various *stress*-induced reduction in locomotion.

Shinba and colleagues submitted male Wistar adult rats to 8 minutes restrain in plastic boxes of 8x15x5 cm dimensions, and injected intramuscular clonidine or saline (control) immediately after. Rats displayed a moderate reduction in spontaneous locomotor activity in a rectangular open space (60 x 25 cm) that was prevented by clonidine injection. “Locomotor reduction [being] related to increased fear/anxiety” (100), the authors concluded that clonidine administered acutely after trauma may be useful in preventing PTSD symptoms. The task used in the article by Shinba and colleagues was designed specifically for this study, but they cited several other groups that used intense IMO to study biological responses to stress (e.g. Carli *et al.*, 1989(104)). Although IMO and their result can be assimilated to sensitization, Shinba and colleagues did not refer to any earlier theoretical work on PTSD models.

The second study was published in the same year by Armario and colleagues, who used immobilization stress to model the sensitization of HPA axis in response to single exposure to intense stressors (99). They submitted adult male Sprague-Dawley rats to one-hour immobilization “in prone position on wooden boards, by attaching their four limbs to metal mounts using adhesive tape”, and tested the effect of subsequent IMO on HPA function.

Stressed rats displayed accelerated termination of the ACTH and corticosterone elevations in response to the second IMO session, an effect that was greatest after >24h (measured at day 1, day 7 and day 13). Exposure to forced swim after IMO did not induce this phenomenon. The authors interpreted their finding as a stressor-specific learned *desensitization* and proposed it could be relevant to study PTSD.

The stress paradigm of Armario and colleagues had been developed earlier to study the effects of acute stress on hormonal (ACTH, growth hormone, thyroid-stimulating hormone, Armario 1989 (105)) and behavioral responses (exploration, forced swim test, (106)), and used identically in 2001 (99).

Relevance for PTSD was discussed in Armario and colleagues’ article on the sole basis of Yehuda and Antelman’s 1993 theoretical article (66): “the present changes in the HPA axis after a single stress session meet some of the most relevant criteria for an animal model of PTSD as stated by Yehuda & Antelman (1993): its appearance with a brief exposure to an unusual situation; its positive relationship with the intensity of the stressor; and its enhancement with time” (99).

Importantly and in contrast with Shinba *et al.* (100), IMO has not been claimed to be an explicit animal model of PTSD by Armario and colleagues, but has only been discussed to represent a potential model to study PTSD. They proposed IMO as a sensitization paradigm that is, moreover, not sufficient by itself to attain face validity. Immobilization stress indeed requires a subsequent stressor to trigger PTSD-like behavioral responses, as indicated by the authors in a subsequent article (107).

Fear conditioning was not mentioned or discussed in any of the two articles (99,100), despite stressor-specific responses to restress described in Marti *et al.*, 2001 (99).

### Fear conditioning (2003-2005)

All of the previous paradigms are based on the sensitization, non-associative learning theory of PTSD. Surprisingly, it is not until 2003 that experimental paradigms using fear conditioning have been claimed to model PTSD. Four articles have been found that are frequently cited by review articles (e.g. Siegmund and Wotjak, 2006; Berardi *et al.*, 2012 (34,82)) and indeed have been found after examination to describe fear conditioning-based experimental models of PTSD. These studies were published by Balogh and Wehner (108), Debiec and Ledoux (109), Pawlyk *et al.* (110), and Rau *et al.* (111).

Balogh and Wehner, researchers in experimental psychology at the university of Boulder, Colorado, published the first of these four studies, in 2003 (108). They submitted adult male mice of 10 strains to classical -pavlovian- cued fear conditioning (1 or 2 pairings of a sound with mild footshocks: 2 seconds 0,7 mA). They then tested animals for fear responses, measured with freezing, in contextual (re-exposure to context without CS or US), cued (CS in a novel environment) and altered contextual settings (pre-CS in the novel environment), at 1 day, 14 days and 60 days. Observing heterogeneity of behavioral responses across mice strains was used by the authors to highlight that variation in stress responses may be useful to study the neurobiology and genetics of memory, and to study anxiety disorders, including PTSD.

In particular, fear responses of several mice (measured by freezing), increased at day 14 in all settings, including altered context, indicative of fear generalization. It is this dimension they proposed useful to study PTSD.

They discussed the links between PTSD and fear conditioning following previous work, as reviewed in chapter 2. In particular, they cited Pitman's 1989 paper to illustrate that "Some [authors] argue that conditioning models are limited in the scope of the symptoms they help describe and are therefore of limited utility", largely ignoring other articles by the same author (e.g. Pitman 1989, Shalev *et al.*, 1992; (51,53)). Kolb, on the other hand, would represent supporters of the conditioning theory "Despite this, reports suggest that conditioning models may help to explain at least some aspects of PTSD-related symptomatology" (108).

Somewhat surprisingly, the value of their paradigm to model anxiety disorders including PTSD is oriented toward the generalized fear behavior displayed by some mouse strains. As such, they 1- asked in introduction "is 14-day hyper-responsiveness observed previously a normal configural learning phenomena exhibited in all strains or does it represent a potentially pathological condition similar to PTSD and occur only in selective genotypes?"; and 2- concluded that regardless of the debate

surrounding the usefulness of conditioning to model PTSD, their result of enhanced fear in the form of generalization in some mouse strains “appear to model several key features of PTSD”.

They used Yehuda and Antelman’s criteria (66) to defend their model: “generalized fear observed 14 days after contextual fear training meets all of the criteria for a suitable model of PTSD. That is, the 14 day generalized fear: (1) develops after exposure to a brief, but poignant stressor; (2) occurs in a dose-dependent fashion (one versus two shock exposures); (3) is not apparent until approximately 1 week after training, but then persists for approximately 3-4 weeks; (4) manifests itself as increased freezing, as well as increased startle responses; (5) is mediated in part by genetic differences” (108).

The second article using fear conditioning to model PTSD was published in 2004 by Debiec, MD-PhD, a physician-scientist in psychiatry and Ledoux, PhD, a leading figure in fear conditioning research. Their article proposed that blocking reconsolidation of conditioned fear memories with propranolol may be of therapeutic interest in PTSD. The initial aim they set was to “reexamine the effects of blockade systemically and within the basolateral amygdala on the consolidation of auditory fear conditioning” (109).

They submitted adult male Sprague-Dawley rats to cued fear conditioning. Rats were given a single conditioning trial consisting of a 30 seconds, 5 kHz, 75 dB tone (CS) that co-terminated with a 1.0 mA, 1-second electrical foot-shock (US). Memories were reactivated by presenting 1 CS to rats one day after conditioning. Systemic and intra-amygdala propranolol infusion immediately after reactivation impaired cued fear expression, while the same infusion immediately after conditioning had no effect. This effect was resistant to passage of time (studied and observed up to 1 month after) and resistant to fear reinstatement. The authors concluded that “noradrenergic transmission is not required for consolidation of auditory fear conditioning, but once a memory is reactivated, noradrenergic signaling plays a critical role in the reconsolidation of retrieved memory”, and that propranolol may be useful for PTSD treatment.

Debiec and Ledoux used fear conditioning to propose treatment for PTSD, but surprisingly did not discuss predictive validity or any other validity criterion of their model. In fact, they only discussed the links with PTSD in an implicit manner: “If the present results are confirmed in studies of humans, propranolol may become a useful tool in treating recurring disruptive emotional memories in [PTSD]” (109). Indeed this theory is reminiscent of the proposal according to which conditioned fear mimics re-experiencing symptoms of PTSD (chapter 2). We have found no evidence for discussion of previous theoretical work in Debiec and Ledoux’ article.

The third article using fear conditioning to model PTSD was published in 2005 by Ross, a physician-scientist (MD-PhD) in psychiatry at University of Pennsylvania and Veterans Affairs medical center, and his colleagues. They used contextual fear conditioning to “provide insight into the neural substrates of disturbed sleep in PTSD” (Pawlyk *et al.*, 2005 (110)).

To mimic the effect of PTSD on sleep, they induced contextual fear conditioning in adult male Sprague-Dawley rats by administering footshocks without (0.5 mA; 0.5 seconds) randomly at 3–6-minute intervals over a 30-minute period in a distinctive context. Then, they tested sleep architecture with polysomnography (electroencephalogram, electromyogram) the next day in a novel or the same chamber. Their main result is that fear conditioning reduced the number and duration of Rapid Eye Movement sleep epochs; a result they interpreted as the potential effect of trauma reminder on sleep in PTSD. They claimed that this was the first report of a days-long effect on sleep architecture of a single session of footshock training in rats: “this is the first report that situational reminders, alone, of a fearful training context can result in alterations of sleep architecture in animals. We believe that this model has great potential for studying the effects of stress, anxiety, and fear on sleep, and especially on their interrelationship in PTSD”(110).

They discussed the relevance of their protocol in the introduction, by likening conditioning and trauma: “fear conditioning might be particularly pertinent to the study of PTSD, which by definition is a disorder occurring in the aftermath of a psychologically traumatic event and must therefore involve learned neurobehavioral responses”. And insisted on the importance of sleep: “we and others have suggested that sleep disturbances, especially recurrent anxiety dreams, are a hallmark of PTSD”, which in fact deals with reexperiencing symptoms, as further indicated by this note “We sought a paradigm in which the recall of fearful memories would influence sleep only when situational reminders are present” (110). This paradigm is reminiscent of the conditioning theory of associative, reexperiencing symptoms (chapter 2), here inducing sleep disorganization. However, none of the articles reviewed in chapter 2 are mentioned by Pawlyk *et al.* Instead, they refer to Pynoos *et al.* (89), for fear conditioning paradigms to model PTSD in humans, while these authors distinguished their protocol from fear conditioning (this chapter).

Finally, the fourth article explicitly using fear conditioning to model PTSD, and often cited for this, was published in 2005 by Fanselow and his colleagues at University of California, Los Angeles (Rau *et al.* 2005, (111)). Fanselow, a scientist in experimental psychology is another leading figure of fear conditioning research. Although it is often cited for modeling PTSD with fear conditioning, it is the non-associative, sensitization dimension of their paradigm, that the authors argued could model PTSD. As a stressor prior to fear conditioning, they submitted adult male Long Evans rats to 15 1-second electrical footshocks of 1mA, over a period of 93 minutes. This stress enhanced the freezing response of rats one day after contextual fear conditioning elicited by 1 shock in a novel context. The authors referred to this effect “stress-induced enhancement of fear learning”. It was resistant to extinction in the first context, and to NMDA receptor blocker APV injected in cerebral ventricles prior to the first stressor.

The initial 15 shocks stress also enhanced cued (auditory) fear responses. Fanselow and colleagues concluded from these experiments that exposure to shock enhances fear learning (111). Interestingly



and because of the resistance to initial extinction and blockade of NMDA-R, they proposed sensitization was independent of learning, and hypothesized that kindling might be responsible for sensitization, referring to Goddard's seminal article ((76); see chapter 2).

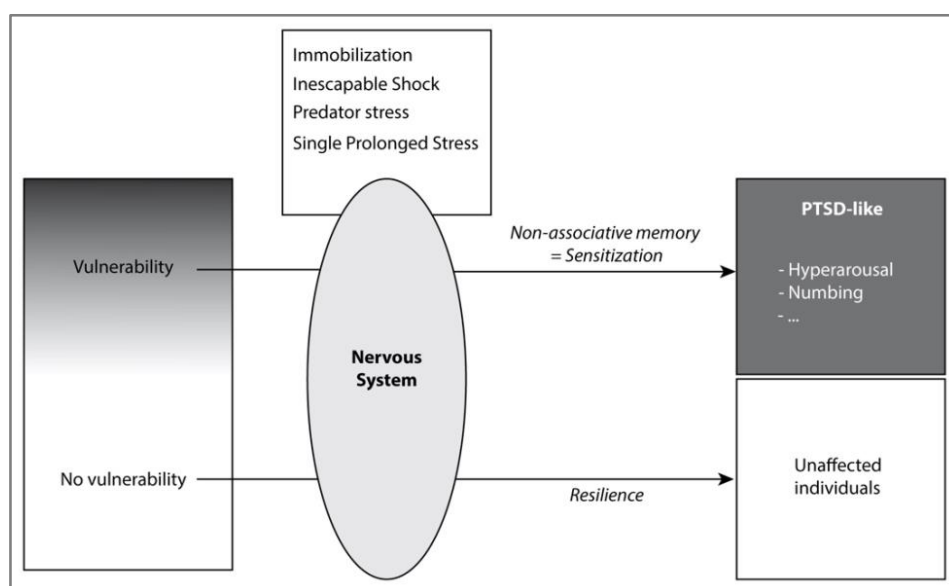
In the article by Rau *et al.*, fear conditioning was only used to assess the sensitization effect of the original stressor, mimicking trauma, as they indicate at the end of the introduction (111). Fanselow and colleagues actually criticized the fear conditioning model: "it is unlikely that simple fear conditioning alone provides an adequate model of all of the complexities of PTSD. In this paper, we develop an animal model that retains the analytical power of Pavlovian fear conditioning but also appears to capture some of the symptoms of PTSD". They stated that "this procedure may serve as a model of certain features of PTSD, namely the ability for a mild stressor (in this case a single shock) to trigger behavior that is more appropriate to the prior trauma (the multi-shock session) and second, the propensity for PTSD patients to form other phobias. Importantly, like PTSD, these effects were induced by exposure to a highly aversive experience but the impact went beyond the context of the original stressor". They also defended that variability in their dataset may pertain to individual differences in PTSD "as in PTSD, there are individual differences in our model in that one of eight rats did not show the sensitization effect".

They cited the group of Krystal for symptoms of PTSD and of Foa for extinction therapy, topics not directly related to theoretical considerations for PTSD modeling (chapter 2).

Overall, these 4 articles have shown diverse standpoints regarding the links between PTSD and fear conditioning. Balogh and Wehner used classical fear conditioning but proposed non-associative generalization to model PTSD (108). Debiec and Ledoux proposed that blocking reconsolidation of conditioned fear pharmacologically might be useful for PTSD treatment without explicitly stating that fear conditioning is a PTSD model (109). Rau *et al.* aimed to demonstrate the potential of initial intense stress resembling the inescapable shock paradigm to sensitize fear conditioning (111). Only Pawlyk used classical contextual conditioning to explicitly model PTSD for its associative dimensions, by describing alterations of sleep following trauma reminder (110). Surprising is also the relative lack of conceptual discussions and references to previous theoretical work in these 4 articles. The heterogeneity in cued vs. contextual conditioning, explicit vs. implicit modeling, and use of fear conditioning paradigms to induce associative or non-associative behavior, adds to this lack of conceptual consideration, resulting in vagueness.

## Summary

Reviewing the 1990s-early 2000s literature showed that experimental model of PTSD directly resulted from theoretical considerations reviewed in Chapter 2 (1980-1993), when authors proposed alternative or complementary paradigms to fear conditioning in order to model PTSD. As a result, creators of original PTSD animal models insisted on the sensitization dimension (Figure 10), even claiming at times the absence of conditioning in their models was an advantage (83). Most of these models were modified versions of earlier models used by the same scientists to study “stress”.



**Figure 10 :**  
**modeling PTSD**  
**with sensitizing**  
**stimuli**

As might have been expected, the development of PTSD models coincided with the beginning of the evaluation of their validity. The creation of animal models of PTSD and evaluation of their validity has been greatly influenced by a single article, published by Yehuda and Antelman in 1993 (“Criteria for rationally evaluating animal models of posttraumatic stress disorder”, (66)). This influential work has been cited by most articles reviewed in this chapter (and has been cited over 200 times until 2019; sources: ISI web of knowledge and Google Scholar). Of note, validity criteria for animal models in psychiatry had started to be formalized during the previous decade (reviewed by Belzung and Lemoine (32)). The first evidence of an explicit mention to “validity criteria” of PTSD models was found in the article describing SPS by Liberzon and colleagues (94).

Moreover, we have found no evidence for the use of classical fear conditioning before 2003, and instead some explicit criticism of this model (e.g. Adamec and Shallow 1993; (83)), leaving a temporal gap between theoretical proposals (chapter 2) and experimentally modeling PTSD with conditioning. Heterogeneity in applications and interpretations of fear conditioning-based models from the beginning has laid the ground for conceptual vagueness. This vagueness aggravated in the following decade. Chapter 4 presents this evolution.

## IV- Evolutions of PTSD models: toward the current use of fear conditioning in PTSD research (2005-present)

*“While fear conditioning alone is not a model of PTSD, it is used to assess abnormalities in fear learning that are associated with PTSD.” Lisieski, Eagle, Conti, Liberzon and Perrine, 2018*

Fear conditioning experiments have gained considerable importance in preclinical research on PTSD, representing one of the leading experimental and conceptual paradigms (introduction).

A bibliometric evaluation indicated that a quarter of all studies referring to animal models of PTSD also mention fear conditioning. Indeed, we have found 544 articles on Pubmed of animal research until 01/01/2019, that contained the terms "model" and "PTSD". Of those, 139 references (25,6%) also contained the terms "fear conditioning" or "conditioned fear" or "cued fear" or "contextual fear" or "pavlovian". In comparison, references to other dominant models of PTSD were found as frequently, at most, including “single prolonged stress” (n= 135; 24,8%), “predator” or “predatory” (n= 83; 15,3%), “inescapable” (n= 46; 8,5%), and “immobilization” (n= 35; 6,4%).

Conditioning had been proposed shortly after the recognition of PTSD to explain part of the condition (chapter 2). However, initial models of PTSD were derived from the sensitization theory, with the late addition of fear conditioning-based models, from year 2003 (see chapter 3).

Nowadays, fear conditioning has become a dominant heuristic in PTSD research (29). Current experiments based on fear conditioning to model PTSD are diverse, ranging from classical fear conditioning to the sequential combination of stressor-based model and *de novo* classical fear conditioning. Seemingly abrupt transitions from original theories, via stressor-based models, to this complex landscape hinders conceptual understanding of the fear conditioning model. It is of paramount importance for progress of research in the field of PTSD to critically study the conceptual spans of different conditioning variants, their relations, and their links with earlier studies (chapter 2 and 3).

This chapter describes developments of the fear conditioning-based models of PTSD from 2005, twenty-five years after the recognition of PTSD (DSM-III, 1980), fifteen years after theoretical proposals to model PTSD with conditioning and sensitization (chapter 2), and following the creation of animal models dedicated to the study of PTSD (see chapter 3). To understand the evolution of the field of PTSD models from largely ignoring fear conditioning in the (1990s-early 2000s; chapter 3) to its current frequent and diverse uses, a qualitative, non-systematic literature review was carried out. Existing review articles were used as a starting point (1–4). Original research articles were studied to clarify a conceptual issue, when necessary.

This review showed that classical fear conditioning has evolved from theoretical proposals (Chapter 2) and early animal models of PTSD (Chapter 3), in three main ways:

- Integrated within stressor-based PTSD models, by modification of the latter, in order to introduce a conditioning dimension
- Integrated within stressor-based PTSD models by juxtaposition of fear conditioning *de novo*
- Alone, mostly in modified versions of classical fear conditioning paradigms.

This chapter presents variants of fear conditioning-based models of PTSD in this order, analyzing their links with previous work, and the conceptual content at stake. Results suggest that heterogeneous uses of fear conditioning with distinct conceptual correlates, and conceptual extrapolation of fundamental research, has led to a state of ambiguity and vagueness. Paradoxically, we found that current theoretical accounts of PTSD are largely based on the fear conditioning heuristic.

### Integration of fear conditioning in dedicated PTSD models

Many researchers have considered fear conditioning useful to model PTSD, but insufficient to account for non-associative aspects of the disorder (chapter 2). As Siegmund and Wotjak stated: “Fear conditioning paradigms are the first choice for memory-related aspects of PTSD-like symptoms. ... Used as the only behavioral readout, however, fear conditioning paradigms resemble symptoms of a specific phobia rather than those of PTSD, and have therefore to be extended by behavioral tests that reflect the non-associative fear component of the disorder, too”. They concluded that “the complexity of brain pathology underlying PTSD [could] only be understood if the complex interrelation between associative and non-associative processing (...) [was] considered” (82).

In this context, fear conditioning has been integrated to animal models based on sensitization that were reviewed in chapter 3, namely, immobilization stress, inescapable shock, predator stress and single prolonged stress. Integration of fear conditioning with previous animal models of PTSD was done either with modifications of stress-based models to test for the conditioning dimension induced by the initial stressor (mimicking trauma), or directly, with the addition of classical fear conditioning *after* stress induction – an experimental setting referred to as “*de novo* fear conditioning” (112).

## Transformation of dedicated PTSD models into fear conditioning paradigms

The first way fear conditioning has been integrated to pre-existing sensitization models of PTSD has been by modifications of experimental protocols, as to be able to induce and measure a fear response to the original stressor or stressor context. Such stressor reminder-induced responses have been conceptualized as *conditioned* responses.

Modified models of PTSD using predator stress have included the conditioning dimension, although initial versions of predator stress were entirely based on the sensitization model (Adamec and Shallow 1993; chapter 3). Some examples are given here, although more have been published (e.g. MacKenzie *et al.* 2010; (113)). Including the conditioning dimension has been achieved by pairing cat/cat odor exposure with context or discrete cues, and testing for conditioned responses, in addition to measuring non-associative, sensitized fear.

### *Predator stress*

Zohar and colleagues modeled trauma in rats with a 10-minute exposure to a soiled cat litter, and tested rats for PTSD-like symptoms of sensitization (elevated plus maze and acoustic startle response -grouped by us under the name “sensitization”) and “trauma-cue vulnerability” (Zohar *et al.*, 2008 (114); no name given in Cohen *et al.*, 2006 (115)) the measure of which was “freezing behavior [in response to] situational/trauma reminder” (a clean litter) (114,115). All parameters were enhanced by exposure to cat litter. Freezing evoked by situational reminder was called “Pavlovian conditioning” in the discussion section of Cohen *et al.* 2006, while no mention was made to conditioning in Zohar *et al.*, 2008 (114,115).

