Université de Montréal

Effects of Fear Conditioning on Pain: Moderation by Mindfulness and the HPA-axis

Par Véronique Taylor

Département de psychologie
Faculté des Arts et Sciences

Thèse présentée à la Faculté des études supérieures et postdoctorales
en vue de l'obtention du grade de Philosophiae Doctor (Ph.D.)
en psychologie recherche – option sciences cognitives et neuropsychologie

Avril 2017

Résumé

Un cercle vicieux par lequel la peur et la douleur se maintiennent explique le développement et maintient de troubles impliquant le conditionnement à la peur. Bien que les processus comportementaux et circuits neurobiologiques du conditionnement à la peur aient été étudiés extensivement, les effets de cet apprentissage sur la douleur sont peu connus. L'objectif de cette thèse était d'étudier les effets du conditionnement à la peur sur la neuro-psychophysiologie de la douleur chez des sujets sains, ainsi que les facteurs modérant ces effets.

Les effets de l'apprentissage de la peur sur la douleur ont été examinés dans l'Étude 1 (N=47) lors d'une tâche de conditionnement classique Pavlovien. Les stimuli conditionnés étaient des images abstraites appariées à une stimulation électrique douloureuse lors de 50% des essais. Les rapports de douleur et réflexes nociceptif de flexion ont été acquis à chaque stimulation, et les réponses électrodermales ont été mesurées à chaque présentation d'image. Nous avons estimé deux paramètres régissant les réponses anticipatoires (électrodermales) de peur pour chaque essai au moyen d'une approche de modélisation computationnelle: les attentes face à l'occurrence d'une stimulation, et l'associabilité des images à la stimulation. Les résultats ont démontré que chacun des paramètres liés à la peur prédisait positivement la douleur ainsi que les réponses nociceptive spinales. Ces effets opéraient en partie directement sur les réponses supraspinales à la douleur, et en partie indirectement par une facilitation de l'influx nociceptif au niveau spinal. Les résultats ont également démontré que la médiation des effets de la peur sur la douleur par l'input nociceptif spinal était plus forte chez les individus rapportant davantage de 'vigilance au danger', et plus faibles chez ceux rapportant plus de détachement émotionnel.

Dans l'Étude 2, nous avons examiné le rôle de l'expérience à long terme en méditation pleine conscience sur les effets de l'apprentissage de la peur sur la douleur. Onze méditants expérimentés ont été testés en utilisant le même protocole expérimental que celui de l'Étude 1. Comparés aux sujets contrôles de l'Étude 1 n'ayant pas d'expérience en méditation, le groupe de méditants a montré une réduction de la douleur rapportée en moyenne lors de la tâche de conditionnement, ainsi qu'une diminution des effets de la peur sur la douleur. Les méditants n'ont pas montré de modulation des processus de bas niveau défensifs ou des mécanismes d'apprentissage à la peur.

Finalement, l'Étude 3 a examiné le rôle des différences inter-individuelles en réactivité de l'axe HPA, opérationnalisé par le niveau de cortisol sécrété pendant la tâche, sur les effets de la peur sur la douleur (N=23). Le protocole expérimental et d'analyses était similaire à celui des Études 1-2 avec l'inclusion d'un SC+ apparié à 100% avec le SI. Les individus ayant une plus grande réponse de cortisol pendant le conditionnement rapportaient en moyenne moins de douleur lors de la tâche, et présentaient une facilitation des réponses défensives spinales par le biais du conditionnement à la peur.

Les résultats de cette thèse appuient le concept d'un cycle vicieux peur/douleur par des données neuropsychophysiologiques, et montrent que celui-ci est modéré par certains traits de personnalité, l'expérience à long-terme en méditation pleine conscience, et les différences individuelles en réactivité de l'axe HPA. Nos résultats appuient également le rôle bénéfique des techniques fondées sur l'acceptation et la pleine conscience pour briser le cycle peur-douleur et prévenir/traiter les manifestations pathologiques de l'exposition répétée à des évènements menaçants (ex: anxiété, douleur chronique).

Mots-clés: conditionnement à la peur, modèles computationnels d'apprentissage associatif, réflexe nociceptif de flexion, méditation pleine conscience, cortisol, douleur

Abstract

A vicious cycle through which fear and pain maintain each other explains the development and maintenance of disorders involving fear conditioning. While the behavioral processes and neurobiological circuits of fear conditioning have been extensively studied, the effects of fear learning on pain remain poorly understood. The objectives of this thesis were to examine the effects of fear conditioning on the neuropsychophysiology of pain, and the factors that could moderate these effects.

The effects of fear learning on pain were examined in Study 1 in 47 human participants during a delay Pavlovian classical fear conditioning task. Conditioned stimuli were abstract visual cues that co-terminated with a painful electric shock on 50% of trials. Pain ratings and the spinal nociceptive flexion reflex were recorded in response to each US, and anticipatory skin conductance responses were recorded to each CS. A computational model of reinforcement learning was fitted to anticipatory SCRs and used to estimate fear learning parameters of expected shock probabilities and associability (uncertainty) to each CS+ paired. Both fear learning parameters positively predicted pain responses. These effects operated in part directly on pain ratings, and in part indirectly by facilitating ascending spinal nociceptive activity. The results also showed that the mediation of the effects of fear learning on pain by spinal nociception was enhanced for individuals reporting more trait harm vigilance, and decreased for individuals reporting more emotional detachment.

In Study 2, we investigated the role of long term mindfulness meditation experience on the effects of fear learning on pain. Eleven experienced meditators (>1000 hours of experience) were tested using the same experimental and analysis protocol as in Study1, and were compared with the meditation-naïve participants from Study1.

Compared to controls, experienced meditators showed an overall reduction in pain ratings during fear learning, as well as reduced effects of learning parameters on pain. No effects of fear learning on lower-level spinal or anticipatory learning responses were observed.

Finally, Study 3 examined how individual differences in HPA axis reactivity, operationalized by the level of cortisol secreted during the task, affected pain modulation induced by fear-learning (N=23). A similar experimental and analysis protocol as in Studies 1-2 was used with an additional visual CS paired with the US on 100% of trials. Individuals with greater cortisol output during fear conditioning reported a global decrease in pain during the task, and showed a facilitation of defensive spinal responses via fear learning mechanisms.

The results of this thesis support the notion of a vicious fear-pain cycle with neuropsychophysiological evidence, and show that this cycle is moderated by certain personality traits, meditation experience, and individual differences in HPA reactivity. Our results also highlight the role of techniques based on acceptation and mindfulness meditation to break the fear-pain cycle and prevent/treat pathological manifestations of repeated threat exposure (eg. anxiety, chronic pain).

Keywords: fear conditioning, reinforcement learning models, nociceptive flexion reflex, mindfulness meditation, cortisol, pain

Table of Contents

List of Tables

Article1

Article2

Article3

List of Figures

General Introduction

Article 1

Article 2

Article 3

General Discussion

List of Abbreviations

ACC: anterior cingulate cortex

Ag - AgCl: Silver-Silver Chloride

AIC: Akaike Information

AMY: amygdala

ANOVA: analysis of variance

ANS: autonomic nervous system

AUCg: area under the curve with respect to ground

AUCi: area under the curve with respect to increase

BA: basolateral amygdala

BDI: Beck Depression Inventory

BIC: Bayesian Information Criterion

BIS: Behavioural Inhibition Scale

CE / CeM / CeA: central nucleus of the amygdala

CG: central gray

CNS: central nervous system

CS-: conditioned stimulus not paired with an unconditioned stimulus

CS: conditioned stimulus

CS+: conditioned stimulus paired with an unconditioned stimulus on a given number of trials

CS100: fear conditioned stimulus using a 100% reinforcement schedule

CS50: fear conditioned stimulus using a 50% reinforcement schedule

DMPFC: dorsomedial prefrontal cortex

EMG: electromyographic recording

FFMQ: Five-Factor Mindfulness Questionnaire

fMRI: functional magnetic resonance imaging

Gi: gigantocellular reticular nucleus

HPA: hypothalamic-pituitary-adrenal

IL: infralimbic cortex

ITC: intercalated cells of the amygdala

LA: lateral amygdala

LC: locus coeruleus

LH: lateral hypothalamus

MAAS: Mindful Attention Awareness Scale

MBSR: mindfulness-based stress reduction

MDVo: ventrocaudal part of the medial dorsal nucleus

MPFC: medial prefrontal cortex

NA: noradrenergic

NFR / RIII-reflex: nociceptive flexion reflex

OFC: orbitofrontal cortex

PAG: periaqueductal gray

PCA: principal component analysis

PCC: posterior cingulate cortex

PCS: pain catastrophizing scale

PFC: prefrontal cortex

PL: prelimbic area

PPC: posterior parietal cortex

PVN: paraventricular nucleus of the hypothalamus

RMS: root mean square

RW: Rescorla-Wagner

RW/PH: Rescorla-Wagner/Pearce-Hall

SCR: skin conductance response

SMA: supplementary motor area

SRD: subnucleus reticularis dorsalis in the caudal medulla

STAI: state trait anxiety inventory

STT: spinothalamic tract

TCI: Temperament and Character Inventory

US: unconditioned stimulus

VAS: visual analog scale

vlPAG: ventrolateral periaqueductal gray

VMpo: ventromedial part of the posterior nuclear complex of the thalamus

VPL: ventroposterior lateral nucleus of the thalamus

To the beauty of Life,

Beautiful things can happen with:
An open-mind,
An open-heart,
Respect,
Discipline,
Love,
Compassion,
Peace of Mind,
Organization,
Work Ethic,
Persistance in the Face of Obstacles,

And mostly with
The BELIEF that anything is possible

Acknowledgments

I would like to thank first and foremost my supervisor Pierre Rainville. His intellectual curiosity and scientific talent, as well as passion for the art and essence of psychology and neuroscience has truly inspired me. The intellectual discussions held together have enriched me personally, and scientifically. The kindness and human aspect of my supervisor, as well as the support for autonomy which he has given me have helped me through this journey. I would also like to thank Mathieu Roy, whose scientific and analytical talent and brillance have given me different points of view from which to study human behavior. He has taught me and given me precious tools and expertise which will serve me in future projects that I undertake.

I would like to thank my mother, for her support and encouragement, love, generosity, and organization. I would like to thank my sister and her husband, for their encouragement and support from their previous PhD journeys. My father's words of encouragement have also supported me to pursue this journey until the end. I also need to thank my friends, for their non-judgemental support and help in the ups and downs of the PhD journey.

The precious new friendships I have made in my karate courses have been a tremendous source of support. I have related and shared my experiences with my thesis with others going through the same academic path, which has helped me grow. I am extremely thankful to have discovered the beautiful art of karate, which instills discipline, hard work, and perseverance. This discipline, taught by the most respectable and admirable

teachers, has given me strength, courage, and belief that anything is possible. The martial arts in combination with the love for my meditation practice, have truly encouraged me to pursue my academic dreams. I have been inspired by great meditation teachers, who have taught me that consistency, discipline, and hard work are more important than tangible results. Through this entire process, I have truly come to realize that the work during a journey is more valuable than the final result.

The members of the Rainville laboratory (Ali Khatibi, Zoha Deldar, Kristina Aurousseau, Anouk Streff, Bérengère Houzé, Natalie Cardinal-Aucoin, Stéphane Caron, Rafi Marouf) who helped me with my experiments and technical difficulties, and with whom I have shared laughs and experiences. I need to thank Louis-Nascan Gill and Christian Mueller, of course, who have valliantly and efficiently tested participants for my experiments on mindfulness and cortisol.

Finally, I would like to express thanks to the participants who bravely sacrificed themselves to experience pain for the sake of science, and without whom psychological scientist's work would not be possible.

General Introduction

Nociception and pain have the adaptive function to teach the organism about potential danger via fear learning mechanisms (McNally, Johansen, & Blair, 2011). Theoretical models of clinical disorders related to fear and/or anxiety of threatening stimuli propose that fear and pain maintain and/or perpetuate each other through a vicious cycle (Crombez, Eccleston, Van Damme, Vlaeyen, & Karoly, 2012; Vlaeyen & Linton, 2000). The interactions underlying this cycle are defined by classical fear conditioning, for which the behavioral, physiological, and neural characteristics have been extensively studied by animal and human research since Pavlov's discoveries in the 20th century (Pavlov & Anrep, 1927). Specifically, the behavioral, physiological, and neural aspects of fear learning have been thoroughly studied using computational approaches (Boll, Gamer, Gluth, Finsterbusch, & Buchel, 2013; LePelley & McLaren, 2004; Li, Schiller, Schoenbaum, Phelps, & Daw, 2011; McNally et al., 2011). These approaches purport that the organism performs neural computations to predict danger in the environment using quantifiable parameters, and updates these predictions at each trial in which relevant information is received. However, while the precise mechanisms through which classical conditioning operates have been established, the effects of fear learning on pain are much less well understood. At a fundamental level, the need to elucidate these effects is essential to contribute to the current state of knowledge in the fields of pain and affective neuroscience. From a clinical perspective, it is critical to understand the effects of fear learning on pain to validate or adjust current models of clinical disorders in order to improve treatment and prevention approaches. Finally, it is also important to understand

factors that moderate (attenuate/potentiate) the effects of fear learning on pain to improve clinical interventions targeting disorders involving fear of threatening stimuli.

To approach these research problems, the studies conducted within this doctoral thesis pursued the following objectives: 1- to determine the effects of classical fear conditioning on the neuro-psychophysiology of pain using computational models of formal associative learning theories 2- to determine individual variables that could moderate (enhance/attenuate) the effects of fear learning on pain. Prior to presenting the experimental findings obtained in these studies, we will describe clinical models of chronic pain to emphasize the clinical significance of elucidating effects of fear learning on pain. We will then describe fear learning mechanisms and their neurobiological bases, as well as a brief overview of formal associative learning computational models used to depict learning processes. We will then summarize the neurophysiology of pain and its modulation. Next, an overview of the beneficial impact of mindfulness meditation experience on pain will be presented. We will also discuss the potential of this practice to reduce the effects of fear on pain and disrupt the fear – suffering cycle, with the intention to benefit clinical interventions to prevent pathological manifestations of fear-pain interactions. Finally, we will discuss the physiology of the stress system and its relationship with pain and pain modulation.

Vicious Fear-Pain Cycle: Fear Avoidance Model of Chronic Pain

To emphasize the clinical relevance of elucidating the modulating effects of fear learning on pain, this section will provide an overview of a behavioral model describing fear avoidance behaviors and their reinforcing role in chronic pain.

The primal emotion of fear is characterized as a response to a perceived threat, accompanied by the activation of the sympathetic so-called 'fight-or-flight' response. It is adaptive in the sense that it allows to escape, confront or cope with threat. Nonetheless, disregulation of the fear system can result in the triggering of fear in the absence of imminent danger, interfering with an individual's general functioning abilities (LeDoux & Phelps, 2008).

In chronic pain disorder, pain becomes the primary source of threatening stimuli. According to the International Association for the Study of Pain, chronic pain is defined as pain that persists beyond a 'normative' time of healing: (generally in clinical practice this corresponds to more than 6 months). It can vary from being perceived in different bodily regions (eg. back, head, viscera) and involving different systems (eg.: gastrointestinal system, nervous system), different temporal characteristics as well as etiologies. Chronic pain is often accompanied by depressive symptoms (feelings of helplessness towards pain, rumination about its potential meaning or consequences), as well as anxious symptoms (apprehension of next painful episode) and fear of encountering the next episode (Crombez et al., 2012; Sullivan & D'Eon, 1990; Vachon-Presseau et al., 2013; Vlaeyen & Linton, 2000; Wall & Melzack, 2006).

These repeated painful episodes become quite threatening to the individual's functioning, daily activities, self-care, and capacity to work (Crombez et al., 2012; Vlaeyen & Linton, 2000). Therefore, fear conditioning with cues associated with pain naturally takes place. Cues, contexts, or settings paired with pain episodes, allowing the individual to predict

pain episode onsets – eg. an individual experienced the onset of a migraine while at a restaurant, or while exercising – acquire aversive properties and trigger behavioral avoidance (Crombez et al., 2012; Vlaeyen & Linton, 2000).

The fear-avoidance (FA) model of chronic pain illustrates the vicious cycle between pain, fear, disabling symptoms / avoidant coping, and suffering (Crombez et al., 2012). This model stipulates that pain triggers fear responses, which results in the instilment of avoidance behaviors towards stimuli associated with painful experiences (Crombez et al., 2012; Vlaeyen & Linton, 2000). These behaviors are reinforced because they are rewarding on a short-term basis (the source of pain is avoided). On a long-term scale, however, they contribute to the maintenance of disability associated with chronic pain (Crombez et al., 2012; Vlaeyen & Linton, 2000). For example, an individual with low back pain could avoid any activity which may trigger or which has been associated with painful experiences, such as exercising or maintaining an active social life. While avoiding these behaviors could temporarily relieve low back pain experiences or prevent its exacerbation, on a longer term, a sedative lifestyle would be physically detrimental (e.g. deconditioning) and actually enhance the potential for experiencing low back pain and disabling symptoms (ex: work absenteeism, isolation, depression, etc) (Crombez et al., 2012; Vlaeyen & Linton, 2000).

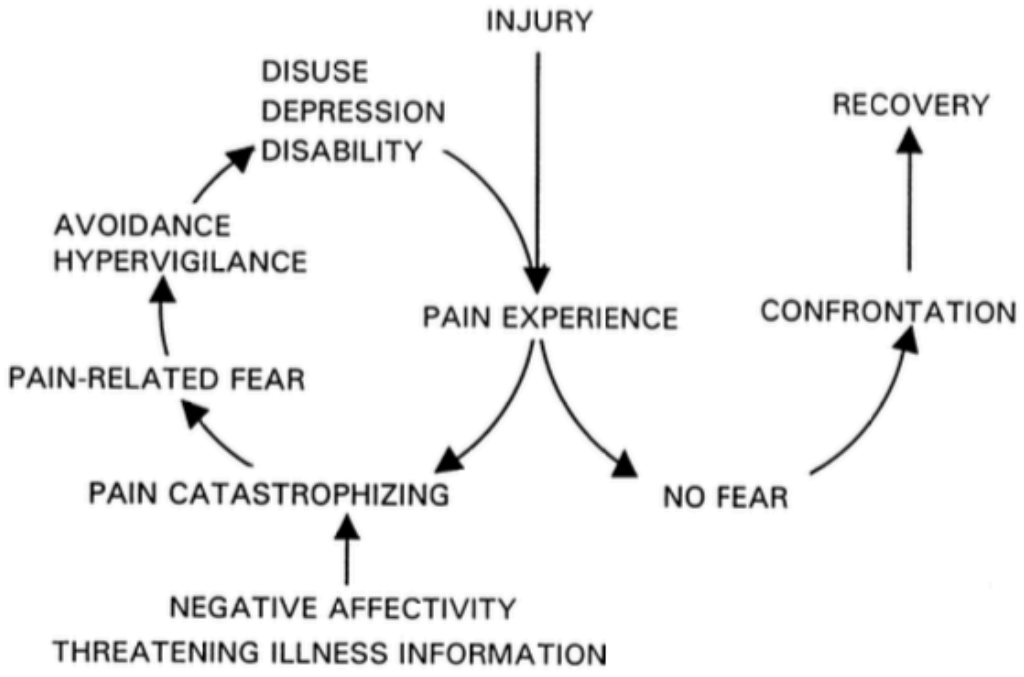

Figure 1. Fear-avoidance model of chronic pain proposed by Vlaeyen and colleagues (2000), demonstrating that if pain is perceived as threatening, fear develops along with the adoption of avoidance behaviors towards activities thought to re-elicit pain. These avoidance tendencies are, in the long run, detrimental to recovery and enhance disabling symptoms.

While this model can account for behavioural consequences of fear and avoidance of pain for the chronicization of pain/suffering, it does not explain the functional consequences of fear learning processes on the actual experience of pain sensations (experientially, and at a neurophysiological level). For instance, if fear conditioning amplified the experience of pain, this could explain, from a neuropsychophysiological point of view, the reinforcing role of fear in the maintenance of pain. A similar reinforcing cycle between fear and suffering is used to explain the development and maintenance of anxiety disorders, such as dental phobia (Armfield, Stewart, & Spencer, 2007). For this reason, the central objective of this thesis is to understand the functional consequences of fear learning on the experience of pain, which would 1- help validate current models of clinical disorders involving classical conditioning, and 2- determine ways to disrupt the vicious cycle between fear and suffering.

Basic affective neuroscience has largely contributed to the understanding of disorders involving associative fear learning. The underlying behavioural neurobiology of classical conditioning has been rigorously studied using paradigms developed in the early 20th century by the work of Pavlov (Pavlov & Anrep, 1927). The next sections will provide an overview of the neurobiology of fear learning, as well as contemporary computational approaches developed to model learning response trajectories.

Classical Fear Conditioning

Construct Definitions

Fear learning can be defined as the process through which an innocuous stimulus (conditioned stimulus 'CS') acquires aversive properties of a threatening stimulus (unconditioned stimulus 'US') after one or several pairings (Pavlov & Anrep, 1927). This association is neurobiologically underlied by a Hebbian learning process in the basolateral amygdala (Kim & Jung, 2006). In other words, 'cells that fire together, wire together' and "When an axon of cell A is near enough to excite B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased" (Hebb, 1949). Thus, the synapse between the cells transmitting sensory information about the conditioned stimulus to basolateral amygdalar cells is strengthened from being active at the same time as the more efficient synapse between cells transmitting information related to the unconditioned stimulus to the basolateral amygdala (Hebb, 1949). The basolateral amygdala projects to the central nucleus of the amygdala, which elicits several responses to threat (neuroendocrine, autonomic, behavioral) by innervating hypothalamus and brainstem sites (LeDoux & Phelps, 2008). An example of a frequently used fear response in experimental paradigms as an index of autonomic arousal or conditioned fear is the skin conductance response (Boucsein, 2012). As such, autonomic arousal can evoke sudomotor nerve activity, which then activates peripheral sweat glands (Boucsein, 2012). This activation can be observed by measuring electrical current conductance at the level of the skin (the inverse of resistance, expressed in siemens):

when sweat glands are activated by sudomotor nerve activity, current resistance decreases between electrodes (and hence, conductance increases) (Boucsein, 2012).

Information related to the CS is relayed from sensory neurons to the amygdala through two routes of transmission: 1- immediately conveyed to the amygdala from the thalamus via the so-called 'direct' thalamo-amygdala pathway (LeDoux & Phelps, 2008), 2- from the thalamus to cortical areas before synapsing onto the amygdala via the 'indirect' thalamo-cortico-amygdala route, presumably underlying response adjustment after an enhanced level of stimulus processing (LeDoux & Phelps, 2008). Figure 2 shows the convergence of CS and US-related information in the amygdala, as well as the response output from the central nucleus of the amygdala.

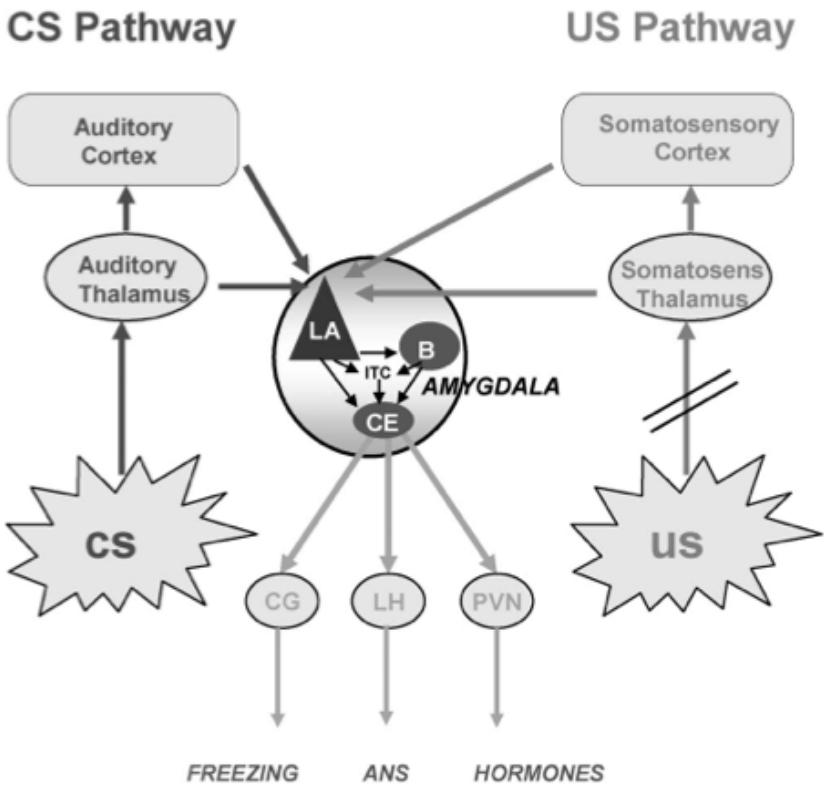

Figure2. Ledoux and Phelps' model (2008) depicting the association of conditioned stimuli (CS) and unconditioned stimulus (US) within the lateral amygdala. The information for each stimulus is relayed through a 'direct' thalamo-amygdalar pathway, and a second 'indirect' thalamo-cortical-amygdala pathway. The lateral amygdala (LA) activates appropriate threat response signals to the central nucleus of the amygdala (CE), directly and by passing through the basal nucleus (B) or the intercalated masses (ITC). The CE activation triggers expressed fear responses, such as freezing (mediated by the central gray; CG), responses of the autonomic nervous system (ANS) from the lateral hypothalamus; LH), as well as hormonal output (mediated by the paraventricular nucleus of the hypothalamus; PVN).