Zoladz and colleagues used a slightly different procedure. To include a conditioning dimension in their paradigm, they modified their model of predator stress by adding exposure to a cue (neutral sound) immediately before exposure to cats (116). They named the process “predator-based fear conditioning”. Trauma was induced by two sessions of 1 hour protected cat exposure and social instability (daily change of littermate). “Traumatic memory” and “general anxiety” behaviors were measured, with exposure to the sound and exposure to the cat exposure environment, and the elevated plus maze test, respectively. Relative to unexposed controls, cat-exposed rats displayed enhanced cued and contextual freezing responses, a phenomenon the authors interpreted as “classically conditioned fear memory”, as well as anxiety-like behavior in the elevated plus maze (116).

As exemplified by these studies, with additional measures of fear of the reminders (114,115) or to the context where rodents were exposed to predators (116), or by pairing of the predator exposure with a neutral cue and subsequent exposure to the cue (116), the modified predator stress paradigm may be considered as a modified form of fear conditioning. There, the UCS is the predator exposure.

### *Single prolonged stress*

The SPS model has also been modified to comprise fear conditioning. Liberzon and colleagues have assessed trauma-related reactions in the SPS paradigm in one review article, by re-exposing animals to forced swim test 7 days after SPS. Rats showed greater immobility in the SPS group but no difference in tail-suspension test (as control for despair), indicating SPS can trigger “trauma”-related fear (117). The authors concluded in the review that “there [was] accumulating evidence that SPS rats exhibit symptoms of increased arousal, such as exaggerated fear responses to *trauma-related* and -unrelated stimuli”. This statement on “trauma-related stimuli” was supported by small evidence (one experiment, published in a review article, with a small number of animals (n=10 vs 10) and scant description of methodology). Nevertheless, if SPS is followed by trauma-related fear, then SPS might be thought of as a modified fear conditioning procedure by itself, where SPS represents the UCS.

### *Inescapable shock*

The most thorough study using a modified sensitization-based model has been published in a seminal article by Siegmund and Wotjak in 2007 ((118); an article cited over 130 times -ISI Web of knowledge). These authors modified the inescapable shock paradigm to integrate contextual fear conditioning, and explicitly evaluated the validity of their model using a battery of behavioral tests. They stated that on the basis of previous work (citing Yehuda and Antelman, 1993; (66)) they “aimed at establishing a mouse model of PTSD that meets the following criteria:

- 1- Face validity: a short stressor should induce symptoms (i) in a dose-dependent manner that (ii) persist or even increase over a considerable length of time, (iii) include core features of PTSD, i.e. signs of exaggerated response to trauma cues and hyperarousal as well as signs of emotional numbing and social withdrawal. The phenotype should (iv) vary between the animals, allowing to assign them to affected (vulnerable) and non-affected (resilient) individuals.
- 2- Predictive validity: Core features of the phenotype should respond to common pharmacological (i.e. chronic SSRI) treatment.
- 3- Utility: a stressor should be utilized that can be systematically varied in intensity without inducing habituation. It should be possible to test the behavior of the mice repeatedly in order to assess long-term extinction (associative) and long-term habituation (non-associative) processes.”

They submitted C57BL6 mice to a single 2-second footshocks of 1.5 mA intensity, representing trauma. When placed back to the same context, shocked mice displayed more freezing than control animals, a finding interpreted as an evidence of contextual fear conditioning, modeling associative memory of trauma, that is, reexperiencing. This effect was observed with a stable intensity during the

entire time range of testing, up to 28 days. It was dependent on the intensity of electrical shocks (from pain threshold to 1.5 mA).

Mice exposed to electrical shocks also displayed greater startle responses to neutral acoustic stimuli, that the authors interpreted as reflecting “fear [or stress] sensitization”, modeling hyperarousal. This effect increased with time, in form of an “incubation”. In the C57BL6N mouse strain, sensitized and conditioned fear persisted on repeated testing at 6 days without habituation or extinction, respectively. In contrast, another strain of mice (C57BL6/O) displayed lesser conditioning and sensitization, and those extinguished and habituated, respectively (119).

Increase of associative and non-associative forms of fear was reversed by administration of fluoxetine in drinking water for 3 weeks followed by 1 week of washout. The authors further separated mice on the basis of their freezing levels in high freezing and low freezing groups, and showed that the effect of fluoxetine was specific to high freezing mice. On the basis of this selectivity and strain differences The authors proposed that their model was valid for displaying individual variability, modeling vulnerability to develop PTSD.

Additional parameters were investigated. Shocked mice showed reduced exploration and novelty-induced suppression of feeding in a “modified holeboard test”, a finding conceptualized as “neophobia”, and interpreted as similar to emotional blunting in PTSD. Shocked mice also showed a moderate increase in immobility in the forced swim test, the authors proposed to reflect depression, comorbid in PTSD. Finally, shocked mice showed reduced social interactions (118).

This modified inescapable shock paradigm has provided a complete model of PTSD, in categorical terms, and is currently used for instance, for therapeutic design (120). It was built on validity criteria previously defined by Yehuda and Antelman (66), as Siegmund and Wotjak stated here (119) and in their 2006 review “we extend[ed] Yehuda’s and Antelman’s criteria by directing the focus of interest to the hypothesized involvement of two important biological processes, conditioning and sensitization, in the development and maintenance of PTSD” (82). They also referred to Balogh and Wehner (108) and Pynoos and colleagues (89) (see chapter 3) to insist on the similarity of their results with persistent conditioned and sensitized fear, respectively.

It must be noted that despite resemblance with inescapable shock models of PTSD such as that Pynoos and colleagues (89), “inescapable shock” was not explicitly mentioned by Siegmund and Wotjak (119).

Modifying original sensitization-based models of PTSD to include conditioning has greatly increased their validity. Displaying associative and non-associative fear memories in response to a single intense stressor experience and with some individual variability provides good construct and face validity. These forms of fear conditioning to model PTSD can be readily understood conceptually.

Semantic vagueness related to “fear conditioning” appeared in these studies, however. This is exemplified, for instance, by articles referring to predator-based fear conditioning as “Pavlovian conditioning” (115), or “[classical fear conditioning]” (116): “in the present work, we have extended our animal model of (...) resulted in a long-lasting, classically-conditioned fear memory for the predator exposure experiences” (116).

Modified fear conditioning protocols created a semantic problem with major implications. Modified and classical conditioning are conceptually close, but the behavioral paradigms are heterogeneous, raising the possibility that underlying mechanisms are distinct. In line with this, one should not be able to extrapolate pathophysiological mechanisms from classical conditioning to modified models. These extrapolations are the main risk of conceptual assimilation of improved PTSD models and classical conditioning, going back to the origin of the problem (insufficient validity of the conditioning model used alone pointed by these authors).

#### *De novo* fear conditioning in dedicated PTSD models

The second way fear conditioning has been combined with stressor-based PTSD models is *de novo* fear conditioning. *De novo* conditioning consists in submitting animals to classical cued and/or contextual fear conditioning after the application of an initial stressor, for instance, single prolonged stress ((121); see chapter 3 for a description of this and other stressors).

#### *De novo* conditioning in stressor-based models of PTSD

An example of *de novo* conditioning is given by an article of Cohen and colleagues (122) for predator stress. Contextualization deficits, according to the authors, could explain re-experiencing and fear generalization symptoms. They took the example of the sound of an helicopter eliciting fear in a safe environment in veterans with PTSD. They used a conditioned discrimination task to demonstrate deficits in memory contextualization after traumatic predator scent stress (10 min exposure to cat soiled litter). Rats were exposed to a differential contextual odor conditioning paradigm: cinnamon odor was paired with an appetitive cue (sweetened water) in a first environment and with footshocks in a second environment. Rats were tested for fear behavior (freezing) in a third, neutral environment with the same odor. Prior exposure to predator scent stress led to greatly increased fear responses (freezing) in response to the cinnamon smell (arena 3). This was interpreted as a contextualization deficit.

On the basis of this contextualization deficit, the conditioning paradigm was proposed to support face validity of the predator stress model. The authors compared this contextualization deficit to “deficient embedding or contextualization of the traumatic events in autobiographical memory [...] one of the main problems in PTSD”. While their rat data suggests a deficit in contextualization of newly acquired



memories, it does not relate to the initial (predator) trauma. This ambiguous interpretation of *de novo* conditioning represents a conceptual extrapolation.

A different way *de novo* fear conditioning is used in animals is to consider the initial stressor as a vulnerability factor, needing a subsequent trauma to precipitate PTSD-like symptoms. There, fear conditioning can be interpreted as the trauma. This type of paradigm is also referred to as “stress-enhanced fear conditioning”.

This paradigm has been applied by Armario and colleagues for IMO (see chapter 3) “we hypothesize that long-term effects of a single exposure to severe stressors could result in relatively transient (few days) behavioral and physiological alterations when the animals are tested under resting conditions, but could render them, in the long-term, more vulnerable to additional superimposed stressors, a characteristic of some PTSD patients” (107). Of note, an article by Andero (a student of Armario), and his colleagues from the group of Ressler, used the same initial stressor (IMO) to model not vulnerability to trauma but trauma *itself* “valid PTSD-like model requires a highly traumatic stress exposure, much more stressful than other mild stressors such as fear conditioning. IMO is one of the most stressful among the emotional models used in the laboratory”. To support their model, one of the argument was that “previous IMO causes PTSD-like impaired fear extinction” (123). Thus, interpretations of *de novo* conditioning can vary, even within the same group of authors.

Another example is found with the use of fear conditioning after SPS. Many studies have been published that have shown extinction deficits after *de novo* cued or contextual fear conditioning following SPS (117,36). The creators of SPS, Liberzon and colleagues (chapter 3), imply by generalization that showing impaired extinction to newly acquired fear is equivalent to demonstrating impaired extinction of the traumatic memory, while they consider SPS as the trauma (117,124).

On the contrary, an article by the same group considered SPS as inducing vulnerability, rather than a trauma precipitating full-blown PTSD, as suggested by the following: “when rats undergo auditory or contextual fear conditioning 7 days after [SPS], they demonstrate impaired retention of extinction learning and the conditioned fear response persists longer than it does with fear conditioning alone. This approach can be useful for modeling PTSD-like symptoms because those who experience multiple traumas, or a trauma early in life, are more susceptible to developing PTSD following a later traumatic event.” (125). Thus, as for IMO, fear conditioning *de novo* after SPS is also interpreted differently by the same group of authors.

### *Clinical supports to links between fear conditioning de novo and PTSD*

Fear conditioning can be carried out in human subjects with their consent, by application of mild electrical shocks to fingers (e.g.) coupled with presentation of CSs such as visual targets or sounds. Fear conditioning *de novo* is justified mainly by *face validity* arguments, based on laboratory observations of increased conditioning and/or impaired extinction in subjects with PTSD (28) that can be replicated in animal models (36). Impaired processing of conditioned fear can be considered as a symptom of PTSD, an interpretation proposed in the first study of this kind, by Grillon, Krystal's colleague (126). Conditioned fear extinction can also be used as a way to model exposure-based (extinction) therapy, as proposed by the same group (127).

Construct validity can be argued. Mechanistic validity is supported for the activation of comparable brain regions in PTSD and fear conditioning, including amygdala, hippocampus and prefrontal cortex (28). Extinction deficit may also represent a pre-existent, vulnerability factor, that may contribute to the development of PTSD.

Predictive validity may also be argued on the basis of facilitated extinction with SSRIs administration (36).

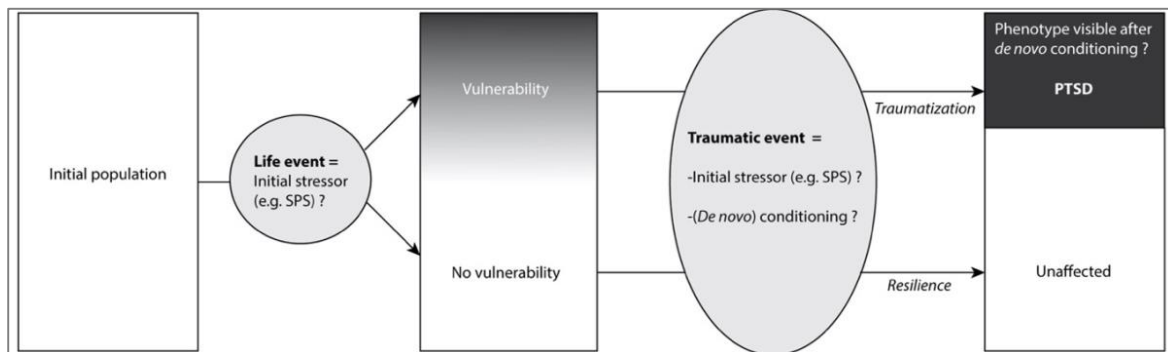
Finally, extinction deficit can be viewed together with fear conditioning as modeling etiology and symptomatic expression of PTSD, in line with initial theories of re-experiencing and avoidance resulting from fear conditioning, and their persistence from impaired extinction, respectively (see chapter 2). This is for instance the position of the authors in the second conditioning *de novo* study in PTSD patients, led by Pitman and colleagues (112). In this case, conditioning is conferred not only *face*, but also *construct* validity.

### *Conceptual issues with de novo conditioning*

The variable conceptual interpretations of fear conditioning by the same group of scientists highlights an ambiguity in the diathesis of PTSD: is fear conditioning the trauma? Impaired extinction a symptom? An epiphenomenon? A general feature that underlies PTSD? Is it acquired in response to trauma or a vulnerability factor? Obviously but importantly, in the case of fear conditioning *de novo* in PTSD, conditioning is not the trauma. With regard to this triggering issue, *de novo* conditioning stands in contrast with earlier work, that directly linked PTSD mnemonic content to conditioning by evoking what was considered conditioned as stress responses to trauma reminders in combat-related PTSD (reviewed in Shalev *et al.*, 1993; (54)) in experimental conditions.

In animals, fear conditioning *de novo* has been considered as a trauma (triggering validity), or its impairments as a symptom of PTSD in a traumatized organism (*face validity*), sometimes with *construct* connotation. Differential use of the task and the concept led to a major ambiguity in the diathesis model of PTSD.

On the other hand, the stressors (predator stress, SPS, IMO, inescapable shock) can be assumed to carry onthopathogenic (vulnerability-inducing preparation, where fear conditioning would be the triggering factor) or triggering validity if the initial stressor represents the trauma (as defined by Belzung and Lemoine; (32)). This ambiguity is illustrated below (Figure 11).



**Figure 11: An ambiguous diathesis with *de novo* fear conditioning.** Fear conditioning *de novo* (after an initial stressor such as Single Prolonged Stress), can be interpreted as a trauma in construct validity, or as a manifestation of PTSD (face validity)

Moreover, lending construct validity to *de novo* fear conditioning in animal models of PTSD represents a form of circular, self-confirmatory reasoning.

Impairments in *de novo* conditioning in the forms of increased fear learning or impaired extinction in PTSD patients can be used to argue in support of the conditioning/extinction deficit theories, but it does not prove them. This was highlighted by Pitman and colleagues “It is tempting to assume that because PTSD by definition is caused by a psychologically traumatic environmental event, any biological abnormality found to accompany PTSD must also have been traumatically induced. However, it is also possible that an abnormality pre-dated the traumatic event and came to be associated with PTSD because it increased the risk of development of the disorder upon traumatic exposure -a psychiatric epitome of gene by environment interaction” (28). These hypotheses cannot be tested directly in Human.

While animal models offer the chance to directly explore conditioning theories in a prospective way, enhanced fear learning or deficient extinction after *de novo* fear conditioning does nothing more than reproducing the limitations of the psychobiological experiments led in PTSD patients. More problematic, if abnormal fear conditioning/extinction is used to assess the validity of a model designed to understand the neurobiology of PTSD, it is very likely that modifications in biological mechanisms underlying fear regulation will be implicated. This type of finding may wrongly lead to conclude on pathogenic mechanisms of PTSD.

## Classical fear conditioning as a PTSD model *per se*

Post-traumatic stress disorder models based on fear conditioning in rodents have appeared in years 2000s (chapter 3). Despite criticism based on their inability to account completely for PTSD symptoms and etiology, classical cued and contextual fear conditioning paradigms have become popular to model some aspects of PTSD. The aim of this section is to give representative examples of various models of PTSD based on classical fear conditioning, and to analyze their conceptual contents.

We have found that classical fear conditioning has been applied using classical conditioning protocols, that can be augmented with biological interventions on experimental subjects (pharmacological, lesional, optogenetic etc.). Many of these models are justified with the validity conferred by deficits in conditioned fear extinction.

Herry and colleagues performed a translational study, including findings in Human and mice (128). The authors explicitly proposed to model PTSD with a fear conditioning paradigm. They submitted adult male C57BL6/J mice to cued (auditory) fear conditioning and extinction. Conditioning was proposed to model traumatic experience, giving construct validity [our note]: “in the laboratory, traumatic aversive experiences are usually induced using the classical auditory fear-conditioning paradigm”. The authors found that animals’ behavior followed a bimodal distribution, with a subset displaying impaired recall of extinction memory (128).

The authors further submitted patients with PTSD to fear conditioning and observed impaired fear extinction measured with subjective, verbal reports, and objective, skin conductance responses. Impaired extinction of fear conditioning was abolished after Eye Movement Desensitization and Reprocessing therapy in the same subjects. The authors used bilateral alternating electrical stimulation of the eyelids in mice in order to mimic stimulations than can be used in EMDR therapy. Compared to controls, the group of mice receiving bilateral stimulation of the eyelid during extinction learning displayed unimodal distribution of low freezing during extinction recall.

Face validity was discussed and defended on the basis of impaired fear extinction recall and individual variability: “these data confirmed previous observations of increased [*de novo*, our note] fear conditioning and delayed extinction in PTSD patients”. Predictive validity was argued because the resemblance of the effect of eyelid stimulation in mice and EMDR in patients on fear extinction recall. This work illustrates the translational potential of fear conditioning to study aspects of PTSD, especially when validity is discussed explicitly. It should be noted, however, that validity and implications of such work are confronted with the same conceptual problems as *de novo* fear conditioning in PTSD (what do fear conditioning and fear extinction deficit represent? Is conditioning a trauma?; this chapter).

Related models of PTSD use variability in fear extinction in the sense that some strains of animals are constitutively prone to extinction deficits. Holmes, Singewald and colleagues found cued fear extinction deficits in the 129S1/SvImJ mouse strain (e.g. Hefner *et al.*, 2008 (129)).

They subsequently proposed that this “poor fear extinction [...] may be useful to model this specific aspect of PTSD” (130). They explained the choice of their model by its mimicking genetic vulnerability to developing PTSD: “with only ~ 9% of individuals who experience a trauma developing full blown PTSD, it is clear that different susceptibility factors predispose individuals to PTSD with genetics likely to be influential”. They added that “The [129S1/SvImJ] mouse strain represents a model of impaired regulation of fear memories seen in PTSD” (130).

Validity criteria are not discussed in O’Connor *et al.* (2019, (130)), but genetic vulnerability refers to construct validity while deficits in fear extinction represents face validity. As in the case of *de novo* fear conditioning (see above), what conditioning itself represents and what impaired extinction may encompass (symptom, pathophysiology, epiphenomenon, constitutive vulnerability) remains unclear.

Another variant of PTSD models based on conditioning consists in combining biological interventions with classical fear conditioning.

For instance, Desmedt and colleagues have proposed to model impairment in restricting fear responses to traumatic context found in PTSD with injections of corticosterone in the dorsal hippocampus after contextual fear conditioning in mice (23).

They submitted groups of adult male C57BL6/J mice to contextual conditioning with electrical footshocks of various intensities (0.3-0.8 mA; 3 seconds), and auditory cues (1kHz, 15 seconds) separated (20-30 seconds) from the shock. Mice injected with corticosterone in the dorsal hippocampus displayed enhanced freezing to context for low-intensity shocks (0.3, 0.5 mA) but reduced freezing to context for high intensity shocks (0.8 mA). Moreover, corticosterone administration after high intensity contextual conditioning increased cued freezing, including responses to cues that were not presented during conditioning but shared some similarities (2kHz), an effect not found for white noise.

The authors interpreted these findings as representing “PTSD-like memory impairments” in that mice showed deficit in restricting fear reactions to appropriate context [D1 criterion of PTSD in DSM-5: inability to remember an important aspect of the traumatic event (2)]. They added “this impairment is very similar to the one observed in PTSD patients in which contextual peritraumatic cues are often forgotten, while salient but irrelevant ones are strongly remembered. These salient cues, and others more or less similar to them, can then induce a strong fear response in contexts different from the traumatic one” (23).

In this work, high intensity shock seemed to have been considered as the traumatic event, but this was not explicitly stated. Confusion in the diathesis was increased by a second set of experiments, in which the authors submitted mice to restraint (resembling IMO) stress *after* contextual fear conditioning, to demonstrate that strong stressor following conditioning could naturally replicate the effects of intra-

hippocampal corticosterone injections. *A priori* fear conditioning raises the same conceptual problems in construct (triggering) validity as *de novo* conditioning. In other words, what is fear conditioning *itself* meant to model?

In summary, classical fear conditioning can also be used to model PTSD, in the sense of :

- 1- Construct validity, considering the conditioning as a traumatic experience, and individual variability as replicating vulnerability to developing PTSD (128).
- 2- Face validity, by specific impairments in extinction, mainly, but also in other dimensions such as contextualization, as exemplified by Kaouane *et al.* (23). The implications of conceptual jumps must be highlighted as well. For instance, articles using fear conditioning to model PTSD without discussing how, or in what way, maintains vagueness and confusion.
- 3- Predictive validity, more occasionally (e.g. Wurtz *et al.*, 2016; (128)).