After being relayed to the amygdala, threat-related information is sent to the medial prefrontal cortex (MPFC). Quirk and colleagues (2006) proposed a neuroanatomical model (Figure 3) underlying conditioned fear expression and extinction emphasizing the functional distinction between dorsal and ventral subdivisions of this region (prelimbic vs infralimbic cortex in the rat, anatomically analogous to the dorsal MPFC/rostral anterior cingulate vs ventral MPFC in humans) (Quirk & Beer, 2006). The model proposed by Quirk et al. (2006) posits that the dorsal MPFC is activated through feed-forward projections from the basolateral amygdala. In turn, through reciprocal efferent excitatory projections, the dorsal MPFC would maintain typically short-lived amygdala responses. This reverbatory activity underlies the maintenance of the mental representation of the CS and the expression of fear responses (Quirk & Beer, 2006).

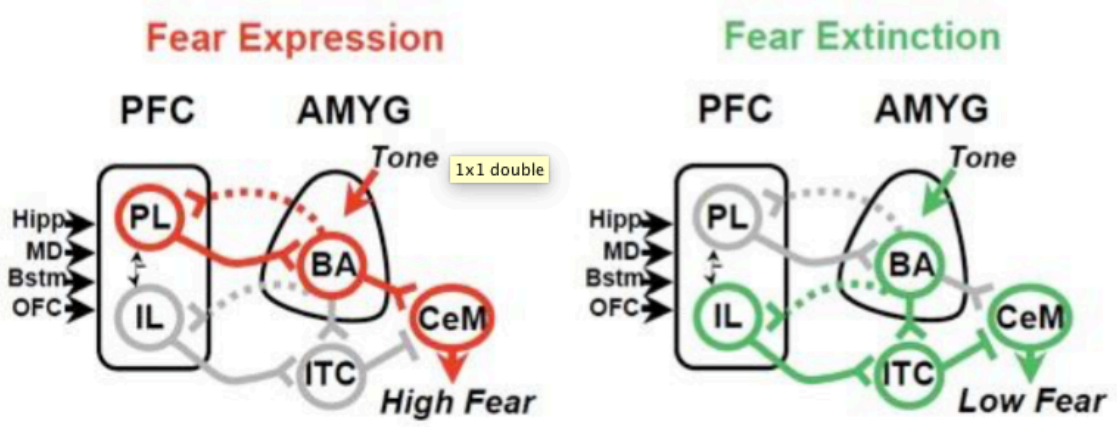

Figure 3. Neuroanatomical model underlying interactions between the prefronal cortex (PFC) and the amygdala (AMYG) underlying fear expression and fear extinction based on the work of Quirk and Milad (source: http://www.md.rcm.upr.edu/quirk/Home.html). The prelimbic area ('PL',corresponding to dorsal medial prefronal cortex (MPFC)/anterior cingulate cortex in humans) has afferent/efferent projections with the basolateral amygdala (BA) and is involved in the maintenance of fear expression. The infralimbic cortex ('IL',ventral MPFC in humans) would be involved in the extinction of conditioned fear, by activating the intercalated cells of the amygdala (ITC) which send inhibitory projections to central nucleus of the amygdala (CeM) which regulates expressed fear behaviors.

By contrast, the more ventral subdivision of the MPFC would be more involved in the extinction of conditioned fear responses and the down-regulation of amygdalar response if the stimulus is no longer perceived as threatening. This mechanism is described as being 'gated' by the hippocampus, which would provide contextual/explicit information to the ventral MPFC concerning the 'safety' of the CS (if it has been presented several times in the absence of the US, for example), and accordingly inhibit amygdalar responses. This mechanism also explains why extinction is viewed as new learning as opposed to a passive degradation of information (Quirk & Beer, 2006).

Furthermore, the brain is organized in such a way as to constantly generate and test predictions about underlying causes of sensory input at multiple levels of integration (Park & Friston, 2013). These predictions are updated in a feed-forward manner, i.e. higher-order areas form and test predictions from incoming input, and are also modulated by descending feedback from higher-order to lower-order areas of processing. From a computational framework of classical conditioning, neural computations are performed by the organism using precise quantifiable parameters and are updated at each trial of learning (McNally et al., 2011). These prediction error signals are particularly biologically relevant with respect to threatening information requiring rapid adaptation to environmental demands. The strength of US connections to the amygdala underlying CS-US plasticity varies as a function of a predictive error signal, i.e. the extent to which the US violated expectations (McNally et al., 2011). Electrophysiological studies recording activity of neurons in the basolateral nucleus of the amygdala have shown that the firing rate in these neurons reflected the underlying prediction error signal calculated using

formal computational laws of associative learning (McNally et al., 2011). Studies in humans have shown that the variance in other behavioral outputs (eg. SCRs) as well as amygdala and/or striatal activity assessed using fMRI during classical conditioning, such as SCRs, have also been explained using computational approaches (Boll et al., 2013; Li, Schiller, et al., 2011).

The next section will provide a brief overview of computational reinforcement learning models and the ways in which parameters such as prediction errors can be estimated from mathematical laws established since the work of Rescorla in the 1970's (Rescorla & Wagner, 1972).

Reinforcement Learning Models

Formal models of classical conditioning are mathematical laws established to predict learning responses as a function of specific parameters (such as prediction errors) (LePelley & McLaren, 2004). The Rescorla-Wagner model (1972) (Equations 1-2) proposed that the strength of the association between a CS and US (V) can best be depicted as a function of the surprising nature of the US. For example, the presentation of a reinforced CS+ (i.e. CS+ followed by a US), may generate expectations that future CS+'s will also be followed by the US. In a context where the US is presented only in a proportion of the CS+ trials, this expectation will be confirmed and strengthened on reinforced trials. In the unreinforced CS+ trials, an error signal will be generated that will weaken the expectations on the following presentations of the CS+. Similarly, an unreinforced stimulus (CS-) will become a safety signal as the subject learns to expect the

absence of the US following this cue.

In other words, an error signal representing the difference between the expected and actual outcome experienced (λ) would determine how much learning the US can convey to the CS (LePelley & McLaren, 2004). The model also includes a constant learning rate parameter (**α)**. This learning rate parameter reflects individual differences (eg. depending on trait anxiety, etc) in updating predictions at each trial. Therefore, the strength of a CS's association with a US, or its expected value (*V*), at a given trial (*t+1),* would correspond to the sum of its expected value from the preceding trial '*t*' (V_t) and the prediction error signal from the preceding trial (δ_t) modulated by the individual learning rate.

$$V_{t+1} = V_t + \alpha * \delta_t \quad \text{(Equation1)}$$

$$\delta_t = \lambda_t - V_t \quad \text{(Equation2)}$$

The Pearce and Hall (1980) (Pearce & Hall, 1980) learning rule later posited that learning was rather driven by aspects related to the processing of the CS (a variable termed 'associability'), such as the level of attention paid to a cue. For example, a cue followed by a US generates uncertainty relative to the CS's threatening consequences. On the following trials, this CS would command more attention, and this cue would become more easily associable to the US. On future learning trials as the CS's reinforcement becomes more easily predictable, the lessened surprise effect would lead to a decrease in

the CS's associability. Under the Pearce Hall learning rule, learning is enhanced for trials in which the CS is a poor predictor of the US and whose consequences are uncertain.

A hybrid Rescorla-Wagner/Pearce-Hall model *combining* both factors related to US processing (prediction error signals, or the extent to which a US violated expectations of occurrence) as well as those related to the CS (eg. cue associability) has been shown to capture more efficiently the variance in learning responses than standard models solely based on prediction errors (Boll et al., 2013; LePelley & McLaren, 2004; Li, Schiller, et al., 2011). In a hybrid reinforcement learning model (Equations 3-5), learning is driven by prediction errors, *and* is dynamically modulated at each trial by associability (***a***). Associability increases following trials with large prediction errors; in other words, there is more to learn when the US is uncertain, and has been suggested to reflect enhanced attentional allocation to the CS (LePelley & McLaren, 2004).

For example, a CS+ newly paired with the US would generate large prediction error signals since the US was not expected following this cue. On the following trial, this CS+'s associability would also be elevated, given the enhanced uncertainty of the CS's consequences conferred by the large preceding surprise signal. On subsequent trials in which the CS is paired with the US, prediction error signals would decrease, and ensuing associability values would also decrease.

In circumstances in which a stable acquisition has been learned between a CS+ and a US, and between a CS- and the absence of a US, a sudden reversal in contingencies between both cues (the CS+ would become CS- and the CS- would become CS+) would lead to

very large PEs and increased allocation of attention to the following CS±'s presentations (i.e. increased associability).

As in the standard RW model, the RW/Pearce-Hall hybrid model describes that the expected value of a CS (***V***) on trial 't+1' is conferred by the sum of the preceding expected value and error signal generated on the preceding trial (modulated by the individual learning rate 'α'). However, in the RW/Pearce-Hall hybrid model, the surprise signal is modulated at each trial by the cue's associability: error signals would have larger impact on learning on a trial in which associability is also high (eg. on a trial in which a CS is paid a large amount of attention, a surprise signal would have a greater impact on learning than on a trial in which a CS's consequences are certain and little attention is paid to the CS). A constant parameter reflecting individual differences in the rate at which associability is updated (γ) is also included in this model.

$$V_{t+1} = V_t + a_t * \alpha * \delta_t \quad \text{(Equation 3)}$$

$$\delta_t = \lambda_t - V_t \quad \text{(Equation 4)}$$

$$a_{t+1} = \gamma * |\delta_t| + (1 - \gamma) * a_t \quad \text{(Equation 5)}$$

These two fundamental variables driving learning, expected shock probabilities (or the counterpart of prediction errors) and associability, likely influence the processing of the US: expectations, attention, and uncertainty are all factors shown to modulate pain (Tracey et al., 2002; Wager et al., 2004). However, the dynamic modulation of pain on a

trial-by-trial basis by expected shock probabilities /associability as they spontaneously develop during learning, remains unknown. Applying mathematical laws which govern learning behaviour to depict fluctuations in pain provides a dynamic trial-level and novel approach to the methods by which pain responses can be modelled and studied. Interestingly, several animal studies showed that a critical pain modulatory region, the periaqueductal gray (PAG), would initially compute error signals from a US and would then relay it to the amygdala (McNally et al., 2011). Indeed, Figure 4 shows that ascending US-related information would, from the PAG, convey error signals to the amygdala and determine the extent to which the US can strengthen learning (McNally et al., 2011). The prediction error-related signal would then be conveyed to the dorsal MPFC, which, through efferent connections to the amygdala, would adjust plasticity depending on other factors (eg.: contextual information).

Importantly, previous work has examined the relationship between latent variables estimated from computational models and neuronal activity/behavior during learning tasks or other paradigms involving painful stimuli (Roy et al., 2014; Seymour et al., 2004; Seymour et al., 2005; Zhang, Mano, Ganesh, Robbins, & Seymour, 2016). As such, previous studies had examined variables estimated using computational modeling to predict neural responses to conditioned cues to painful stimuli or to pain relief (Seymour et al., 2005) during learning. Pain-related predictions have also been shown to reflect BOLD-signal changes in the striatum derived from temporal difference models (Seymour et al., 2004). Other tasks involving instrumental learning with painful stimuli found prediction-error – related signals encoded in the periaqueductal gray (Roy et al., 2014),

consistent with previous literature (for review see McNally et al., 2011). While these studies examined pain-related learning signals in terms of brain function using computational modeling, they did not examine the relationship between modeling of learned anticipatory processes on behavioral/psychophysiological pain outcomes themselves.

Few studies have examined the relationship between parameters estimated from computational modeling and subjective behavioral measures, though previous studies have examined the link between modeled expectations and subjective happiness during tasks with monetary rewards (Ruttledge, Skandali, Dayan, & Dolan, 2015a, 2015b). Examining correspondence between computationally modeled processes and self-reported behavior is a critical step in bridging the gap between computational methods and behavior. As previous studies had not addressed the direct link between anticipatory processes modeled using formal associative learning theory and pain, these dynamic interactions remain to be examined, which constitutes the main scope of this thesis.

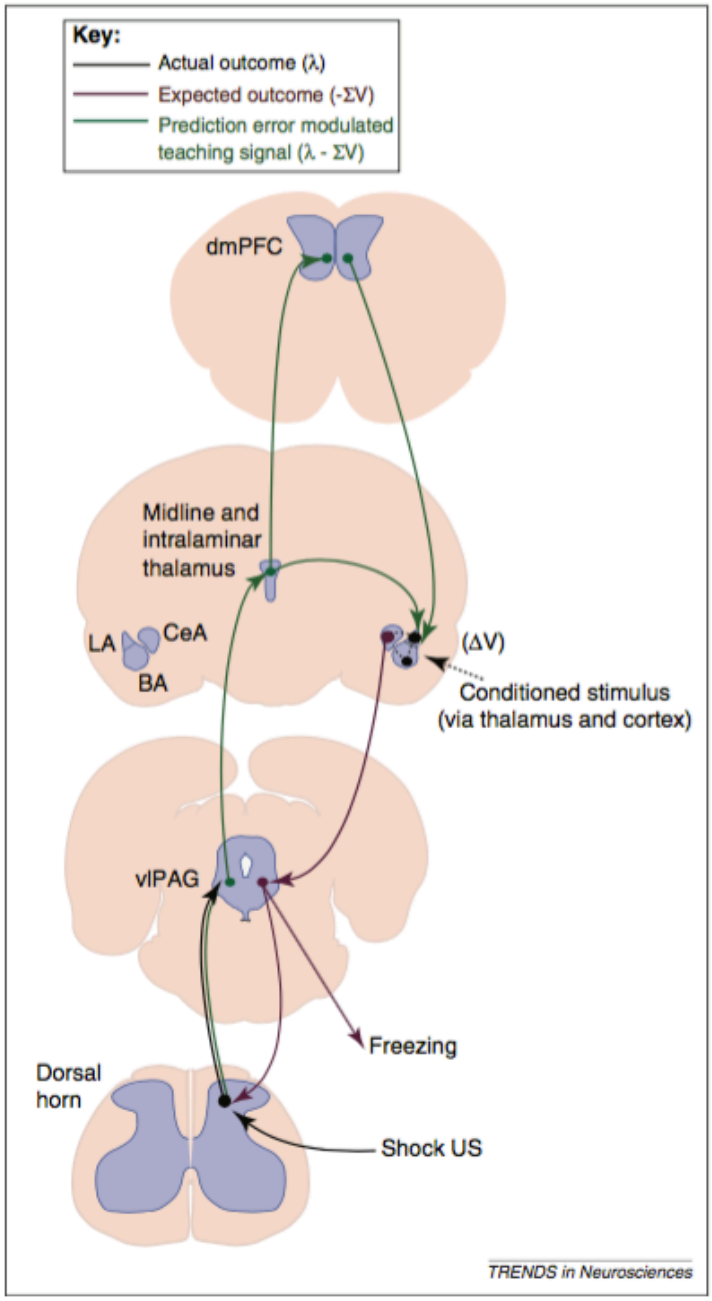

Figure 4. Neuroanatomical model for ascending and descending pathways involved in error signal encoding during fear conditioning. During fear learning, the weaker synapses between CS-related information (eg. auditory) and the amygdala (dashed black arrow) would be strengthened (ΔV) when co-occurring with stronger ascending US input to these neurons. Periaqueductal gray neurons would convey prediction error signals to the

amygdala. In turn, descending paths from the cortex and the amygdala having encoded prediction error signals during fear conditioning would modulate behavioral responses (eg. freezing) and nociceptive signals at the spinal cord. Unconditioned stimulus: US, vlPAG: ventrolateral periaqueductal gray, BA: basolateral amygdala, LA: lateral amygdala, CeA: central nucleus of the amygdala, dMPFC: dorso-medial prefrontal cortex. From McNally and collaborators (2011).

The advances in neurosciences over the last two decades now allow to start examining how brain circuits underlying expectations and associability affect the nociceptive system dynamically, leading to ongoing pain modulation during fear-learning in humans. The next section will describe the underlying neurophysiological mechanisms of pain and pain modulation.

Neurophysiology of Pain and Pain Modulation

The International Association Society for Pain describes pain as an "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage", while nociception refers to the neural encoding of stimuli that are noxious, i.e. that have the potential to cause tissue damage. Pain is therefore a conscious experience resulting from several neural processing stages: transduction, transmission, modulation, and perception (Beaulieu, 2013).

At the periphery, noxious stimuli of different modalities (eg. thermal, mechanical, and chemical) are transduced by nociceptors, which can be specific to a modality of stimuli or polymodal (Beaulieu, 2013). The nociceptive signal is then relayed to the central nervous systems (CNS) through nociceptive fibers, of which two categories exist. A-delta fibers are lightly myelinated, and are generally associated with brief and well-localized pain sensation. In contrast, C-fibers (80% of pain fibers) are unmyelinated, have slower conduction velocity, and are associated with slower and more diffuse pain sensation (Beaulieu, 2013).

Nociceptive information is first transmitted the dorsal horn of the spinal cord. Modulation (facilitation or inhibition) of the nociceptive signal can occur in the CNS at different levels of transmission, including at the level of the spinal cord, and at a higher-order cerebral level (Beaulieu, 2013). A major descending pain control pathway involves the periaqueductal gray area (PAG), which integrates input from the hypothalamus and the amygdala, and other higher-order sources (Fields & Basbaum, 2006). Neurons from the PAG project to brainstem sites (rostro-ventral medulla or dorsolateral pontine tegmental area) to control (up or down-regulate) nociceptive transmission at the dorsal horn of the spinal cord (Fields & Basbaum, 2006).

Pain perception occurs from the relay of nociceptive information to cortical areas through the spinothalamic tract (Beaulieu, 2013). Through this tract, the noxious signal ascends to the thalamus and is relayed onto the anterior cingulate cortex (ACC) and the insula, thought to reflect the affective/unpleasant dimension of pain (Price, 2000). The nociceptive signal is also relayed from thalamic nuclei to primary and secondary sensory cortices, described as underlying the perception of sensory/discriminant aspects of pain (Price, 2000). From sensory cortices, projections exist to parietal, insular, amygdala and hippocampal formation areas, indicating an integration between several cerebral regions in contributing to pain affect (Price, 2000). There also exist pathways transmitting noxious information from the spinal cord to brainstem/limbic sites including a spinohypothalamic and a spinopontoamygdaloid which may reflect raw or primary affective (eg. autonomic, arousal, homeostatic) responses to noxious stimuli (Price, 2000).

Another dimension of pain has been described as ‘secondary pain affect’, and is distinct from pain unpleasantness. Secondary pain affect rather refers to the cognitive elaboration of pain concerning its meaning in terms of consequences for the future, for functioning, etc. (Price, 2000). “Secondary pain affect is sustained by pain unpleasantness and may depend on ACC-prefrontal cortical interactions that add further cognitive evaluation to emotions associated with pain” (Price, 2000). Figure 5 illustrates the transmission of the nociceptive signal from the periphery to the cortex and different stages of processing.

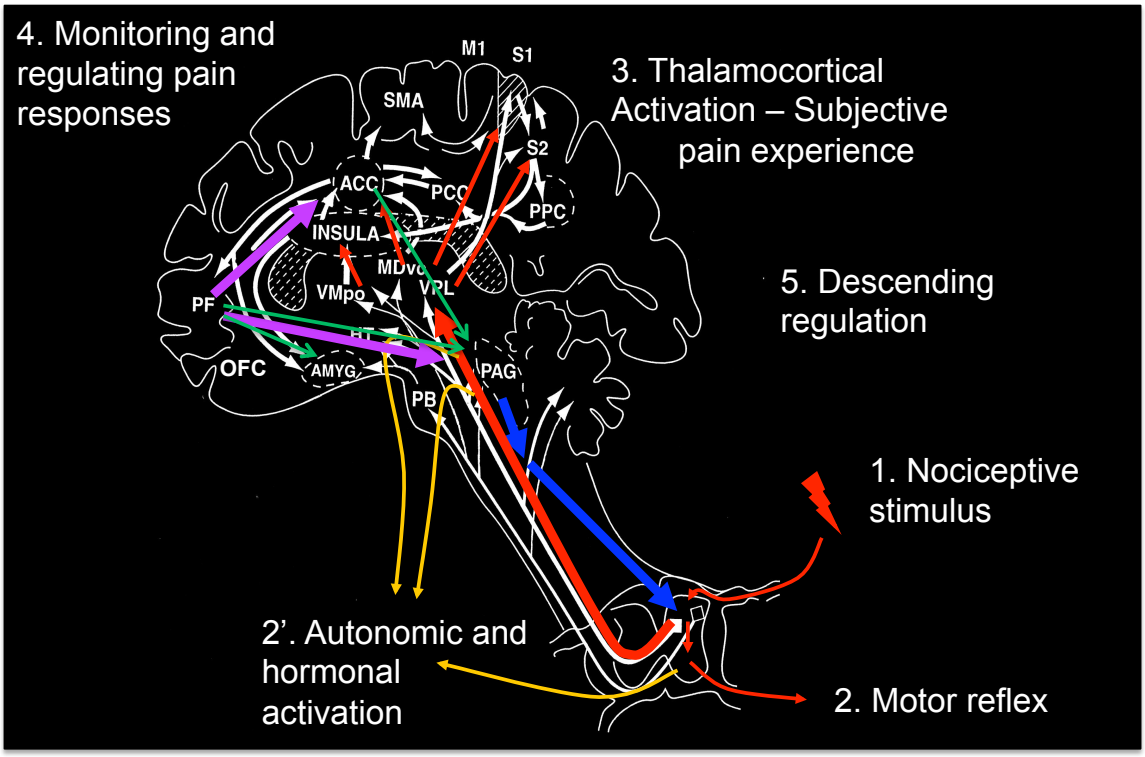

Figure 5. Spinothalamic transmission of the nociceptive signal. The nociceptive signal triggered by the nociceptive stimulus (1) and integrated at the dorsal horn of the spinal cord from which defensive motor reflex responses can be observed (2). Information is integrated at brainstem (parabrachial nucleus: PB) and limbic sites (amygdala: AMYG, hypoathalamus: HT) from which autonomic and endocrine responses are generated (2'). The signal is then relayed to the thalamus and cortical areas (insula, anterior cingulate cortex: ACC, primary and secondary sensory cortices: S1 and S2) resulting in the conscious perception of pain (3). Information is also relayed to the prefrontal cortex (PF), involved in monitoring and regulating pain responses (4). Prefrontal and other cortical sites can activate descending pain controls (5) originating from the periaqueductal gray (PAG) to modulate the signal at the spinal cord. M1: primary motor cortex, SMA: supplementary motor area, OFC: orbito-frontal cortex, VMpo: ventromedial part of the

posterior nuclear complex of the thalamus, MDVo: ventrocaudal part of the medial dorsal nucleus, VPL: ventro-posterior lateral nucleus of the thalamus, PCC: posterior cingulate cortex, PPC: posterior parietal cortex. Adapted from Price (2000).

In addition, as illustrated in Figure 6, different pain and nociception-related responses reflect activity at various levels of integration as the nociceptive signal ascends to the cortex (Rainville, 2013). Different responses and modulatory processes can be observed as the nociceptive signal ascends from the spinal cord to different levels of integration, such as at the brainstem (eg. autonomic arousal), the diencephalon (eg. associative aversive learning), and the telencephalon (pain ratings) (Rainville, 2011, 2013). At the spinal level, the lower limb flexion reflex or withdrawal reflex is a protective response from noxious stimuli (Sandrini et al., 2005). This response is also called the nociceptive flexion reflex, and is often used as a neurophysiological tool to index spinal responding to nociceptive stimuli. It can be elicited by electrical stimulation of the sural nerve and assessed from electromyograhic recordings of the lower limb of the biceps femoris, for example. The threshold intensity at which it is elicited also generally correlates with that eliciting pain thresholds (Sandrini et al., 2005).