### Fear conditioning as a theoretical model: a paradox

Despite extensive theoretical work criticizing classical fear conditioning as a model for PTSD, and the development of dedicated, alternative models, classical conditioning has gained considerable importance in the field, somewhat disconnected from earlier literature. This paradox is expressed both in experimental reports (previous section) as well as in theoretical accounts (this section). Neurobiological accounts of PTSD reported in general media and review articles are largely based on the fear conditioning/fear circuit theory.

On the Wikipedia page dedicated to PTSD, the “Physiopathology” section says:

“The basolateral nucleus (BLA) of the amygdala is responsible for the comparison and development of associations between unconditioned and conditioned responses to stimuli, which results in the fear conditioning present in PTSD. The BLA activates the central nucleus (CeA) of the amygdala, which elaborates the fear response, (including behavioral response to threat and elevated startle response). Descending inhibitory inputs from the medial prefrontal cortex (mPFC) regulate the transmission from the BLA to the CeA, which is hypothesized to play a role in the extinction of conditioned fear responses” (131).

This paragraph reveals a direct assimilation of PTSD with fear conditioning, with the consequence of direct extrapolations from fundamental research on fear conditioning to PTSD. Indeed, neurobiological mechanisms listed here are identical to that given for cued fear and extinction learning expression (20,26,132).

In a review article entitled “*Pavlovian fear memory circuits and phenotype models of PTSD*”, Johnson and colleagues proposed that “pavlovian fear [has] a significant potential to begin to explain

why some individuals form stronger and longer lasting Pavlovian fear memories to traumatic events”, implying again that PTSD is an expression of pavlovian fear conditioning, similar to the original analogy (38).

Another example is found in an article written for the general medical audience by Ressler and colleagues, entitled “*An Integrated Neuroscience Perspective on Formulation and Treatment Planning for Posttraumatic Stress Disorder, An Educational Review*” (Ross *et al.*, JAMA Psychiatry, 2017 (29)). The authors proposed that “any contemporary neuroscience formulation of PTSD should include an understanding of fear conditioning (...) fear conditioning and other elements of basic learning theory offer a framework for understanding how traumatic events can lead to a range of behaviors associated with PTSD”. They further added that “any conversation about the neurobiology of PTSD needs to begin with what happens in the brain following a traumatic event (...) We study this process through a behavior called fear (or threat) conditioning” (29), illustrating the central place given to fear conditioning in explaining PTSD for a wide audience .

These influential theoretical accounts illustrate that currently, fear conditioning is used to explain the neurobiology of PTSD, without discussing its validity as an animal model.

## Summary and interim discussion

Fear conditioning has not been used initially to model PTSD, and applied alone, has been criticized early for lacking construct and face validity (124).

However, it has subsequently been integrated to model PTSD in various ways. Sensitization-based models have been modified to include a conditioning dimension, and those have become fear conditioning paradigms by themselves. *De novo* (classical) fear conditioning has also been applied in these sensitization-based models. Classical fear conditioning paradigms alone (cued and contextual) have started to be used as PTSD models. Finally, fear conditioning has become a widely accepted theoretical account of neurobiological mechanisms implicated in PTSD.

Modifications of stressor-based models are rooted in initial experimental work (chapter 3), while the return of classical fear conditioning to model PTSD theoretically and experimentally is reminiscent of early conceptual work (on conditioning and sensitization dimensions of PTSD; chapter 2).

Classical fear conditioning is used to model PTSD, in the sense of face validity by specific impairments in extinction, mainly, but in also in other dimensions such as contextualization. Claims for construct and predictive validities are inconsistent, but some authors propose the initial conditioning models a traumatic experience. It should be highlighted that classical fear conditioning alone is not claimed to model all symptoms of PTSD, but only some aspects. Admitting to model only associative memory-based aspects is pragmatic positioning but the debate of conditioning vs. sensitization remains unaddressed.

Stressor-based models have been modified, from original sensitization models, to include conditioning. They represent the closest approximation of PTSD, with good construct, face and predictive validity, and in a sense, a continuum with classical fear conditioning (e.g. the paradigm developed by Siegmund and Wotjak (119) is similar to contextual conditioning (120)).

Uses of fear conditioning in PTSD modeling, both experimental and theoretical, are heterogenous. This has led to conceptual vagueness and ambiguity.

*De novo* fear conditioning raises the issue of how original stressor and conditioning are conceptualized, bringing ambiguity to the diathetic model of PTSD. If conditioning is considered a trauma, then original stressors are *preparatory* to subsequent vulnerability (Figure 11). Alternatively, conditioning is used to reveal deficits in fear extinction as a face validity argument. Impaired extinction can in turn be considered a symptom, a constitutional vulnerability factor, a general feature that underlies PTSD, or even an epiphenomenon of PTSD.



Conceptual problems are similar with PTSD models based on classical fear conditioning applied alone. What do conditioning and fear expression/extinction represent? Difficulty in interpreting these models conceptually is reinforced by the frequent lack of conceptual discussion in scientific articles.

Animal models that were modified from original sensitization-based models to include conditioning represent the closest approximation of PTSD, with good construct, face and predictive validity. The main problem identified for these models is semantic. Indeed, in these models, behavioral fear responses to trauma reminders have been interpreted as reflecting “classical fear conditioning” (116). Conceptual assimilation will naturally result from using the same name as employed in more standard protocols, dominant in fundamental research, with the risk of extrapolating results. Extrapolation is the main risk of conceptual assimilation of improved PTSD models and classical conditioning, going back to the origin of the problem (validity of the conditioning model).

As a result, we have found that neurobiological narratives derived from fear conditioning literature is applied without any modification, or caution, to account for the pathophysiology of PTSD.

In the last three chapters, qualitative results of literature reviews were presented. In order to complement these results with an unbiased, quantitative approach, an automated network analysis was performed. It is presented in chapter 5.

## V- Network analysis of semantic content

Qualitative analyses of the literature related to PTSD, fear conditioning, and animal models of PTSD, have suggested that original theories, dedicated animal models of PTSD, and integration of conditioning in heterogeneous ways, all under the influence of some seminal papers (e.g. Yehuda and Antelman 1993) have gradually shaped the field. We have proposed that difficulties in interpreting the models based on fear conditioning could result from these diverse evolutions of the fear conditioning-based model.

However, apart from chapter 2, that could thoroughly describe a limited number of articles, it has been necessary in chapter 3, and even more so chapter 4, to select relevant and illustrative articles among hundreds of publications. Although we cautiously cross-referenced the literature and studied articles in depth, subjectivity and selection bias cannot be excluded. This qualitative approach may therefore have led to self-confirmation of the investigators' opinions.

In order to try and verify the main conclusions of this work, a complementary quantitative approach has been applied to examine the evolution of the field of PTSD modeling. Automated network analysis was applied on entire corpuses of literature, to provide quantitative, unbiased data. The results of this analysis confirmed the main qualitative results of chapters 2 to 4, namely, they identified the links between pivotal publications and the gradual integration of fear conditioning to PTSD models, to become central in the field, on the basis of heterogeneous conceptual links.

### General methodology

Scientific search engine ISI web of knowledge was searched for relevant terms. This platform was chosen because we have observed its yield is higher, especially with better referencing of articles published prior to year 2000 (observation on references used for chapter 2). Results of the search were exported in "full record and cited references".

Text corpus were uploaded in the open source text mining platform Cortext Manager, with which semantic network mapping was performed.

Algorithms extract semantic nodes and edges, and display them in 2 dimensions, as described by Rule *et al.*, 2015 (133). Cortext was created by Jean-Philippe Cointet, an author of this article. Cortext procedures are available online.

The methods have been described by Cointet and colleagues (133). In brief, structured lists of texts obtained as described above were analyzed with natural language processing algorithms to perform text parsing, that is, extracting terms and structure of the sentences, and the position of terms in sentences. The extraction process proceeded in four steps: first, a part-of-speech tagger was run over the

full corpus. A text-chunking algorithm was then used to identify noun phrases corresponding to a predefined set of grammatical sequences. Extracted multi-terms were gathered under the same class when their stemmed version was identical (“city” and “cities” are then indexed as “city”). Similarly, semantically coherent multi-terms were then gathered into the same class. Different term classes were then indexed and their frequency denoted. From this list, the 1,000 most frequent multi-terms were extracted from each corpus.

After text parsing, network mapping proceeded in four steps: 1) term co-occurrence matrices were built for different time points (years); 2) matrices were aggregated by defined time periods to suppress temporality dimension, and proximity between term pairs was calculated on the basis of their contextual relatedness, yielding a weighted semantic network with terms as nodes, and edge weights indexing proximity. 3) The semantic network was next filtered to conserve only edges above a threshold. The threshold is automatically computed so that the final network is interconnected, and its total number of edges is minimized. 4) Louvain community detection algorithm was applied to identify cohesive subgraphs, i.e. categories of terms.

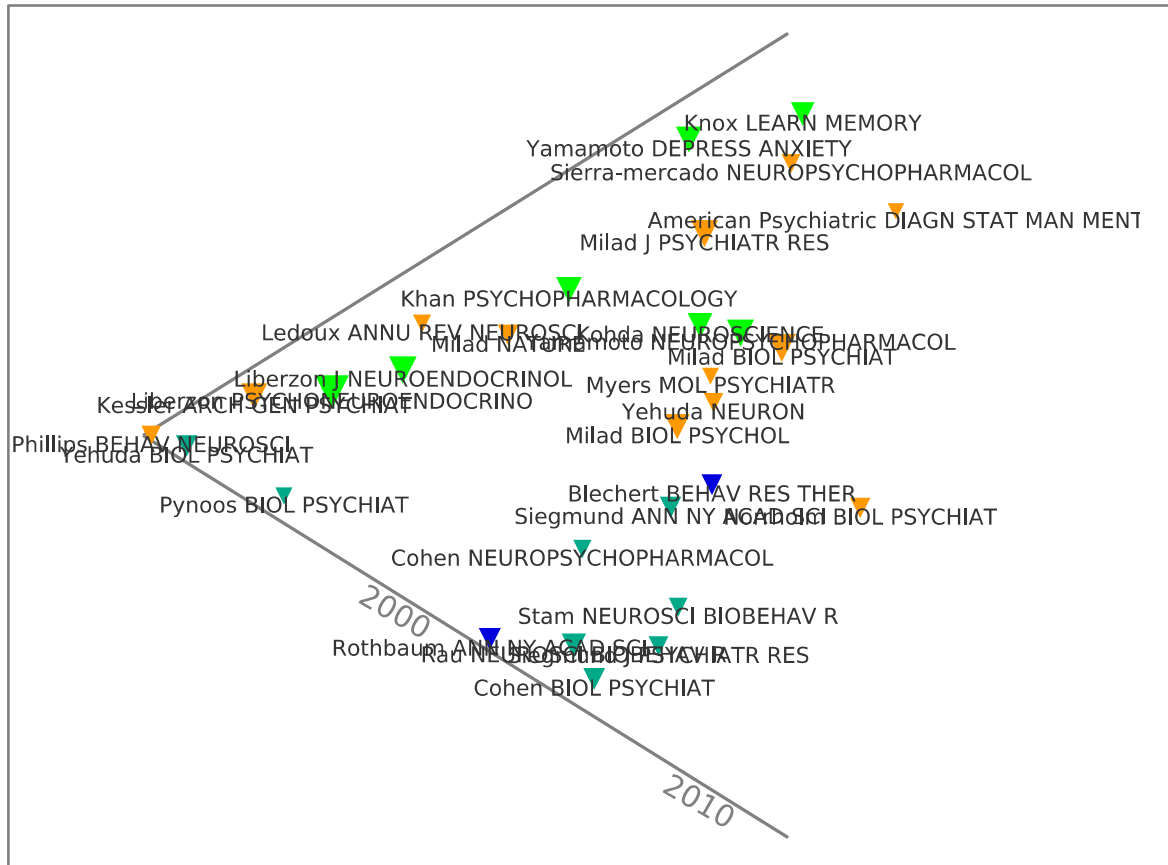
To obtain visual representations of the results, network mapping was applied. Force directed layout was used to spatialize our semantic networks. The nodes were then colored according to the cluster to which they belong. In graphical representations, nodes’ sizes are proportional to terms’ frequency in the corpus. Edges thickness represent proximity weights. This Natural Language Processing approach allows automated extraction and visualization of semantic/conceptual links across large amounts of articles, across time, in an unbiased way.

## Historical overview of modeling PTSD in animals

The first aim was to compare the general findings of qualitative analysis of the key articles and of their links (chapters 2-4). A co-citation network of articles was constructed with graphical representation across time. In this analysis, the more two articles are jointly cited, the stronger their link will be.

ISI web of knowledge was searched for “Fear conditioning” (topic) + “PTSD” (topic) + “animal model” (topic) from 1980-present. This combination of keywords was chosen because for this analysis, source articles must be limited to relevant references (here articles dealing with the subject of this thesis, that is, animal models of PTSD using fear conditioning). The search yielded 272 references, starting from 1992. Articles represented in the network must be significantly cited among the 272 articles, but they do not have to belong to this corpus. Result of this analysis is presented below (Figure 12).

For clarity, only the 30 most frequent co-cited references were extracted. Nodes represent individual articles, their color the cluster to which they belong their size the relative number of co-citations (e.g. Liberzon *et al.*, 1997 (94), bright green on far left), the thickness of edges the occurrence of co-citations.



**Figure 12: Historical network mapping of citations 1992-2019** in the field of animal models of PTSD with fear conditioning.

This graph is consistent with the main results of this thesis. The main trend is of some original articles in 1990s and early 2000s, but with the vast majority of pivotal studies located between 2005 and 2010. Co-citation analysis identified similar central articles, thematic clustering, and historical links.

Cluster analysis identified the main trends in modeling PTSD identified in chapter 3 and 4. Light green corresponds to SPS literature, dark green to inescapable shocks and predator stress models, while orange nodes are related to classical fear conditioning.

Moreover, the temporal sequence of original sensitization-based models of PTSD (1990s, chapter 3), followed by adaptations to become modified fear conditioning (chapter 4), can be identified :

- Inescapable shock: Pynoos *et al.*, 1996 ((89) ; one of the two original IS models of PTSD), then Siegmund and Wotjak 2006 (the review (82) preceding their seminal 2007 article including fear to trauma context (119)),

- Single prolonged stress: Liberzon *et al.*, 1997 ((94); the original SPS article), then Yamamoto *et al.*, 2009 (the first study that tested for trauma-related fear after SPS; (117)),
- Predator stress: Cohen *et al.*, 2004 ((134)without conditioning) then Cohen *et al.*, 2006 ((115); including a conditioning dimension).

The 1993 article by Yehuda and Antelman (66) is at the origin of the graph, linked with inescapable shock and predator stress literatures. While inescapable stress (Pynoos *et al.*, 1996; (89)) relates to Yehuda and Antelman's work from the start, this was not the case for predator stress (83). This discrepancy is explained by the absence of original predator stress literature in this 30-article graph. In contrast, this analysis failed to identify a link between Yehuda and Antelman article and SPS literature, while Liberzon and colleagues explicitly claimed this influence (94).

Remarkably, modeling PTSD with fear conditioning *per se, de novo* (127) and by extrapolation (127), are grouped in a single cluster. This result suggests that those secondary uses of fear conditioning have evolved jointly, but apart from modified sensitization-based protocols, as indicated in the previous chapter. The origins of this cluster can be traced back to the work of Ledoux (Phillips *et al.*, 1992; Ledoux 2000; (20,22)), followed by work of Quirk and his mentees including Milad, and Sierra-Mercado. Their articles included fear extinction neurobiology and fear conditioning *de novo* in PTSD (127,135) and fundamental research on fear conditioning and extinction (132).

## Evolution of semantic content and links

Having validated the main trends in the literature by comparison of qualitative and quantitative analyses, the second aim of this chapter was to analyze the evolution of conceptual links in PTSD models literature across time. Special attention was given to concepts related to fear conditioning.

ISI web of knowledge was searched for "PTSD" (topic) + "animal model" (topic) from 1980-present, that is, the same search criteria as above, except "fear conditioning" was omitted. This combination of keywords was chosen to be more inclusive than for the previous section. While the latter needed to include only articles directly relevant to fear conditioning in modeling PTSD, the second analysis required to place fear conditioning within the entire PTSD modeling context. The search yielded 860 references.

Network mapping of keywords from these 860 references was computed by merging correlation matrices in time intervals, balanced for the number of citations included. Mapping was limited to 150 terms. Of note, it is not possible with Cortext to choose time boundaries, beyond the number of time windows. Therefore, time windows presented here do not necessarily correspond to historically relevant periods. The results are presented below (Figure 13 to 17).

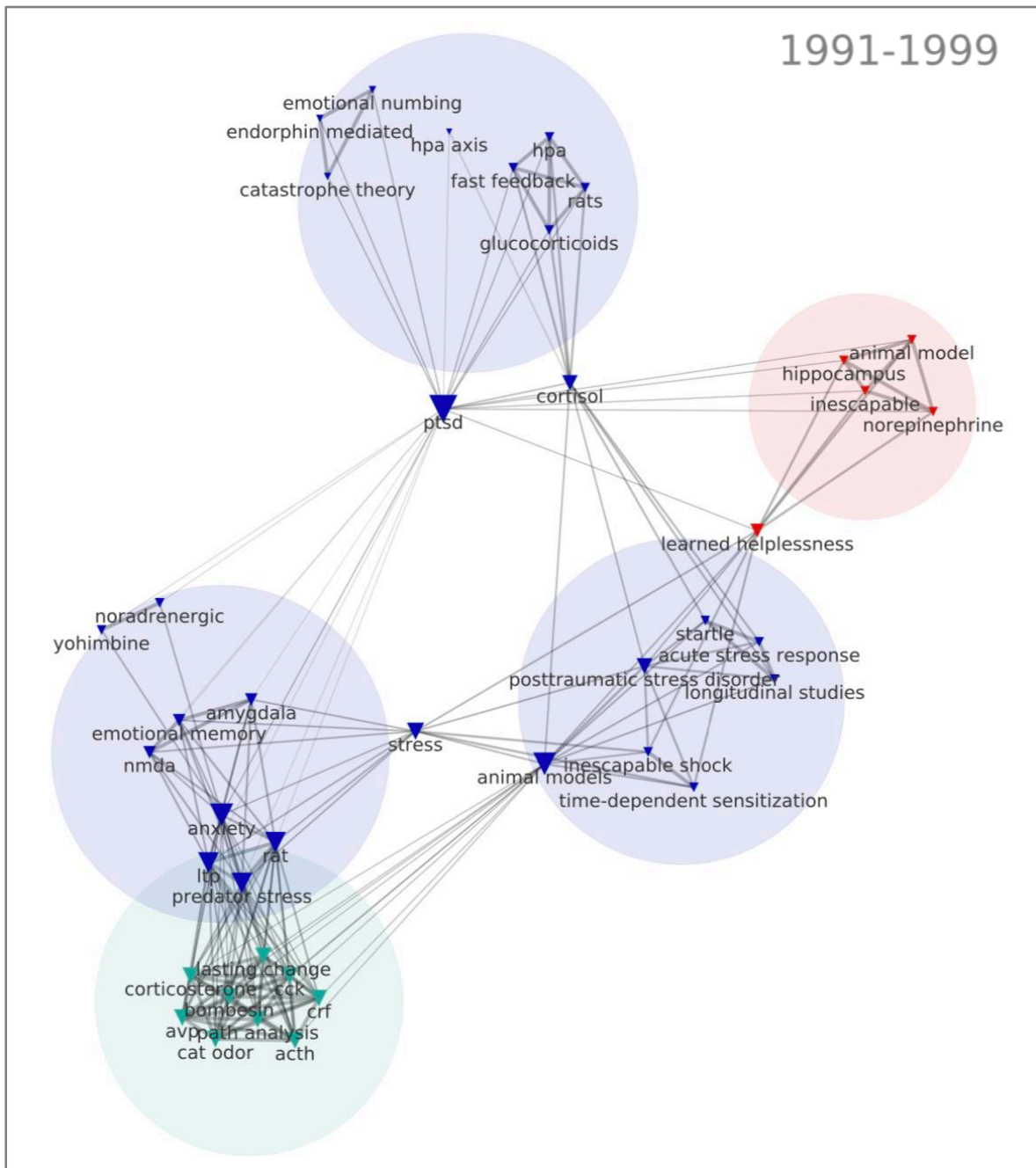


Figure 13: Semantic network mapping for keywords of articles mentioning “PTSD” and “animal model” (1991-1999).

Mapping of constructs employed in articles published in the 1990s yielded two clusters containing inescapable shock/time-dependent sensitization models and two clusters containing predator stress (chapter 3). Central constructs included stress, cortisol and learned helplessness.

Most constructs were related to neurobiological mechanisms, and were similar to those identified in chapter 2: stress hormone systems: the “HPA axis” (CRF, ACTH, corticosterone), norepinephrine and the fear system (amygdala, hippocampus), to which the animal models are linked. References to synaptic plasticity were also found (LTP, NMDA, lasting change).