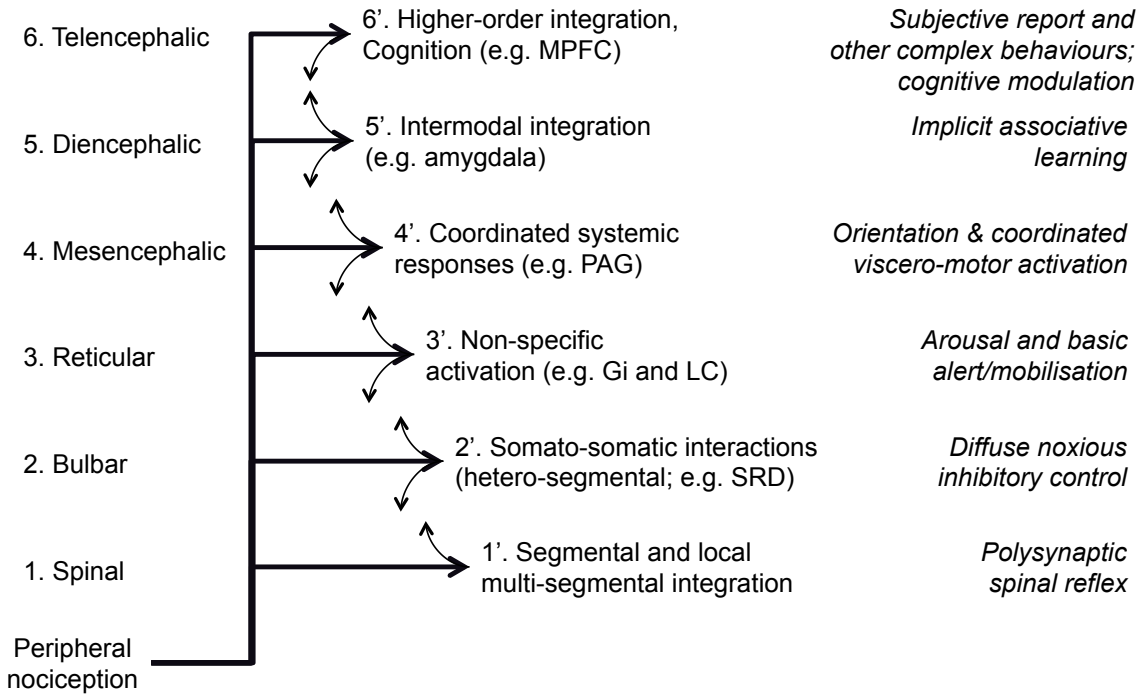

Figure 6. Organization of the nociceptive system as a hierarchy. Nociceptors at the periphery activate neurons relaying the nociceptive signal to the spinal cord. The signal is further relayed through multiple ascending pathways reaching several target sites at different levels (brainstem, diencephalon, and telencephalon). Integration processes occurring at each level are reflected in different responses or modulatory processes. Inter-level interactions can occur via feedback and feedforward connections between levels. From Rainville (2013). MPFC: medial prefrontal cortex; PAG: periaqueductal gray; LC: locus coeruleus; Gi: gigantocellular reticular nucleus; SRD: subnucleus reticularis dorsalis in the caudal medulla.

Combining spinal responses to nociceptive stimuli with pain ratings reflecting higher-order perceptual processing of pain allows to determine the specific levels (spinal, supraspinal, or a combination of both) at which pain is modulated. The *selective* pain modulation at a higher-order level of perceptual processing would indicate modulation at a supraspinal level that may reflect interactions between cerebral targets of pain transmission pathways. An observed modulation at the level of spinal responses to noxious stimuli would implicate the recruitment of descending pain controls. Of course, 'supraspinal' processes could also be a direct reflection of afferent spinal input, which could be determined using a mediation analysis framework to examine the proportion of variance in higher-order level responses explained by afferent bottom-up (spinal) facilitation.

Pain provides necessary information to adaptively respond to environmental harm. In the same way, fear is a vital emotion that adaptively allows the organism to learn, adapt, and survive in the environment filled with challenges, threat, and constant change. Therefore, to reduce dysfunctional cyclic interactions between fear and pain, the objectives should not be aimed at *eliminating* either of these vital teaching signals, but rather to break the maladaptive relationship between fear and pain. Mindfulness meditation is an excellent candidate tool to achieve this effect, because its focus is to welcome states of aversion rather than to suppress or change them.

The next section will provide an overview of this practice and its potential role in disrupting the fear – pain cycle.

Mindfulness Meditation: Role In Breaking the Fear-Pain Cycle

Increasingly integrated in modern medical practice, mindfulness meditation is a form of mental cognitive/affective training that has been demonstrated for its efficacy to improve psychological well-being, and attenuate pathological symptoms of chronic pain and stress/affect-related disorders (anxiety, major depression) (Baer, 2003; Brown & Ryan, 2003; Grossman, Tiefenthaler-Gilmer, Raysz, & Kesper, 2007; Keng, Smoski, & Robins, 2011; Morone, Greco, & Weiner, 2008; Weber, Jermann, Lutz, Bizzini, & Bondolfi, 2012). Mindfulness involves paying attention purposefully to the present-moment (Bodhi, 2005). An important aspect of mindfulness meditation is the welcoming of any event (thoughts, sensations, perceptions) entering the field of awareness, without judgement or attempt to eliminate/suppress these events (Bishop, 2004). The method emphasizes that distractions/ruminations/cognitive elaborations be acknowledged and observed, while returning to an initial object of focus (eg.: the posture or the breath).

Mindfulness is a core aspect of acceptance-based forms of clinical therapies. Exposure-based therapies aimed at cultivating attitudes of ***acceptance*** towards pain have proven efficacious in reducing detrimental effects of pain on physical and psychological health (Crombez et al., 2012; McCracken & Eccleston, 2005; Viane et al., 2003). Indeed, the notion of acceptance has been defined as "halting the dominant search for a definitive cure for pain and reorienting one's attention toward positive everyday activities and other rewarding aspects of life" (Crombez et al., 2012).

Acceptance stances towards pain could potentially reduce its negative impact by attenuating the so-called 2nd dart of pain. As such, pain has been described in phenomenological terms as consisting in a '1st' and a '2nd dart' of suffering (Bodhi, 2005). The '1st dart of suffering would correspond to the raw emotional and sensory aspects of pain experience, i.e. pain unpleasantness and sensory-discriminant pain dimension, mainly reflected by the activation of spinothalamic tract targets (ACC, insula, S1 and S2). The 2nd dart of suffering would correspond to the ruminations generated by the painful experience about potential meanings/consequences of pain and its causes. This aspect of pain could be compared to the secondary pain affect dimension described by Price (2000), which is supported by prefrontal cortical activation. The repeated practice of mindfulness would foster the elimination/attenuation of the 'second dart' of suffering by training the mind to pay attention to pain objectively and in the present-moment, detached from any form of cognitive elaboration or rumination about the meaning of pain in terms of past events and consequences for the future (Bodhi, 2005).

Mindfulness was first implemented as part of an 8-week stress-reduction program (MBSR) in clinical settings by Jon Kabat-Zinn for patients suffering from chronic pain (Kabat-Zinn, Lipworth, & Burney, 1985). Effects of such interventions were shown to produce clinical improvements in disabling symptomatology associated with chronic pain (McCracken, Gauntlett-Gilbert, & Vowles, 2007). The precise mechanisms, however, through which mindfulness attains these beneficial effects remain unclear. Since mindfulness meditation cultivates acceptance and the welcoming of aversive states/pain as opposed to maladaptive strategies such as avoidance, it is quite possible that

mindfulness benefits chronic pain management symptomatology by breaking the cycle between fear and pain.

In addition, the pain attenuating impact of long-term (Grant, Courtemanche, & Rainville, 2011; Grant & Rainville, 2009; Perlman, Salomons, Davidson, & Lutz, 2010) and short-term (Zeidan, Gordon, Merchant, & Goolkasian, 2010; Zeidan et al., 2011) practice of mindfulness meditation has been scientifically proven in experimental settings. However, the mechanisms underlying the hypoalgesic effects of meditation practice on pain still remain undetermined. The hypotheses are that mindfulness reduces pain affect due to its present-moment focused awareness qualities, thus eliminating any influence from mental events preceding pain (fear and anticipatory processes) and those following it (cognitive elaboration, fear of the meaning of pain in terms of potential consequences for the organism).

In line with these hypotheses, neuroimaging studies have shown that hypoalgesia resulting from meditation are associated with changes in brain activity during the anticipation of pain in long-term meditators (Brown & Jones, 2010; Gard et al., 2012; Lutz, McFarlin, Perlman, Salomons, & Davidson, 2013). However, it cannot be determined from these studies whether meditation attenuates pain by dampening anticipatory behaviors per se, or by diminishing the ***impact of anticipation and fear on pain.*** There is evidence that mindfulness meditation practice does not dampen fear conditioned SCR acquisition (Holzel et al., 2016), and is not associated with reduced amygdala responses to emotionally aversive pictures (Taylor et al., 2011). It is therefore

more likely that mindfulness meditation influences the impact of fear on pain, rather than fear learning processes per se.

Brain imaging results also show that long-term meditators exhibit reductions in prefrontal areas and enhanced activity in cerebral target sites of the spinothalamic tract during pain, as well as reduced prefrontal-ACC connections (Gard et al., 2012; Grant et al., 2011; Lutz et al., 2013). These results suggest that hypoalgesia related to contemplative practice occurs from reduced cognitive elaboration of pain affect, thereby reducing the 2nd 'dart' of suffering (secondary pain affect) which sustains the aversive experience. The result of these studies suggest that mindfulness attenuates pain solely by reducing higher-order cortico-cortical interactions between areas related to cognitive elaboration of pain and targets of spinothalamic pain transmission, and does not engage descending inhibitory pain controls (Gard et al., 2012; Grant et al., 2011; Lutz et al., 2013). This hypothesis has not formally been tested to date using subjective ratings in conjunction with assessments of spinal nociceptive processing (nociceptive flexion reflex). Thus this thesis was also aimed at elucidating the level(s) at which pain modulating effects of this practice take place: 1-uniquely at a higher –order perceptual level of pain processing, 2- by engaging descending pain controls inhibiting transmission at the spinal cord, 3- or a combination of both mechanisms.

By reducing the impact of fear on pain, the propensity of fear to be reinforced in this cycle would also be reduced, as well as the risk for developing pathological avoidance to threat. This is why interventions such as mindfulness meditation, exposing individuals to painful or threatening experiences and training to dissociate these with any fear-related

thoughts about interpretations or meaning of pain, may aid in breaking the fear-pain cycle and contribute to the treatment of chronic pain and pain-related anxiety.

Thus, known for its stress-reducing benefits (Baer, 2003), individual factors such as mindfulness meditation experience are hypothesized to decrease the effects of fear on pain. By contrast, it is possible that individual factors underlying the regulation of stress-related responses, at the neurohormonal level, also moderate the relationship between fear conditioning and pain.

Stress Hormones and Pain Modulation

When a threat is perceived by the organism, attempts to achieve homeostasis are made and a cascade of neuronal and neuroendocrine events is triggered, involving sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis activation (Cacioppo, Tassinary, & Berntson, 2007). The stress response is characterized by a so-called first and second wave response (Sapolsky, Romero, & Munck, 2000). The first-wave response is rapid acting (within seconds) and is characterized by sympathetic nervous system activation inducing catecholamine release (adrenaline and noradrenaline) (Sapolsky et al., 2000). The first-wave response also involves the release of corticotropin-releasing factor from the hypothalamus and adrenocorticotropic hormone from the pituitary gland (Sapolsky et al., 2000). The slower and longer-lasting second-wave response involves the peripheral release of the glucocorticoid hormone cortisol (within minutes) from the adrenal cortex (Sapolsky et al., 2000). The 'stress hormone' cortisol is the end-product of HPA-axis activation. This steroid hormone has anti-inflammatory properties and is

implicated in the breakdown of glucose and mobilization of energy stores to effectively cope with sources of threat (Cacioppo et al., 2007). The stress response activation is adaptive on an acute basis to cope with threat, but excessive prolongation, dysregulation, or inability to shut off the stress response from central negative feedback mechanisms can result in poor health outcomes and psychiatric conditions (Cacioppo et al., 2007).

The neurocircuitry of the stress system shares several regions with the circuitry of fear learning and pain systems. For instance, "The LC-NA (locus coeruleus – noradrenergic) system activates and is activated by the amygdala, which, acting in conjunction with the hippocampus and the anterior cingulate and prefrontal cortices, mediate focused attention of a perceived threat, define the affective state of the individual and regulate fear- related behaviors " (p.305) (Cacioppo et al., 2007). In addition, cortisol crosses the blood brain barrier and binds to two main types of glucocorticoid receptors (mineralocorticoid and glucocorticoid receptors) in several brain areas such as the hippocampus and amydgala, as well as the prefrontal cortex (Lupien & McEwen, 1997). It is therefore not surprising that glucocorticoids influence cognition, learning, memory and affective processes (Lupien & McEwen, 1997).

Recent evidence also shows that pain responses in the brain and pain unpleasantness reports are lower in individuals showing stronger stress-reactivity as measured by cortisol (Vachon-Presseau et al., 2013). This is consistent with stress-induced analgesia (Fanselow, 1986) and may be mediated by various processes including, but not restricted to, NA and/or HPA-axis activity. However, whether individual differences in HPA-axis

activity only induced pain modulation at a higher-order level of processing, or whether descending pain inhibitory controls were also engaged remains undetermined. In addition, given the shared neurocircuitry between the stress system, stress hormone receptor distribution, fear learning, and pain systems (LeDoux & Phelps, 2008; Lupien & McEwen, 1997; Price, 2000), it is also possible that pain modulating influences of stress hormones operate by targeting fear learning mechanisms and their effects on pain. In other words, it is possible that effects of fear conditioning on pain varies from individual to individual depending on the underlying activity of the HPA-axis.

Previous studies examining the relationship between cortisol and anticipatory conditioned responses found a positive relationship in men, but not in women (Jackson, Payne, Nadel, & Jacobs, 2006; Zorawski, Blanding, Kuhn, & LaBar, 2006; Zorawski, Cook, Kuhn, & LaBar, 2005). The same results were found following psychosocial stress (Jackson, Payne, Nadel, & Jacobs, 2006; Zorawski, Blanding, Kuhn, & LaBar, 2006), which has been explained by interactions with sex hormones (Merz & Wolf, 2017). No study to date, however, has examined the relationship between HPA-axis activation, and the effects of fear learning on pain.

Presentation of empirical articles of this thesis

Three experimental studies were conducted within this doctoral thesis in an attempt to shed light on the effects of fear learning on pain, as well as predisposing factors of vulnerability and resilience to the maintenance of fear-pain interactions. In the first

article, the objectives were to determine effects of fear learning on the dynamic modulation of pain at different levels of nociceptive processing using computational reinforcement learning modeling. Individual personality traits were also examined as moderators of the effects of fear on pain. In the second article of this thesis, the aim was to examine the role of long-term mindfulness meditation experience on fear learning, pain modulation, and as a moderator in the effects of fear conditioning on pain at different levels of nociceptive processing (spinal and supraspinal). Finally, in the third article of this thesis, the role of HPA-axis activation during fear learning, as measured by salivary cortisol, was examined as a modulator of pain and as a moderator of the impact of fear learning on pain and spinal nociception.

In all three studies, classical delay fear conditioning paradigms were used to assess fear learning with visual cues as conditioned stimuli and noxious electrical shocks to the right sural nerve as the unconditioned stimulus. Computation reinforcement learning models were used in all three studies, to determine fear learning parameters (expectations/associability) at each CS+ trial paired with a shock. These parameters were then used as predictors of pain responses to the US using multi-level regressions. Measures of pain responses included subjective ratings, reflecting higher-order perceptual processes, and the nociceptive flexion reflex (NFR), reflecting spinal nociceptive activity. Individual trait factors relevant to affective processes (self-reported personality variables, meditation experience, HPA-axis activation) were entered as moderators of the relationship between fear learning parameters and pain outcomes.

Article 1: Learned Expectations and Uncertainty Facilitate Pain during Classical Conditioning

Taylor, V., Chang, L., Rainville, P., Roy, M. Learned Expectations and Uncertainty Facilitate Pain during Classical Conditioning. *Pain* (accepted, In Press).

Contribution of authors: VT, PR and MR designed the study; VT acquired the data; VT and MR analyzed the data and applied computational models with the help of LC; VT drafted the manuscript and all authors revised and approved the article.

Abstract

Pain spontaneously activates adaptive and dynamic learning processes affecting the anticipation of, and the responses to, future pain. Computational models of associative learning effectively capture the production and ongoing changes in conditioned anticipatory responses (e.g. skin conductance response, SCR), but the impact of this dynamic process on unconditional pain responses remains poorly understood. Here, we investigated the dynamic modulation of pain and the nociceptive flexion reflex by fear learning in healthy human adult participants undergoing a classical conditioning procedure involving an acquisition, reversal and extinction phase. Conditioned visual stimuli (CS+) co-terminated with a noxious transcutaneous stimulation applied to the sural nerve on 50% of trials (unconditioned stimuli; US). Expected pain probabilities and cue associability were estimated using computational modeling by fitting a hybrid learning-model to SCR elicited by the CS+. Multi-level linear regression analyses confirmed that trial-by-trial changes in expected pain and associability positively predict ongoing fluctuations in pain outcomes. Mediation analysis further demonstrated that both expected probability and associability affect pain perception through a direct effect and an indirect effect mediated by descending modulatory mechanisms affecting spinal nociceptive activity. Moderation analyses further showed that hyperalgesic effects of associability were larger in individuals reporting more harm vigilance and less emotional detachment. Higher harm vigilance was also associated with a stronger mediation of hyperalgesic effects by spinal processes. These results demonstrate how dynamic changes in pain can be explained by associative learning theory and that resilient attitudes towards

fear/pain can attenuate the adverse impact of adaptive aversive learning processes on pain.

Keywords: Fear conditioning, reinforcement learning models, pain, nociceptive flexion reflex

Significance Statement: Pain has an important function in teaching the organism to escape potential sources of harm, yet the dynamics of pain modulation as a function of associative learning remains unknown. Here we used computational modeling to predict pain during classical conditioning in healthy human participants. Our results show that pain is markedly enhanced when it is either highly probable or uncertain, and that inter-individual traits related to harm vigilance and emotional detachment can influence the strength of learning effects on pain. These findings demonstrate that pain is constantly under the influence of learning and suggest an experimental model explaining how associative learning could contribute to the central maintenance of pain observed in several chronic pain syndromes.

Introduction

Pain has an important teaching function: past pain episodes shape our current reactions to pain, which in turn influences our future responses to painful events. The influence of learning on pain perception may be particularly important when individuals are subjected to successive episodes of acute pain, as observed in many chronic pain syndromes (Baliki, Geha, Fields, & Apkarian, 2010; Borsook, Maleki, Becerra, & McEwen, 2012; Flor & Turk, 1999). Unfortunately, we still know very little of the dynamic influence that learning continuously exerts on pain perception during repeated exposure to painful stimuli.

Prior studies using conditioned cues to manipulate expectations about pain (Jensen, Kirsch, Odmalm, Kaptchuk, & Ingvar, 2015; Montgomery & Kirsch, 1997) have shown that pain perception generally increases following cues that predict the occurrence of noxious stimuli or signal more intense stimulation (i.e. "conditioned hyperalgesia"). However, these studies examined averaged pain responses following an initial conditioning phase during which participants are assumed to have acquired stable cue-pain associations, thereby treating learning as a static process. Here, by contrast, we opted to examine the dynamic influence of learning over pain as associations are formed and updated at every trial. More specifically, we employed computational methods to extract trial-by-trial values of latent variables reflecting core associative learning processes. We predicted that these latent learning variables would explain trial-by-trial fluctuations in pain ratings and spinal nociceptive flexion reflexes induced by noxious electrical stimulations during classical conditioning.

In their simplest form, computational models posit that associative learning is driven entirely by prediction errors, i.e. the difference between expected and experienced outcomes. In typical conditioning paradigms, expectations about outcomes, or the valuation processes underlying the assessment of upcoming reward/punishment magnitude and probability (LePelley & McLaren, 2004), can be inferred from indirect measures (e.g. anticipatory skin conductance responses (SCRs)). By fitting the model to the data, the values of the latent variable (e.g. expectations) that best predict the indirect indexes of learning (e.g. anticipatory SCRs), can be estimated for each trial. However, in many conditions, simple models based solely on prediction errors provide an incomplete account of associative learning. Recent studies have shown that hybrid models comprising an associability term provide a better account of anticipatory SCRs (Li, Schiller, et al., 2011) and self-reported pain expectations (Boll et al., 2013) than standard learning models relying only on prediction errors/expectations. Hybrid models posit that the rate at which expectations are updated following outcomes (i.e. the "learning rate") varies as a function of each trial's informational value. This additional variable, called "associability", increases when predictions are unreliable (i.e. there is more to learn when outcomes are difficult to predict), and has been suggested to involve increased attentional demands associated with uncertainty about the outcomes (LePelley & McLaren, 2004). Recent brain imaging studies have shown that these two fundamental learning variables – associability and expectations – are associated with activity in different brain networks, confirming that they may reflect at least partly distinct neural processes (Boll et al., 2013; Li, Schiller, et al., 2011). Here, we predicted that the dynamic influence of associability

and expectations would provide a more comprehensive account of ongoing effects of aversive learning on pain responses.

In this study, participants underwent a classical delay-conditioning task during which one of the two predictive cues (CS+) was associated with a 50% probability of being followed by a painful electric shock (US). Anticipatory SCRs to the predictive cues were used to extract trial-by-trial estimates of associability and expected shock probability (henceforth referred to as EShock). Because associability normally decreases as participants gradually learn the fixed probability of pain during acquisition, cue-outcome associations were reversed during the experiment to transiently decouple pain predictions and associability. Moreover, to examine how learning exerts its effects at various levels of nociceptive processing, we recorded spinal nociceptive flexion reflexes (NFRs) in addition to pain ratings in response to the painful electric shocks (Rhudy, Williams, McCabe, Rambo, & Russell, 2006; Roy, Piche, Chen, Peretz, & Rainville, 2009; Sandrini et al., 2005). We then examined the relationship between learning variables derived from SCRs to predictive cues, and pain ratings and NFRs in response to subsequent electric shocks. Finally, we explored the influence of several relevant personality traits on the relationship between learning variables and pain responses to identify individual factors affecting the magnitude of conditioned hyperalgesia.

Methods

Participants

The sample consisted of 47 healthy young adults between 19 and 32 years of age (25 male, 22 female;) recruited from advertisements in local University settings (Université de Montréal as well as McGill and Concordia Universities). Ethical approval for the study was obtained by the ethics research committee of the Centre de Recherche de l'Institut Universitaire de Gériatrie de Montréal (CRIUGM).

Potential participants were considered eligible to take part in the study upon meeting the following criteria: no pregnancy, no psychological/psychiatric condition (such as major depressive disorder and substance abuse), no medication intake (except for oral contraceptives), no pain-related diseases (such as chronic pain or neuropathic pain), and no regular use of anti-inflammatory or analgesic medications. Potential participants were invited to visit the Laboratory of the Neuropsychophysiology of Pain (UdeM, Canada) for a screening and familiarization session to assess their pain thresholds and physiological signals (skin conductance and NFR) and for a second visit to complete the experimental paradigm. Nine participants were not retained following the familiarization session for one of the following reasons: extreme pain thresholds, excessive use of alcohol, drugs, or analgesic medication, discomfort with the nature of the noxious stimuli (electrical stimulations), or absent/unstable skin conductance or NFRs to the painful stimuli. Fifty participants participated in the experimental session, but three subjects were

excluded from data analysis due to poor electrodermal signal or very inconsistent NFRs. Finally, computational learning model fits were extremely deviant for 2 participants (with predicted SCR values below or over 10 SDs from the mean), yielding a remaining total of 47 participants included in the analyses.

Stimuli

Visual stimuli were presented on a computer screen monitor with E-Prime2 Professional (Psychology Software Tools, Sharpsburg, PA). The CSs (cue1 and cue2) consisted in coloured fractal images (circles filled with computerized random colors and shape patterns) presented for 2s on a black background. The unconditioned stimuli (US) co-terminated with CS presentation, and consisted of a 30 ms transcutaneous electrical stimulation (trains of ten 1-millisecond pulses at 333 Hz) delivered with an isolated DS7A constant current stimulator (Digitimer Ltd, Welwyn Garden City, United Kingdom) triggered by a train generator (Grass Medical Instruments, Quincy, MA) and controlled by a computer running E-Prime2 Professional. Stimulation electrodes were positioned on degreased skin on the retromalleolar path of the right sural nerve. NFR thresholds were assessed based on the NFR staircase thresholding method previously described (Willer, 1977). The value corresponding to 135% of the threshold intensity was calculated to be administered as the US intensity in the fear conditioning paradigm.

Measures and Dependent Variables

Physiological measures were recorded using BIOPAC Systems Inc. and Acqknowledge data acquisition software (version 4.2).

Pain Ratings

A visual analog scale (VAS) was used to indicate the level of pain elicited by each electrical stimulation (0: no pain to 100: extremely painful). The VAS consisted in a graduated horizontal bar shown on the computer screen with a cursor moved using a computer keyboard response pad. Pain ratings were normalized across trials for each participant before data analysis.

Electromyographic (EMG) Recording

EMG was recorded using two pre-gelled electrodes on degreased (and shaved if necessary) skin at the level of the right biceps femoris. A ground electrode was placed on the right tibial bone. The EMG signal was amplified 1000 times, sampled at 1000 Hz, and band-passed filtered (100 -500 Hz). The EMG signal was transformed online using the root mean square transform (computed over 20 consecutive samples). Finally, the RMS was integrated offline over 90- 180ms post-shock onset, and was defined as the raw NFR scores. Raw NFR scores were then normalized into z-scores across all trials of the conditioning task for each participant.

Electrodermal Recording

Electrodermal activity was recorded using two electrodes placed on the palmar surface of the left hand. The signal was amplified (5 μs/volt) and bandpass filtered (1-5 Hz). The signal was temporally smoothed offline at 500 ms. Using SCRalyze (Bach, Flandin, Friston, & Dolan, 2010), the skin conductance response (SCR) was assessed to CS- and CS+ unpaired. SCRs were determined using a general-linear model-based approach, by

convolving a standard canonical SCR basis function to event onsets. This function was then regressed onto the acquired data, and beta values estimating the goodness of fit of the model onto the data were computed. In order to obtain an SCR estimate for each CS trial, one model per trial was conducted, a procedure shown to be effective in estimating trial-by-trial responses in timeseries data (Mumford, Turner, Ashby, & Poldrack, 2012). For each model, a regressor was entered with the event onset for the trial of interest, and another regressor with all other CSs onsets was included. Shock onsets and pain rating periods were also entered as regressors of non-interest to account for residual variance in the data. Thus, these analyses yielded an estimate of SCR amplitude for each trial (henceforth referred to simply as 'SCR' for the sake of conciseness).

Testing procedure

For their initial screening session, participants provided informed consent and were asked a series of questions concerning demographic variables. They were then prepared for electrophysiological recordings after which they were submitted to the NFR thresholding procedure. Finally, they were given a battery of self-report questionnaires to fill out. Trait anxiety was assessed using the State-Trait Anxiety Inventory (STAI) (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). Dispositional mindfulness was assessed using the Five Factor Mindfulness Questionnaire (Baer, Smith, Hopkins, Krietemeyer, & Toney, 2006) due to its inverse relationship with pain catastrophizing (Schutze, Rees, Preece, & Schutze, 2010), and due to the role of mindfulness meditation in attenuating pain perception and developing resilience in the management of chronic pain (Grant &

Rainville, 2009; Kabat-Zinn et al., 1985; Zeidan et al., 2010). This 39-item questionnaire is composed of five sub-scales assessing different dimensions of dispositional mindfulness: 'Observe' (ability to observe inner experiences), 'Describe' (ability to describe inner experiences), 'Aware' (acting with awareness), 'Non-judgment' of and 'Non-reactivity' to experiences. Dispositional mindfulness was also assessed using the 15-item Mindful Attention Awareness Scale (MAAS) "designed to assess a core characteristic of dispositional mindfulness, namely, open or receptive awareness of and attention to what is taking place in the present." (Brown & Ryan, 2003). In addition, the Pain Catastrophizing Scale (PCS) (Sullivan, Bishop, & Pivik, 1995) was administered, which is a 13-item questionnaire assessing the degree to which individuals catastrophize about their pain with three subscales: pain magnification, pain rumination, and helplessness towards pain. Depressive symptoms were assessed using the Beck Depression Inventory (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961), and punishment sensitivity was assessed using the Behavioral Inhibition/Activation Scale (Carver & White, 1994). Finally, the Temperament and Character Inventory (Cloninger, 1994) was administered to assess several different personality facets, and our focus was on its following subscales due to their relevance to fear/pain processing and trait mindfulness: harm avoidance (sum of scores on the subscales of 'Anticipatory worry & Pessimism vs Uninhibited optimism', 'Fear of Uncertainty', 'Shyness with strangers', and 'Fatigability & asthenia'), self-transcendence (sum of scores on 'Self-forgetful vs Self-Conscious Experience', 'Transpersonal Identification vs Self-Differentiation', and 'Spiritual Acceptance vs Rational Materialism'), and self-directedness (sum of scores on 'Responsibility vs blaming', 'Purposefulness vs lack of goal-direction',

'Resourcefulness', 'Self-acceptance vs Self-striving', and 'Enlightened Second Nature').

Participants were invited to return a few days later for a second visit to complete the experiment. After being prepped for electrophysiological recordings, the procedure for NFR thresholding was conducted to determine the intensity of electrocutaneous stimulation administered during the task. Before the start of the task, 2 trials of each CS (without any shocks) were presented, as well as a 'baseline' block of 10 stimulations at the individually determined intensity. Participants then underwent the fear conditioning paradigm (Figure 1), which was adapted from previous work (Schiller, Levy, Niv, LeDoux, & Phelps, 2008). Prior to completing the learning task, participants were instructed to observe and pay attention to the stimuli presented on the computer screen, and that they may or may not receive electrical stimulations (following which they would provide a pain rating). Thus, no explicit instructions were given as to the cue-shock contingencies or reversal of these contingencies.

The fear conditioning paradigm included phases of acquisition (Blocks1 and 2), reversal (Blocks1 and 2, in which stimuli assigned as CS+/CS- in the acquisition phase were reversed), and extinction (presentation of CSs alone). In the acquisition and reversal blocks, one image was paired and co-terminated with the shock at a contingency rate of 50% (CS+), and the other was never paired with the shock (CS-). Each US was followed with an interval (jittered between 4 and 8 seconds; to allow the recording of a SCR to the US) and the VAS. The inter-trial intervals consisted of a white cross centered on a black background (duration jittered between 9, 10, 11, and 12 seconds)

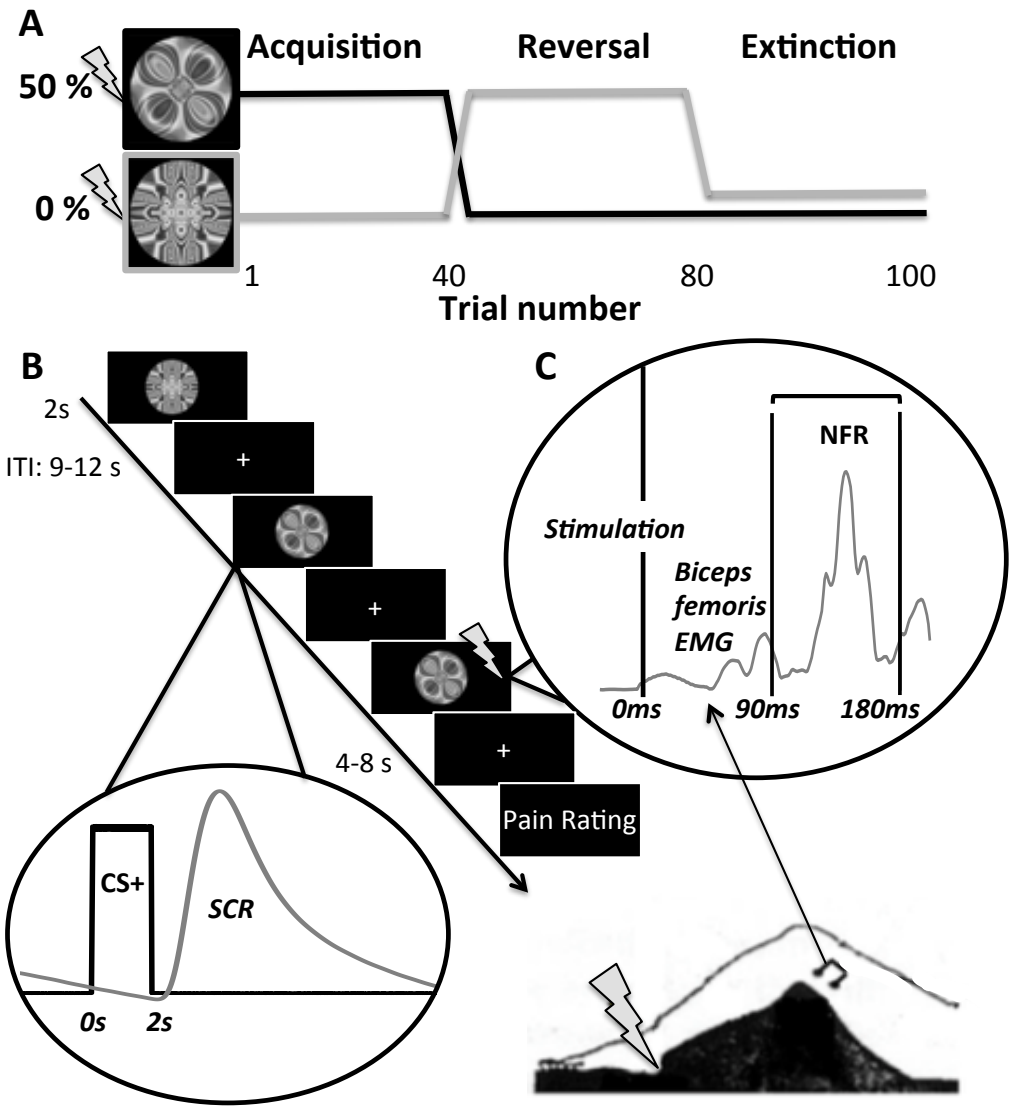

Figure 1. Experimental paradigm. A) In the initial acquisition stage (trials 1- 40), one cue was associated with a 50% chance of being followed by an electric shock (CS+), while the other cue was associated with a 0% chance of shock (CS-). In the reversal stage, the reinforcement contingencies between the two cues were reversed, such that the previous CS+ became the new CS- and the previous CS- became the new CS+. In the extinction phase, both cues were associated with a 0% chance of shock. B) Example of each type of trial (CS-, CS+, and CS+paired). Each trial began with the presentation of one of the two cues. On reinforced (CS+paired) trials, the presentation of the cue co-terminated with an

electric shock (30 ms) to the right sural nerve and participants were asked to rate their pain after a jittered interval of 4-8s. Then, after another jittered inter-trial interval (ITI) of 9-12s, the following cue was presented. During unreinforced (CS- or CS+unpaired) trials, there were no pain ratings, and fear conditioned responses to visual cues were assessed by examining skin conductance responses (SCR; with a typical latency between 0.5 and 2s) from electrodermal activity recordings. C) Electromyographic (EMG) activity was recorded using electrodes placed on the biceps femoris. The NFR was observable at a latency of 90-180 ms post-stimulation onset.

Acquisition and reversal blocks consisted of 40 trials (20 CS-, 10 CS+ unpaired, 10 CS+ paired) and lasted 13 minutes each. Trials were presented in a pseudo-random order, with the constraint that there were no more than 2 consecutive presentations of the same trial type. Also, the first trial of each block always consisted of a paired CS+, and the second always consisted of a CS- to instantiate learning contingencies at the onset of the block. The assignment of the CS+ in the acquisition phase (stimulus A or B) was counter-balanced across subjects. The extinction block lasted 10 minutes and consisted of 40 trials of unreinforced CSs (20 trials for each image). The assignment of the CS+ in the acquisition phase (stimulus A or B) was counter-balanced across subjects. A final block of 10 stimulations without any CS was then administered in order to account for non-specific changes in the NFR as a function of time. At the end of the experiment, electrodes were removed and participants completed a post-experimental interview assessing their awareness of CS-US pairings adapted from previous studies (Bechara et al., 1995; LaBar, LeDoux, Spencer, & Phelps, 1995). They were then debriefed and remunerated 15$/hour for their time.

Data Analyses

Self-Report Questionnaire Analyses

In order to reduce the number of individual trait dimensions, a principal component analysis using an oblique rotation method was conducted on the different self-report questionnaire data scales using SPSS Version 21.0. Data included in the analyses were BDI scores (square root transformed to correct for a positively skewed distribution),

Mindful Awareness Attention Scale (MAAS) scores, Pain Catastrophizing Scale (PCS) scores (sum of scores on the magnification, rumination, and helplessness towards pain), Behavioural Inhibition Scale scores (BIS), scores on each Five-Factor Mindfulness Questionnaire subscale, trait anxiety scale were scores, as well as the TCI subscales of harm avoidance, self-transcendence, and self-directedness.

The first three components extracted explaining a total of 62% of the variance in the data were retained in order to use as moderators of the fear-conditioning-induced modulation of pain. Factor loadings onto the questionnaire dimensions are illustrated in Table 1. The first factor loaded positively onto pain catastrophizing, trait anxiety, harm avoidance, punishment sensitivity (BIS), depressive symptoms, and negatively onto 'Non-reactivity to inner experiences'. This factor was labeled as 'Harm vigilance', since it combines attributes specific to catastrophizing pain attitudes, avoidance behaviors, anxiety and emotional volatility. The second factor loaded positively onto FFMQ 'Describing experiences', 'Observing experiences', self-transcendence, and negatively onto 'Non-judgment of experiences'). This factor was labeled as 'Emotional Detachment', since it combined the dimensions of trait mindfulness oriented towards cultivating separation between the self and emotional experiences, distinct from adopting an accepting attitude towards inner experiences. The last factor loaded positively onto 'Acting with Awareness', 'Non-judgment of experiences', self-directedness, present-moment awareness, and negatively onto depressive symptoms, harm avoidance, and trait anxiety. This factor was labeled as 'Acceptance/positive affect', since it combined aspects related to trait mindfulness involved in emotional acceptance and living in the present moment, low anxiety/negative affect and tendencies to avoid harm.

Table 1 Individual Trait Dimensions and their Loadings onto Factors obtained from the Principal Component Analysis

	Principal Component Analysis Factors		
	Harm Vigilance	*Emotional Detachment*	*Acceptance/ Positive Affect*
Individual Trait Dimensions			
Pain Catastrophizing (PCS)	0.64		
Describing Experience (FFMQ)		0.66	
Observing Experience		0.77	
Acting with Awareness			0.78
Non-judgmental (FFMQ)		-0.44	0.68
Non-reactivity (FFMQ)	-0.64		
Trait Anxiety (STAI)	0.70		-0.56
Self-Transcendence (TCI)		0.73	
Self-Directedness (TCI)			0.68
Harm Avoidance (TCI)	0.90		-0.42
Present Moment Awareness (MAAS)			0.74
Depressive Symptoms (BDI)			-0.68
Punishment Sensitivity (BIS)	0.89		

Computational Modeling

Different computational learning models (Rescorla-Wagner and Pearce-Hall hybrid) were (LePelley & McLaren, 2004) fitted to trial-by-trial SCR data to unreinforced cues (CS- and CS+ unpaired), from which fear learning parameters to CS+paired trials were estimated. The following models were tested: a Rescorla-Wagner model (RW model; driven by prediction errors), and a RW/Pearce-Hall hybrid model (RW/PH hybrid), in which the expected value or probability of shock at each trial is computed as a function of prediction errors AND in which the learning rate is dynamically modulated by associability at each trial. Finally, an inter-cue dependent RW/PH hybrid model was conducted, which was a variant of the RW/PH hybrid model in which EShock and associability were updated for the cue presented at each trial, as well as for the unpresented cue. For example, if cue1 were presented at a given trial with the US, the assumption could be made that cue2 would not be associated with the US; thus, at this trial, EShock and associability were updated for cue2 using the opposite outcome as for cue1 (i.e. no shock).

Learning Model Selection. Model fit indices to SCR data were extracted for each subject: Akaike Information Criteria (AIC), and Bayesian Information Criteria (BIC). Non-parametric paired samples comparisons (Wilcoxon test) were conducted on AIC and BIC to compare model fit indices between the RW/PH hybrid, the inter-cue dependent RW/PH hybrid, and the RW models. Model fits were superior for the inter-cue dependent RW/PH hybrid model compared to the other two models (*ps* < .05, AIC and BIC indices

were smaller for the RW/PH hybrid model vs the RW model, and AIC/BIC indices were significantly or marginally significantly smaller for the inter-cue dependent RW/PH hybrid vs the RW/PH hybrid models, see Table 2). The use of the AIC to evaluate model fits to the data allowed to compare the fit of different models while taking into account differing levels of model complexity, or the number of free parameters estimated in a given model. The fact that the RW/PH hybrid models had a superior fit to our data than the RW model indicates that learning was better modeled as a function of both associability and prediction error, and that learning was accelerated following enhanced prediction errors and decelerated following smaller errors. In addition, the superior fit to our data of an inter-cue dependent RW/PH hybrid model compared to a RW/PH hybrid model indicates that participants' learning was dependent on the structure of our task, and that learning about the cue that was *not* presented on a given trial occurred based on information obtained from the cue that *was* present on that trial.

Table 2 Wilcoxon Signed Rank Test Statistics for Comparison of Computational Model AIC/BIC fit Indices

	RW Model		RW/ (PH) Hybrid	
	AIC	BIC	AIC	BIC
RW/Pearce-Hall (PH) Hybrid	Z= -4.61**	Z= -5.97**	-	-
Inter-cue dependent RW/PH Hybrid	Z= -2.30*	Z=-5.78**	Z=-1.76#	Z=-5.78**

Notes. Significant effects of predictors are indicated on the graph with asterisks

*$p < .05$ **$p < .001$, #$p = 0.079$

RW: Rescorla-Wagner; PH: Pearce-Hall.

Model Descriptions. For the Rescorla-Wagner model (Equations 1-2), ***expected shock probabilities*** (denoted as *V* in the following equations) on a given trial (*t*) were updated as a function of the ***prediction error*** (δ) obtained on the preceding trial. The prediction error - discrepancy between the ***actual outcome*** (λ) administered on a given trial and the expected outcome **-** was modulated by a constant learning rate (***α***). Pain administration was coded as 1 and absence of pain as 0.

$$V_{t+1} = V_t + \alpha * \delta_t \quad \text{(Equation1)}$$

$$\delta_t = \lambda_t - V_t \quad \text{(Equation2)}$$

For the RW/PH hybrid model (Equations 3-5), expected shock probabilities were modeled in the same way as in the RW model, but the learning rates were dynamically modulated by an associability term (*a*). The associability term was updated as a function of the prediction error's absolute value (the surprising quality of the outcome, whether it be unexpected pain or unexpected pain omissions), and modulated by a constant term (**γ**).

$$V_{t+1} = V_t + a_t * \alpha * \delta_t \quad \text{(Equation 3)}$$

$$\delta_t = \lambda_t - V_t \quad \text{(Equation 4)}$$

$$a_{t+1} = \gamma * |\delta_t| + (1 - \gamma) * a_t \quad \text{(Equation 5)}$$

More specifically, the RW/PH hybrid inter-cue dependent model depicted the nature of our fear conditioning paradigm involving 2 distinct CSs, i.e. the CS+ paired with the US while the other cue (CS-) predicted the absence of pain in a given learning phase. Thus, fear learning parameters were not necessarily updated independently from one another.

For example, on the presentation of a cue paired with a US, an assumption could easily be made that the other cue was systematically not associated with the US. In other words, the unpresented cue at each trial would be updated according to a prediction error computed by attributing the 'opposite' outcome. Therefore, the RW/PH hybrid inter-cue dependent model consisted of a variant of the RW/PH hybrid model by attributing specific parameters to the cue presented on each trial (*c_pres*) and the cue that was not presented on that trial (*c_unpres*).

$$Vc\_pres_{t+1} = Vc\_pres_t + ac\_pres_t * \alpha c\_pres * \delta c\_pres_t \quad \text{(Equation 6)}$$

$$\delta c\_pres_t = \lambda_t - Vc\_pres_t \quad \text{(Equation 7)}$$

$$ac\_pres_{t+1} = \gamma c\_pres * \left| \delta c\_pres_t \right| + (1- \gamma c\_pres) * ac\_pres_t \quad \text{(Equation 8)}$$

In the same way, associability and expected pain on each trial were updated for the cue that had not been presented (*c_unpres*) by attributing it the opposite outcome, denoted by $\left| 1- \lambda_t \right|$.

$$Vc\_unpres_{t+1} = Vc\_unpres_t + ac\_pres_t * \alpha c\_unpres * \delta c\_unpres_t \quad \text{(Equation 9)}$$

$$\delta c\_unpres_t = \left| 1- \lambda_t \right| - Vc\_unpres_t \quad \text{(Equation 10)}$$

$$ac\_unpres_{t+1} = \gamma c\_unpres * \left|\delta c\_unpres_t\right| + (1- \gamma c\_unpres) * ac\_unpres_t \quad \text{(Equation 11)}$$

Following previous recommendations (Daw, 2011), expected shock probabilities and associability values at each trial for each subject were computed from the following fixed parameters (corresponding to the model's free parameters averaged across subjects):

$\alpha c\_pres = 0.19$, $\alpha c\_unpres = 0.22$, $\gamma c\_pres = 0.21$, $\gamma c\_unpres = 0.33$, $V_{0=} 0.35$, $a_{0=} 0.49$.

Figure 2B illustrates Trial-by-trial SCRs to unreinforced cues (CS- and CS+ unpaired) and predicted SCR estimations to reinforced and unreinforced cues from the inter-cue dependent RW/PH hybrid model. Expected shock probabilities (C) as well as associability (D) related to each cue are also shown in Figure 2.

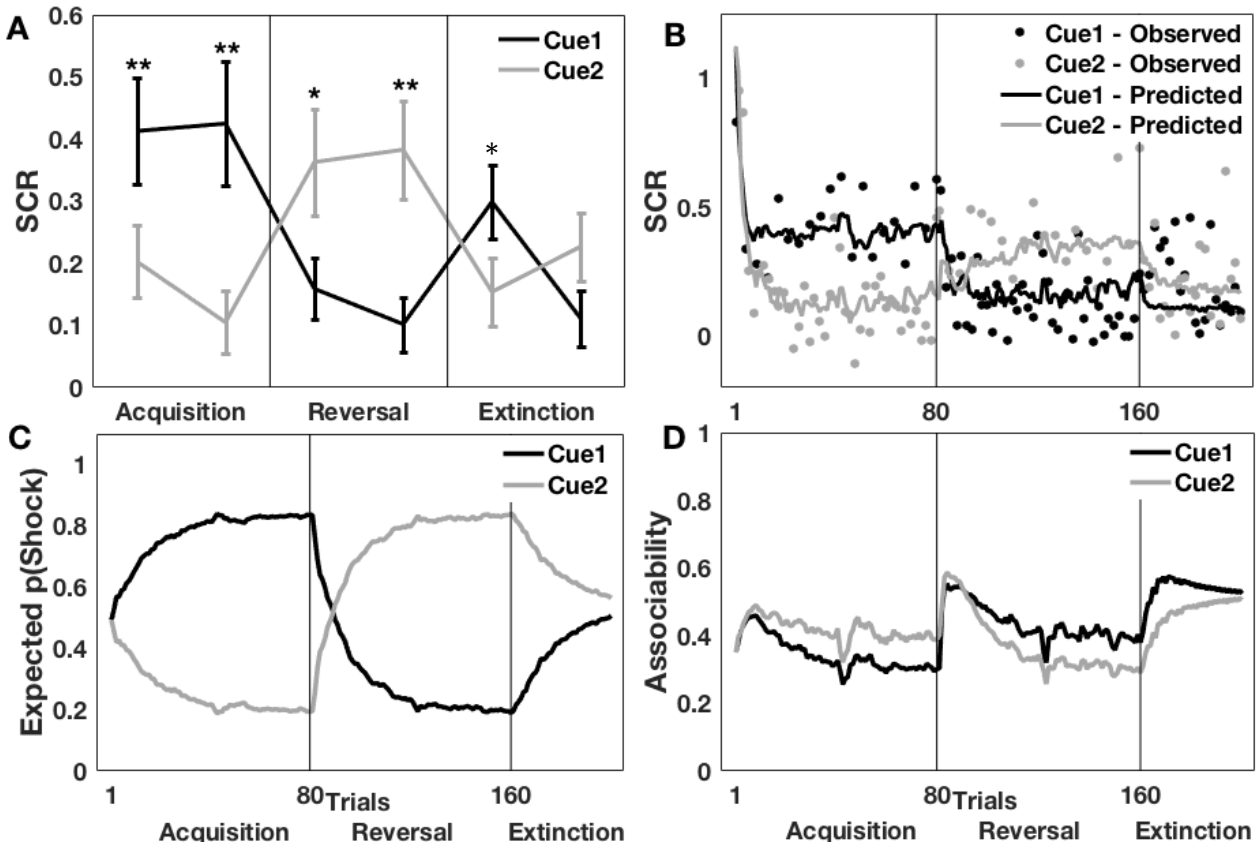

Figure 2. Anticipatory skin conductance responses (SCR) and associability and expected probability of shock (expected p(shock)) estimates throughout the acquisition, reversal, and extinction phases of the experiment. A) Anticipatory skin conductance responses (SCR) for all unreinforced (CS- and CS+unpaired) trials of the experiment, averaged (± SEM) across the whole group for the early (first half) and late (second half) phases of acquisition, reversal, and extinction (*$p < .05$, **$p < .01$, paired t-tests). B) SCRs predicted from the computational learning model (lines) and observed SCRs (dots). Note that while anticipatory SCRs cannot be measured for reinforced trials (CS+paired) due to the temporal contiguity between the CS and the US, computational estimates can be derived from these trials, and used to predict pain responses (see Figure 3 C and D) C and D) Trial-by-trial expected p(shock) (C) and associability (D) estimates, averaged over the whole group.

Results

Effects of conditioning on anticipatory SCRs

In order to demonstrate the efficacy of our paradigm to elicit conditioned fear responses, we first examined anticipatory SCRs in response to the two predictive cues, averaged within the first (early) and second (late) halves of the acquisition, reversal and extinction phases of the experiment (Schiller et al., 2008). As expected, results of a 2 (cue 1, cue 2) x 6 (acquisition-early/late, reversal-early/late, extinction-early/late) ANOVA revealed a significant Cue X Phase interaction ($F_{(1.47, 71.86)}$ =11.36, p<.0001 with Greenhouse-Geisser Correction; Figure 2A). Follow-up paired t-tests revealed that SCRs to the CS+ were higher than SCRs to CS- during conditioning (acquisition-early, $t_{(46)}$= 3.15, p=.003; acquisition-late, $t_{(46)}$= 3.41, p=.001; reversal-early, $t_{(46)}$= 2.46, p=.018; reversal-late, $t_{(46)}$= 2.97, p=.005). Moreover, conditioned SCRs decreased significantly at reversal and extinction when cues stopped to be paired with shocks (cue 1 acquisition-late vs. reversal-early: $t_{(46)}$= 3.07, p=.004; cue 2 reversal-late vs. extinction-early: $t_{(46)}$= 3.55, p=.001). SCRs were also significantly higher for cue 1 than cue 2 during early-extinction ($t_{(46)}$= 3.42, p=.001), suggesting that participants may have expected another reversal at the onset of the extinction phase.