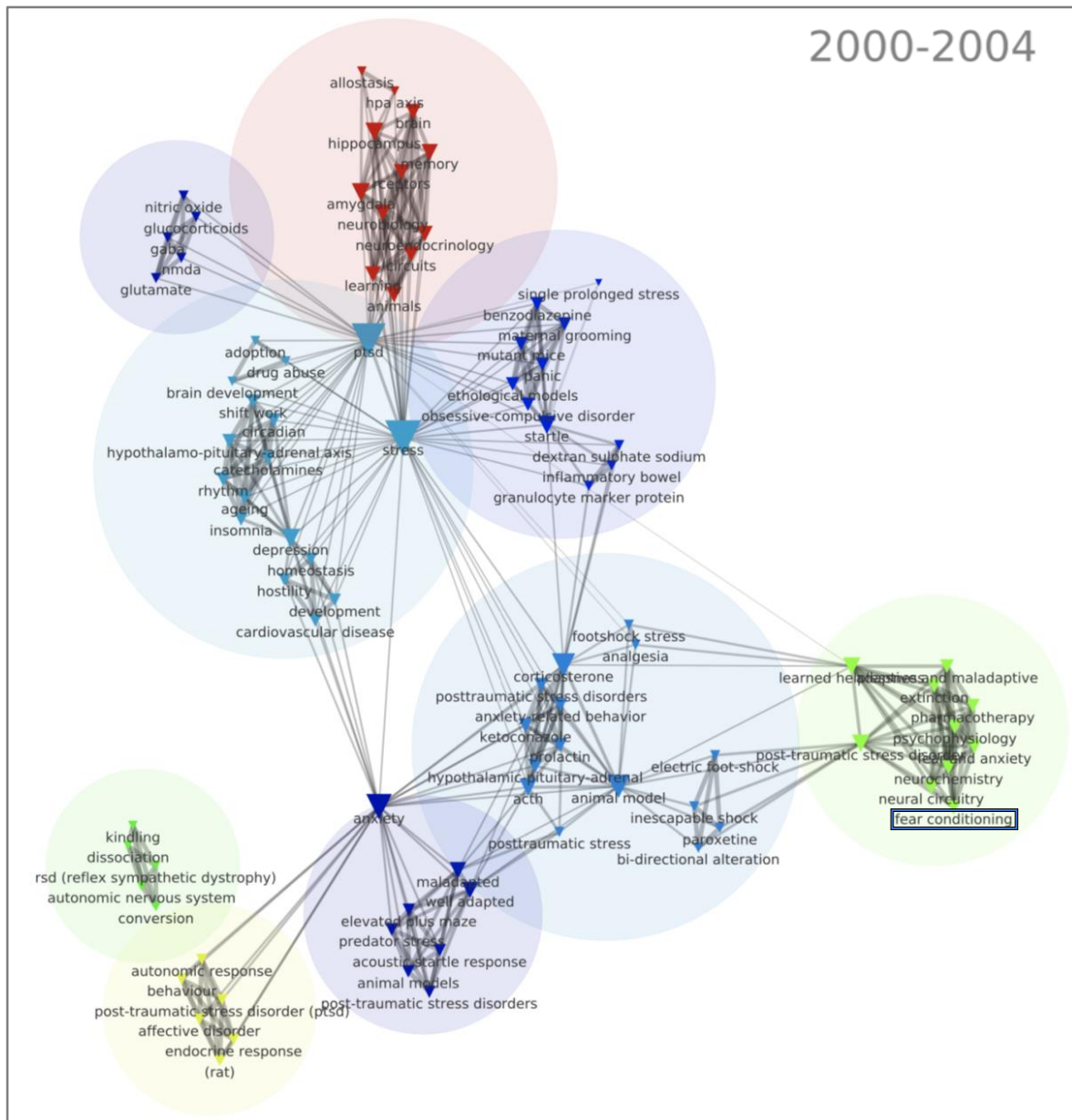


Figure 14: Semantic network mapping for keywords of articles mentioning “PTSD” and “animal model” (2000-2004).

Network of keywords employed in the 2000s organized mainly around the constructs of stress and anxiety. The increasing number of clusters suggests that the field expanded and complexified. Predator stress, inescapable shock, single prolonged stress and learned helplessness, appeared in distinct clusters. Neurobiological constructs were comparable to those found above. Apart from the HPA axis, most neurobiological constructs appeared in clusters independent of PTSD models, suggesting that brain mechanisms were not directly investigated in these models. Fear conditioning appeared for the first time in a map, in a peripheral position (bottom right; box) linked, among others, to the neurobiological constructs of neural circuitry, neurochemistry, and extinction. This is consistent with our observation that fear conditioning appeared in PTSD models in 2003-2005.

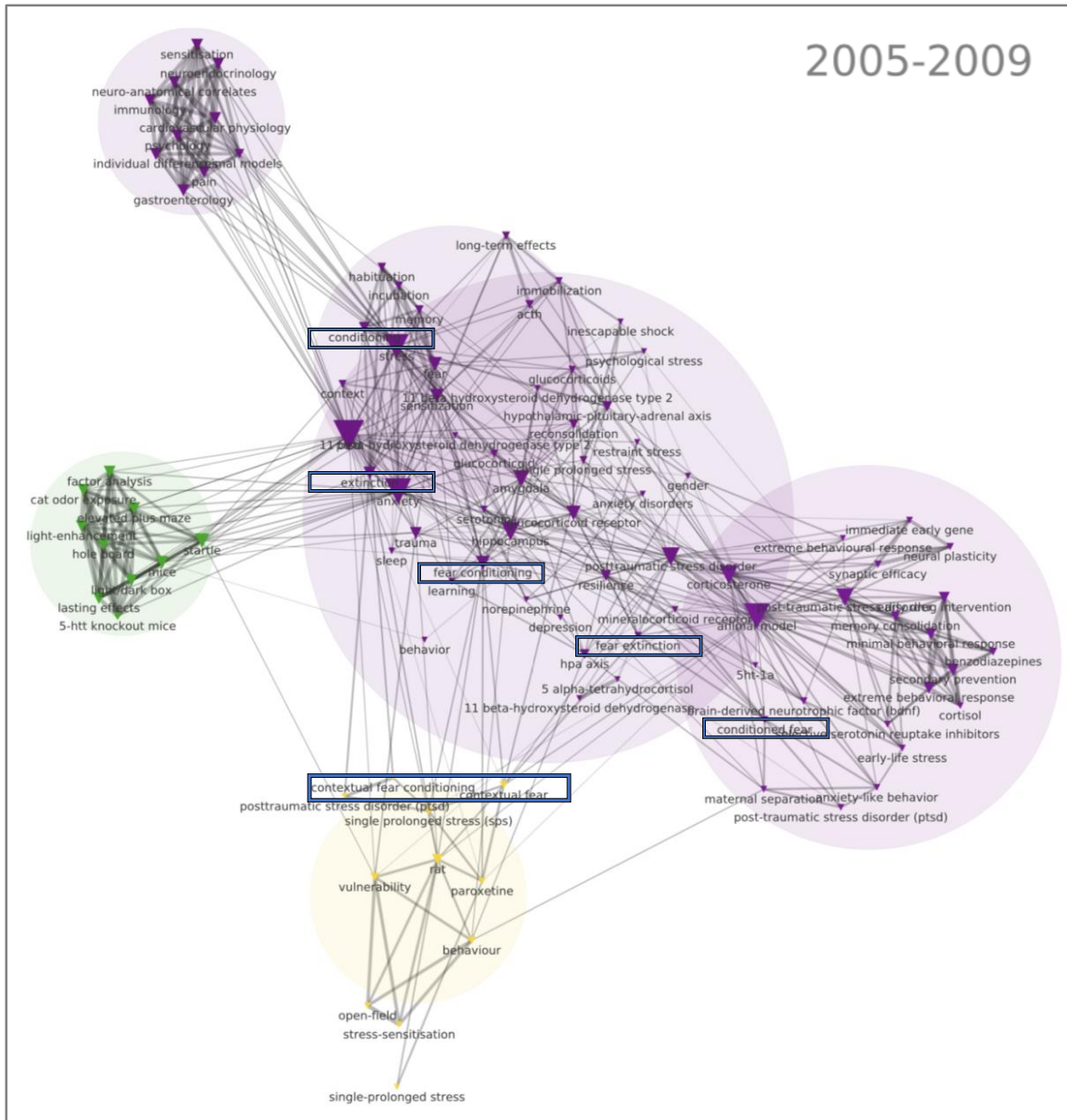


Figure 15: Semantic network mapping for keywords of articles mentioning “PTSD” and “animal model” (2005-2009).

The mapping obtained for the second half of years 2000s revealed a degree of complexification, with a less organized network. As for the 2000-2004 period, this may be the result of the expansion of the field, but might also reflect the emergence of a genuine disorganization.

Models of PTSD positioned in the network included predator stress, inescapable shock, single prolonged stress and immobilization. It is striking that fear conditioning appeared in many more instances, reflecting a quantitative expansion, and under various names (conditioned fear, conditioning, contextual fear, contextual fear conditioning, extinction, fear conditioning, fear extinction; boxes), suggesting the appearance of heterogeneity. These observations are in line with results described in chapters 3-4.



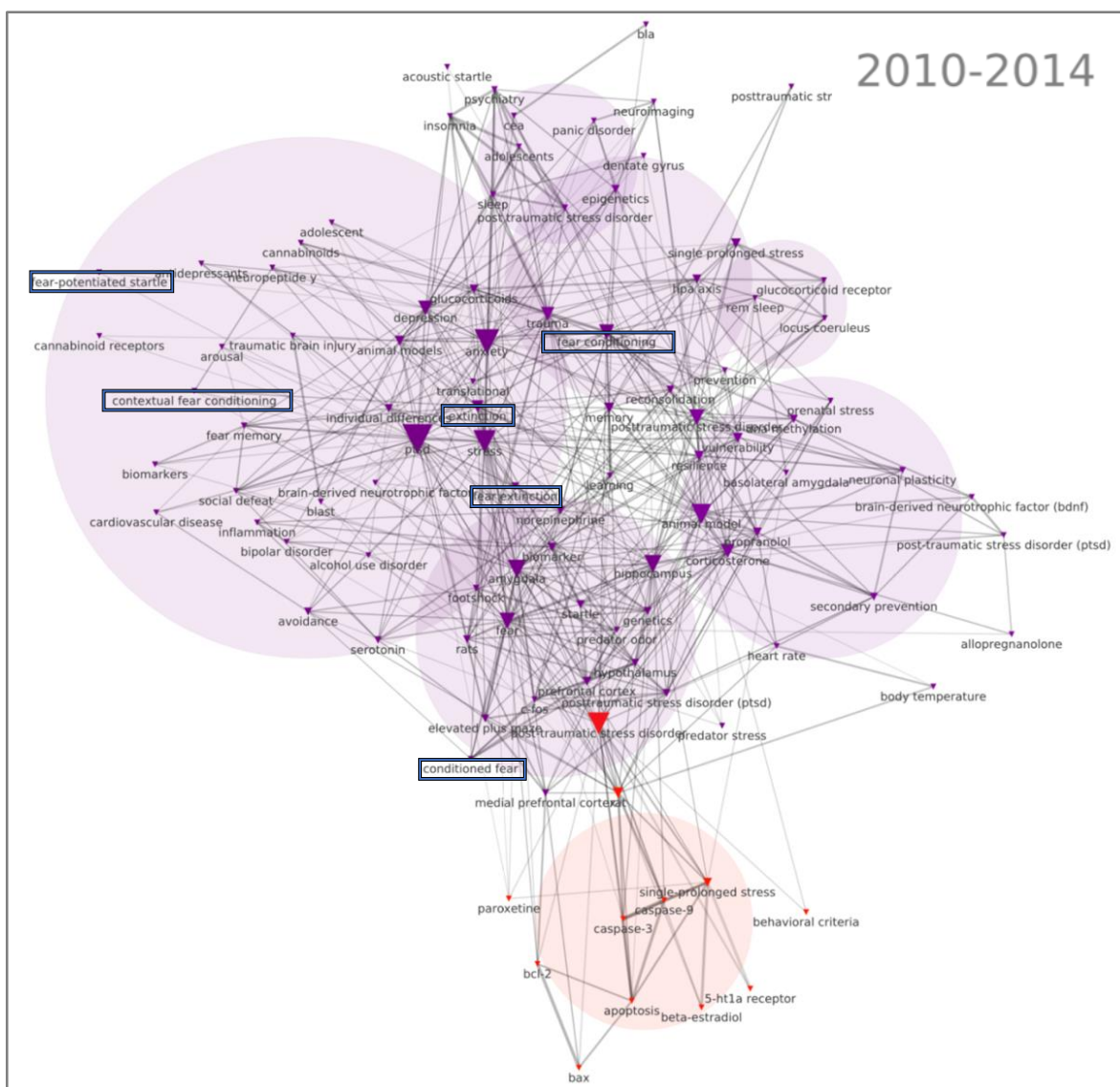
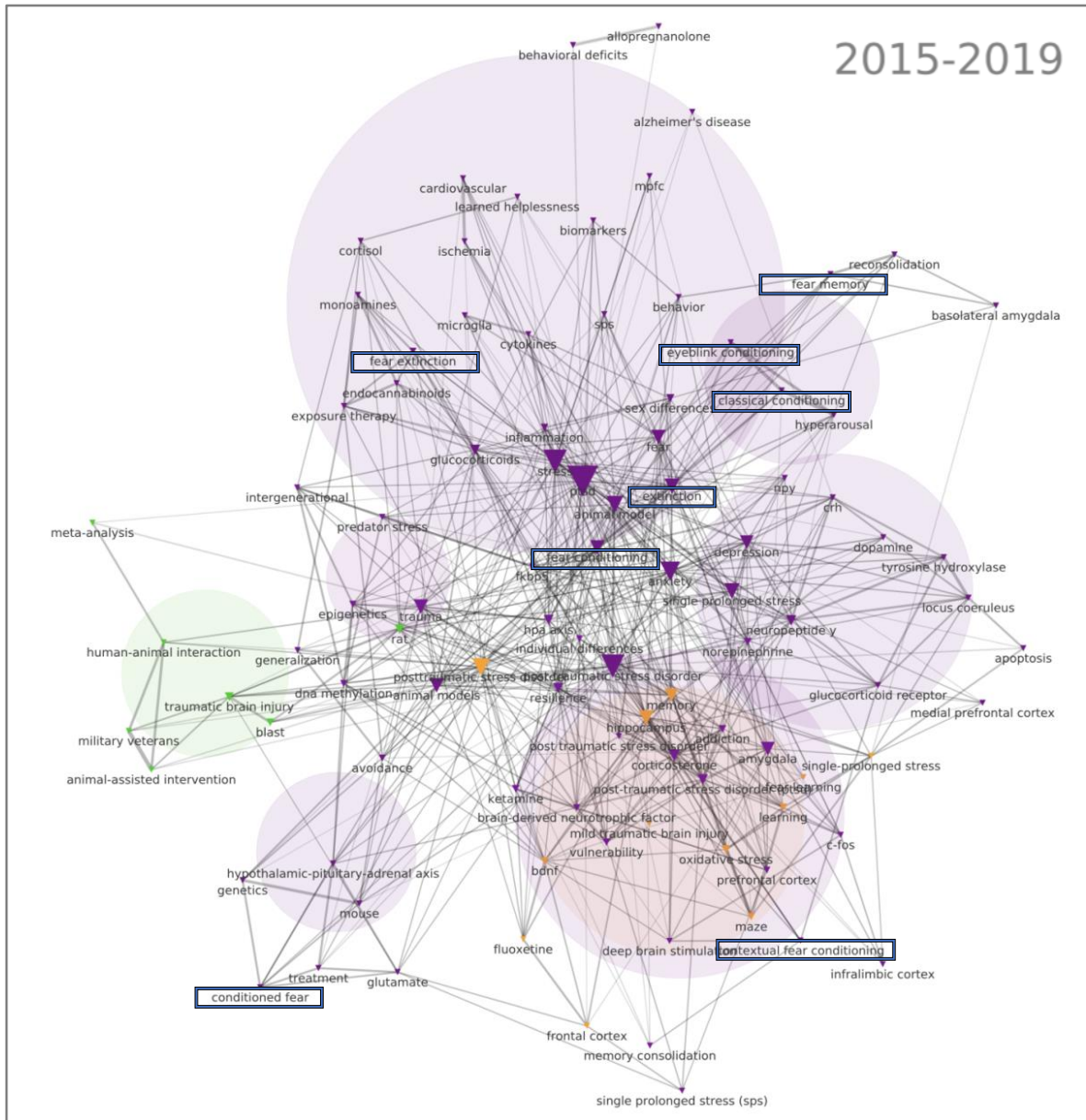


Figure 16: Semantic network mapping for keywords of articles mentioning “PTSD” and “animal model” (2010-2014).



**Figure 17: Semantic network mapping for keywords of articles mentioning “PTSD” and “animal model” (2015-2019).**

Figure 16 and 17 show that the disorganization observed for the 2005-2009 period has actually increased after 2009. Although a larger number of articles might have accounted for this trend, the combination of this result with the description of heterogeneous uses of fear conditioning to model PTSD (chapter 4), suggested that indeed the development of animal models of PTSD has been paralleled by an increasing use of fear conditioning in various, disorganized ways. Moreover, fear conditioning and related constructs appeared gradually in a more central position in the network maps, suggesting the influence of the fear conditioning heuristic has risen in the field of PTSD.

## Summary

Network mapping of co-citation and keywords has complemented the qualitative analysis used in chapters 2-4 with a quantitative approach. Main conclusions of previous chapters are supported by this automated language processing.

Inspection of the historical map of the most frequent co-citation pairs constructed on the basis of articles published between 1980-2019 (topics of animal models, PTSD, and fear conditioning) indicated that sensitization-based models of PTSD have first appeared, following the theoretical article by Yehuda and Antelman (66). This article proposed criteria to evaluate animal models of PTSD and represents the conclusion of a decade of theoretical accounts of PTSD pathophysiology. Sensitization models have then evolved to become modified forms of fear conditioning. Models of PTSD using unmodified classical fear conditioning, alone or *de novo* have evolved separately, following early work by Ledoux and colleagues on cued and contextual fear conditioning (22).

Plotting network maps of keywords of articles published between 1980-2019 across time (topics of animal models and PTSD) indicated that the construct of fear conditioning has gradually become more central in the field of PTSD modeling, but in various semantic forms and with diverging links. Moreover, the organization of networks has decreased with passage of time.

These unbiased results are in line with conclusions of chapter 4, and suggest that heterogeneous use of fear conditioning, with conceptual ambiguity and vagueness, has contributed to blur the lines in the field of PTSD modeling.

## VI- Contextual analysis

*“The dearth of clinical neurobiologic research on PTSD stands in contrast to a number of investigations of the behavioral, biochemical, and neurophysiologic effects of fear and stress in laboratory animals. These studies provide insight into certain neural processes that either contribute to the origin of PTSD or alternatively may be involved in the maintenance of pathologic features.”*  
Charney, Deutch, Krystal, Southwick, and Davis, 1993

Expansion and heterogeneous use of fear conditioning, with conceptual ambiguity and vagueness, has contributed to obscure the field of PTSD modeling. But why did fear conditioning transition from seldom use to model PTSD to its current dominant position?

Indeed, explaining PTSD with fear conditioning despite limitations identified by three decades of discussions and criticism (chapter 2, 3) represents a clear paradox (chapter 4).

The aim of this chapter was to formulate tentative explanations of this paradox, that include :

- The influence of the scientific context, with
  - The construction of the fear learning theories, in a biological psychiatry framework
  - The development of functional brain imaging in Human, and
  - The influence of a limited number of researchers
  
- The influence of the political context, with
  - History of wars,
  - Research funding
  - The American dominance

### Scientific context

The development of the conditioning model of PTSD has paralleled scientific interest in stress and the neurobiology of fear and memory. Neurobiological theories of PTSD and fear conditioning, that are correlated, have themselves evolved with scientific tools in the context of broader, dominant scientific paradigms.

## Construction of the fear learning theory

Experimental work aiming at deciphering working principles of the emotion/fear neuronal circuit has started to develop in the 1980s and 1990s, and exploded after 2000. According to Ledoux, this progress has been permitted by putting aside the vague notion of the “limbic system” and the subjective aspects of emotions. Ledoux and others (including Davis and Fanselow) had then the opportunity to apply quantitative analysis on the relatively simple behavioral paradigms of fear conditioning in rodents (20).

Coupling rodent behavior -mainly cued auditory fear conditioning in rat- with direct interventions on the central nervous systems (lesion, pharmacological activation/inactivation or neurotransmitter-receptor modulation), and using anatomical tract-tracing, these authors have delineated an “emotion circuit” in the brain, with the amygdala playing a central role (19,20). Interestingly, the notion of a brain circuit underlying a behavioral function implicitly carries the notion of a hard-wired system, as stated by Ledoux (80). Similar to this was Ledoux’ notion of “quick-and-dirty” thalamic inputs that converge onto the basolateral amygdala with the “slower but more accurate cortical inputs” (80). The fear circuit of Ledoux has rapidly attracted interest of researchers in the field of PTSD ((52,53); chapter 2).

Conditioning eliciting fear responses to conditioned cues that are stable over time has led to the idea that some form of learning occurred, and because the “fear circuit” was thought to underlie fear expression, that long-lasting biological changes occurred in this fear circuit (74).

The concept of persistent (“long-term”) synaptic plasticity was proposed around the same time to underly memory (136). Kandel and colleagues had shown that the gill/siphon-withdrawal reflex of sea slug *Aplysia* could be habituated (i.e. decreased) in a prolonged way (1 week) by application of repeated sensory stimulation. Sensory stimulation was simulated in the isolated abdominal ganglion preparation by electrical stimulation of sensory nerves (inputs). Stimulation with the same temporal structure as used for habituation produced a lasting decrease of post-synaptic potential amplitude in motor neurons known to mediate withdrawal reflex (137). Kandel and colleagues proposed that such long-term “decrement” of synaptic potentials mediates “habituation” of the gill/siphon-withdrawal reflex. Similarly, withdrawal reflex can be “sensitized” (i.e. enhanced in a non-associative way) by noxious stimulations. Kandel proposed that “molecular mechanisms for this short-term form of synaptic plasticity “[could] be extended to explain both long-term memory and classical conditioning” (138).

Long-term potentiation (LTP) of synaptic inputs in the hippocampus (known to be central in spatial memory) has subsequently been proposed to underly memory formation (136).