The conventional demonstration of the conditioned fear-responses shown in Figure 2A was further expanded to a trial-by-trial analysis, allowing for the estimation of EShock and Associability, the two key parameters of the hybrid learning-model. Estimates of each parameter were optimized using computational modeling. The global pattern of

CS+/CS- discriminative learning was clearly captured by the model in the acquisition and reversal phases as shown by the time-course of the predicted SCR (Figure 2B). Learning parameters were then extracted from the optimized model for each trial and each subject according to the individual time-series of CS+/CS- and US (see group averages in Figure 2C-D). EShock and Associability for the reinforced trials (i.e. paired CS+), reproduced in Fig.3A, provided learning-related predictors of responses to the noxious electrical stimulation.

Effects of conditioning on responses to electric shocks

The time-course of mean shock-evoked pain and NFR responses displayed in Figure 3B did not reveal a global pattern of modulation across the early vs late parts of the acquisition and reversal phases using a conventional analysis based on trial and group averaging (ANOVA, p's > .05). However, both responses appeared to be consistently lowest on the first trials of the acquisition and reversal phases, as compared to their immediate neighboring trials (first vs. second acquisition trial: $F_{(1, 46)} = 6.72$, p=.013; last acquisition trial vs. first reversal trial: $F_{(1, 46)} = 12.97$, p=.001; and first vs. second reversal trial: $F_{(1, 46)} = 18.74$, p<.001). Not surprisingly, computational modeling also indicates that these key learning trials show very large shifts in EShock Probability and Associability (Figure 3A). Notably, these shifts are visible in the group averages because learning starts or contingencies change consistently in all subjects in those specific trials. This implies that trial and group averaging may mask dynamic effects and that the individual pattern of trial-by-trial fluctuations in pain responses may relate to immediate adjustments in the ongoing learning processes.

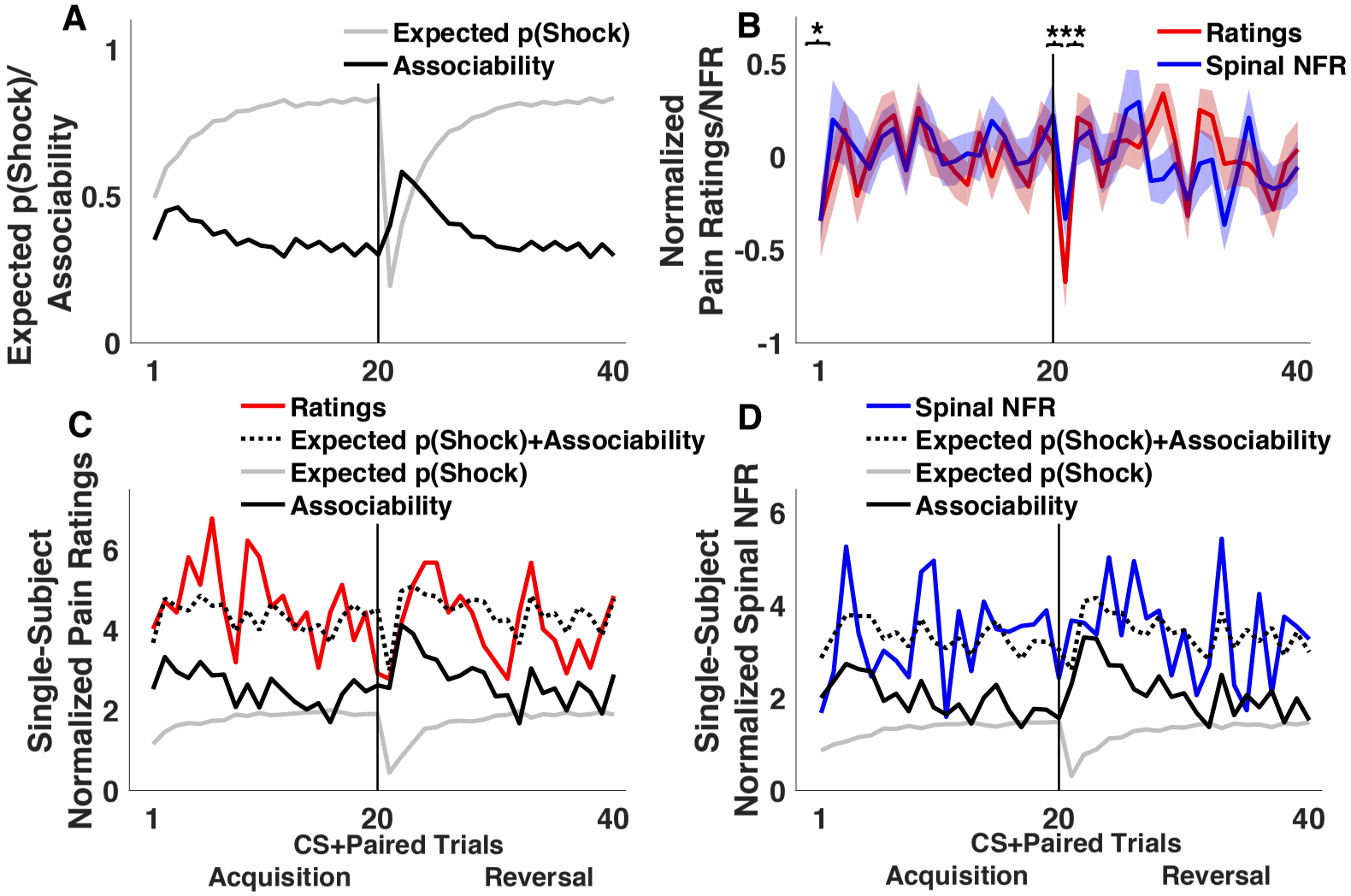
A
Expected p(Shock)
Associability
Expected p(Shock)/
Associability
1
0.5
0
1
20
40
B
*
***
Ratings
Spinal NFR
Normalized
Pain Ratings/NFR
0.5
0
-0.5
-1
1
20
40
C
Ratings
Expected p(Shock)+Associability
Expected p(Shock)
Associability
Single-Subject
Normalized Pain Ratings
6
4
2
0
1
20
40
CS+Paired Trials
Acquisition
Reversal
D
Spinal NFR
Expected p(Shock)+Associability
Expected p(Shock)
Associability
Single-Subject
Normalized Spinal NFR
6
4
2
0
1
20
40
CS+Paired Trials
Acquisition
Reversal

Figure 3. Relationship between expected shock probabilities (expected p(shock)), associability, pain ratings and nociceptive flexion reflexes (NFR) for reinforced (CS+paired) trials. A) Average associability and expected p(shock)) estimates. B) Average pain ratings and NFR amplitudes (lines), with shaded areas representing standard errors of the mean. C and D) Relationship between pain ratings, NFR amplitudes and associability/expected p(shock)) estimates for two individual subjects. Trial-by-trial associability and expected p(shock) estimates were weighted by their regression coefficients in order to illustrate the multi-level regressions effects reported in Table 3. For parcimony, the intercepts of the regression models predicting pain ratings and NFRs from expected p(shock) and associability estimates (see Table 3) were removed from observed pain ratings and NFRs. Pain responses were consistently lowest on the first trials of the acquisition and reversal phases, as compared to their immediate neighboring trials (first vs. second acquisition trial; last acquisition trial vs first reversal trial; first reversal trial vs second reversal trial). ***$p<0.001$, *$p<0.05$

The effects of EShock and Associability on pain responses were examined using multilevel regression analyses in which fear learning parameters at each trial were entered at the first level and subjects at the second level. Specifically, we predicted self-reported pain and NFR scores at each trial from EShock and Associability to shock-predicting cue (CS+paired) using multi-level regressions as implemented in Hierarchical Linear Modelling (HLM) software. Results are shown in Table 3 and confirmed that both EShock (*Beta* = 0.68, $t = 4.16$, $SE = 0.16$, $R^2 = 0.28$, $p < .001$) and Associability (*Beta* = 1.18, $t = 4.23$, $SE = 0.28$, $R^2 = 0.29$, $p < .001$) positively predicted pain ratings and NFRs (*Beta* = 0.82, $t = 4.52$, $SE = 0.18$, $R^2 = 0.32$, $p < .001$; *Beta* = 1.60, $t = 4.75$, $SE = 0.34$, $R^2 = 0.34$, $p < .001$ for effects of EShock and Associability respectively). As can be observed in Figure 3, the combined contribution of EShock and Associability derived from the learning model allows making a prediction that explains a significant amount of the trial-by-trial variance in pain and the NFR (Figures 3C and D, respectively).

Table 3 Multi-level regression analysis on pain ratings and NFR scores predicted by fear learning parameters

	Beta	*SE*	*t*	R^2	*p*
Dependent Variable: Pain Ratings to US					
LEVEL-1 Predictors					
				-	<.001
Intercept	-1.09	0.24	-4.66		***
Expected Shock(US)				0.28	<.001
Probabilities	0.68	0.16	4.16		***
				0.29	<.001
Associability	1.18	0.28	4.23		***
Dependent Variable: NFR scores to US					
LEVEL-1 Predictors					
				-	<.001
Intercept	-1.19	0.21	-5.64		***
Expected Shock(US)				0.32	<.001
Probabilities	0.82	0.18	4.52		***
				0.34	<.001
Associability	1.60	0.34	4.75		***

Notes. Significant effects of predictors are indicated on the graph with asterisks (***p<.001)

Moreover, while EShock was relatively low in the first few trials of the acquisition and reversal phases, associability rapidly peaked after the surprising first cue-shock pairings of both phases. The combined influence of EShock and associability therefore paints a very dynamic and complex portrait of learning effects on pain. Indeed, pain appears to be increased when shocks are either expected with a high probability or when uncertainty is high. In contrast, participants experienced less pain when they were most certain that they would not receive an electric shock; i.e. at the first reinforced trials of the reversal phases (see dip at reversal in Figure 3B). Average effects of expected p(shock) (A) and Associability (B) on pain ratings and NFRs are illustrated in Figure 4.

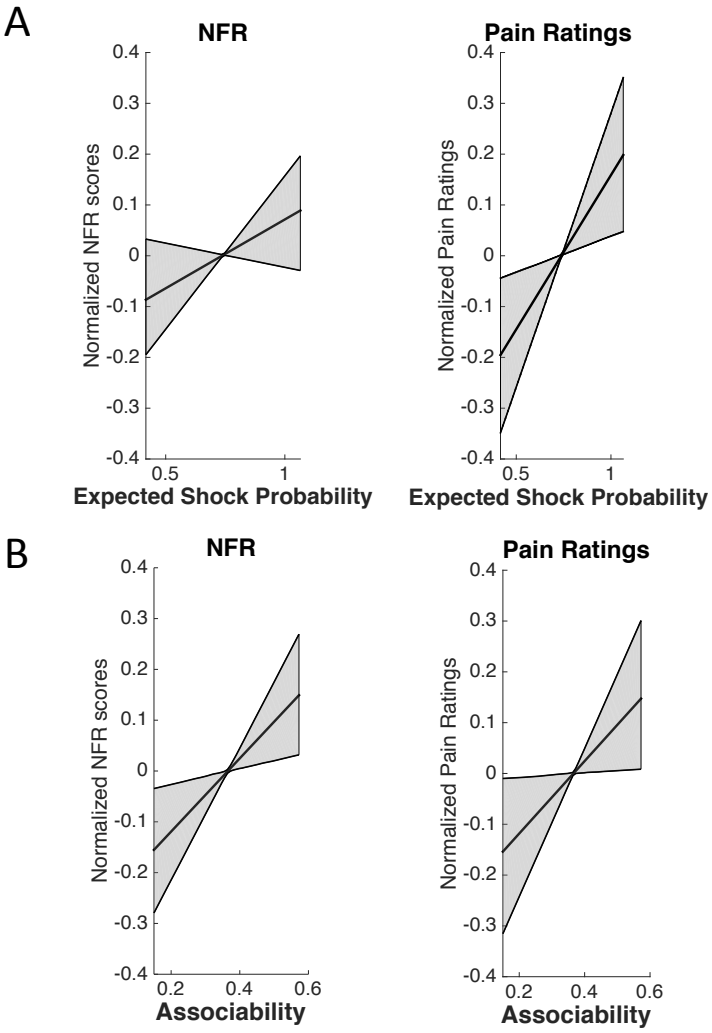

Figure 4. Average effect of expected p(shock) (A) and Associability (B) on pain ratings and NFRs. The shaded gray area shows the 95% confidence interval for the regression slopes. Variability in the intercept values across participants has been removed for display purposes. ***p<0.001, *p<0.05

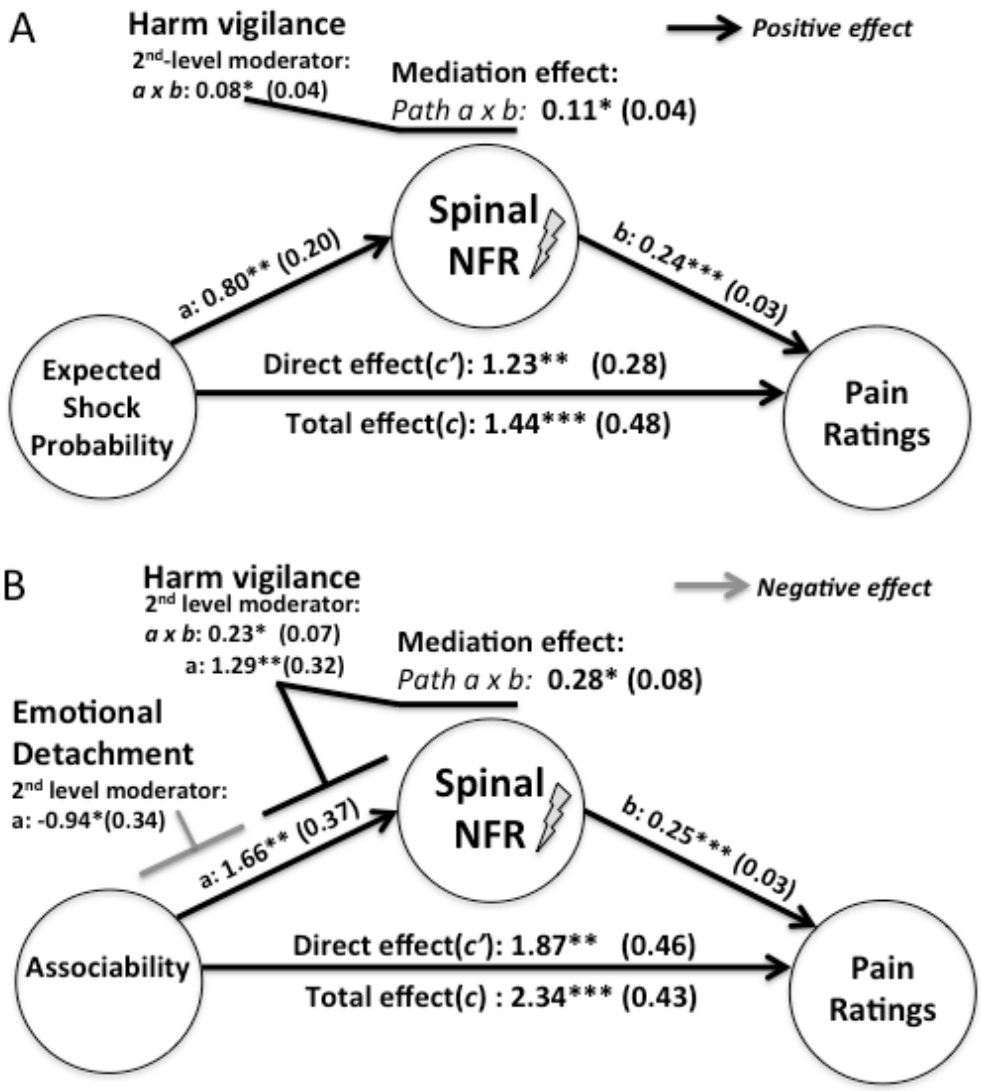

Figure 5. Multi-level mediation models of the effects of expected probability of shock (expected p(shock)) and associability on pain ratings. Path coefficients are shown for each path (*a*, *b*, *c*, *c'*) and mediation effects (*a x b*) with standard errors in parentheses. A) NFRs partially mediated the effect of expected probability of shock (expected p(shock)) on pain ratings. Harm vigilance increased NFRs mediating effects (mediation term a x b). B) NFRs partially mediated the effect of associability on pain ratings. Harm vigilance increased the effects of associability on NFRs (path *a*), as well as NFRs mediating effects

(mediation term *a x b*). Emotional detachment decreased the effects of associability on NFRs (path *a*). Average effect of expected p(shock)/Associability on pain ratings and NFRs are shown in Figure 4. ***p<0.001, *p<0.05

Learning processes affected both pain perception and the spinal nociceptive response and the possible relation between those modulatory effects was further assessed in multi-level mediation analyses. Given that the modulation of pain perception is often assumed to reflect at least in part the involvement of cerebro-spinal mechanisms affecting spinal nociception (Tracey & Mantyh, 2007), we tested the hypothesis that pain modulation by learning variables (EShock or associability) was mediated by the corresponding changes in the NFR (implemented with custom code written in Matlab, http://wagerlab.colorado.edu/tools, see Figure 5). Moreover, in order to account for the significant negative relationship between EShock and associability (*Beta* = -1.05, *SE* = 0.02, *t* = -61.72, $p < .001$), each variable was regressed onto the other and the residuals (i.e. EShock controlling for associability and vice-versa) were entered as predictors in the two mediation models tested. Results showed that the NFR was a significant mediator of the effects of both learning variables on pain ratings ($c = 1.44$, $SE = 0.28$, $p < .001$, $ab = 0.11$, $SE = 0.04$, $p = .012$, for the effect of EShock on ratings and its mediation by NFRs; $c = 2.34$, $SE = 0.43$, $p < .001$, $ab = 0.28$, $SE = 0.08$, $p = .001$ for the effect of associability on ratings and its mediation by NFRs). However, the *c* path remained significant after accounting for the NFR mediation (*c'* in Figure 5) indicating that effects of both learning processes on pain perception could be explained in part, but not entirely, by the descending modulation of spinal nociception.

Finally, in order to examine the influence of personality traits on learning-induced pain modulation, we first performed a PCA on scores of several psychological questionnaires (listed in Table 1). This allowed reducing the dimensionality of the data to three

personality components: harm vigilance, emotional detachment, and acceptance (see methods). These three variables were then tested as second-level moderators in our mediation models. Results showed that harm vigilance significantly increased 1 - the effects of associability on NFRs (path *a*, Figure 5), as well as 2 - the NFR mediation between associability and pain ratings (path *ab*, Figure 5), and 3 - the NFR mediation between EShock and pain ratings (path *ab*, Figure 5). Moreover, emotional detachment also decreased the strength of the relation between associability and NFRs (path *a*, Figure 5). None of the personality components were significantly correlated with the fixed parameters of the learning model (all $ps > 0.05$) suggesting that the effects of personality factors on pain processing could not be explained simply by underlying inter-individual differences in associative learning. This indicates that personality traits influence how learning affects pain processing.

Discussion

Pain plays an important role in teaching us about potential sources of harm in our environment. Pain-evoking stimuli further trigger associative learning mechanisms that constantly refine our predictions about what is most likely to cause us pain. Here, we employed computational modeling to demonstrate that associative learning produces transient states of conditioned hyperalgesia that are paradoxically induced by both pain predictability and uncertainty. Indeed, the only moment when participants did not appear to suffer from hyperalgesic effects is when they were the most certain that they would not receive a painful electric shock. Effect sizes were large (Tabachnick & Fidell, 2007), suggesting that the influence of learning on pain processing is considerable and may potentially have an important impact on pain perception in our day-to-day lives.

When only considering the averaged effects of trial number on pain processing, the hyperalgesic effects of conditioning could only be observed as the difference between the relatively low pain ratings and NFRs in response to the first shocks of the acquisition and reversal phases of the experiment, and the higher pain indexes observed throughout the rest of the experiment. However, results from computational modeling revealed that the apparent lack of learning effects after the first cue-pain pairings was in fact caused by opposing effects of expected pain (EShock) - which steadily rises as participants are exposed to repeated cue-pain associations - and associability - which tends to decrease as predictions become more accurate. Because these two parameters were estimated by fitting the learning models to anticipatory SCRs, and not to unconditioned responses to electric shocks, the opponency between EShock and associability effects cannot be due to

over-fitting of the learning model, and therefore likely reflects the workings of learning mechanisms that are affecting both anticipatory SCRs and responses to electric shocks. For the same reason, the strong and significant relationship between our computational estimates of associability/EShock and both measures of pain processing provide a convincing additional validation of the selected hybrid learning model.

According to reinforcement learning theories, EShock and associability reflect qualitatively different learning processes. Expected probability of shock simply refers here to the subjective probability of receiving an electric shock and therefore broadly reflects the learning process that is generally implied in most fear conditioning studies (Jensen et al., 2015). However, in contrast with more traditional analyses splitting acquisition and reversal phases in early and late phases (see Figure 2A), EShock is estimated on a trial-by-trial basis. Thus, rather than considering the overall reinforcement rate (e.g. 50%) across blocks of pseudo-random trials, modern implementations of learning models consider the effective and unique sequence of reinforcement experienced by the subject on a trial-by-trial basis. Computational modeling therefore provides EShock estimates that are better tailored to the unique sequence of reinforcement that is experienced by each subject.

In contrast with EShock, the associability term used in our learning model reflects the informational value of the outcome with respect to reinforcement contingencies. Associability is therefore expected to go down as predictions become more and more accurate (i.e. reduced prediction error), and to rise when participants have recently been surprised by an unexpected outcome (i.e. on trials that follow a large prediction error).

Indeed, after having been surprised, attention towards the outcome of the following trial is increased because it may confirm or disconfirm a potential change in contingencies. Recent brain imaging studies have reported that different brain regions may encode EShock (ventral striatum) and associability (amygdala and basolateral amygdala) (Boll et al., 2013; Li, Schiller, et al., 2011) during the presentation of the outcome during aversive conditioning. Our data suggest that the output of these two systems may ultimately converge onto a single effector system responsible for allocating attentional resources to the processing of the unconditioned stimulus (US).

Finally, the present study demonstrated that learning effects on pain were partly mediated by spinal nociceptive processes, indicating that conditioned hyperalgesia at least partly relies on descending cerebro-spinal modulatory pathways that gate the transmission of ascending nociceptive signals at the spinal level. Still, a significant part of learning effects on pain was not mediated by spinal nociceptive processes, and could therefore reflect higher-order (i.e. supra-spinal) processes affecting pain perception as a function of its predictability. Interestingly, inter-individual differences in harm vigilance and emotional detachment specifically affected the portion of learning effects that was mediated by spinal nociceptive processes. Indeed, participants that were more harm vigilant and/or less emotionally detached displayed stronger spinal facilitation, which in turn contributed more to the hyperalgesic effects observed in pain perception. Thus, the parsing of learning effects into EShock/associability and spinally mediated/unmediated effects allowed us to reveal the precise mechanisms by which predisposing personality traits may influence conditioned hyperalgesia.

By contrast, the facilitatory effects of associability on pain responses was *reduced* in individuals with elevated dispositions to adopt detached and non-reactive attitudes towards their inner and emotional experiences. However, here the moderating effect was only found on the NFR, suggesting that in the context of learning, emotional detachment may reduce the reactivity to the noxious stimulus without having indirect consequences on pain perception. Previous studies have suggested that trait mindfulness is inversely related to pain catastrophizing in a chronic pain patient sample and that it moderates the relationship between catastrophizing and reported pain intensity (Schutze et al., 2010). The present findings should motivate further investigation of the impact of emotional regulation training on aversive learning processes to unravel potential benefits in preventing learning-induced pain facilitation (Crombez et al., 2012).

It is important to highlight that, although it would also have been possible to directly model *pain outcomes* using computational modeling, this approach would address a different question and viewpoint as to that of modeling anticipatory SCRs and using model estimates to predict pain outcomes. In the latter approach, model estimates are derived from anticipatory processes to the occurrence/absence of pain, and are then used to predict pain outcomes. Variation in pain outcomes therefore reflects dynamics underlying anticipation of the occurrence of pain. The former approach would constitute a different question, i.e. to examine whether expectations and prediction signals would adequately fit pain outcomes using a specific model over another. Examining this question however, would not take into account the entire reinforcement learning history at all trials, or would have limited data to do so given that signals would only be fitted to

pain-predicting cues. This question is qualitatively different as examining the relationship between dynamic processes underlying learned anticipatory SCRs and pain outcomes, but would nonetheless provide invaluable information in understanding dynamic pain modulation during learning and should be addressed in future studies.

In conclusion, the present study is the first to demonstrate that pain perception is under the constant influence of learning processes that dynamically control the sensory gating of painful stimuli as a function of each individual's unique reinforcement history. This suggests that when an individual is submitted to repeated episodes of pain, a significant proportion of the pain perceived may become rapidly facilitated by learning and attentional factors. A better understanding of the psychological and neural mechanisms underlying learning effects on pain could therefore provide important insights into the sequence of psychological and neural events that lead to pain chronicity, and hopefully indicate novel ways of breaking the vicious circle by which expected and/or uncertain pain causes more pain.

Acknowledgements

This project was supported by a grant awarded to Dr. Pierre Rainville from the *Canadian Institutes of Health Research* (CIHR) destined to investigate the neural and psychophysiological processes underlying normal and dysfunctional pain modulation, and a doctoral scholarship from the Natural Science and Engineering Research Council of Canada and a Mind and Life Varela Award (to V.A.T). We thank Dr Ali Khatibi and Mathieu Laramée for their help with data acquisition. The authors have no financial or other relationships that might lead to a conflict of interest.

References

Bach, D. R., Flandin, G., Friston, K. J., & Dolan, R. J. (2010). Modelling event-related skin conductance responses. *Int J Psychophysiol, 75*(3), 349-356.

Baer, R. A., Smith, G. T., Hopkins, J., Krietemeyer, J., & Toney, L. (2006). Using self-report assessment methods to explore facets of mindfulness. *Assessment, 13*(1), 27-45.

Baliki, M. N., Geha, P. Y., Fields, H. L., & Apkarian, A. V. (2010). Predicting value of pain and analgesia: nucleus accumbens response to noxious stimuli changes in the presence of chronic pain. *Neuron, 66*(1), 149-160.

Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A. R. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science, 269*(5227), 1115-1118.

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., & Erbaugh, J. (1961). An inventory for measuring depression. *Arch Gen Psychiatry, 4*, 561-571.

Boll, S., Gamer, M., Gluth, S., Finsterbusch, J., & Buchel, C. (2013). Separate amygdala subregions signal surprise and predictiveness during associative fear learning in humans. *Eur J Neurosci, 37*(5), 758-767.

Borsook, D., Maleki, N., Becerra, L., & McEwen, B. (2012). Understanding migraine through the lens of maladaptive stress responses: a model disease of allostatic load. *Neuron, 73*(2), 219-234.

Brown, K. W., & Ryan, R. M. (2003). The benefits of being present: mindfulness and its role in psychological well-being. *J Pers Soc Psychol, 84*(4), 822-848.

Carver, C. S., & White, T. L. (1994). Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: The BIS/BAS Scales. *Journal of Personality and Social Psychology, 67*, 319-333.

Cloninger, C. R. (1994). Temperament and personality. *Curr Opin Neurobiol, 4*(2), 266-273.

Crombez, G., Eccleston, C., Van Damme, S., Vlaeyen, J. W., & Karoly, P. (2012). Fear-avoidance model of chronic pain: the next generation. *Clin J Pain, 28*(6), 475-483.

Daw, N. D. (2011). Trial-by-trial data analysis using computational models. In M. R. Delgado, E. A. Phelps, & T. W. Robbins (Eds.), *Decision Making, Affect, and Learning*

Attention & Performance XXIII (pp. 3-38). New York: Oxford University Press.

Flor, H., & Turk, D. (1999). Cognitive and Learning Aspects. In P. D. Wall & R. Melzack (Eds.), *Textbook of Pain* (pp. 256-272). Edinburgh: Churchill Livingstone.

Grant, J. A., & Rainville, P. (2009). Pain sensitivity and analgesic effects of mindful states in Zen meditators: a cross-sectional study. *Psychosom Med, 71*(1), 106-114.

Jensen, K., Kirsch, I., Odmalm, S., Kaptchuk, T. J., & Ingvar, M. (2015). Classical conditioning of analgesic and hyperalgesic pain responses without conscious awareness. *Proc Natl Acad Sci U S A, 112*(25), 7863-7867.

Kabat-Zinn, J., Lipworth, L., & Burney, R. (1985). The clinical use of mindfulness meditation for the self-regulation of chronic pain. *J Behav Med, 8*(2), 163-190.

LaBar, K. S., LeDoux, J. E., Spencer, D. D., & Phelps, E. A. (1995). Impaired fear conditioning following unilateral temporal lobectomy in humans. *J Neurosci, 15*(10), 6846-6855.

LePelley, M. E., & McLaren, I. P. (2004). Associative history affects the associative change undergone by both presented and absent cues in human causal learning. *J Exp Psychol Anim Behav Process, 30*(1), 67-73.

Li, J., Schiller, D., Schoenbaum, G., Phelps, E. A., & Daw, N. D. (2011). Differential roles of human striatum and amygdala in associative learning. *Nat Neurosci, 14*(10), 1250-1252.

Montgomery, G. H., & Kirsch, I. (1997). Classical conditioning and the placebo effect. *Pain, 72*(1-2), 107-113.

Mumford, J. A., Turner, B. O., Ashby, F. G., & Poldrack, R. A. (2012). Deconvolving BOLD activation in event-related designs for multivoxel pattern classification analyses. *Neuroimage, 59*(3), 2636-2643.

Rhudy, J. L., Williams, A. E., McCabe, K. M., Rambo, P. L., & Russell, J. L. (2006). Emotional modulation of spinal nociception and pain: the impact of predictable noxious stimulation. *Pain, 126*(1-3), 221-233.

Roy, M., Piche, M., Chen, J. I., Peretz, I., & Rainville, P. (2009). Cerebral and spinal modulation of pain by emotions. *Proc Natl Acad Sci U S A, 106*(49), 20900-20905.

Sandrini, G., Serrao, M., Rossi, P., Romaniello, A., Cruccu, G., & Willer, J. C. (2005). The lower limb flexion reflex in humans. *Prog Neurobiol, 77*(6), 353-395.

Schiller, D., Levy, I., Niv, Y., LeDoux, J. E., & Phelps, E. A. (2008). From fear to safety and back: reversal of fear in the human brain. *J Neurosci, 28*(45), 11517-11525.

Schutze, R., Rees, C., Preece, M., & Schutze, M. (2010). Low mindfulness predicts pain catastrophizing in a fear-avoidance model of chronic pain. *Pain, 148*(1), 120-127.

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Sullivan, M. J. L., Bishop, S. R., & Pivik, J. (1995). The Pain Catastrophizing Scale: Development and Validation. *Psychological assessment, 7*(4), 524-532.

Tabachnick, B. G., & Fidell, L. S. (2007). *Using Multivariate Statistics, 6th Edition*. Boston: Pearson.

Tracey, I., & Mantyh, P. W. (2007). The cerebral signature for pain perception and its modulation. *Neuron, 55*(3), 377-391.

Willer, J. C. (1977). Comparative study of perceived pain and nociceptive flexion reflex in man. *Pain, 3*(1), 69-80.

Zeidan, F., Gordon, N. S., Merchant, J., & Goolkasian, P. (2010). The effects of brief mindfulness meditation training on experimentally induced pain. *J Pain, 11*(3), 199-209.

Article2: Impact of Meditation Experience on Fear-Conditioned Pain Modulation

Taylor, V., Roy, M., Gill, L.-N., Mueller, C., Rainville, P. Impact of Meditation Experience on Fear-Conditioned Pain Modulation. Submitted to Psychosomatic Medicine.

Contribution of authors: VT, MR and PR designed the study; VT recruited experienced meditators and acquired the data with the help of L.-N. Gill and C. Mueller; VT and MR analyzed the data; VT drafted the manuscript and all authors revised and approved the article.

Abstract

Mindfulness-based practice is a form of cognitive/affective training that may help reduce suffering by attenuating maladaptive anticipatory processes. The hyperalgesic effects of pain expectation and uncertainty was assessed outside formal meditation in 11 experienced meditators (>1000h) compared to meditation-naïve controls during a fear-conditioning paradigm involving two visual stimuli (CS+/CS-), one of which (CS+) co-terminated with a noxious electrical stimulus (US) on 50% of trials. A Rescorla-Wagner/Pearce-Hall hybrid model was fitted onto the conditioned skin conductance responses (SCRs) using computational modelling to estimate two learning parameters – expected shock probability and associability (i.e. uncertainty). Meditators reported less pain but had comparable spinal motor responses (nociceptive flexion reflex; NFR) to the US. Importantly, meditators also displayed comparable discriminative SCRs to the CS+ vs CS-. However, multilevel mediation analyses revealed that meditators exhibited reduced hyperalgesic effects of fear learning on higher-order pain responses but comparable effects on the NFR. These results suggest that mindfulness affects higher-order perceptual processes independent from descending inhibitory controls. Furthermore, mindfulness reduced hyperalgesic effects of fear-conditioning without affecting conditioned learning as evidenced by normal discriminative anticipatory responses. These results provide evidence that mindfulness may help reduce the maladaptive reinforcing cycle between fear and pain reported in some chronic pain patients.

Keywords: mindfulness meditation, pain, nociceptive flexion reflex, fear conditioning

Introduction

Cultivated through the practice of meditation, mindfulness has gained worldwide scientific interest for its accessibility and proven effectiveness in attenuating symptoms of pathologies related to chronic pain as well as affect, anxiety and stress (Baer, 2003; Brown & Ryan, 2003; Kabat-Zinn et al., 1985; Keng et al., 2011; Morone et al., 2008). This state of awareness involves intentionally paying attention to the present-moment and monitoring mental/physical events in a detached and accepting manner (Bodhi, 2005). Findings from neuroimaging studies suggest that the hypoalgesic influence of the cognitive/affective training of mindfulness meditation is particular because it selectively affects higher-order brain centers linked to cognitive/affective elaboration of pain and not primary sensory aspects of nociceptive pain (Gard et al., 2012; Grant et al., 2011). More specifically, one of the premises underlying mindfulness is that relief from suffering occurs by detaching oneself from past events and future scenarios (Bodhi, 2005), which is accurately in line with the anticipation-mediated hypoalgesic effects of meditation (Brown & Jones, 2010; Gard et al., 2012; Lutz et al., 2013).

Indeed, the mechanisms of action of the pain-attenuating effects of mindfulness has been suggested to operate by reducing neural activity during pain anticipation (Brown & Jones, 2010; Lutz et al., 2013). Electrophysiological and functional imaging studies using MRI have reported changes in anticipatory brain activity that may contribute to the reduced pain sensitivity of experienced meditators (Lutz et al., 2013). It remains unclear, however, whether meditation-related pain modulation is due to diminished anticipatory

processes per se or reduced impact of anticipation on pain. In addition, due to a lack of low-level (spinal) assessments of nociceptive transmission in previous studies examining pain modulation by meditation, it remains unknown whether anticipation-mediated or general effects of mindfulness on pain target uniquely higher-order perceptual centers, descending inhibitory control mechanisms, or both.

In a previous independent report, we showed that learned expectations and uncertainty during the anticipation of pain facilitate pain during classical conditioning (Taylor, Chang, Rainville, & Roy, In Press). We demonstrated that pain facilitation during fear conditioning can be explained by specific parameters (expectations and uncertainty) estimated from computational modeling of anticipatory responses (skin conductance responses; SCRs) to fear conditioned cues (CS+). Computational models of reinforcement learning are thought to best depict trial-by-trial variations in anticipatory behavior as a function of predictions, or expectations, formed about the occurrence of pain, as well as the associability of conditioned cues (LePelley & McLaren, 2004). The latter factor, 'associability', is highest when predictions are unreliable, i.e. when the absolute magnitude of prediction errors experienced in previous trials is elevated. In other words, there is more to learn when contingencies are uncertain; therefore, associability is thought to reflect enhanced attention allocation to cues most informative about uncertain environmental contingencies (LePelley & McLaren, 2004). We showed that both learned expectations and uncertainty (associability) positively predicted trial-by-trial fluctuations in pain perception and spinal nociceptive flexion reflex (NFR) responses to noxious electrical US (Taylor et al., In Press). In addition, we showed that resilient attitudes

towards fear/pain (i.e. such as low pain catastrophizing and elevated dispositional mindfulness) attenuated the hyperalgesic impact of adaptive aversive learning processes on pain.

Here, we examined the impact of extensive mindfulness meditation experience on the dynamic (trial-by-trial) pain modulating effects of fear learning at the spinal and supraspinal levels of pain processing. The main objective of the present study was to determine whether effects of mindfulness meditation practice are due to an absence of learned anticipatory responses or an absence of impact of pain anticipation on pain-evoked responses. The secondary aim of this study was to investigate whether general hypoalgesic effects of mindfulness meditation occur selectively by targeting pain responses at a higher order level of processing (i.e. perceptual), by influencing descending inhibitory control systems, or both.

Given evidence that mindfulness meditation training does not eliminate fear conditioned anticipatory SCRs (Holzel et al., 2016) or emotional amygdala responses to negative affective pictures (Taylor et al., 2011), we hypothesized that mindfulness meditation experience would not affect anticipatory SCRs to conditioned cues but would reduce the impact of fear learning parameters (EShock and associability) on pain (Taylor et al., In Press). Finally, since mindfulness meditation practice is associated with reduced brain function in regions related to cognitive/affective elaboration of pain and enhanced activation of regions related to sensory aspects of pain (Gard et al., 2012; Grant et al., 2011), we hypothesized that mindfulness meditation training would reduce the

hyperalgesic impact of fear learning only at a higher-order perceptual level of nociceptive processing (Grant & Rainville, 2009).

Methods

Participants

Meditators were recruited from Zen and Boddhicita meditation centers in Montreal. The group consisted of 11 experienced meditators with a minimum of 1000 hours of practice involving the cultivation of mindfulness (ranging between 1050 and 9500 hours; 7 male, 4 females, aged between 28 and 68 years). Eight meditators practiced traditional Zen meditation, which involves open-monitoring meditation (Lutz, Slagter, Dunne, & Davidson, 2008) practiced by concentrating on the breath and monitoring thoughts/feelings/sensations without attempting to follow or grasp them. Two other meditators practiced meditation from the 'Boddhicita' tradition, and one from the 'Kadampa' tradition, which also involve open-monitoring meditation performed by concentrating on mantras and the breath, and monitoring thoughts/feelings/sensations in a detached manner. The control group (N = 51, 24 males, 27 females, aged between 19 and 61 years) had no prior meditation training/experience and was recruited from advertisements in local University settings (Université de Montréal, McGill and Concordia Universities). The control group consisted of 47 participant described in a previous report to which we added four participants matching the age of the older meditators. Due to participant number inequality between groups, all of the analyses described in the present report were also conducted by comparing the group of experienced meditators with a subsample of 11 subjects taken from the large control group and selected to match the meditators for age, sex and number of years of education. The present study used the same methods and experimental protocol developed and

described in detail in our recent study. Here, we reproduce the description of the main aspects of the experimental and analytical procedures and refer the reader to this previous report for additional details.

Potential participants were considered eligible to take part in the study upon meeting the following criteria: no pregnancy, no psychological/psychiatric condition (such as major depressive disorder and substance abuse), no medication intake (except for oral contraceptives), no pain-related diseases (such as chronic pain or neuropathic pain), and no regular use of anti-inflammatory or analgesic medications. Potential participants were invited to visit the Laboratory of the Neuropsychophysiology of Pain (UdeM, Canada) for a screening and familiarization session to assess their pain thresholds and physiological responsivity (skin conductance and NFR) and for a second visit to complete the experimental paradigm. Twelve participants (nine meditation-naïve controls and three experienced meditators) were not retained following the familiarization session for one of the following reasons: extremely low/high pain thresholds, excessive use of alcohol, drugs, or analgesic medication, discomfort with the nature of the noxious stimuli (electrical stimulations) or oversensitivity of skin at the site of electrical stimulation, or absent/unstable skin conductance or NFRs to the painful stimuli. Sixty-five participants participated in the experimental session, but three meditation-naïve control subjects were excluded from data analysis due to poor electrodermal signal or very inconsistent NFRs.

Stimuli

The conditioned stimuli (cue1 and cue2) consisted of visual stimuli, i.e. colored fractal

images (randomly colored and patterned circles). Cues were presented in the center of a black screen for a duration of 2 s on a computer monitor with E-Prime2 Professional (Psychology Software Tools, Sharpsburg, PA). Transcutaneous electrical stimulation were used as unconditioned stimuli (US), and each consisted of a train of ten 1-millisecond pulses (at 333 Hz). US were administered using an isolated DS7A constant current stimulator (Digitimer Ltd, Welwyn Garden City, United Kingdom) and were triggered by a train generator (Grass Medical Instruments, Quincy, MA). US administration was controlled by the computer delivering the visual CSs.

The US was administered via two stimulation electrodes placed on the participants' cleaned skin, at the level of the right sural nerve's retromalleolar path. The thresholding procedure for the was assessed as previously described, using the staircase thresholding method (Willer, 1977). As in our previous work (Taylor et al., In Press), the stimulus intensity corresponding to 135% of the NFR threshold was used as the US intensity delivered during the fear conditioning paradigm. Ten stimulations were administered at this intensity before the conditioning experiment in order to ensure that the NFR was consistently elicited and to account for any habituation effects.

Measures and Dependent Variables

BIOPAC Systems Inc. and Acqknowledge data acquisition software (version 4.2) were used to acquire physiological measures. Pain ratings were recorded using E-Prime2.

Pain Ratings. A visual analog scale (VAS) was used to assess the pain level elicited by each US. Anchors were set as 0: no pain to 100: extremely painful. Shown on the computer screen following each US, this scale consisted of a graduated horizontal bar with a moving cursor (using response keys on a computer keyboard).

Electromyographic (EMG) Recording. Two pre-gelled electrodes on exfoliated and shaved (if necessary) skin on the right biceps femoris, and a ground electrode on the right tibial bone were used to record EMG activity. The EMG signal was sampled at 1000 Hz, was amplified 1000 times was filtered online (bandpass filtering: 100 -500 Hz). In addition, the EMG signal was transformed online to its root mean square (RMS) value over bins of 20 consecutive samples. Finally, the integral of the EMG RMS signal was computed offline over 90-180ms following shock administration, and consisted in the metric used as raw NFR scores.

Electrodermal Recording. Two electrodes placed on the on the left palm of the hand were used to record electrodermal activity. The electrodermal signal was amplified (5 μS/volt) and filtered online (bandpass filtering: 1-5 Hz). The signal was smoothed temporally offline using the mean value over a 500 ms moving window.

Self-report Questionnaires. The State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983) was used to assess trait and state anxiety. The Five Factor

Mindfulness Questionnaire was used to assess participants' dispositional levels of mindfulness (Baer et al., 2006). This questionnaire is composed of 39 items and five sub-scales, which assess different facets of trait mindfulness: 'Observe' (tendency to observe experiences), 'Describing' (aptitude of describing experiences), 'Acting with awareness', as well as acting with 'Non-reactivity' and 'Non-judgment' to experiences. The Mindful Attention Awareness Scale (MAAS) was also used to assess trait mindfulness dispositions, which is a 15-item scale "designed to assess a core characteristic of dispositional mindfulness, namely, open or receptive awareness of, and attention to, what is taking place in the present." (Brown & Ryan, 2003). The Pain Catastrophizing Scale (PCS) was administered (Sullivan et al., 1995), which is a 13-item questionnaire with three different subscales accessing different aspects of attitudes towards pain: pain magnification, pain rumination, and helplessness towards pain. The Beck Depression Inventory was used to assess depressive symptomatology (Beck et al., 1961), and the Behavioral Inhibition/Activation Scale was used to assess punishment sensitivity (Carver & White, 1994).

Finally, participants completed the Temperament and Character Inventory (Cloninger, 1994) which assesses different dimensions of personality from 7 subscales: Cooperativeness, Persistence, Reward Dependence, Novelty Seeking, harm avoidance, self-transcendence, self-directedness.

Testing procedure

Participants provided informed consent for their initial screening session and were prepared for electrophysiological recordings. They were then submitted to the NFR thresholding procedure and completed the self-report questionnaire battery.

Participants returned a few days following their first visit for a second session to complete the experiment. They were first prepared for electrophysiological recordings, and were submitted to the procedure for NFR thresholding to determine the intensity of electrocutaneous stimulation administered during the task. A block of 10 stimulations was administered as a baseline measure of pain processing at the determined intensity, and a CS habituation block was administered which consisted of 2 trials of each CS shown without any shock delivery.

The fear conditioning paradigm (Figure 1), which was adapted from previous work (Schiller et al., 2008), was then administered. Prior to completing the task and as in our previous work (Taylor et al., In Press), participants were instructed to observe and pay attention to stimuli that would appear on the screen. They were also told that they may or may not receive electrical stimulations (following which they would need to provide a pain rating). Thus, no explicit instructions with respect to the task structure (cue-shock contingencies or reversals) had been given.

The fear conditioning task included an acquisition phase (composed of 2 blocks), a reversal phase (composed of 2 blocks) in which cue-shock pairings were reversed (the cue previously assigned as the CS+ became the CS-, whereas the cue previously assigned as the CS- became the CS+) and an extinction phase (1 block) in which CSs were presented alone. In reversal and acquisition blocks, one cue co-terminated with the US for 50% of trials (CS+), while the other cue was presented alone (CS-). A random time interval of 4-8 sec followed each US, to allow the recording of a SCR to the US, following which participants provided their pain rating on the VAS (of a variable duration period until the participant had finished responding, approximately 10 s). The 9-12 sec inter-trial intervals were signaled with a white cross centered on a black background.

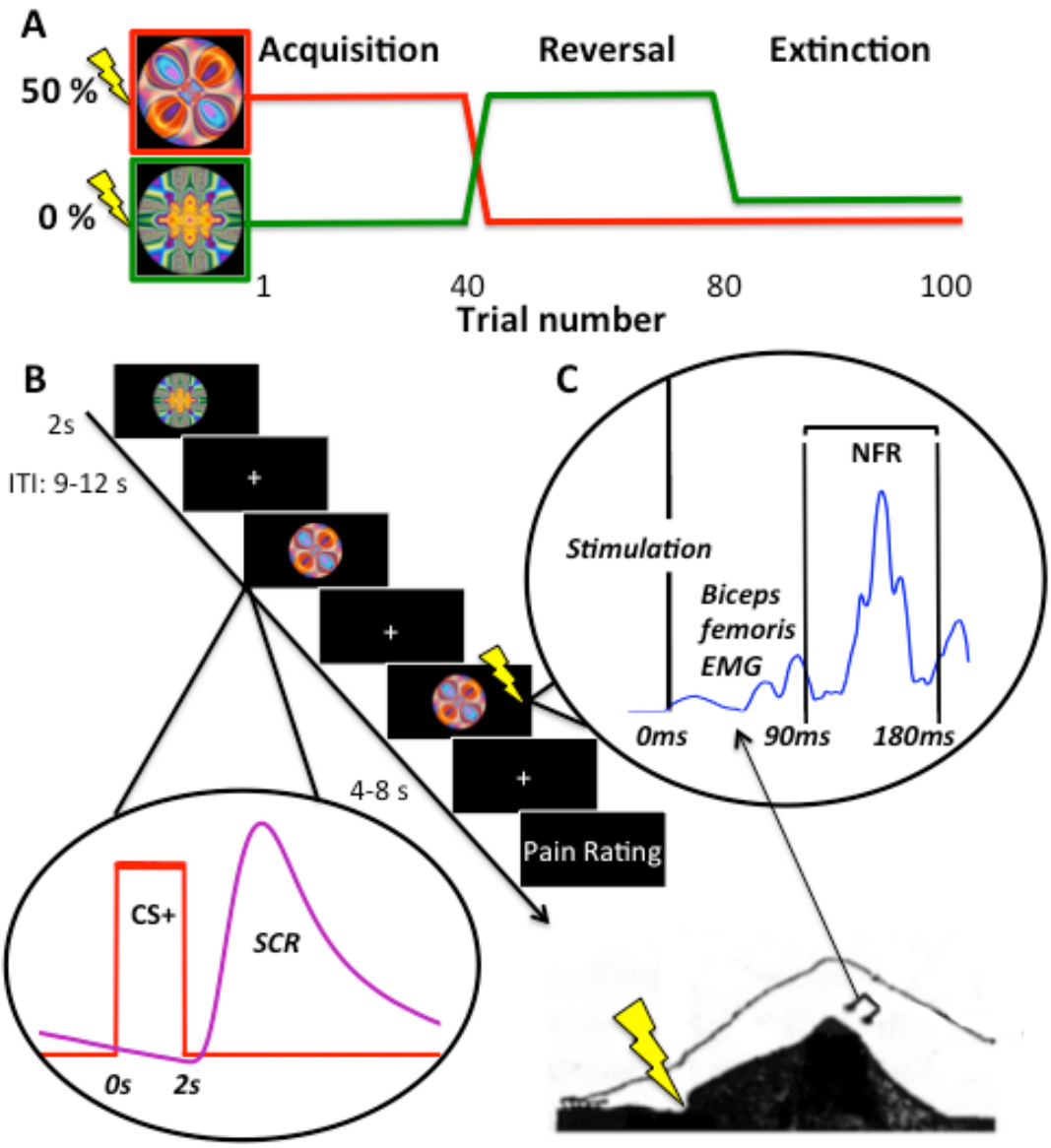

Figure 1. Experimental paradigm. A) In the acquisition phase (trials 1- 40), one cue had a 50% chance of co-terminating with an electric shock (CS+), while the other cue was never presented (0% chance) with the shock (CS-). In the reversal phase, cue-shock contingencies between the two cues were reversed: the cue previously assigned as the CS+ became the CS-, while the cue previously assigned as the CS- became the CS+. In the extinction phase, both cues had a 0% chance of being presented with the shock. B) An example of each type of trial (CS-, CS+, and CS+paired) is shown: each trial started with

the presentation of one of the two cues for a 2s duration. On trials involving shock administration ('CS+paired' trials), the cue co-terminated with an electric shock (30 ms) to the right sural nerve and participants rated their pain after a time interval (randomized between 4-8s). The next trial began following a random inter-trial interval (ITI) of 9-12s. No pain ratings were assessed for unreinforced trials (CS+unpaired and CS-), and skin conductance responses (SCR; with a typical latency between 0.5 and 2s) to visual cues were obtained from electrodermal activity recordings. C) The electromyographic (EMG) signal was recorded with electrodes at the level of the right biceps femoris (above the sural nerve stimulated) in order to assess the nociceptive flexion reflex (NFR), observable at a 90-180 ms latency following electrical stimulation onset.