This synaptic memory theory has soon been speculated to underly conditioned fear learning, accounting for the creation of traumatic memory traces (52,53,60). Kolb wrote in 1987, citing Kandel and Schwartz, 1982 (138): “Kandel’s studies on sensitization learning are particularly relevant to questions related to posttraumatic stress disorder (...) it involves postsynaptic facilitation mediated by axo-axonic synapses” (52).

Evidence for LTP of thalamic inputs to the amygdala was later found in acute brain slices prepared from animals submitted to prior auditory fear conditioning (139). Such findings as well as evidence for LTP *in vivo* induced in the amygdala by fear conditioning (20) have led many to consider this model as one of the best to study the links between neural circuits and behavior (25). From these seminal studies, fear conditioning has become one of the leading paradigms in neuroscience (see also Figure 5 in Introduction chapter), amplified by technological advances, such as optogenetic manipulations in behaving animals (25).

While fear conditioning was developing as a central paradigm to study memory and emotions (20), behaviorism and biological psychiatry were becoming dominant paradigms in psychiatry. Behaviorism followed the work of Skinner, Rescorla and others, and tried to explain Human behavior with conditioning theories. Biological psychiatry, explaining mental disorders with biological (molecular) dysfunction of the brain developed with the discovery of antipsychotic agents (chlorpromazine 1950s) and SSRIs (fluoxetine 1980s).

On these grounds, fear conditioning and extinction have drawn a lot of attention from the mental-health care community. Yehuda and Ledoux indeed wrote “One idea that arose was that PTSD might reflect strong associative learning akin to Pavlovian fear conditioning ...Part of the attraction of fear conditioning was that much was concurrently being learned about the neurobiology of this behavioral paradigm from animal studies” (37). Moreover “in the case of PTSD, there [was] a greater potential to accurately model the disorder because the major precipitating factors [were] known, i.e., PTSD occurs in response to severe and unusual stressful or traumatic situations” (Yehuda and Antelman, 1993; (66)).

The medical promise was (and still is) that understanding the neural circuits of fear conditioning and extinction would facilitate the development of novel psychological and biological therapies (medication, neuromodulation) for PTSD (29).

In experimental sciences, scientific knowledge is tightly linked with development of novel measurement tools. The use of *in vitro* and *in vivo* recordings of the fear circuit has led to the LTP theory of fear conditioning and PTSD. Similarly, hormones were heavily studied in PTSD after its recognition,

likely because dosage of cortisol, noradrenaline etc. could be readily performed, and because stress and hormones were a leading scientific topic. Non-invasive imaging of the human is also one such tool.

#### Development of brain imaging in Human

Functional imaging of the Human brain with fMRI was invented in the early 1990s by Belliveau, Kwong and their colleagues. The technique rests on the basic principle that magnetic properties of blood hemoglobin molecules change with their oxygenation levels, giving access to imaging contrast measurable with MRI (the Blood-Oxygen-Level Dependent signal). Because brain activity consumes oxygen, it was postulated that such BOLD signal could provide indirect ways to measure brain activity non-invasively in Humans engaged in a variety of experimental paradigms (140).

Thus, it has become possible to submit Humans to fear conditioning, including subjects with PTSD, while monitoring their brain activity

Coupling fear conditioning with fMRI in PTSD subjects has been a subject of intense research, but can be grossly summarized as over-activation of the amygdala and low prefrontal activation and/or low functional connectivity with the amygdala. These findings in PTSD have been widely used in support of the analogy between fear conditioning and PTSD. The analogy has been extended to encompass the link between a deficient neuronal circuit of fear extinction on the one hand, and PTSD on the other hand, with potential mechanistic validation of the fear conditioning model (e.g. Milad *et al.*, 2009 (127)).

Considering the scientific framework in which these theories were developed can help to understand some of their limitations. Because of the theoretical framework in which these brain imaging studies were carried out, there is a risk of self-confirmation and extrapolation. This is suggested by this quote from Yehuda and Ledoux (2007) : “Because PTSD is a clinical syndrome in which an initial fear response does not abate, the neuroimaging findings showing exaggerated amygdala responses recapitulate, but do not explain, the nature of the brain disturbance in PTSD” (37).

Epistemologically, preclinical research has concentrated a lot of efforts on fear conditioning in rodents and neural circuits at play, including amygdala and prefrontal cortex. It followed that fMRI research in PTSD patients primarily focused on the same questions.

The second issue is methodological. Detecting the activation of brain regions and functional connectivity with fMRI often rests on analysis by regions of interest. On top of designing clinical paradigms that resemble preclinical experiments, region of interest analysis may lead to a form of confirmation bias. Moreover, gross activation of brain areas as studied with fMRI suggests the implication of a brain area in fear conditioning/PTSD, but does not confirm mechanistic similarity.

Third, the application of brain imaging to study PTSD may have stimulated the expansion of *de novo* conditioning paradigms in patients. *De novo* conditioning has most likely expanded because it



offered controlled experimental conditions, useful in psychophysiological and functional imaging studies (e.g. fMRI; Milad *et al.*, 2009 (127)), and in contrast with earlier, non-controlled experiments that directly linked PTSD to trauma reminders in combat-related PTSD (reviewed by Shalev *et al.*, 1993 (54)). *De novo* conditioning in animals has contributed to the conceptual vagueness in the field of PTSD.

Synergistic interplay between the fear conditioning paradigm and biological theories of PTSD may have benefited from the same authors participating in both preclinical and clinical research, in a *translational* way (e.g. Milad *et al.*, 2002, Milad *et al.*, 2009; (127,141)).

### A small number of influential researchers

Throughout this thesis, qualitative and quantitative analyses of the literature have highlighted the great influence of a limited number of scientists. In particular, physician-scientists have played a great role in the development of fear conditioning-based PTSD models. Two groups have particularly contributed: one at Yale University, the other at Dartmouth Medical School. We present here a selection of names that have stood out as first or last authors of the studies cited in this manuscript.

#### *Yale University*

A first influential group was constituted by clinician-scientists affiliated to Yale University School of Medicine and to the National Center for Post-Traumatic Stress Disorder, Department of Veterans Affairs in West Haven, Connecticut :

- Dennis S Charney, MD
- John H. Krystal, MD
- Steven M. Southwick, MD
- Rachel Yehuda, PhD (Psychology)

Along with scientist Michael Davis, PhD,

This first group, led by Dr Charney and Dr Krystal has recognized limitations of fear conditioning to model PTSD by itself, but has considered it useful in theoretical accounts, when sensitization was included as well (chapter 2). They, and their students, have contributed to develop fear conditioning-based theories of PTSD.

Their main contribution was initially theoretical (chapter 2), while they subsequently contributed clinical research, including some work with *de novo* fear conditioning in subjects with PTSD (142).

Kerry Ressler, MD-PhD, has been a student of Michael Davis, studying molecular determinants of synaptic plasticity in the fear-potentiated startle paradigm (e.g. Ressler *et al.*, 2002 (143)). Dr. Ressler then went on to become a leading figure in PTSD research and as a psychiatry professor, a champion of the fear conditioning theory (29,39).



### *Dartmouth Medical School*

A second research group that played a key role in developing fear-conditioning theories of PTSD was formed by clinician-scientists at Dartmouth Medical School and the Veterans Administration Medical Center in Manchester, New Hampshire, and was made of :

- Roger K. Pitman, MD
- Scott P. Orr, PhD (Psychology)

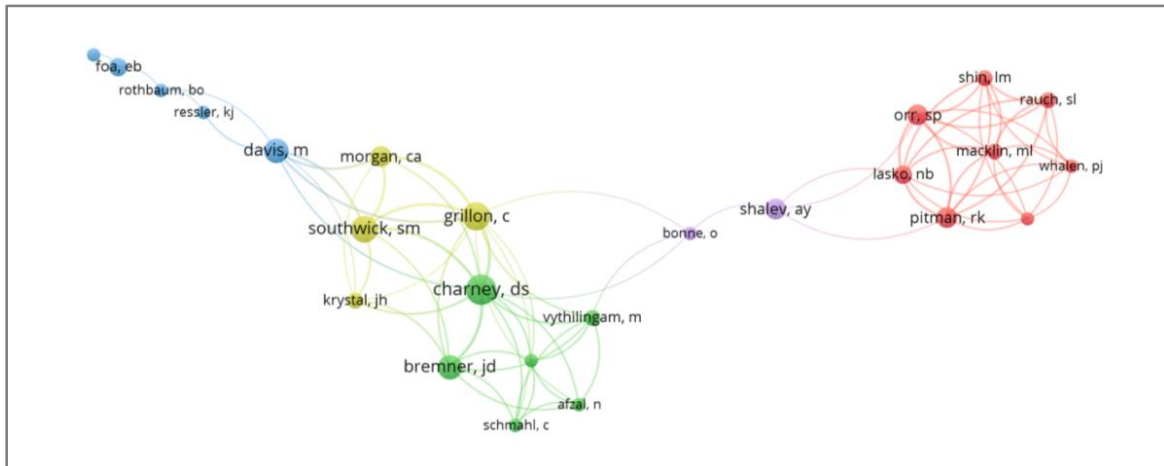
In collaboration with Arieh Y. Shalev, MD, from the department of Psychiatry, Center for Traumatic Stress at the Hadassah University Hospital, Jerusalem, Israel.

This second group, led by Dr. Pitman, has strongly defended the direct application of fear conditioning to explain and model PTSD (chapter 2), although it identified its limitations in explaining non-associative memory components (55). Interestingly, this only theoretical article of theirs criticizing the conditioning paradigm in PTSD has had direct consequences in promoting the sensitization-based PTSD model of predator stress (chapter 3). Orr, Pitman and Shalev later participated in clinical research with *de novo* fear conditioning in PTSD patients (135,144).

Trainees of this group also played great roles in building the fear conditioning narrative of PTSD. Work by Mohammed R. Milad, PhD has been cited extensively in this thesis, reflecting his influence in the field of fear conditioning and extinction in PTSD. After doctoral studies studying neural correlates of fear extinction in rats with Dr. Quirk, a student of Ledoux (141), Milad, indeed worked as an associate professor of psychiatry with Dr. Pitman at Harvard Medical School.

In order to obtain an objective and visual representation of leading scientists in the field of fear conditioning theories of PTSD, and of their interactions, a co-authorship network maps were constructed. The methodology was identical to that described above (chapter 5), except that inputs to the algorithm were author lists.

Articles lists and metadata were obtained by searching on the ISI web of knowledge for “PTSD” + “fear conditioning” as topics. The time period was defined as between years 1992-2005, because, 1) no article was found before 1992 with this search, and, 2) because of the period time during which fear conditioning theories of PTSD were developed (theoretical accounts until the use of classical fear conditioning to model PTSD; chapters 2 to 4). The size of nodes represent the number of appearances in articles used to construct the graph, and the edge thickness the number of co-appearances (co-authorship). Automatedly-defined clusters were color-coded. Results are presented below (Figure 18).



**Figure 18: Co-authorship network mapping of articles mentioning “PTSD” and “fear conditioning” (1992-2005).**

Figure 18 represents the network map of authors of articles published between 1992-2005. This result confirmed qualitative estimates of the leading figures of the convergence between PTSD and fear conditioning research. Pitman, Orr and colleagues on the one hand, and Charney, Davis (and student Ressler), Krystal, Southwick and colleagues, on the other hand, tended to cluster on the map, with strong co-authorship links within clusters. Connections between clusters were made by clinician-scientists Bonne and Shalev (Hadassah University Hospital, Jerusalem, Israel).

Remarkable is the fact that all authors mentioned in this section, who began their careers with work on PTSD and fear conditioning, have become leading academic figures:

- Dennis S. Charney became Dean and Professor of Neuroscience and Pharmacology at the Icahn School of Medicine at Mount Sinai, New York,
- John H. Krystal, Professor of Translational Research and Professor of Psychiatry and of Neuroscience; Chair of the Department of Psychiatry, Yale University Medical School,
- Scott P. Orr, Associate Professor of Psychology at Harvard Medical School,
- Roger Pitman, Professor of Psychiatry at Harvard Medical School,
- Steven M. Southwick, Professor of Psychiatry at Yale University Medical School,
- Arieh Shalev, after a career in at Hadassah University Hospital, Israel, became a Professor of Psychiatry at New York University,
- Rachel Yehuda became Professor of Psychiatry and Neuroscience, the Director of the Traumatic Stress Studies Division at the Mount Sinai School.

These career advancement may have been the result of, 1) their neurobiological inclination, which was the dominant theory in psychiatry in the 1980s (this chapter), and, 2) their early interest in studying the newly-described disorder that subsequently became intensely investigated. It cannot be

excluded that all clinician-scientist trainees in psychiatry in the 1990s had such prominent academic careers, although this seems unlikely.

One anecdote told by Dr. Krystal at the ISTSS 2015 meeting and reported in the *European Journal of Psychotraumatology* in 2016 (145) illustrates the paramount importance of interactions between scientists and their scientific backgrounds, in specific places, in the construction of PTSD science. Dr. Krystal related the history of his locus coeruleus theory of hyper-arousal in PTSD (chapter 2): “Dr. Krystal described an incident from his past which had initially led him to believe in the wrong idea, and how that transformed into his newly formed opinion. In medical school, he was working with monkeys in a laboratory led by D. Eugene Redmond that studied the activity of the locus coeruleus. Around the same time, he was also taking a medical school class on PTSD taught by Robert Jay Lifton [an anti-war psychiatrist who played a key role in PTSD recognition (3); our note]. As the professor laid out the symptoms of PTSD, Dr. Krystal made a link to the consequences of stimulating the locus coeruleus. This compelled him to postulate that perhaps, PTSD was linked to the activation of the locus coeruleus. However, as the years passed, Dr. Krystal was able to update this opinion through observation and experience. He noticed that medications like clonidine, as well as more powerful sedatives such as benzodiazepines, were not very effective in treating PTSD. In fact, major tranquilizers and antipsychotic medications make most people with PTSD feel listless and anhedonic. Furthermore, by looking at the data from the resilience study of the special forces training, it was reasonable to conclude that the assumption of dampening arousal being adaptive was not true. The research group found that the more resilient special forces actually showed signs of higher noradrenergic activation than their less resilient counterparts, but they were able to turn off arousal more effectively than soldiers who were less resilient. The lesson to be learned from these results was that the problem of arousal was not its activation in itself but rather the inability to dampen it down when appropriate” (145).

This anecdote points toward the importance of the synergy of people and scientific and political contexts. Indeed, the construct of PTSD and its care is tightly linked to geopolitical history.

### Geopolitical context

Three inter-dependent geopolitical factors have contributed to the creation of PTSD and of the fear conditioning narrative of PTSD: history of war, the implication of the USA (and to a lesser extent Israel) and scientific research funding.

## War and politics

Posttraumatic Stress Disorder was created following Vietnam veterans activism to recognize specific war-related disorders in 1970s. With help from medical supporters, this led to the creation of a Committee on Reactive Disorders to report to the DSM-III task force, in 1975. The committee was made of 3 DSM-III task force members, two psychiatrists, and Spitzer (chair of the task force). They were instructed by Spitzer to work with Robert Lifton, Chaim Shatan (psychiatrists and supporters of the Vietnam veterans cause) and Jack Smith (a veteran). Through Smith, patients participated for the first time in a DSM revisions committee. Lifton, Shatan and Smith defended and obtained the integration of the “Post-traumatic stress disorder” into the American psychiatric nomenclature as a formal diagnostic entity with the publication of DSM-III in 1980 (3).

Thus, the construct of PTSD resulted from a highly political process in a post-war context. In turn, clinical observations made on Vietnam veterans served as a basis for early theoretical modeling of PTSD as a result of fear conditioning (chapter 2).

Before this, wars had already stimulated theoretical considerations regarding unusual mental states observed in combat survivors. Indeed, clinical entities comparable to PTSD had been recognized under various names following historical events, mainly wars: railway spine from the Industrial revolution, soldier’s heart from the American Civil War, shell shock and war/traumatic neurosis from the First World War, combat fatigue and traumatic neurosis of war from the Second World War (146). The history of war is therefore constitutive of the notion of psychological trauma and of resulting disorders.

As Pitman stated in 2012 “A day scarcely passes that one does not see a mention of post-traumatic stress disorder (PTSD) in the media. However, this has not always been the case. In American history, post-traumatic psychopathology has been recognized under various names following wars: soldier’s heart from the American Civil War, shell shock from the First World War, combat fatigue from the Second World War and delayed stress from the Vietnam War. However, between these wars, the condition was all but forgotten” (28).

This citation is interesting in two ways. First it suggests that in 2012, PTSD was considered a subject of great interest in the media in the USA. Second, it denotes the personal links of the author with war, as he seems to rejoice that psychopathology is no longer forgotten between wars.

Dr. Pitman, according to his personal institutional profile, "served as a psychiatrist in the U.S. Navy during the Vietnam era and went on to complete a 30-year career in the Department of Veterans Affairs prior to moving to [the Massachusetts General Hospital]" (147).

Thus, scientific and academic contexts may have shaped the mind of PTSD scientists, but personal and national links with history and wars might have been instrumental in stimulating development of PTSD research. Other examples can be found in links with the Holocaust.

Dr. John Krystal's father, Dr. Henry Krystal, was a Polish-born survivor of the Holocaust, and a psychiatrist who, after immigrating to the USA, led a career of research on psychological trauma (148). This family history must have stimulated John Krystal's interest for psychiatry and psychological trauma, as he actually reported (145,149).

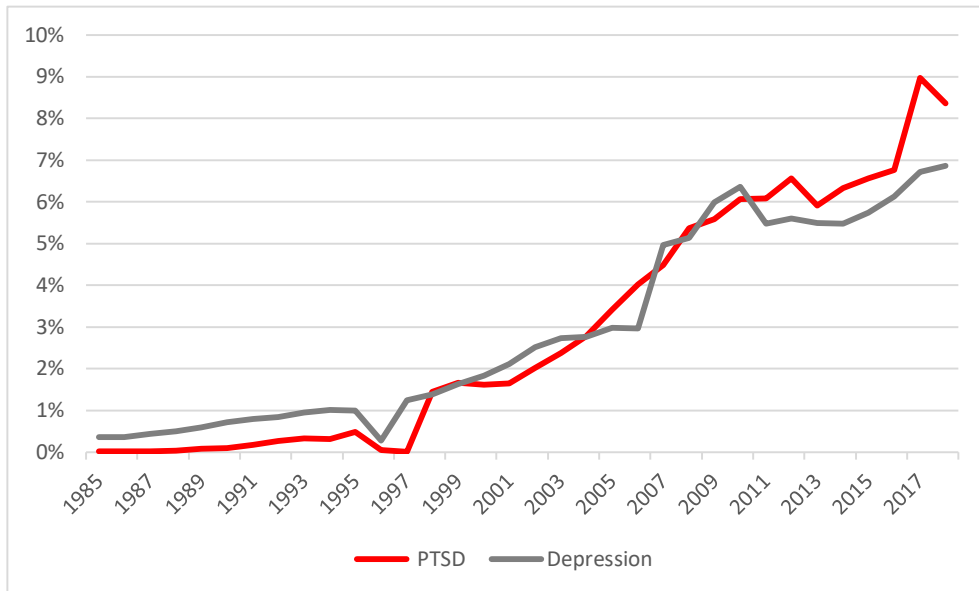
In a related manner, Dr Yehuda grew up in a neighborhood populated by many Holocaust survivors, and this has stimulated her research interests, perhaps in her career choices, but certainly in designing clinical research with Holocaust survivors (150).

Large-scale traumatic events have influenced general politics as seen for the integration of PTSD into DSM-III. Research directions may be influenced by historical events directly (this chapter), but also indirectly, through research funding that are attributed to specific topics.

### Research funding

Following recognition of PTSD as a disorder, PTSD clinics were created and run throughout the USA by the Veterans Affairs department, with massive financial support from the federal government (67). Scientific research on PTSD resonated in Veterans Affairs centers, as it was stimulated by clinical issues encountered by healthcare professionals, and enabled by substantial financial resources (67). We have identified researchers whose scientific and theoretical contributions were instrumental in the advent of animal models and fear conditioning as PTSD models (chapters 2-4). Many have worked for the Veterans Affairs Department, in clinical and/or research centers.

Did subsequent historical events influence research funding for PTSD? For instance, biomedical research on PTSD accelerated in the 2000s (Figure 5), which temporally follows 9/11, the first significant attacks on the USA territory. We attempted to identify putative links between historical events and how research funding dedicated to the study of PTSD has evolved. The NIH RePORT database (151) was searched from 1985 (its creation) to 2018. Depression was used as a comparison. Results are presented below (Figure 19). Because depression research is funded by an order of magnitude more than PTSD research (in 1.46 billion dollars vs. 110 million dollars in 2018), the data were normalized to the sum of funding during the entire period.



**Figure 19 : Trends of funding of PTSD and depression research.** Data from the National Institutes of Health RePORT database (151), normalized by the sum of total funding during the entire study period.

The graph suggests that indeed regain of interest in PTSD following 2001 was accompanied by increased financial support from NIH to study PTSD. Whereas depression and PTSD research funding followed a similar trend, a closer look at the 1998-2006 period showed that during 1998-2003, research on depression grew while PTSD plateaued. After plateauing, PTSD research funding increased steadily from 2002 while national efforts to promote research on depression slowed down. This increase of funding for PTSD continued in the late 2000s, probably strengthened by the increasing number of PTSD cases represented by returning soldiers from Afghanistan and Iraq wars (2001-2014 and 2003-2011, respectively). The trough in the graphs seem to correspond to the December 2015-January 2016 shutdown of the Federal Congress.