Each acquisition and reversal block (about 13 minutes each) was composed of 40 trials: 20 CS-, 10 CS+ unpaired, 10 CS+ paired. Trials were pseudo-randomly presented across participants, but no more than 2 identical consecutive trial types were presented. To emphasize cue-shock contingencies at the onset of each block, each block's first trial always consisted of a paired CS+, and the second always consisted of a CS-. The cue assigned as the CS+ in the acquisition phase was counter-balanced across subjects. The extinction block (approximately 10 minutes) consisted of 40 trials (20 trials per cue) presented without any shocks. Lastly, a 10 electrical stimulation block (without any cue) was administered in order to ensure that habituation or sensitization of the NFR did not occur. All subjects showed clear NFR responses in this post-experimental block of US trials (this is not reported further).

Electrodes were removed at the end of experimental procedure. A post-experimental interview adapted from previous studies (50, 51) was conducted to evaluate participants' awareness of CS-US pairings, after which they were debriefed and compensated with the equivalent of 15$/hour.

Data Analyses

Sample Description

To examine characteristics of control and meditator participants, independent-samples t-tests were conducted on several demographic and personality trait variables: pain

catastrophizing subscales, TCI subscales, FFMQ subscales, trait and state anxiety, depressive symptoms, and punishment sensitivity (BIS). Pain and NFR threshold intensity were also examined using independent samples t-tests. Pain sensitivity and NFR activity at baseline (10 baseline trials at 135% of NFR threshold intensity) were also compared between groups using TRIAL x GROUP ANOVAs.

Effects of Meditation on Pain and the NFR

Effects of meditation group were examined on pain responses (pain ratings and NFRs) to the US during the fear conditioning task using a TRIAL x GROUP ANOVAs. One meditator subject was missing pain ratings for one block of the fear conditioning task; thus, his pain ratings were replaced with the group mean value at each trial of the task.

Analyses of Fear Conditioned Skin Conductance Responses

Using SCRalyze (Bach et al., 2010), the skin conductance response (SCR) was assessed to CS- and CS+. Since the painful shocks (US) also produced strong unconditioned SCRs overlapping with the response to the CS+ in reinforced trials (i.e. CS+ paired with US), the conditioned SCR was assessed using only the unreinforced CS+ trials (50%; see procedure below). Trial-by-trial SCR amplitude estimates (henceforth denoted as SCRs for the sake of clarity) were determined using a general-linear model-based approach using the same analytical software and procedure as in our previous independent report (Taylor, Chang, Rainville, & Roy, submitted). Finally, beta values for each unreinforced

trial (CS+ unpaired and CS-) were entered into multi-level regressions analyses (implemented with custom code written in Matlab, http://wagerlab.colorado.edu/tools), by entering CS-type as the first-level predictor, and meditation group as the second-level predictor, to test potential group differences in conditioned responses.

Computational Modeling of Fear-Conditioning

After confirming the global pattern of discriminative learning from the SCR data, different computational learning models were fitted to individual trial-by-trial SCR data to the unreinforced cues (CS- and CS+ unpaired). This allowed estimating the fear learning parameters to the CS+ paired trials and, in the second part of the analysis, to assess how these parameters predicted ongoing fluctuations in the shock-evoked pain responses. The following models were tested: Rescorla-Wagner (RW model; in which learning is driven as a function of prediction errors), a RW/Pearce-Hall hybrid model (RW/PH hybrid; learning is driven by prediction errors and associability dynamically modulates the learning rate trial-by-trial). Last, an inter-cue dependent RW/PH hybrid model was tested. This model is a variant of the RW/PH hybrid model in which the expected shock probability and associability are updated both for the cue that was presented at trial *t*, as well as for the cue that was not presented on this trial. The RW/PH hybrid inter-cue dependent model, which was the best-fitted model to the data as in our previous report (Taylor et al., In Press), is described in the equations below (equations (1-6)). A thorough description of model equations used for the RW and RW/PH models is provided in our previous report.

Model Description. Expected shock probabilities ('*V'* in equations 1-6) on trial '*t'* were updated from the *prediction error* (δ) computed on the preceding trial. The prediction error, or the difference between the outcome experienced (λ) on a given trial and the outcome expected from prior trials**,** was modulated by a constant term (learning rate 'α'). Administration of shock was coded as 1, whereas the absence of US was coded as 0. An associability term (*a*) dynamically modulated learning rates. The associability term, also specific to each cue, was updated as a function of the absolute value of the prediction error, which refers to the surprising nature of the outcome (unexpected US or unexpected US omissions). The associability term was also weighted by a constant term (γ).

Furthermore, the RW/PH hybrid model applied took into account the inter-cue dependency. Because our fear conditioning paradigm involved 2 distinct CSs, i.e. the CS+ paired with the US while the other cue (CS-) predicted the absence of pain in a given learning phase, fear learning parameters may not have been necessarily updated independently for each CS. For example, when presented with a cue paired with a US (reinforced CS+), an implicit inference could be made that the second cue was not paired with the US. As such, expectations regarding the unpresented cue could be updated at each trial as a function of a prediction error computed using the 'opposite' outcome as that obtained with the presented cue. Thus, the RW/PH hybrid inter-cue dependent model was a variant of the RW/PH hybrid model using specific parameters with respect to the cue presented at a given trial (*c_pres*) as well as to the cue that was not presented on this trial (*c_unpres*). The model is formally described with the following equations:

$$Vc\_pres_{t+1} = Vc\_pres_t + ac\_pres_t * \alpha c\_pres * \delta c\_pres_t \quad \text{(Equation 1)}$$

$$\delta c\_pres_t = \lambda_t - Vc\_pres_t \quad \text{(Equation 2)}$$

$$ac\_pres_{t+1} = \gamma c\_pres * |\delta c\_pres_t| + (1- \gamma c\_pres) * ac\_pres_t \quad \text{(Equation 3)}$$

The cue that had not been presented (*c_unpres*) on a given trial was attributed the opposite outcome as to the cue that was presented: $|1- \lambda_t|$.

$$Vc\_unpres_{t+1} = Vc\_unpres_t + ac\_pres_t * \alpha c\_unpres * \delta c\_unpres_t \quad \text{(Equation 4)}$$

$$\delta c\_unpres_t = |1- \lambda_t| - Vc\_unpres_t \quad \text{(Equation 5)}$$

$$ac\_unpres_{t+1} = \gamma c\_unpres*|\delta c\_unpres_t|+(1- \gamma c\_unpres)*ac\_unpres_t \quad \text{(Equation 6)}$$

As previously recommended (Daw, 2011), learning parameters (expected shock probabilities and associability values) at each trial for each subject were computed using fixed parameters, i.e. the model's free parameters averaged across subjects, in order to minimize noise levels from subject-level free parameter estimation: $\alpha c\_pres = 0.15$, $\alpha c\_unpres = 0.34$, $\gamma c\_pres = 0.17$, $\gamma c\_unpres = 0.35$, $V_0 = 0.31$, $a_0 = 0.56$.

Predicting Pain Responses from Expected shock probability and Associability

To predict pain responses from fear learning parameters, EShock and associability values for each CS+ paired trial were extracted and used as first-level predictors in multi-level mediation analyses (implemented with custom code written in Matlab,

http://wagerlab.colorado.edu/tools). Meditation group was entered as a second-level moderator variable for each path of the mediation model. Furthermore, to account for the significant negative relationship between associability and EShock (*Beta* = -1.10, *SE* = 0.02, *t* = -63.36, *p* = .0005), each predictor variable was regressed onto the other in order to use the residuals (i.e. EShock controlling for associability and vice-versa) as predictors in the two multi-level models tested. Finally, since pain modulation is often presumed to reflect an involvement (at least partly) of cerebro-spinal mechanisms influencing spinal nociception (Tracey & Mantyh, 2007), we tested the hypothesis that pain modulation by learning parameters (EShock or associability) was mediated by changes in the NFR. Pain ratings and raw NFR scores were normalized (Rhudy & France, 2007) across all trials of the conditioning task for each participant to conduct these analyses. Thus, two mediation models were tested: 1- including EShock as the first-level predictor, (normalized) pain ratings as the dependent variable, (normalized) NFRs as the mediator, and impact of meditation group as the second-level moderator, and 2- idem as 1) but using Associability as the first-level predictor. In a case in which a 2nd level group difference was marginally significant on the direct effects of Associability on pain ratings, Bayesian analyses were conducted to obtain the Bayes factor representing the odds for and against the null hypothesis (Gallistel, 2009). In addition, follow-up simple multi-level regression analysis on the direct effects of Associability on pain ratings was also conducted using the residuals of pain ratings after having regressed out NFRs as the dependent variable, Associability as the first-level predictor, and meditation group as the 2nd level moderator.

Results

The group comparisons below are presented between experienced meditators and the large control cohort of participants. Due to size inequality of groups and a difference in age ($t_{(57)} = -4.53$, $p < 0.001$) between the large control cohort and experienced meditators, all of the analyses were validated and conducted additionally in comparison to the matched control subsample. All results are reported for the large control group and statistical conclusions were corroborated with the matched subsample. The only instance in which this was not the case is with respect to the effects of meditation experience on pain modulation by fear learning, described and discussed below.

Sample Description and Group differences in Personality Traits

Table 1 illustrates demographic and questionnaire variables for the experienced meditators and the control groups. The group of meditators scored higher on Describing inner experiences, acting with Non-reactivity (*ps*<0.05), and on the TCI scale assessing Self-Transcendence (*p*<0.0001).

Table1.
Characteristics of the Experienced Meditators, Control Group and Age-Matched Control Subsample

	Group		
	Meditators (n=11)	**Controls (n=51)**	**Age-Matched Control Subsample (n=11)**
Sex	7M; 4F	24M; 27F	7M; 4F
Age	45 (13)	28 (11)	41 (16)
Pain threshold intensity (mA)	4.19 (2.3)	3.86 (2.8)	3.67 (2.2)
NFR threshold intensity (mA)	8.6 (2.8)	7.7 (2.7)	7.9 (2.2)
135% NFR threshold (mA)	11.4 (3.6)	10.7 (3.8)	10.9 (2.6)
Baseline pain ratings at 135% of NFR threshold	**18.7 (9.6)**	**36.7 (19.8)****	**37.5 (15.4)****
Baseline NFR (EMG RMS integral)	0.002 (0.002)	0.003 (0.004)	0.002 (0.002)
Hours of meditation experience	4056 (2574)	NA	NA
Magnification of Pain (PCS)	**1.9 (1.6)**	**4 (2.6)***	2.6 (2.8)
Helplessness towards Pain (PCS)	4.9 (4.4)	7 (5.0)	3.5 (3.1)
Rumination About Pain (PCS)	**5.2 (3.0)**	**7.8 (3.9)***	**5.9 (3.4)**
Describing Experience (FFMQ)	**31.8 (4.9)**	**28.1 (5.9)***	**26.5 (6.8)***
Observing Experience (FFMQ)	**31.4 (3.1)**	**26.6 (6.3)***	26.9 (7.5)
Acting with Awareness (FFMQ)	27 (3.1)	26.1 (4.6)	27.4 (5.1)
Non-judgmental (FFMQ)	29.1 (4.5)	28.6 (5.8)	29.5 (4.5)
Non-reactivity (FFMQ)	**25.2 (2.7)**	**22.4 (3.9)***	**22.5 (3.5)***
Present Moment Awareness (MAAS)	4.4 (0.4)	4.2 (0.6)	4.2 (0.8)
Self-Transcendence (TCI)	**95.2 (10.8)**	**73.5 (16.6)*****	**70.9 (14.1)*****
Self-Directedness (TCI)	110.1 (14.8)	106.6 (16.9)	113.1 (16.4)
Harm Avoidance (TCI)	79.1 (13.5)	83.6 (17.8)	78.1 (11.7)
Cooperativeness (TCI)	**146.1 (14.7)**	**134.9 (15.9)***	138.7 (10.8)
Persistence (TCI)	130.7 (17.8)	124.2 (20.9)	129.1 (24.1)
Novelty Seeking (TCI)	98.8 (12.29)	105.5 (13.9)	95.3 (11.9)
Reward Dependence (TCI)	102.6 (15.2)	100.1 (14.5)	94.3 (13.3)
State anxiety (STAI)	29.9 (10.4)	35.4 (7.7)	26 (4.8)
Trait Anxiety (STAI)	34.9 (4.6)	35.4 (7.7)	31.5 (5.6)
Depressive Symptoms (BDI)	**1.8 (2.0)**	**5.2 (5.5)***	3.1 (3.7)
Punishment Sensitivity (BIS)	17.7 (2.9)	19.1 (3.7)	18.5 (4.4)

Significant Group effect (T-Test) compared to Experienced Meditators: *$p<0.05$, **$p<0.01$, ***$p<0.0001$. Means are shown with standard deviations in parentheses, except for the variable sex for which number of males (M) and females (F) are presented.
NFR: nociceptive flexion reflex. EMG RMS: root mean square of the electromyographic signal. PCS: pain catastrophizing scale, FFMQ: five factor mindfulness questionnaire. MAAS: mindful awareness attention scale. TCI: temperament and character inventory. STAI: state-trait anxiety inventory. BDI: beck depression inventory. BIS: behavioral inhibition scale.

Effects of Meditation Experience on Pain-Evoked Responses

Effects of Meditation Experience on Baseline Pain Assessments and NFR Threshold

Independent samples *t*-tests indicated that meditators did not exhibit any difference compared to their control counterparts with respect to the electrical stimulation intensity corresponding to the NFR threshold ($t_{(60)}$=-0.98, p=.33) or pain threshold ($t_{(60)}$=-0.20, p=.84). Meditators and controls also received similar stimulus intensity levels (135% of the NFR threshold) during the fear conditioning task ($t_{(60)}$=-0.425, p=.675). One-way ANOVAs revealed that baseline NFR responses to the US assessed before fear conditioning were similar between meditators and controls ($F_{(60)}$=0.90, p=.35). However, for comparable US intensities and NFR responses, meditators reported lower levels of perceived pain at baseline ($F_{(60)}$=10.82, p=.002; see Means and standard deviations in Table 1).

Effects of Meditation Experience on Pain during Fear Conditioning

First, there were no significant group differences in the free individual parameters of the learning model (all *p's*<0.05: αc_pres = 0.20 (SD = 0.34), αc_unpres = 0.23 (SD=0.36), γc_pres = 0.21 (SD=0.33), γc_unpres = 0.39 (SD = 0.42), V_0 = 0.38 (SD=0.38), a_0 = 0.50 (SD=0.29) for controls; αc_pres = 0.30 (SD = 0.39), αc_unpres = 0.35 (SD=0.41), γc_pres = 0.28 (SD=0.42), γc_unpres = 0.36 (SD = 0.43), V_0 = 0.39 (SD=0.38), a_0 =0.50 (SD=0.31) for meditators). This was replicated when comparing the group of meditators to the age-matched control subsample.

Follow-up analyses to conventional null hypothesis testing using Bayesian statistics to assess odds for and against the null hypothesis revealed enhanced odds for the null hypothesis relative to against the null hypothesis for all learning parameters. Bayes factors indicating the likelihood that results were observed under the null hypothesis ranged from 5.75:1 to 14.6:1, representing substantial to strong odds for the null hypothesis (Jeffreys, 1961). This pattern of results was confirmed when comparing the group of meditators to the age-matched controls. These results suggest that fear-learning processes were not affected by meditation experience, and indicates that meditation experience did not influence basic associative learning mechanisms.

Pain outcome measures (pain ratings, NFR) to unconditioned stimuli through the entire fear learning paradigm were then compared between groups to determine the impact of meditation experience on pain sensitivity (Figure 2A-B). A mixed-measures TRIAL (40 US trials) X GROUP (Meditators, Controls) ANOVA, performed on each dependent variable, revealed a significant GROUP effect on pain ratings ($F_{(1, 60)}$=6.31, p=.015, partial eta squared = 0.10) such that experienced meditators rated the US as less painful compared to their control counterparts. The power estimated post-hoc for the group difference in pain ratings was of 0.70. In contrast, meditation experience (GROUP) had no significant effect on the NFRs ($F_{(1, 60)}$=0.79, p=.377). This replicates effects reported above in the baseline measures before fear-conditioning.

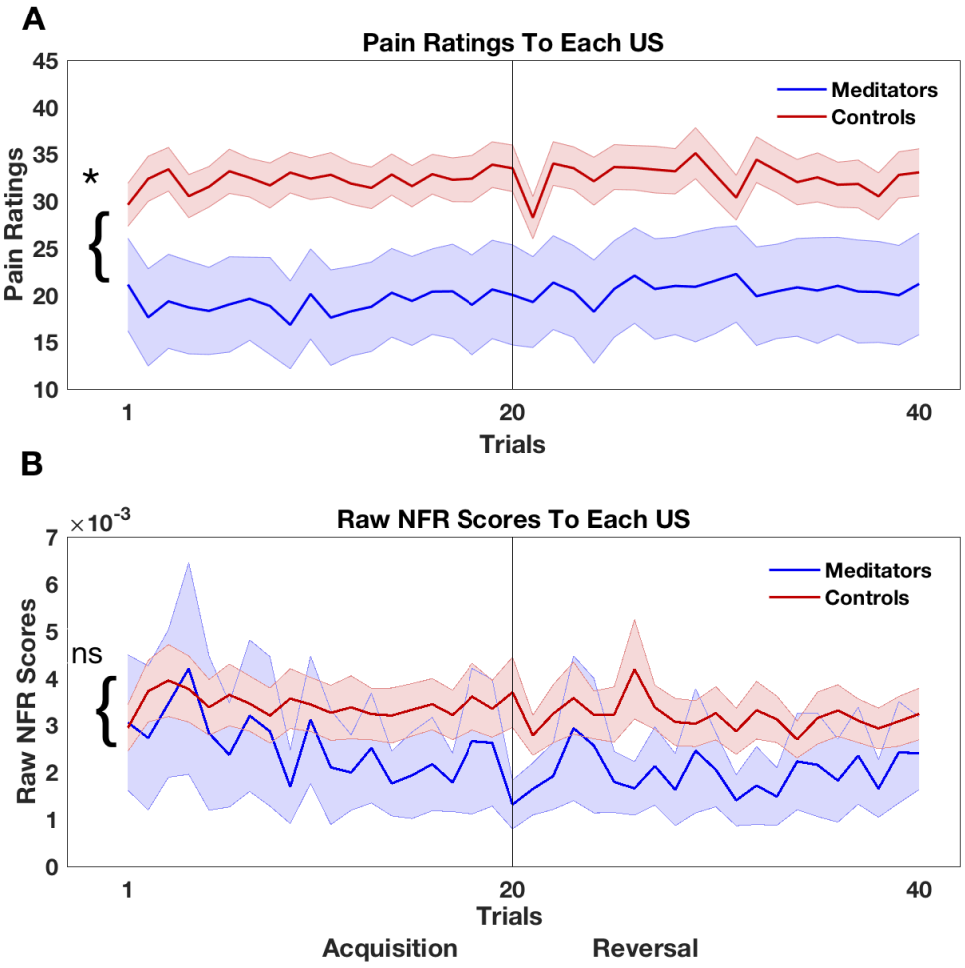

Figure 2. Mean (SEM) pain responses (A) and NFRs by group for each CS+paired trial during the fear conditioning task. (* $p<0.05$).

In sum, our data indicate that meditation reduced the perceived pain induced by the US before and during fear conditioning, but had no significant impact on the NFR. In the following sections, we tested the effect of meditation on fear-learning processes and on the trial-by-trial modulation of pain and the NFR by the learning parameters Eshock and associability.

Effects of Meditation Experience on Fear conditioning of the SCR.

The results of multi-level regression analyses revealed significant increases in SCRs to the CS+ compared to the CS- (Beta = 0.22, SE = 0.06, p = 0.0004; see Figure 2). There was no significant group differences in discriminant SCRs (Beta = -0.13, SE = 0.10, p = 0.13, Figure 3). These results indicate enhanced SCRs elicited by the CS+ (M = 0.296, SE = 0.096) than the CS- (M = 0.140, SE = 0.054) across both groups of participants. These results indicate successful fear learning acquisition of fear conditioned responses across both groups of participants, and that meditation experience did not have a statistically significant impact on fear learning.

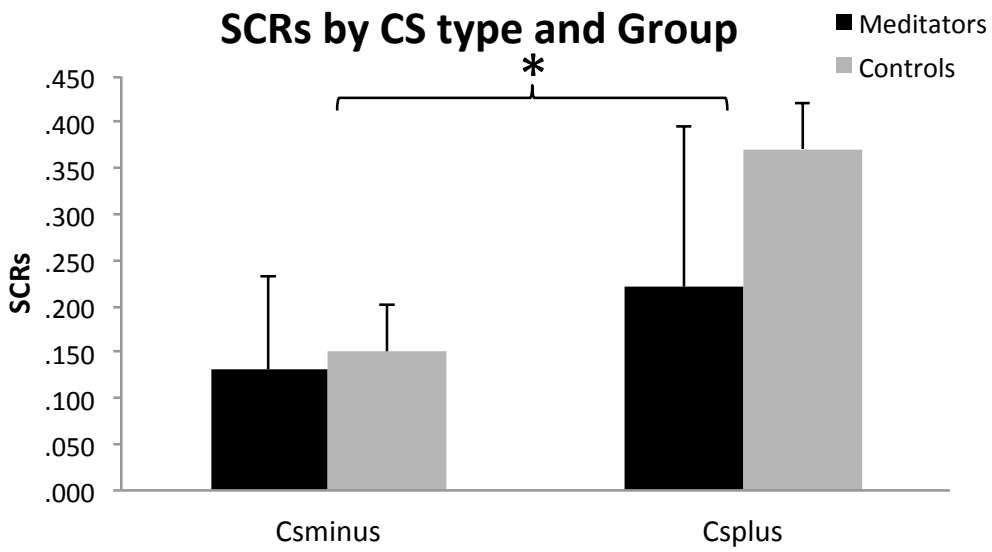

Figure 3. Mean (SEM) SCRs by CStype (CS+ and CS-) for meditators and control participants. Multi-level analyses revealed that no significant group differences were found in discriminatory SCRs, but that there was an overall effect of stimulus type on SCRs (CS+ > CS-) ($p<0.05$).

SCR: skin conductance response. CS: conditioned stimulus.

Effects of Meditation Experience on Pain Modulation by Learning Parameters

The effects of both fear-learning parameters on pain and the NFR were examined at the trial level (first level) and then at the group level (second level) to assess the moderating effect of meditation on pain/NFR modulation by fear.

At the first level, as reported in our recent study (Taylor et al., submitted), expected shock probability significantly predicted pain ratings (i) directly, i.e. after taking into account the mediating effects of NFRs (path c': *Beta* = 0.79, *SE* = 0.25, *t* = 3.18, *p* = 0.002) and (ii) indirectly via mediation effects of the NFR (path a x b: *Beta* = 0.09, *SE* = 0.03, *t* = 3.07, *p* = 0.003). The same pattern of results was found in the model of Associability (path c': Beta = 0.93, *SE* = 0.35, *t* = 2.63, *p* = 0.011; path a x b: *Beta* = 0.19, *SE* = 0.06, *t* = 3.02, *p* =0.004). In models conducted with the age-matched control subsample, first-level indirect effects of EShock on pain ratings were replicated (path a x b: *Beta* = 0.13, *SE* = 0.06, *t* = -2.19, *p* = 0.042) but not first-level direct effects of EShock on pain ratings (path c': *Beta* = 0.33, *SE* = 0.48, *t* = 0.69, *p* = 0.50). Similarly, first-level direct (path c': *Beta* = -0.06, *SE* = 0.54, *t* = -0.12, *p* = 0.91) and indirect (path a x b: Beta = 0.15, SE = 0.13, t = 1.17, p = 0.26) effects of Associability on pain ratings did not reach statistical significance using the age-matched control subsample.