Research funding is influenced by history outside of science, but obviously by scientific context itself. Biological psychiatry has become a dominant paradigm starting from the 1980s, leading to development of experimental psychiatry and animal models. An article published in 2001 before 9/11, reported on a national workshop organized to facilitate modeling of psychiatric disorders with animal models. “The National Institute of Mental Health convened a workshop to discuss the relationship between existing behavioral models of anxiety and the clinical profile of anxiety disorders. A second goal of this workshop was to outline various approaches towards modeling components of anxiety disorders” and [it was] concluded that: “The probability of developing comprehensive animal models that accurately reflect the relative influences of factors contributing to anxiety disorder syndromes [was] quite low” but that “ample opportunity remain[ed] to better define and extend existing models and behavioral measures related to specific processes that may be disrupted in anxiety disorders and to

develop new models that consider the impact of combined factors in determining anxious behaviors.” (Shekar 2001). Investing on topics already stimulated by scientific and political contexts may result in amplification of scientific trends.

We speculate that the increase of funding dedicated to PTSD research, because of historical reasons, has interacted with clinical interest in the condition and with scientific interest in fear conditioning, amplifying the attraction towards fear conditioning-based models.

#### An American history

This thesis has highlighted the influence of history of science and politics on the construction of neurobiological theories of PTSD. In addition to time, *place* is also critical, because research is fostered by direct interactions between individuals and their scientific and cultural backgrounds.

All chapters implicitly contained the notion of a paramount influence of the USA in constructing this conceptual framework. Be it the creation of PTSD (this chapter), the development of the fear conditioning theory (by Davis and Ledoux), the early theoretical modeling of PTSD with fear conditioning (chapter 2), or the development of PTSD research in animals and Human using fear conditioning (chapters 3-4), all have emerged in the USA. This mirrors the world-wide financial, political and scientific influence of the USA during the 20<sup>th</sup> century. Ironically, the first explicit animal model of PTSD was published by a Canadian team (83).

A second country that played a role in the development of PTSD models using fear conditioning is the State of Israel. Clinician-scientist Shalev, MD has played an important role in developing pioneering theoretical models of PTSD that included fear conditioning. He and his colleague Bonne represent the link between the two groups of American researchers who contributed most to the fear conditioning theory of PTSD (this chapter).

Moreover, Israeli scientists Cohen and Zoladz have contributed several seminal studies in the field of PTSD modeling in animals with predator stress models and their modification to include the fear conditioning dimension (chapter 4).

These two leading countries in the field of PTSD modeling are linked by a collective trauma, the Holocaust. This link is exemplified in the personal accounts of Krystal and Yehuda (149,150).

## Summary

This chapter suggests that the birth and development of fear-conditioning as a model of PTSD have resulted from a complex synergy between individuals, scientific paradigms and geopolitical history, in the USA.

Scientific development of fear learning and synaptic plasticity theories coincided with clinical and general audience interest in the newly-described post-traumatic stress disorder. Co-incidence was materialized in theoretical intersection with the implication of individuals in both clinical and fundamental research. With respect to this personification of research, it is striking that only a small number of clinically-involved researchers have played critical roles in shaping the field. Biological psychiatry has also provided a fertile ground for the expansion of the fear conditioning theory of PTSD.

Technical advancements in biomedical research have contributed to the creation of theoretical paradigms. While fear conditioning had not been considered as an animal model of PTSD in the 1990s, it is tempting to think that fMRI experiments have contributed to its return in the 2000s. These experiments, using *de novo conditioning* in patients, were interpreted by fear conditioning supporters as evidence for the implication of the fear circuit in PTSD. Simultaneously, animal models of PTSD using fear conditioning have emerged and expanded.

This chapter also indicated that individual and collective links (geographic, political) with PTSD might have stimulated biomedical research in the field. Wars and collective traumata such as the Holocaust attracted attention and funding to the study of PTSD. We speculate that the interaction between individuals, historical events and scientific context in the USA has led to the creation and expansion of the fear conditioning model in PTSD.



## VII- Discussion

*It is scarcely possible to read a grant proposal nowadays without encountering the buzz word 'translational'. Pitman, Rasmusson, Koenen, Shin, Orr, Gilbertson, Milad and Liberzo; 2012.*

### Main findings

Fear conditioning is a dominant experimental and theoretical model of PTSD. Heterogeneous forms of fear conditioning are used to model PTSD and criticism against their validity have been formulated, raising the question of how fear conditioning has become a model of PTSD and with what theoretical implications. The aim of this work was to better understand the history of conceptual links between PTSD and fear conditioning, in order to place current research in a clearer conceptual framework.

A historical method was used, first with qualitative analyses, i.e. manual reviews of the literature, systematic whenever possible, and of selected articles when necessary (high number of articles). Qualitative analyses were complemented with quantitative analyses of entire literature corpuses (automated network mapping). Both approaches converged towards the same conclusions. An analysis of the historical context itself was carried out, to formulate theories regarding causes of conceptual evolutions. The main findings of this thesis are summarized here.

We found that fear conditioning has been linked to PTSD by analogy soon after the recognition of PTSD (1980-1993). The analogy was made between trauma and conditioning, and between reexperiencing and conditioned responses. This analogy was attractive because it concerned the two specific features of PTSD: trauma and reexperiencing. Experimental parallels in PTSD patients, evoking stress responses to trauma reminders, lent support to the theory. To account for clinical features beyond reexperiencing and avoidance, non-associative memory theories of sensitization and kindling were put forth. Long-term changes in the neural circuit of fear and the hormonal stress system, by synaptic plasticity, were proposed to underly conditioning and sensitization, respectively. We proposed that conditioning-based theories of PTSD emerged from interactions between fear conditioning, synaptic plasticity, interest in PTSD, within small groups of scientists, in a special historical context.

Explicit animal models of PTSD were found in articles published from 1993. Experimental models of PTSD directly resulted from previous theoretical considerations. Intriguingly, in the 1990s creators of PTSD animal models focused only on the sensitization dimension. Most animal models were then modified versions of models previously used to study responses to "stress". The development of PTSD models coincided with the beginning of the evaluation of their validity, and this trend was profoundly influenced by a single article, published by Yehuda and Antelman in 1993.

We have found no evidence for the use of classical fear conditioning to model PTSD before 2003. From there onward, however, fear conditioning-based models have rapidly expanded to their dominant position in the field of PTSD neurobiology. Expansion was observed in several directions. Sensitization-based models have been complemented to include a conditioning dimension, becoming modified fear conditioning paradigms by themselves. *De novo* (classical) fear conditioning has been applied in sensitization-based models to test for face validity, on the ground of deficient fear extinction in patients with PTSD. Last, classical fear conditioning paradigms alone have started to be used as PTSD models.

The diversity observed in fear conditioning-based models of PTSD has confirmed the problem of heterogeneity identified at the beginning of this thesis. Conceptual analysis of the different models has shown that vagueness has resulted from this heterogeneity, and from loose definitions of constructs at stake. Modeling PTSD with a combination of intense stress and classical fear conditioning, or with fear conditioning, an adaptive learning, sets an ambiguity in the diathesis of PTSD: what is the trauma? On the other hand, some modified fear conditioning paradigms display good construct, face and predictive validity. Because these models are referred to as “fear conditioning”, or even as “classical fear conditioning” (while they are somewhat different from classical fear conditioning), they carry the risk of conceptual assimilation. In other words, conclusions made on mechanisms of classical fear conditioning may be abusively applied to dedicated models of PTSD.

Finally, we proposed that the implication of the same group of scientists who formulated the fear conditioning/PTSD analogy, the invention of fMRI with studies of the “fear circuit” in PTSD, and a renewed interest and funding to study PTSD following 9/11, all contributed to sustain the expansion and heterogeneous content of fear conditioning-based models of PTSD, and, in turn, to PTSD.

### Internal validity and limitations

The aim of this thesis was to study the history of conceptual links between fear conditioning and PTSD. In order to try and be as objective as possible, and restrict allegiance and confirmation biases, complementary methods were applied. Traditional reviews of the literature, were complemented with text mining methods borrowed from the digital humanities. Complementary approaches yielded similar results, providing consistent answers to the questions asked.

However, the main limitation of the present work is the inevitably subjective nature of its methodology. Although automated algorithmic analysis and systematic reviews of the literature were performed, the object of the analysis is conceptual, and difficult to standardize.

- Main trends of articles published between 2005-present were obtained by studying secondary sources (review articles) and individual articles, selected to be representative of a trend (chapter 4). With this method, confirmation bias cannot be ruled out. A systematic review is warranted of all 242 articles published between 2005-2018 on the topic of [“fear conditioning” + “PTSD” + “animal model”] (ISI web of knowledge). When considering PTSD and fear conditioning, around 1000 articles would need to be analyzed manually, representing several months of work by itself. These systematic reviews were beyond the scope of this thesis.
- Subjectivity is obvious in analysis of plain text, but it also applies to analysis of text mining (chapter 5). Despite being unbiased in their construction (generated by algorithms), network maps were inspected visually, with qualitative interpretations. The complexity of the results is beyond simple interpretation by human investigators. We have tried and describe main trends and objective observations in semantic network maps in order to reduce subjectivity. Other methods could complement our analysis, such as topic modeling or correlation between weighted vectors of semantic cluster and vectors of semantic content in individual articles (133).
- Analysis of the context in which the fear conditioning model of PTSD has been developed (chapter 6) is partly speculative. This analysis does not claim to provide answers, but proposes hypotheses. Additional work is needed to analyze more thoroughly the historical and anthropological determinants of scientific theories, similar to what has been done for the “invention” of PTSD (67).

The second limitation is the search of sources in electronic databases. Articles published before the 1990s are often missing from main databases (Pubmed, ISI web of knowledge). We have used Google Scholar, that provides references on a more liberal basis, and performed extensive investigation of reference lists in relevant articles. However, we cannot rule out that early references were missed because of this methodology. In particular, articles that have a greater impact on later scientific research are better referenced. On the contrary, articles presenting more confidential theories might be absent. As a result of this methodology, alternative theories might have been missed.

A third potential caveat is the approach chosen to study the 1993-2005 literature, dealing with PTSD modeling. Our work may lead to think that the fear conditioning theory had disappeared while sensitization-based animal models of PTSD were created (chapter 3). However, the conditioning model was still discussed in theoretical articles by authors identified in chapter 6 (152,153). For clarity, we did not present the overlap of theoretical studies of PTSD mechanisms that included fear conditioning and actual animal models that used sensitization. Reviewing in-depth theoretical articles between 1993-2005 may help to clarify this issue.

## External validity

No study of the kind presented in this manuscript was found.

Many authors have analyzed and reviewed the experimental supports to fear conditioning theories in PTSD. We have cited some of these (e.g. (28,29,39)). VanElzakker *et al.* have dedicated a review article to what they considered a key role of extinction deficit in PTSD (*From Pavlov to PTSD: The extinction of conditioned fear in rodents, humans, and anxiety disorders* (154)). Its content is similar to what has been described in chapter 4. It confirms the influence of the fear conditioning and extinction. VanElzakker and colleagues proposed a fear conditioning framework to understand PTSD, with a central role attributed to extinction deficits, and experimental support drawn from animal research on PTSD and fMRI experiments in healthy and PTSD subjects, combined with psychophysiological experiments, including *de novo* fear conditioning (154).

Boscarino and Figley (155) reviewed early clinical work on PTSD and acknowledged the great importance of Veterans Affairs centers in generating research material from large cohorts of patients. We and others (67) have made a similar observation.

Lissek and van Meurs, like many, considered PTSD as a learning and memory disorder. They reviewed all the associative and non-associative theories of PTSD and their experimental support. Interestingly, results of their systematic review is in line with observations made in chapters 2 and 4 (156). Fear learning was divided into associative and non-associative dimensions. Associative learning comprised 1) resistance to extinguish conditioned fear, 2) Mowrer's two-stage learning, 3) stimulus generalization, 4) hyper-conditionability, and 5) associative-learning deficits leading to contextual anxiety. Non-associative learning included 1) habituation, and, 2) sensitization (including kindling). This list is similar to what we extracted from theoretical articles published after the recognition of PTSD (chapter 2). They described experimental work lending support to the extinction deficit theory, consisting in psychophysiological tasks combined with fMRI, that suggest a role for the PFC in pathological extinction. This is consistent with propositions made in chapter 4 of this manuscript. Moreover, Lissek and van Meurs criticized the centrality of conditioning paradigms in clinical research for PTSD, and suggested to assess several learning aspects within single research protocols (156).

No study has been published on the history of animal models of PTSD, although many articles have been written on the models and their validities. The most thorough review article has been published by Deslauriers *et al.* in 2018 (36). They reviewed over 600 papers to assess the validity and the main results obtained with stressor-based models of PTSD. We have used this article as a reference throughout this thesis.

Somewhat surprisingly, we found that the concept of conditioning was had been used to account for the consequences of psychological trauma existed before the recognition of PTSD, as early as WWII. Morphy wrote in 1938 an article dealing with body-mind interactions in the context of mental disorders, reviewing psychobiological and psychoanalytic theories. He wrote “many instances of this conditioning can be found in the records of war neurosis”(43).

Similarly, Sargant and Slater, published a study on « acute war neurosis » and wrote : “the degree to which recovery is complete [after military hospital treatment] is more doubtful. It seems that these patients have gone through a process for which the term conditioning may be used, for lack of a better expression” (157).

In these articles, the meaning of conditioning is more similar to learning, with induction of non-associative traces. Pre-existence of conditioning theories does not change the main conclusions of this thesis, but the fact that conditioning theories of PTSD appeared in the 1980 may need to be examined. Because this thesis focused on *bona fide* PTSD, articles published before 1980 were beyond its scope.

## Perspectives

The present work opens opportunities for conceptual and experimental changes, and for building more efficiently on the extensive literature on the topics of fear conditioning and PTSD.

### Clarification of concepts

First, conceptual vagueness, ambiguity and semantic assimilations have likely hindered progress in understanding the neurobiology of PTSD, despite great scientific and financial investments.

It is necessary for the full exploitation of this massive investment that scientists 1) use unambiguous denomination of their experimental paradigms and conceptual constructs and 2) explicitly discuss the conceptual span and limitations of their work. If editing constrains prevent lengthy discussions of concepts in scientific journals, supplementary material may be used for this purpose. As with experimental methods, disclosure of conceptual considerations will be needed if we are to claim validity for animal models in psychiatry.

Moreover, researchers in the field could agree on definitions of the main constructs. We speculate that semantic clarifications would be sufficient as a first step towards a better outcome of translational research for PTSD.

### Using animal models dedicated to PTSD

Animal models of PTSD have to date contributed no significant improvement in managing PTSD in the clinical setting. Pitman and colleagues have recently pointed to this gap between scientific developments and clinical benefits: “it is scarcely possible to read a grant proposal nowadays without encountering the buzz word ‘translational’. If translational is taken to mean the cross-fertilization that can be achieved when results from animal research are used to inform human studies and vice versa, then PTSD research has been highly fruitful in this regard. However, if translational is taken to mean the conversion of research results into novel and effective treatments, the story is different. The currently most effective treatment for PTSD, viz., cognitive behavioural therapy, was conceived entirely on psychological grounds. Trials of the most effective drugs for PTSD, viz., SSRIs, were based on these drugs’ observed anti- depressant effect” (28).

Several suggestions may be made to improve this situation and go beyond current limitations :

- To use multiple models, in order to increase validity. In a categorial view, preclinical studies should primarily focus on animal models of maladaptive fear/stress reactions (158)(but see below). State-of-the art techniques have been applied in classical fear conditioning paradigms,

but not to stressor-based models of PTSD (158). Semantic vagueness should be overcome by direct testing of neurobiological hypotheses in dedicated PTSD models.

- To regularly assess the validity of models and recognize what can be modeled or not (previous section), and only experiment on reasonably valid models. The inescapable shock model with contextual conditioning appears as a good choice ((118); see chapter 4).
- To create review committees dedicated to this regular evaluation. Experts could assess the level of scientific evidence, and the relevance of the constructs and animal models.
- To develop treatment-driven translational research. Findings in animal models should be validated as much as possible in Human patients (biomarkers, mechanisms, treatments; (158)).

Moreover, regular and alternative evaluations of nosological entities should give feedback to fundamental work in animals. Animal models are indeed entirely determined by the definitions of diseases.

The link of PTSD with fear conditioning may be written in its own name

The recognition of PTSD in DSM-III (1) and its definition has led to the creation of animal models. We speculate that the name chosen for the disorder is indicative of conceptual constructs determined by stress and behavioral theories, the direct consequence of which is modeling PTSD with fear conditioning. “Post- Traumatic- Stress- Disorder” :

- *Post traumatic*: this suggests the persistence of the trauma, a “hidden” memory. According to Young, the recognition of a mnemonic trace has been central to the “invention” of the PTSD construct (67). The concept of a traumatic memory provides a temporal link between trauma and symptoms, but also a nosological link between symptoms that are otherwise unspecific (67).
- *Stress disorder*: with this name, the concept of “stress” is considered to recapitulate the full spectrum of post-traumatic symptoms, as a consequence of the traumatic trace. The memory can express itself in front of trauma reminders, or spontaneously. Trauma itself is often conceptualized as a stress. We have seen that “PTSD” is constructed in animals with the application of intense stressors to model traumatic experience. In DSM-III, the trauma was defined as “a stressor” (1). As a result, we are facing a *post-stress, stress disorder*.

By juxtaposition of these entities (stress → memory → stress), a logical link can be traced between PTSD and behavioral paradigms in which negative memory traces are imprinted in animals confronted

with a negative-valence stimulus, and stress behavior is readily induced by memory cues. That is, fear conditioning. This readily explains the drive to use fear conditioning to model PTSD, theoretically and experimentally. A provocative way of formulating this hypothesis is that fear conditioning was bound to become animal models of PTSD because of the disorder's name and conceptual content.

It is likely that this did not happen by chance. In fact, the definition of the disease seems heavily impregnated by behaviorism from its creation, and, in a way, self-prophetic. The name of the disorder is reflective of this theoretical influence. We have seen that conceptual accounts of war-related disorders and conditioning actually pre-existed the creation of PTSD. Interestingly, during preparatory work for DSM-III preparation, the original proposal of the Vietnam Veterans Working group was "post-combat disorder". Their views were confronted with opinions from clinicians and researchers, among which researchers on stress, including Mardi J. Horowitz (Scott 1990; (3)). Other causes of trauma were added, and the name proposed by the committee for the new diagnosis entity was "catastrophic *stress* disorder". Finally, Spitzer, chair of the DSM-III task force, Andreasen, Committee chair, set the disorder's name into "post-traumatic stress disorder" (3).

#### Other perspectives on post-traumatic conditions

Because clinical and preclinical research interact, current limitations of the fear conditioning model in improving clinical care are leading to reconsider the PTSD landscape. The 2015 panel discussion of the International Society for Traumatic Stress Studies gathered some of the most prominent experts in the field. They highlighted the limitations of the fear-based model of PTSD in research and therapy. Here we use this expert opinion material to highlight translational how fundamental and clinical research evolve together, and to show that other perspectives on post-traumatic conditions may lead to global progress. It is noteworthy that three of the six panelist (Krystal, Southwick and Yehuda) have been identified in chapter 6 as key scientists who contributed to the advent of PTSD models, including fear conditioning.

"Dr. Yehuda opened the discussion by pointing out that confronting new truths occurs in the context of both structured research and clinical practice. The process of connecting with data, including anecdotes from patients, naturally leads to revisiting assumptions and formulating new hypotheses" (145).

Thomas Neylan criticized the importance of fear-based models:

"Dr. Neylan began his discussion by stating that he has changed his mind about the future potential of the dominant model in our field: fear conditioning. He no longer shares the commonly endorsed notion that the fear conditioning -extinction learning- exposure therapy model will lead us to accelerated discovery in our field. He provided an example of dialysis as an analogy for the fear-conditioning model



to explain his position. In the 1960s, the field of nephrology was advancing rapidly culminating in the development of dialysis which offered treatment for the first time for patients with end-stage kidney failure (...). Following the development of dialysis, the pace of discovery in the field of nephrology plateaued outside of the separate field of organ transplantation. The field has produced incremental refinements and dissemination, but has not enjoyed a period of rapid scientific achievement and treatment development that characterized the early days of dialysis. Dr. Neylan expressed the concern that exposure therapy which is based on fear- conditioning model of PTSD is our form of dialysis” (145) On the same occasion, Dr Krystal also expressed concern: “The fear-based model that has advanced the field is beginning to look a bit outdated. We need to think in other ways and test them out” (145).

Instead, PTSD specialists recognized the critical importance of existential modifications induced by trauma. Southwick stated that “While fear-based models of therapy have helped countless trauma survivors, these models do not fully address trauma-related concerns such as moral injury, guilt, and shame” (145).

These dimensions of moral and cognitive injuries have long been considered central in post-traumatic by many authors above stress reactions in psychological accounts of PTSD (62,63,67). It might be argued that PTSD definition and treatments should give more credit to this theory, although these aspects of PTSD may not be prone to modelling in animals.

Mental disorders need to be regularly re-defined in light of novel knowledge, and this is critical for translational research progress. However, one problem is that changes in clinical definitions mean a moving target for the validity of animal models. A solution has been proposed to this problem and to enhance translational value of animal research: the Research Domain Criteria (RDoC) (159).

#### A dimensional framework

The RDoC is not a new nosography; it has been created to facilitate comparisons between clinical and fundamental research. It describes mental conditions not as a monolithic groups of symptoms, (categorical classification such as the DSM), but instead as varying combinations (a matrix) of constructs described along a span of functioning from normal to pathological. Such an approach is referred to as “dimensional” (160).

The RDoC matrix is organized around six domains of human functioning: negative valence systems, positive valence systems, cognitive systems, systems for social processing, arousal/regulatory systems and sensorimotor systems. For instance, the negative valence system includes acute threat (*fear*) as a construct (159). Constructs are meant to be measurable using several different methods including molecular, genetic, neurocircuit and behavioral assessments (159), with the important notion that pathology is considered as quantitatively but not qualitatively different from the unaffected condition.

The domains are said to “reflect contemporary knowledge about major systems of emotion, cognition, motivation, and social behavior” (159). Indeed, the RDoC assumes a direct link between dimensions of psychiatric disorders and neurobiological constructs, on the basis of interspecies similarities in behavior. Neurobiological dimensions correspond to specific brain circuits as indicated by NIMH “the current set of constructs is focused on (and constrained by) circuit definitions” (159). In turn, a better understanding of the functioning of these circuits is expected to inform on pathological dimensions and therapeutic design.

Applied to modeling PTSD, this means for instance the involvement of the negative valence system of fear. This perspective would solve the controversy over the fear conditioning model of PTSD. Such a shift from unified, categorial validation of animal models of PTSD towards dimensional modeling is actually the proposal of Ressler and colleagues in their 2017 review. They divided DSM-5 criteria into constructs corresponding to dimensions of PTSD (29). Considering fear conditioning as a dimensional construct indeed offers an alternative solution to the validity issue.

Some concerns and questions regarding the translational value of RDoC should be raised, however. Neuroscience is a recent research discipline, and the neurobiology of highly complex phenomena such as cognition, emotions and behavior remain speculative and, often, anthropomorphic. Contemporary knowledge, that is said to serve as a basis for RDoC, is indeed limited.

Moreover, despite the efforts to increase robustness, precision and translational value of constructs, the RDoC are no exception to the conceptual and semantic vagueness problem. This is exemplified by the use of ill-defined construct of “fear”. Joseph LeDoux, the most notorious researcher on neurobiology of fear, has himself proposed that defensive behavior such as freezing and the subjective experience of fear in human may be entirely distinct phenomena, relying on distinct neurobiological mechanisms: “There is no need for conscious feelings of fear to intervene. The circuit function is the intervening variable. Yet, I and others [Davis and Fanselow, our note] muddied the waters by continuing to call the circuits involved in detecting and responding to threats the fear system.” (Ledoux, *Coming to terms with fear*; 2014 (161)).

Are mental disorders just a constellation of symptoms? And mental states a juxtaposition of brain circuit states? This may seem contradictory, while complex dynamics resulting from interactions between heavily interconnect brain areas are currently the subject of intense investigation (162). Likewise, what is a neuron “circuit” and can all mental states, behavior and disorders be explained by a circuit-theory? On a related note: is pathology too much of the normal thing? This is debated, supported by some (163), but not all researchers (158).

Circuit-based theories largely respond to technical advances, i.e. neuroimaging in human and tract tracing-based, opto- and pharmacogenetics methods in animals. Although these techniques are revolutionary for the study of the brain, it is known that circuit representations are simplified, to provide didactic representations of highly complex brain systems.

Thus, the RDoC might not be as robust and scientifically-informed as claimed by the NIMH, and does not solve key conceptual issues in modeling psychiatric disorders in animals.

## Outlook

By providing material to understand the history of conceptual links between PTSD and fear conditioning, this work places current research on the neurobiology of PTSD in a clearer conceptual framework.

Future conceptual clarifications of animal models will help to make the most of abundant, sophisticated and costly research that, so far, has brought no therapeutic solution. Differences between several forms of fear conditioning need to be acknowledged explicitly, and neurobiological mechanisms examined independently in each model, before extrapolations can be made. Moreover, clinical work revising the nosology of post-traumatic symptoms may open new research avenues. Overall, a better grasp of concepts and conceptual history will help select relevant information, and refine and create experiments to study PTSD.

“... the importance of existential acceptance of trauma effects and searching for meaning and post-traumatic growth [is] clearly much more difficult to put into a translational framework using animal models. We must not mistake expediency for universality. At the same time, we must use paradigms that allow scientific development while recognizing their limitations.” T. Neylan (145).

## Conclusions et perspectives

La peur conditionnée est fréquemment utilisée pour modéliser le trouble de stress post-traumatique chez l'animal. Puisque le protocole expérimental de peur conditionnée classique a été inventé au début du XX<sup>ème</sup> siècle, et que ce protocole présente des limites de validité, nous avons cherché pourquoi et de quelle manière la peur conditionnée avait été portée au rang de modèle dominant du TSPT.

L'objectif de ce travail de thèse était donc de comprendre l'évolution des liens conceptuels entre peur conditionnée et TSPT, en appliquant une méthodologie qualitative et quantitative issue des domaines de l'histoire et de la philosophie des sciences. Les deux approches ont apporté des résultats concordants.

Le premier point de convergence conceptuelle entre peur conditionnée et TSPT a été théorique, par analogie entre conditionnement et psychotraumatisme d'une part, et peur conditionnée et symptômes de reviviscence d'autre part. Cette convergence est intervenue rapidement dans la littérature scientifique (1982) après l'inclusion du TSPT dans la nosographie internationale (DSM-III, 1980 ; (1)).

L'analyse du contexte historique suggère que la convergence conceptuelle des construit de « peur conditionnée » et de « TSPT » a résulté de la coïncidence de l'intérêt clinique pour le TSPT et de travaux de neurobiologie fondamentale réalisés sur les circuits neuronaux de la peur conditionnée (Ledoux) et sur les mécanismes de plasticité synaptique, offrant une explication biologique à la mémoire (Kandel). La rencontre entre ces domaines de recherche a été incarnée par un nombre restreint de psychiatres- et psychologues-chercheurs, aux États-Unis. Cette dynamique a probablement été favorisée par des initiatives fédérales pour encourager la recherche sur le TSPT, et par des liens personnels de ces chercheurs avec la question du psychotraumatisme.

Les chercheurs ont néanmoins identifié au début des années 1990 que la peur conditionnée ne pouvait expliquer seule tous les symptômes du TSPT. La sensibilisation, ou mémoire non-associative, a alors été proposée pour expliquer les symptômes d'hyper-reactivité.

La période initiale de formulations théoriques (1980-1993) a été suivie par la proposition d'expériences menées sur le rongeur pour modéliser le TSPT. De manière surprenante, nous avons trouvé que l'ensemble des expériences initialement proposées pour modéliser le TSPT se basaient sur la théorie de la sensibilisation (1993-2003). Cette longue période d'exclusion de la peur conditionnée a été suivie par l'apparition de modèles explicites de PTSD basés sur le conditionnement de peur, dans sa forme classique, mais également dans des formes modifiées (2003-2005).

Par la suite (2005-présent), la peur conditionnée est devenue polysémique. Des modèles animaux de sensibilisation ont été adaptés pour inclure une dimension de peur conditionnée modifiée. De plus, le conditionnement de peur classique, appliqué *après* le stress de sensibilisation, a été mis en

place pour étudier les déterminants d'une altération de l'extinction de la peur dans ces modèles. Le concept d'extinction insuffisante de la peur est central dans la théorie moderne du TSPT. Nous avons proposé que l'implication des scientifiques à l'origine des travaux théoriques sur la peur conditionnée dans le TSPT, et le développement de technologies permettant l'étude dynamique de « circuits neuronaux de la peur (conditionnée) » chez des sujets humains, ont suscité un intérêt nouveau pour la peur conditionnée dans le TSPT. Cet intérêt a rapidement été décuplé, toujours dans un contexte géopolitique de traumatismes collectifs, impliquant les États-Unis.

L'avènement du modèle théorique de peur conditionnée en modèle expérimental dominant révèle néanmoins le problème de l'hétérogénéité des modèles (peur conditionnée modifiée, classique, *de novo*). Cette hétérogénéité s'accompagne d'un flou conceptuel majeur, d'une diathèse ambiguë (qui de la sensibilisation ou du conditionnement modélise le trauma ?), et d'extrapolations de mécanismes biologiques sur la base d'assimilations sémantiques.

Une clarification sémantique et conceptuelle des modèles animaux du TSPT sera nécessaire pour permettre d'extraire d'éventuelles informations pertinentes pour le traitement du trouble.

D'autres propositions ont été formulées, comme l'utilisation d'une approche dimensionnelle de la pathologie mentale, par le NIMH (159). Sur la base de construits bio-comportementaux quantifiables liés à des bases biologiques objectives, l'espoir de cette approche est de dépasser les limites de l'approche catégorielle (DSM), et de faciliter la recherche translationnelle. Toutefois, nous suggérons que l'approche dimensionnelle est confrontée aux mêmes limites fondamentales que l'approche catégorielle : les liens entre circuits neuronaux et activité mentale sont incertains, marqués par le flou conceptuel, et empreints d'anthropomorphisme.

Enfin, nous avons émis l'hypothèse que la définition originale du TSPT, et son nom, avaient par leurs influences théoriques contraint la recherche fondamentale et clinique dans le cadre conceptuel du stress (un *trouble de stress post-stress*). De fait, les théories psychologiques et comportementales développées dans les années 1970 aux États-Unis accordaient une large place au concept de stress. L'influence directe des théories du stress et de ses tenants sur la définition du trouble a ainsi été documentée par Scott, en 1990 (3).

Le futur de la recherche dans le domaine du TSPT devrait bénéficier d'une part de clarifications conceptuelles de ses modèles animaux, et d'autre part de l'exploration d'autres cadres théoriques, pour définir et traiter les conditions post-traumatiques (145).

## References

1. DSM-III: Diagnostic and statistical manual of mental disorders. Fifth edition. Arlington, VA : American Psychiatric Association. 1980.
2. DSM-5: Diagnostic and statistical manual of mental disorders. Fifth edition. Arlington, VA : American Psychiatric Association. 2013.
3. Scott WJ. PTSD in DSM-III: A Case in the Politics of Diagnosis and Disease. *Social Problems*. 1990 Aug;37(3):294–310.
4. North CS. The evolution of PTSD criteria across editions of DSM. *Annals of Clinical Psychiatry* 2016;28(3):197-208
5. Kessler RC. Posttraumatic Stress Disorder in the National Comorbidity Survey. *Archives of General Psychiatry*. 1995 Dec 1;52(12):1048.
6. Smith TC, Wingard DL, Ryan MAK, Kritz-Silverstein D, Slymen DJ, Sallis JF, et al. PTSD Prevalence, Associated Exposures, and Functional Health Outcomes in a Large, Population-Based Military Cohort. *Public Health Reports*. 2009 Jan;124(1):90–102.
7. Treatment for Posttraumatic Stress Disorder in Military and Veteran Populations: Final Assessment. *Military Medicine*. 2014 Dec;179(12):1401–3.
8. Kilpatrick DG, Resnick HS, Milanak ME, Miller MW, Keyes KM, Friedman MJ. National Estimates of Exposure to Traumatic Events and PTSD Prevalence Using *DSM-IV* and *DSM-5* Criteria: *DSM-5* PTSD Prevalence. *Journal of Traumatic Stress*. 2013 Oct;26(5):537–47.
9. Breslau N, Kessler RC, Chilcoat HD, Schultz LR, Davis GC, Andreski P. Trauma and Posttraumatic Stress Disorder in the Community: The 1996 Detroit Area Survey of Trauma. *Archives of General Psychiatry*. 1998 Jul 1;55(7):626.
10. Brunello N, Davidson JRT, Deahl M, Kessler RC, Mendlewicz J, Racagni G, et al. Posttraumatic Stress Disorder: Diagnosis and Epidemiology, Comorbidity and Social Consequences, Biology and Treatment. *Neuropsychobiology*. 2001;43(3):150–62.
11. Shalev AY, Gevonden M, Ratanatharathorn A, Laska E, van der Mei WF, Qi W, et al. Estimating the risk of PTSD in recent trauma survivors: results of the International Consortium to Predict PTSD (ICPP): Estimating the risk of PTSD in recent trauma survivors: results of the International Consortium to Predict PTSD (ICPP). *World Psychiatry*. 2019 Feb;18(1):77–87.
12. Davidson JRT, Stein DJ, Shalev AY, Yehuda R. Posttraumatic Stress Disorder: Acquisition, Recognition, Course, and Treatment. (*The Journal of Neuropsychiatry and Clinical Neurosciences*. 2004;16:135–147.
13. The Management of Post-Traumatic Stress Working Group. VA/DoD Clinical Practice Guideline for the Management of Post-Traumatic Stress. Department of Veterans Affairs Department of Defense; 2010.
14. World Health Organization. Guidelines for the management of conditions specifically related to stress. Geneva: WHO; 2013.
15. The American Psychological Association. Clinical practice guidelines for the treatment of PTSD. 2017. 139 p.

16. Post-traumatic stress disorder -The management of PTSD in adults and children in primary and secondary care. Royal College of Psychiatrists; 176 p.
17. Baldwin DS, Anderson IM, Nutt DJ, Allgulander C, Bandelow B, den Boer JA, et al. Evidence-based pharmacological treatment of anxiety disorders, post-traumatic stress disorder and obsessive-compulsive disorder: A revision of the 2005 guidelines from the British Association for Psychopharmacology. *Journal of Psychopharmacology*. 2014 May;28(5):403–39.
18. Mills KL, Hlth B, Teesson M, Ross J, Peters L. Trauma, PTSD, and Substance Use Disorders: Findings From the Australian National Survey of Mental Health and Well-Being. *Am J Psychiatry*. 2006;7.
19. Davis M. The role of the amygdala in fear-potentiated startle: implications for animal models of anxiety. *Trends in Pharmacological Sciences*. 1992 Jan;13:35–41.
20. LeDoux JE. Emotion Circuits in the Brain. *Annual Review of Neuroscience*. 2000;23(1):155–84.
21. Watson JB, Rayner R. Conditioned emotional reactions. *Journal of Experimental Psychology*. 1920 Feb;III(1):1–14.
22. Phillips RG, LeDoux JE. Differential Contribution of Amygdala and Hippocampus to Cued and Contextual Fear Conditioning. :12.
23. Kaouane N, Porte Y, Vallee M, Brayda-Bruno L, Mons N, Calandreau L, et al. Glucocorticoids Can Induce PTSD-Like Memory Impairments in Mice. *Science*. 2012 Mar 23;335(6075):1510–3.
24. Quirk GJ, Mueller D. Neural Mechanisms of Extinction Learning and Retrieval. *Neuropsychopharmacology*. 2008 Jan;33(1):56–72.
25. Herry C, Johansen JP. Encoding of fear learning and memory in distributed neuronal circuits. *Nature Neuroscience*. 2014 Dec;17(12):1644–54.
26. Sotres-Bayon F, Quirk GJ. Prefrontal control of fear: more than just extinction. *Current Opinion in Neurobiology*. 2010 Apr;20(2):231–5.
27. Dejean C, Courtin J, Rozeske RR, Bonnet MC, Dousset V, Michelet T, et al. Neuronal Circuits for Fear Expression and Recovery: Recent Advances and Potential Therapeutic Strategies. *Biological Psychiatry*. 2015 Sep;78(5):298–306.
28. Pitman RK, Rasmusson AM, Koenen KC, Shin LM, Orr SP, Gilbertson MW, et al. Biological studies of post-traumatic stress disorder. *Nature Reviews Neuroscience*. 2012 Nov;13(11):769–87.
29. Ross DA, Arbuckle MR, Travis MJ, Dwyer JB, van Schalkwyk GI, Ressler KJ. An Integrated Neuroscience Perspective on Formulation and Treatment Planning for Posttraumatic Stress Disorder: An Educational Review. *JAMA Psychiatry*. 2017 Apr 1;74(4):407.
30. Willner P. The validity of animal models of depression. *Psychopharmacology*. 1984 Apr;83(1):1–16.
31. Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. *Nature Neuroscience*. 2010 Oct;13(10):1161–9.

32. Belzung C, Lemoine M. Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression. *Biology of Mood & Anxiety Disorders* [Internet]. 2011 Dec. Available from: <https://biolmoodanxietydisord.biomedcentral.com/articles/10.1186/2045-5380-1-9>
33. Goswami S, Rodríguez-Sierra O, Cascardi M, Paré D. Animal models of post-traumatic stress disorder: face validity. *Frontiers in Neuroscience* [Internet]. 2013(7);89. Available from: <http://journal.frontiersin.org/article/10.3389/fnins.2013.00089/abstract>
34. Berardi A, Trezza V, Campolongo P. Modeling specific phobias and posttraumatic stress disorder in rodents: the challenge to convey both cognitive and emotional features. *Reviews in the Neurosciences*. 2012;23(5–6).
35. Borghans B. Animal models for posttraumatic stress disorder: An overview of what is used in research. *World Journal of Psychiatry*. 2015;5(4):387.
36. Deslauriers J, Toth M, Der-Avakian A, Risbrough VB. Current Status of Animal Models of Posttraumatic Stress Disorder: Behavioral and Biological Phenotypes, and Future Challenges in Improving Translation. *Biological Psychiatry*. 2018 May;83(10):895–907.
37. Yehuda R, LeDoux J. Response Variation following Trauma: A Translational Neuroscience Approach to Understanding PTSD. *Neuron*. 2007 Oct;56(1):19–32.
38. Johnson LR, McGuire J, Lazarus R, Palmer AA. Pavlovian fear memory circuits and phenotype models of PTSD. *Neuropharmacology*. 2012 Feb;62(2):638–46.
39. Mahan AL, Ressler KJ. Fear conditioning, synaptic plasticity and the amygdala: implications for posttraumatic stress disorder. *Trends in Neurosciences*. 2012 Jan;35(1):24–35.
40. Burghardt NS, Bauer EP. Acute and chronic effects of selective serotonin reuptake inhibitor treatment on fear conditioning: Implications for underlying fear circuits. *Neuroscience*. 2013 Sep;247:253–72.
41. Lebrón-Milad K, Tsareva A, Ahmed N, Milad MR. Sex differences and estrous cycle in female rats interact with the effects of fluoxetine treatment on fear extinction. *Behavioural Brain Research*. 2013 Sep;253:217–22.
42. Gunduz-Cinar O, Flynn S, Brockway E, Kaugars K, Baldi R, Ramikie TS, et al. Fluoxetine Facilitates Fear Extinction Through Amygdala Endocannabinoids. *Neuropsychopharmacology*. 2016 May;41(6):1598–609.
43. Morphy AG. A review of recent literature on neuroses and psychoneuroses. *The Canadian Medical Association Journal*. 1938 Sep;39(3):282–6.
44. Beckers T, Krypotos A-M, Boddez Y, Effting M, Kindt M. What's wrong with fear conditioning? *Biological Psychology*. 2013 Jan;92(1):90–6.
45. Flandreau EI, Toth M. Animal Models of PTSD: A Critical Review. In: Vermetten E, Baker DG, Risbrough VB, editors. *Behavioral Neurobiology of PTSD*. 2017. p. 47–68.
46. Dougherty DD, Brennan BP, Stewart SE, Wilhelm S, Widge AS, Rauch SL. Neuroscientifically Informed Formulation and Treatment Planning for Patients With Obsessive-Compulsive Disorder: A Review. *JAMA Psychiatry*. 2018 Oct 1;75(10):1081.
47. Pavlov IP. *Conditioned Reflexes. An investigation of the physiological activity of the cerebral cortex*. Oxford University Press; 1927.



48. Kolb LC, Multalipassi LR. The conditioned emotional response: a sub-class of the chronic and delayed post-traumatic stress disorder. *Psychiatric Annals*. 1982;12:979–87.
49. Brett EA, Ostroff R. Imagery and Posttraumatic Stress Disorder: An Overview. *American Journal of Psychiatry*. 1985 Apr;142(4):417–24.
50. Keane TM, Zimering RT, Caddell JM. A Behavioral Formulation of Posttraumatic Stress Disorder in Vietnam Veterans. *the Behavior Therapist*. 1985;8(1):9–12.
51. Pitman RK. Post-Traumatic Stress Disorder, Hormones, and Memory. *Biological Psychiatry*. 1989;26:221–3.
52. Kolb LC. A Neuropsychological Hypothesis Explaining Posttraumatic Stress Disorders. *American Journal of Psychiatry*. 1987 Aug;144(8):989–95.
53. Shalev AY, Rogel-Fuchs Y, Pitman RK. Conditioned Fear and Psychological Trauma. *Biological Psychiatry*. 1992;31:863–5.
54. Shalev AY, Rogel-Fuchs Y. Psychophysiology of the posttraumatic stress disorder: from sulfur fumes to behavioral genetics.: *Psychosomatic Medicine*. 1993 Sep;55(5):413–23.
55. Pitman RK, Orr SP, Shalev AY. Once Bitten, Twice Slay: Beyond the Conditioning Model of PTSD. *Biological Psychiatry*. 1993;33:145–6.
56. van der Kolk B, Greenberg M, Boyd H, Krystal J. Inescapable shock, neurotransmitters, and addiction to trauma: Toward a psychobiology of post traumatic stress. *Biological Psychiatry*. 1985 Mar;20(3):314–25.
57. van der Kolk BA. The drug treatment of post-traumatic stress disorder. *Journal of Affective Disorders*. 1987 Sep;13(2):203–13.
58. van der Kolk BA. The trauma spectrum: The interaction of biological and social events in the genesis of the trauma response. *Journal of Traumatic Stress*. 1988 Jul;1(3):273–90.
59. Kosten T.R., Krystal J. (1988) Biological Mechanisms in Posttraumatic Stress Disorder Relevance for Substance Abuse. In: Galanter M. et al. (eds) *Recent Developments in Alcoholism*. Springer, Boston, MA
60. Krystal JH, Kosten TR, Southwick S, Mason JM, Perry BP, Giller. Neurobiological Aspects of PTSD: Review of Clinical and Preclinical Studies. *Behavior Therapy*. 1989;20:177–98.
61. Charney DS. Psychobiologic Mechanisms of Posttraumatic Stress Disorder. *Archives of General Psychiatry*. 1993 Apr 1;50(4):294.
62. Foa EB, Steketee G, Rothbaum BO. Behavioral/cognitive conceptualizations of post-traumatic stress disorder. *Behavior Therapy*. 1989;20(2):155–76.
63. Foa EB, Zinbarg R, Rothbaum BO. Uncontrollability and unpredictability in post-traumatic stress disorder: An animal model. *Psychological Bulletin*. 1992;112(2):218–38.
64. Keane TM, Kaloupek DG. Imaginal Flooding in the Treatment of a Posttraumatic Stress Disorder. *Journal of Consulting and Clinical Psychology*. 1982;50(1):138–40.
65. Davey GCL, De Jong PJ, Tallis F. UCS Inflation in the aetiology of a variety of anxiety disorders: Some case histories. *Behaviour Research and Therapy*. 1993 Jun;31(5):495–8.

66. Yehuda R, Antelman SM. Criteria for Rationally Evaluating Animal Models of Posttraumatic Stress Disorder. *Biological Psychiatry*. 1993;33:479–86.
67. Young A. *The Harmony of Illusions – Inventing Post-Traumatic Stress Disorder*. Princeton University Press, NJ. 1997. 328 p.
68. Malloy PF, Fairbank JA, Keane TM. Validation of a Multimethod Assessment of Posttraumatic Stress Disorders in Vietnam Veterans. *Journal of Consulting and Clinical Psychology*. 1983;51(4):488–94.
69. Pitman RK. Psychophysiological Assessment of Posttraumatic Stress Disorder Imagery in Vietnam Combat Veterans. *Archives of General Psychiatry*. 1987 Nov 1;44(11):970.
70. Blanchard EB, Kolb LC, Pallmeyer TP, Gerardi RJ. A psychophysiological study of Post Traumatic Stress Disorder in Vietnam veterans. *Psychiatric Quarterly*. 1982;54(4):220–9.
71. Pallmeyer TP, Blanchard EB, Kolb LC. The psychophysiology of combat-induced post-traumatic stress disorder in Vietnam veterans. *Behaviour Research and Therapy*. 1986;24(6):645–52.
72. McFall ME, Murburg MM, Ko GN, Veith RC. Autonomic Responses to Stress in Vietnam Combat Veterans With Posttraumatic Stress Disorder. *Biological Psychiatry*. 1990;27:1165–75.
73. Boudewyns PA, Hyer L. Physiological Response to Combat Memories and Preliminary Treatment Outcome in Vietnam Veteran I-’ISD Patients Treated with Direct Therapeutic Exposure. *Behavior Therapy*. 1990;21:63–87.
74. LeDoux JE, Romanski L, Xagoraris A. Indelibility of Subcortical Emotional Memories. *Journal of Cognitive Neuroscience*. 1989 Jul;1(3):238–43.
75. Mowrer OH. *Learning theory and behavior*. Hoboken, NJ, US: John Wiley & Sons Inc.; 1960.
76. Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. *Experimental Neurology*. 1969 Nov;25(3):295–330.
77. Goddard GV. Development of epileptic seizures through brain stimulation at low intensity. *Nature*. 1967;214:1020–1.
78. Krystal JH. Animal models for posttraumatic stress disorder. In: *Biological assessment and treatment of posttraumatic stress disorder*. Griller E.L., Spiegel D. American Psychiatric Press Inc.; 1990. p. 3–26. (Progress in psychiatry).
79. Adamec RE. Anxious personality in the cat: Its ontogeny and physiology. In: *Psychopathology and the Brain*. New York: Raven Press; 1991. p. 153–68.
80. LeDoux JE. Emotions: clues from the Brain. *Annual Review of Psychology*. 1985;46:209–35.
81. Miserendino MJD, Carherine B. Sasanee, Melia KR, Davis M. Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala. *Nature*. 1990 Jun 21;345:716–8.
82. Siegmund A. Toward an Animal Model of Posttraumatic Stress Disorder. *Annals of the New York Academy of Sciences*. 2006 Jul 1;1071(1):324–34.

83. Adamec RE, Shallow T. Lasting effects on rodent anxiety of a single exposure to a cat. *Physiology & Behavior*. 1993 Jul;54(1):101–9.
84. Adamec R. Transmitter Systems Involved in Neural Plasticity Underlying Increased Anxiety and Defense— Implications for Understanding Anxiety Following Traumatic Stress. :11.
85. Adamec RE, Stark-Adamec C, Livingston KE. The Development of Predatory Aggression and Defense in the Domestic Cat (*Felis catus*). I. Effects of Early Experience on Adult Patterns of Aggression and Defense. *Behavioral and Neural Biology*. 1980;30:389–409.
86. Hamilton DR. Immunosuppressive effects of predator induced stress in mice with acquired immunity to *hymenolepis nana*. *Journal of Psychosomatic Research*. 1974 Jun;18(3):143–50.
87. Speed N, Engdahl B, Schwartz J, Eberly R. Posttraumatic Stress Disorder as a Consequence of the POW Experience: *The Journal of Nervous and Mental Disease*. 1989 Mar;177(3):147–53.
88. Servatius RJ, Ottenweller JE, Natelson BH. Delayed startle sensitization distinguishes rats exposed to one or three stress sessions: Further evidence toward an animal model of PTSD. *Biological Psychiatry*. 1995 Oct;38(8):539–46.
89. Pynoos RS, Ritzmann RF, Steinberg AM, Goenjian A, Prisecaru I. A behavioral animal model of posttraumatic stress disorder featuring repeated exposure to situational reminders. *Biological Psychiatry*. 1996 Jan;39(2):129–34.
90. Ottenweller JE, Servatius RJ, Tapp WN, Drastal SD, Bergen MT, Natelson BH. A chronic stress state in rats: Effects of repeated stress on basal corticosterone and behavior. *Physiology & Behavior*. 1992 Apr;51(4):689–98.
91. Servatius RJ, Ottenweller JE, Gross JL, Natelson BH. Persistent plasma cholesterol elevations are produced by one or three stressor exposures in rats fed a normal laboratory diet. *Physiology & Behavior*. 1993 Jun;53(6):1101–4.
92. Antelman SM, Caggiula AR, Kocan D, Knopf S, Meyer D, Edwards DJ, et al. One experience with ‘lower’ or ‘higher’ intensity stressors, respectively enhances or diminishes responsiveness to haloperidol weeks later: implications for understanding drug variability. *Brain Research*. 1991 Dec;566(1–2):276–83.
93. Weiss JM, Stone EA, Harrell N. Coping behavior and brain norepinephrine level in rats. *Journal of Comparative and Physiological Psychology*. 1970;72(1):153–60.
94. Liberzon I, Krstov M, Young EA. Stress-restress: Effects on ACTH and fast feedback. *Psychoneuroendocrinology*. 1997 Aug;22(6):443–53.
95. Liberzon, López, Fligel, Vázquez, Young. Differential Regulation of Hippocampal Glucocorticoid Receptors mRNA and Fast Feedback: Relevance to Post-Traumatic Stress Disorder. *Journal of Neuroendocrinology*. 1999 Jan;11(1):11–7.
96. Liberzon I, Young EA. CNS oxytocin binding: effects of stress and glucocorticoids. In: *Biological psychiatry*. 1995.
97. Charney DS, Steven M. Southwick, Krystal JH, Deutch AY, Murburg MM, Davis M. Neurobiological mechanisms of PTSD. In: *Catecholamine Function in Posttraumatic Stress Disorder: Emerging Concepts*. 42nd ed. Arlington, VA, US: American Psychiatric Association.; 1994. p. 131–58. (Progress in psychiatry).

98. Antelman SM. Time-dependent sensitization as the cornerstone for a new approach to pharmacotherapy: Drugs as foreign/stressful stimuli. *Drug Development Research*. 1988;14(1):1–30.
99. Marti O, Garcia A, Velles A, Harbuz M, Armario A. Evidence that a single exposure to aversive stimuli triggers long-lasting effects in the hypothalamus-pituitary- adrenal axis that consolidate with time. *European Journal of Neuroscience*. 2001;8.
100. Shinba T, Shinozaki T, Mugishima G. Clonidine immediately after immobilization stress prevents long-lasting locomotion reduction in the rat. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2001 Nov;25(8):1629–40.
101. van Dijken HH, Mos J, van der Heyden JAM, Tilders FJH. Characterization of stress-induced long-term behavioural changes in rats: Evidence in favor of anxiety. *Physiology & Behavior*. 1992 Nov;52(5):945–51.
102. Van Dijken HH, Van Der Heyden JAM, Mos J, Tilders FJH. Inescapable footshocks induce progressive and long-lasting behavioural changes in male rats. *Physiology & Behavior*. 1992 Apr;51(4):787–94.
103. Van Dijken HH, Tilders FJH, Olivier B, Mos J. Effects of anxiolytic and antidepressant drugs on long-lasting behavioural deficits resulting from one short stress experience in male rats. *Psychopharmacology*. 1992 Dec;109(4):395–402.
104. Carli M, Prontera C, Samanin R. Effect of 5HT1A agonists on stress-induced deficit in open field locomotor activity of rats : evidence that this model identifies anxiolytic-like activity. *Neuropharmacology*. 1989;28:471–6.
105. Armario A, Jolin T. Influence of intensity and duration of exposure to various stressors on serum TSH and GH levels in adult male rats. *Life Sciences*. 1989 Jan;44(3):215–21.
106. Armario A, Gil M, Marti J, Pol O, Balasch J. Influence of various acute stressors on the activity of adult male rats in a holeboard and in the forced swim test. *Pharmacology Biochemistry and Behavior*. 1991 Jun;39(2):373–7.
107. Belda X, Fuentes S, Nadal R, Armario A. A single exposure to immobilization causes long-lasting pituitary-adrenal and behavioral sensitization to mild stressors. *Hormones and Behavior*. 2008 Nov;54(5):654–61.
108. Balogh SA, Wehner JM. Inbred mouse strain differences in the establishment of long-term fear memory. *Behavioural Brain Research*. 2003 Mar;140(1–2):97–106.
109. Dębiec J, Ledoux JE. Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amygdala. *Neuroscience*. 2004 Jan;129(2):267–72.
110. Pawlyk AC, Jha SK, Brennan FX, Morrison AR, Ross RJ. A rodent model of sleep disturbances in posttraumatic stress disorder: The role of context after fear conditioning. *Biological Psychiatry*. 2005 Feb;57(3):268–77.
111. Rau V, DeCola JP, Fanselow MS. Stress-induced enhancement of fear learning: An animal model of posttraumatic stress disorder. *Neuroscience & Biobehavioral Reviews*. 2005 Jan;29(8):1207–23.

112. Orr SP, Metzger LJ, Lasko NB, Macklin ML, Peri T, Pitman RK. De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. *Journal of Abnormal Psychology*. 2000;109(2):290–8.
113. Mackenzie L, Nalivaiko E, Beig MI, Day TA, Walker FR. Ability of predator odour exposure to elicit conditioned versus sensitised post traumatic stress disorder-like behaviours, and forebrain  $\Delta$ FosB expression, in rats. *Neuroscience*. 2010 Aug;169(2):733–42.
114. Zohar J, Matar MA, Ifergane G, Kaplan Z, Cohen H. Brief post-stressor treatment with pregabalin in an animal model for PTSD: Short-term anxiolytic effects without long-term anxiogenic effect. *European Neuropsychopharmacology*. 2008 Sep;18(9):653–66.
115. Cohen H. The Contribution of an Animal Model Toward Uncovering Biological Risk Factors for PTSD. *Annals of the New York Academy of Sciences*. 2006 Jul 1;1071(1):335–50.
116. Zoladz PR, Fleshner M, Diamond DM. Psychosocial animal model of PTSD produces a long-lasting traumatic memory, an increase in general anxiety and PTSD-like glucocorticoid abnormalities. *Psychoneuroendocrinology*. 2012 Sep;37(9):1531–45.
117. Yamamoto S, Morinobu S, Takei S, Fuchikami M, Matsuki A, Yamawaki S, et al. Single prolonged stress: toward an animal model of posttraumatic stress disorder. *Depression and Anxiety*. 2009 Dec;26(12):1110–7.
118. Siegmund A, Wotjak CT. Hyperarousal does not depend on trauma-related contextual memory in an animal model of Posttraumatic Stress Disorder. *Physiology & Behavior*. 2007 Jan;90(1):103–7.
119. Siegmund A, Wotjak CT. A mouse model of posttraumatic stress disorder that distinguishes between conditioned and sensitised fear. *Journal of Psychiatric Research*. 2007 Nov;41(10):848–60.
120. Legrand M, Troubat R, Brizard B, Le Guisquet A-M, Belzung C, El-Hage W. Prefrontal cortex rTMS reverses behavioral impairments and differentially activates c-Fos in a mouse model of post-traumatic stress disorder. *Brain Stimulation*. 2019 Jan;12(1):87–95.
121. Takahashi T, Morinobu S, Iwamoto Y, Yamawaki S. Effect of paroxetine on enhanced contextual fear induced by single prolonged stress in rats. *Psychopharmacology*. 2006 Nov 3;189(2):165–73.
122. Cohen H, Liberzon I, Richter-Levin G. Exposure to extreme stress impairs contextual odour discrimination in an animal model of PTSD. *The International Journal of Neuropsychopharmacology*. 2009 Apr;12(03):291.
123. Andero R, Brothers SP, Jovanovic T, Chen YT, Salah-Uddin H, Cameron M, et al. Amygdala-Dependent Fear Is Regulated by Opr11 in Mice and Humans with PTSD. *Science Translational Medicine*. 2013 Jun 5;5(188):188ra73-188ra73.
124. Lisieski MJ, Eagle AL, Conti AC, Liberzon I, Perrine SA. Single-Prolonged Stress: A Review of Two Decades of Progress in a Rodent Model of Post-traumatic Stress Disorder. *Frontiers in Psychiatry* [Internet]. 2018(9):196. Available from: <http://journal.frontiersin.org/article/10.3389/fpsy.2018.00196/full>
125. Souza RR, Noble LJ, McIntyre CK. Using the Single Prolonged Stress Model to Examine the Pathophysiology of PTSD. *Frontiers in Pharmacology* [Internet]. 2017(8):615. Available from: <http://journal.frontiersin.org/article/10.3389/fphar.2017.00615/full>

126. Grillon C, Morgan III CA. Fear-Potentiated Startle Conditioning to Explicit and Contextual Cues in Gulf War Veterans With Posttraumatic Stress Disorder. *Journal of Abnormal Psychology*. 1999;108(1):134–42.
127. Milad MR, Pitman RK, Ellis CB, Gold AL, Shin LM, Lasko NB, et al. Neurobiological Basis of Failure to Recall Extinction Memory in Posttraumatic Stress Disorder. *Biological Psychiatry*. 2009 Dec;66(12):1075–82.
128. Wurtz H, El-Khoury-Malhame M, Wilhelm FH, Michael T, Beetz EM, Roques J, et al. Preventing long-lasting fear recovery using bilateral alternating sensory stimulation: A translational study. *Neuroscience*. 2016 May;321:222–35.
129. Hefner K, Whittle N, Juhasz J, Norcross M, Karlsson R-M, Saksida LM, et al. Impaired Fear Extinction Learning and Cortico-Amygdala Circuit Abnormalities in a Common Genetic Mouse Strain. *Journal of Neuroscience*. 2008 Aug 6;28(32):8074–85.
130. O'Connor RM, McCafferty CP, Bravo JA, Singewald N, Holmes A, Cryan JF. Increased amygdalar metabotropic glutamate receptor 7 mRNA in a genetic mouse model of impaired fear extinction. *Psychopharmacology*. 2019 Jan;236(1):265–72.
131. Posttraumatic stress disorder. In: Wikipedia [Internet]. 2019. Available from: [https://en.wikipedia.org/w/index.php?title=Posttraumatic\\_stress\\_disorder&oldid=903525872](https://en.wikipedia.org/w/index.php?title=Posttraumatic_stress_disorder&oldid=903525872)
132. Sierra-Mercado D, Padilla-Coreano N, Quirk GJ. Dissociable Roles of Prelimbic and Infralimbic Cortices, Ventral Hippocampus, and Basolateral Amygdala in the Expression and Extinction of Conditioned Fear. *Neuropsychopharmacology*. 2011 Jan;36(2):529–38.
133. Rule A, Cointet J-P, Bearman PS. Lexical shifts, substantive changes, and continuity in State of the Union discourse, 1790–2014. *Proceedings of the National Academy of Sciences*. 2015 Sep 1;112(35):10837–44.
134. Cohen H, Zohar J, Matar MA, Zeev K, Loewenthal U, Richter-Levin G. Setting Apart the Affected: The Use of Behavioral Criteria in Animal Models of Post Traumatic Stress Disorder. *Neuropsychopharmacology*. 2004 Nov;29(11):1962–70.
135. Milad MR, Orr SP, Lasko NB, Chang Y, Rauch SL, Pitman RK. Presence and acquired origin of reduced recall for fear extinction in PTSD: Results of a twin study. *Journal of Psychiatric Research*. 2008 Jun;42(7):515–20.
136. Bliss TVP. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*. 1993 Jan 7;361:31–9.
137. Carew TJ, Kandel ER. Acquisition and Retention of Long-Term Habituation in Aplysia: Correlation of Behavioral and Cellular Process. *Science*. 1973;182:1158–60.
138. Kandel ER, Schwartz JH. Molecular Biology of Learning: Modulation of Transmitter Release. *Science*. 1982 Oct 29;218:433–43.
139. McKernan MG, Shinnick-Gallagher P. Fear conditioning induces a lasting potentiation of synaptic currents in vitro. *Nature*. 1997 Dec;390(6660):607–11.
140. A History of fMRI | MGH/HST Martinos Center for Biomedical Imaging [Internet]. Available from: <http://www.nmr.mgh.harvard.edu/history-fMRI>
141. Milad MR, Quirk GJ. Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature*. 2002 Nov;420(6911):70–4.

142. Bremner JD, Vermetten E, Schmahl C, Vaccarino V, Vythilingam M, Afzal N, et al. Positron emission tomographic imaging of neural correlates of a fear acquisition and extinction paradigm in women with childhood sexual-abuse-related post-traumatic stress disorder. *Psychological Medicine*. 2005 Jun;35(6):791–806.
143. Ressler KJ, Paschall G, Zhou X, Davis M. Regulation of Synaptic Plasticity Genes during Consolidation of Fear Conditioning. *The Journal of Neuroscience*. 2002 Sep 15;22(18):7892–902.
144. Peri T, Ben-Shakhar G, Orr SP, Shalev AY. Psychophysiological assessment of aversive conditioning in posttraumatic stress disorder. *Biological Psychiatry*. 2000 Mar;47(6):512–9.
145. Yehuda R, Spiegel D, Southwick S, Davis LL, Neylan TC, Krystal JH. What I have changed my mind about and why. *European Journal of Psychotraumatology*. 2016 Dec;7(1):33768.
146. Crocq M-A, Crocq L. From shell shock and war neurosis to posttraumatic stress disorder: a history of psychotraumatology. *Clinical research*. 2000;2(1):9.
147. Roger Pitman | Massachusetts General Hospital Psychiatry Academy [Internet]. Available from: [https://mghcme.org/faculty/faculty-detail/roger\\_pitman](https://mghcme.org/faculty/faculty-detail/roger_pitman)
148. Roberts S. Henry Krystal, Holocaust Trauma Expert, and Survivor, Dies at 90. *The New York Times* [Internet]. 2015 Oct 14; Available from: <https://www.nytimes.com/2015/10/15/science/henry-krystal-holocaust-trauma-expert-dies-at-90.html>
149. Krystal J. My Path to Psychiatry. *Psychiatrics News* [Internet]. 2017 Oct 19; Available from: <https://psychnews.psychiatryonline.org/doi/abs/10.1176/appi.pn.2017.10b1>
150. Rachel Yehuda — How Trauma and Resilience Cross Generations [Internet]. The On Being Project. Available from: <https://onbeing.org/programs/rachel-yehuda-how-trauma-and-resilience-cross-generations-nov2017/>
151. Query Form - NIH RePORTER - NIH Research Portfolio Online Reporting Tools Expenditures and Results [Internet]. Available from: <https://projectreporter.nih.gov/reporter.cfm>
152. Shalev AY, Bonne O, Eth S. Treatment of Posttraumatic Stress Disorder: A Review. *Psychosomatic Medicine*. 1996;18.
153. Rasmusson AM, Charney DS. Animal Models of Relevance to PTSD. *Annals of the New York Academy of Sciences*. 1997 Jun;821(1 Psychobiology):332–51.
154. VanElzakker MB, Kathryn Dahlgren M, Caroline Davis F, Dubois S, Shin LM. From Pavlov to PTSD: The extinction of conditioned fear in rodents, humans, and anxiety disorders. *Neurobiology of Learning and Memory*. 2014 Sep;113:3–18.
155. Boscarino JA, Figley CR. Understanding the Neurobiology of Fear Conditioning and Emergence of Posttraumatic Stress Disorder *Psychobiology: Commentary on Blanchard et al.* *The Journal of Nervous and Mental Disease*. 2012 Sep;200(9):740–4.
156. Lissek S, van Meurs B. Learning models of PTSD: Theoretical accounts and psychobiological evidence. *International Journal of Psychophysiology*. 2015 Dec;98(3):594–605.
157. Sargant W, Slater E. Acute war neuroses. *The Lancet*. 1940 Jul 6;236(6097):1–2.

158. Flores Á, Fullana MÀ, Soriano-Mas C, Andero R. Lost in translation: how to upgrade fear memory research. *Molecular Psychiatry*. 2018 Nov;23(11):2122–32.
159. NIMH » Research Domain Criteria (RDoC) [Internet]. Available from: <https://www.nimh.nih.gov/research/research-funded-by-nimh/rdoc/index.shtml>
160. Demazeux S, Pidoux V. Le projet RDoC: La classification psychiatrique de demain ? *médecine/sciences*. 2015 Aug;31(8–9):792–6.
161. LeDoux JE. Coming to terms with fear. *Proceedings of the National Academy of Sciences*. 2014 Feb 25;111(8):2871–8.
162. Buzsáki G. Neural Syntax: Cell Assemblies, Synapsembles, and Readers. *Neuron*. 2010 Nov;68(3):362–85.
163. Calhoun GG, Tye KM. Resolving the neural circuits of anxiety. *Nature Neuroscience*. 2015 Oct;18(10):1394–404.