At the second-level, in the model of Expected shock probability, the significant group differences in the total effect (i.e. direct and indirect) of EShock on pain ratings, as well

in the path of the relationship between EShock and NFRs, were significant in the analysis performed with the large control group but did not reach significance using the matched control subsample. Hence, these differences are displayed and explained in Figure 4 but are not discussed further. However, meditators showed a reduction in the direct effect of EShock on pain ratings after accounting for the mediating effect of the NFR (moderator effect on path c': *Beta* = -1.63, *SE* = 0.65, *t* = -2.51, *p* = 0.015, partial eta squared = 0.08).

In the model of Associability, meditators showed significant reduction in the direct effect of associability on pain ratings (moderator effect on path c': *Beta* = -2.36, *SE* = 0.93, *t* = -2.54, *p* = 0.014, partial eta squared = 0.09, estimated power (post-hoc) = 0.65). This effect was replicated yet fell slightly short of statistical significance in the age-matched control subsample (moderator effect on path c': *Beta* = -2.20, *SE* = 1.09, *t* = -2.02, *p* = 0.057). Nonetheless, the Bayes Factor obtained for this group difference was of 3.56:1 against the null hypothesis, representing substantial odds against the null hypothesis of no difference between groups (Jeffreys, 1961). Follow-up simple multi-level regression analyses on the direct effect of Eshock on pain ratings indicated that meditators showed a significant reduction in the direct effect of associability on pain ratings (2^{nd} level moderator effect: *Beta* = -2.11, *SE* = 1.02, *t* = -2.01, *p* = 0.047). Table 2 shows 2^{nd} level moderator effects of meditation group on each path of the mediation models.

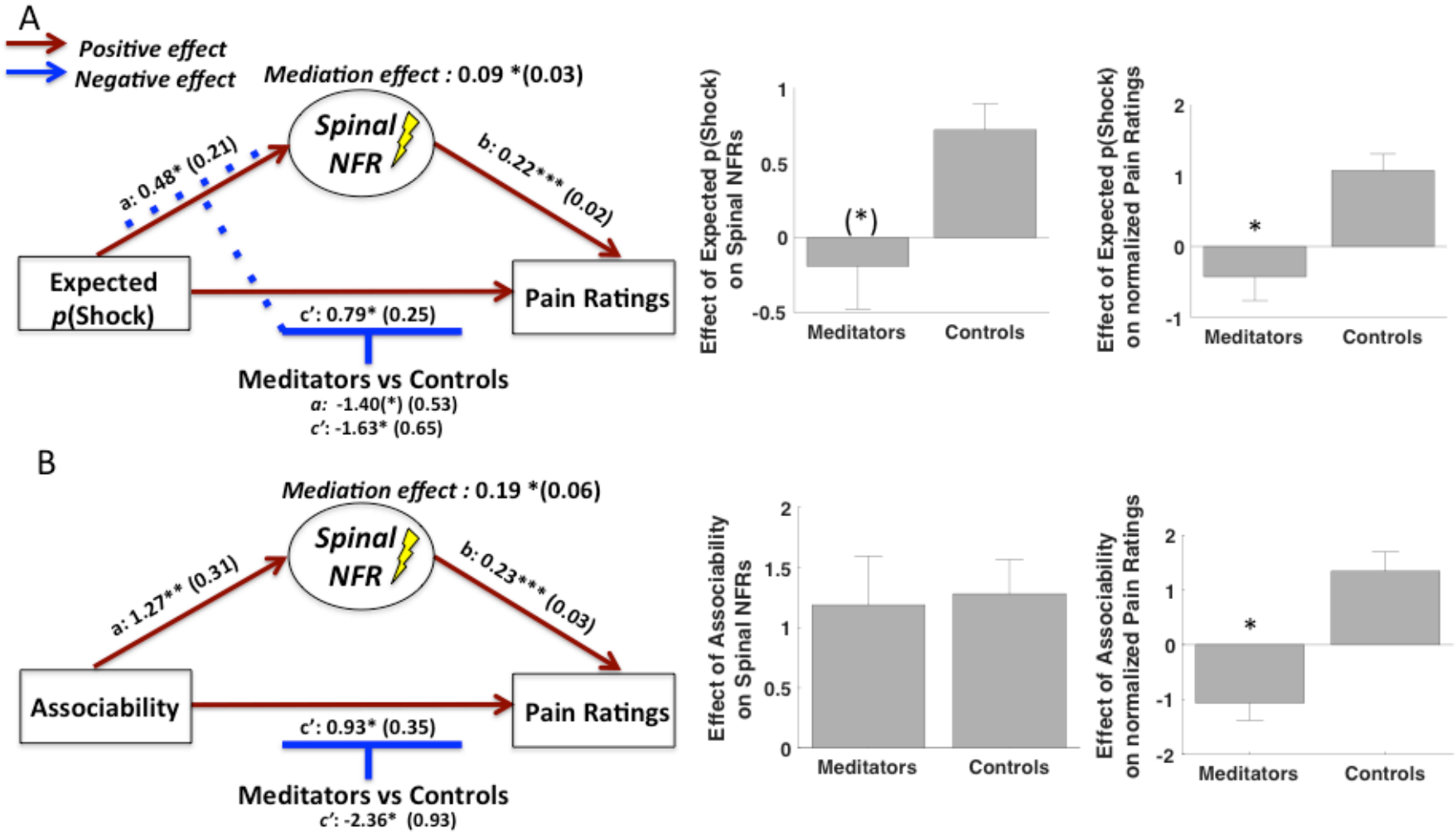
A
Positive effect
Negative effect
Mediation effect : 0.09 *(0.03)
Spinal NFR
a: 0.48* (0.21)
b: 0.22*** (0.02)
Expected p(Shock)
Pain Ratings
c': 0.79* (0.25)
Meditators vs Controls
a: -1.40(*) (0.53)
c': -1.63* (0.65)
Effect of Expected p(Shock) on Spinal NFRs
(*)
Meditators
Controls
Effect of Expected p(Shock) on normalized Pain Ratings
*
Meditators
Controls
B
Mediation effect : 0.19 *(0.06)
Spinal NFR
a: 1.27** (0.31)
b: 0.23*** (0.03)
Associability
Pain Ratings
c': 0.93* (0.35)
Meditators vs Controls
c': -2.36* (0.93)
Effect of Associability on Spinal NFRs
Meditators
Controls
Effect of Associability on normalized Pain Ratings
*
Meditators
Controls

Figure 4. Multi-level mediation models of the effects of (A) expected probability of shock (expected p(shock)) and (B) associability on pain ratings. Both models confirmed an overall direct effect on pain (path c') and an indirect effect (mediation) through changes in spinal nociception (path ab). Coefficients are shown for each path and mediation effects with standard errors in parentheses. The negative moderating effects of meditation group are shown with blue lines. Bar graphs illustrate the moderating effect of the group on Beta values for the direct effects on the NFR and on pain. Meditators showed significant decreases in the direct effects of both (A) expected p(shock) and (B) associability on pain ratings.

**$p<0.001$, *$p<0.05$, (*) $p<0.05$ not confirmed with an age-matched control subsample. NFRs: nociceptive flexion reflex.

Table 2

Mediation Model of Expected Shock Probabilities as a predictor of Pain Ratings with NFRs as a Mediator and Meditation Group as the 2nd level Moderator

Mediation Model of Expected Shock Probabilities				
	Beta	*SE*	*t*	*p*
2nd level Effects of Meditation Group on each path				
path a	-1.40	0.53	-2.63	(*)0.011
path b	-0.01	0.07	-0.18	0.862
path c	-1.50	0.64	-2.36	(*)0.022
path c'	-1.63	0.65	-2.51	*0.015
path ab	0.08	0.08	0.99	0.328
Mediation Model of Associability				
path a	-0.21	0.81	-0.26	0.795
path b	-0.04	0.07	-0.64	0.528
path c	-2.01	0.89	-2.27	(*)0.027
path c'	-2.36	0.93	-2.54	*0.014
path ab	0.09	0.16	0.59	0.557

**$p<0.001$, $p<0.05$, (*) $p<0.05$ not confirmed with an age-matched control subsample

NFR: nociceptive flexion reflex

Figure 5 shows mean fear learning parameters to CS+ paired trials averaged across both groups of subjects (A). As can be seen in an exemplar subject from each group of participants, fear learning parameters (weighted by regression coefficients) do not predict the pain rating trajectory during learning for the experienced meditator (B) but accurately depict the pain rating time-course of the control participant (C).

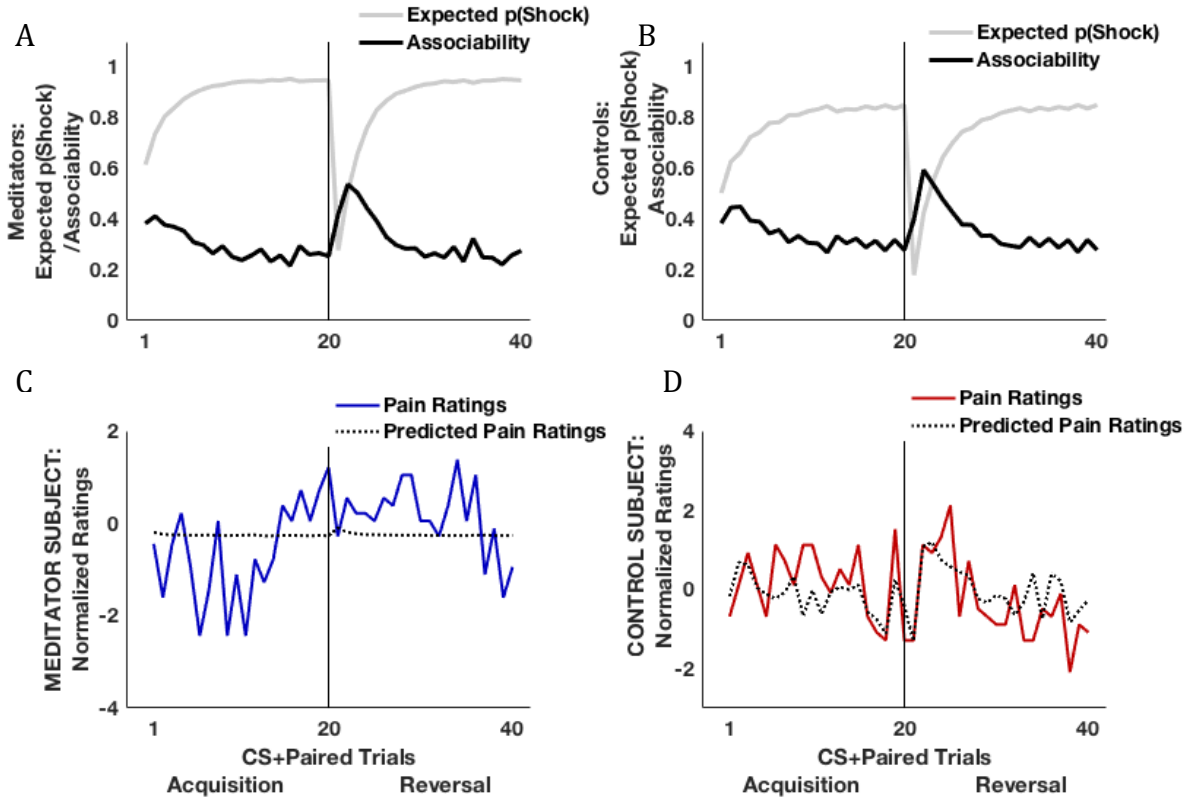

Figure 5. Relationship between expected shock probabilities (expected p(shock)), associability, and pain ratings for reinforced (CS+paired) trials in a meditator and a control participant. A-B) Average associability and expected p(shock)) estimates in experienced meditators and controls respectively. C) Relationship between pain ratings and associability/expected p(shock)) estimates for a control participants (C), and a meditator subject D). Trial-by-trial associability and expected p(shock) estimates were weighted by their regression coefficients in order to illustrate the multi-level regressions. NFR: nociceptive flexion reflex. CS: conditioned stimulus.

Discussion

The results of the present study can be summarized as follow. First, an overall hypoalgesia (decreased pain ratings) to the noxious US was observed both before and during fear conditioning in meditators. Second, meditation experience did not dampen the discriminant anticipatory responses (SCR), indicating normal fear-learning processes. Rather, our data show that meditation experience reduced the hyperalgesic effects of anticipatory processes on pain. These effects are further detailed in the following paragraphs.

First, as hypothesized, the overall hypoalgesia we observed during the fear conditioning task are directly in line with previous reports that meditation experience reduces pain perception and sensitivity (Gard et al., 2012; Grant & Rainville, 2009; Perlman et al., 2010). The fact that we did not observe group differences in nocifensive spinal reflexes to nociceptive stimuli demonstrates that the general hypoalgesic effects of meditation do not operate by activating inhibitory descending control of pain. Rather, our results support the notion that the hypoalgesic effects of meditation selectively target cognitive/affective elaboration. This is consistent with neuroimaging studies on the pain-modulating effects of meditation showing reduced activity in brain regions associated with the mental elaboration/evaluation of pain but not in regions receiving nociceptive signals directly from the spino-thalamo-cortical pathways (Gard et al., 2012; Grant et al., 2011). These results are directly in line with premises taught in meditation: aversive experiences are welcomed and are not suppressed or changed, but they are not further elaborated upon

(Bodhi, 2005).

Second, the fact that extensive mindfulness experience did not yield any detectable differences with respect to the production of anticipatory SCRs to the conditioned cues is consistent with previous results (Holzel et al., 2016). Specifically, Holzel and collaborators (2016) showed that short-term meditation experience, i.e. 8-week mindfulness-based stress-reduction (MBSR) program, did not affect fear conditioned SCRs assessed pre- and post-training (Holzel et al., 2016). The hypoalgesic impact of meditation experience we observed on pain perception did not lead to reduced anticipatory responses. Thus, the meditation-related reduced neural activity during the anticipation of pain observed by others (Brown & Jones, 2010; Lutz et al., 2013) does not reflect an absence of anticipatory processes at a psychophysiological level. Rather, our data show that previous findings of hypoalgesic effects of meditation via reduced neuronal anticipation (Brown & Jones, 2010; Lutz et al., 2013) may reflect a reduced effect of anticipation on pain rather than a reduced ability to learn about pain and to predict its occurrence. Our findings further show that individuals with extensive meditation experience exhibit preserved basic associative learning mechanisms. In other words, mindfulness training may attenuate the aversive quality of unconditioned stimuli, but does not interfere with the 'teaching function' provided by noxious events in terms of forming predictions about the occurrence of impending harm, or allocating attention to critical moments informative of CS-US contingencies (LePelley & McLaren, 2004). These results show that mindfulness meditation does not achieve its attenuating effects on pain by abolishing fear conditioned anticipatory behaviors altogether. This finding is also

in line with the premise that mindfulness promotes the acceptance of all (aversive, neutral or positive emotional) feelings/sensations as opposed to the *suppression* of low-level aversive emotional responses (Taylor et al., 2011).

Finally, our results indicate that the reduction of anticipation-mediated hyperalgesia by mindfulness meditation operates by disrupting the *influence* of associative learning on pain responses mainly at a supraspinal level of processing. Our results suggest that mindfulness meditation experience does not abolish the critical ability to learn from associative cues in the environment to predict impending harm or to allocate more attention to associative cues when uncertainty is high. Specifically, meditation experience reduced expectations about the probability of occurrence of impending harm, on pain perception directly. The attenuation of the hyperalgesic influence of expectations at higher-order levels of pain processing possibly reflects a detached or non-reactive stance towards the probability of the upcoming aversive event.

With respect to associability, meditation experience also reduced the effects of this parameter on higher order pain perception directly. Associability is encoded in the amygdala (Li, Schiller, et al., 2011) and is thought to reflect attention allocation, vigilance to cues informative of CS-US contingencies at moments critical to learning (i.e. following large prediction errors) (LePelley & McLaren, 2004). The hyperalgesic effects of associability on pain may provide an important 'teaching function' in the sense that pain perception is enhanced in trials critical to the association between environmental

predictive cues and sources of harm: the information may be better integrated if the US is more saliently/aversively experienced. Thus, meditation experience may preserve from such pain enhancement only at the beginning of a learning phase, when associability/uncertainty is highest.

In control participants with no prior mindfulness meditation experience, the pain modulating effects we observed in our previous report (Taylor et al., In Press) of learned expectations and uncertainty (associability) during fear conditioning may explain the central maintenance of pain and pathological manifestations of repeated exposure to noxious stimuli. The results of the present study suggest that the extensive practice of mindfulness meditation may efficiently prevent repeated pain exposure from escalating into chronic and centrally maintained hypersensitivity to pain. Our findings provide further support for the integration of mindfulness meditation in clinical interventions targeted towards the treatment/management of chronic pain (Kabat-Zinn et al., 1985; Morone et al., 2008).

Nonetheless, the present study is not without its limitations: the cross-sectional nature of the design does not allow us to draw causal inferences on the effects of the practice of meditation per se on pain or fear-conditioning effects on pain. Thus, future longitudinal studies should be conducted to examine the effects of meditation experience, pre and post a meditation training intervention, on pain and pain modulation by classical conditioning. In addition, the sample size for experienced meditators was limited. Nonetheless, the lack

of power induced by the low sample size would not have been problematic in detecting significant differences in the absence of real effects, but would have rather impaired the ability to detect significant group differences. Therefore, in cases in which non-significant differences were obtained, follow-up Bayesian analyses to conventional null-hypothesis testing were conducted, and determined enhanced odds in support of the null hypothesis of no group differences. Nevertheless, further studies examining the effects of fear conditioning on pain should be conducted in larger participant groups.

In conclusion, our results show that meditation experience 1- achieves its hypoalgesic effects by selectively targeting higher-order perceptual mechanisms rather than by activating descending inhibitory controls, 2 – does not alter the anticipatory learning process but rather 3 – reduces the interaction between anticipatory processes and pain perception at higher-order levels of processing. Importantly, this is achieved without compromising the adaptive value of pain signal in aversive leaning. Our results may contribute to the validation of mindfulness-based interventions for the treatment of disorders related to affect, stress and pain, particularly with respect to the central maintenance of pain resulting from repeated exposure to noxious stimuli.

References

Bach, D. R., Flandin, G., Friston, K. J., & Dolan, R. J. (2010). Modelling event-related skin conductance responses. *Int J Psychophysiol, 75*(3), 349-356.

Baer, R. A. (2003). Mindfulness training as a clinical intervention: a conceptual andempirical review. *Clin. Psychol. Sci. Pract., 10*(2), 125-143.

Baer, R. A., Smith, G. T., Hopkins, J., Krietemeyer, J., & Toney, L. (2006). Using self-report assessment methods to explore facets of mindfulness. *Assessment, 13*(1), 27-45.

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., & Erbaugh, J. (1961). An inventory for measuring depression. *Arch Gen Psychiatry, 4*, 561-571.

Bodhi, B. (2005). *In the Buddha's Words*. Boston: Wisdom Publications.

Brown, C. A., & Jones, A. K. (2010). Meditation experience predicts less negative appraisal of pain: electrophysiological evidence for the involvement of anticipatory neural responses. *Pain, 150*(3), 428-438.

Brown, K. W., & Ryan, R. M. (2003). The benefits of being present: mindfulness and its role in psychological well-being. *J Pers Soc Psychol, 84*(4), 822-848.

Carver, C. S., & White, T. L. (1994). Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: The BIS/BAS Scales. *Journal of Personality and Social Psychology, 67*, 319-333.

Cloninger, C. R. (1994). Temperament and personality. *Curr Opin Neurobiol, 4*(2), 266-273.

Daw, N. D. (2011). Trial-by-trial data analysis using computational models. In M. R. Delgado, E. A. Phelps, & T. W. Robbins (Eds.), *Decision Making, Affect, and Learning*

Attention & Performance XXIII (pp. 3-38). New York: Oxford University Press.

Gallistel, C. R. (2009). The importance of proving the null. *Psychol Rev, 116*(2), 439-453.

Gard, T., Holzel, B. K., Sack, A. T., Hempel, H., Lazar, S. W., Vaitl, D., et al. (2012). Pain attenuation through mindfulness is associated with decreased cognitive control and increased sensory processing in the brain. *Cereb Cortex, 22*(11), 2692-2702.

Grant, J. A., Courtemanche, J., & Rainville, P. (2011). A non-elaborative mental stance and decoupling of executive and pain-related cortices predicts low pain sensitivity in Zen meditators. *Pain, 152*(1), 150-156.

Grant, J. A., & Rainville, P. (2009). Pain sensitivity and analgesic effects of mindful states in Zen meditators: a cross-sectional study. *Psychosom Med, 71*(1), 106-114.

Holzel, B. K., Brunsch, V., Gard, T., Greve, D. N., Koch, K., Sorg, C., et al. (2016). Mindfulness-Based Stress Reduction, Fear Conditioning, and The Uncinate Fasciculus: A Pilot Study. *Front Behav Neurosci, 10*, 124.

Jeffreys, H. (1961). *Theory of probability, 3rd ed.* Oxford, England: Oxford University Press.

Kabat-Zinn, J., Lipworth, L., & Burney, R. (1985). The clinical use of mindfulness meditation for the self-regulation of chronic pain. *J Behav Med, 8*(2), 163-190.

Keng, S. L., Smoski, M. J., & Robins, C. J. (2011). Effects of mindfulness on psychological health: a review of empirical studies. *Clin Psychol Rev, 31*(6), 1041-1056.

LePelley, M. E., & McLaren, I. P. (2004). Associative history affects the associative change undergone by both presented and absent cues in human causal learning. *J Exp Psychol Anim Behav Process, 30*(1), 67-73.

Li, J., Schiller, D., Schoenbaum, G., Phelps, E. A., & Daw, N. D. (2011). Differential roles of human striatum and amygdala in associative learning. *Nat Neurosci, 14*(10), 1250-1252.

Lutz, A., McFarlin, D. R., Perlman, D. M., Salomons, T. V., & Davidson, R. J. (2013). Altered anterior insula activation during anticipation and experience of painful stimuli in expert meditators. *Neuroimage, 64*, 538-546.

Lutz, A., Slagter, H. A., Dunne, J. D., & Davidson, R. J. (2008). Attention regulation and monitoring in meditation. *Trends Cogn Sci, 12*(4), 163-169.

Morone, N. E., Greco, C. M., & Weiner, D. K. (2008). Mindfulness meditation for the treatment of chronic low back pain in older adults: a randomized controlled pilot study. *Pain, 134*(3), 310-319.

Perlman, D. M., Salomons, T. V., Davidson, R. J., & Lutz, A. (2010). Differential effects on pain intensity and unpleasantness of two meditation practices. *Emotion, 10*(1), 65-71.

Rhudy, J. L., & France, C. R. (2007). Defining the nociceptive flexion reflex (NFR) threshold in human participants: a comparison of different scoring criteria. *Pain, 128*(3), 244-253.

Schiller, D., Levy, I., Niv, Y., LeDoux, J. E., & Phelps, E. A. (2008). From fear to safety and back: reversal of fear in the human brain. *J Neurosci, 28*(45), 11517-11525.

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Sullivan, M. J. L., Bishop, S. R., & Pivik, J. (1995). The Pain Catastrophizing Scale: Development and Validation. *Psychological assessment, 7*(4), 524-532.

Taylor, V. A., Chang, L., Rainville, P., & Roy, M. (In Press). Learned Expectations and Uncertainty Facilitate Pain Processing During Classical Conditioning. *Pain*.

Taylor, V. A., Grant, J., Daneault, V., Scavone, G., Breton, E., Roffe-Vidal, S., et al. (2011). Impact of mindfulness on the neural responses to emotional pictures in experienced and beginner meditators. *Neuroimage, 57*(4), 1524-1533.

Tracey, I., & Mantyh, P. W. (2007). The cerebral signature for pain perception and its modulation. *Neuron, 55*(3), 377-391.

Willer, J. C. (1977). Comparative study of perceived pain and nociceptive flexion reflex in man. *Pain, 3*(1), 69-80.

Article 3: Cortisol Modulates Pain during Pavlovian Fear Learning

Taylor, V.[1,4,6], Roy, M.[3] , Gill, L.-N. [1,4], Mueller, C.[5], Lupien, S., Rainville, P. Salivary Cortisol Modulates Pain during Pavlovian Fear Learning. Manuscript in preparation.

Contribution of authors: VT, MR and PR designed the study; VT recruited experienced meditators and acquired the data with the help of L.-N. Gill and C. Mueller; VT and MR analyzed the data and applied computational models with the help of LC; VT drafted the manuscript and SL, PR, and MR revised and approved the article. SL provided particular support regarding analyses of cortisol data and aspects of the manuscript related to the literature on stress and cortisol.

Abstract

Stress involves increased activity of the hypothalamic-pituitary-adrenal (HPA) axis and has been associated with decreased pain processing, yet the literature on this topic in humans is scarce. For instance, HPA activity, assessed using salivary cortisol, may be associated with pain regulation at higher-order perceptual levels, with the recruitment of descending control systems affecting spinal nociception, or a combination of both processes. In addition, the stress system may affect pain indirectly by changing the gain of distinct pain modulatory mechanisms such as those involved in fear learning. This study examined the moderating impact of spontaneous HPA-axis activity on pain and spinal nociceptive flexion reflexes (NFR) during Pavlovian fear learning. The Pavlovian fear learning task included a visual cue (CS+) paired with a noxious electrical stimulation (unconditioned stimulus; US) on 50% of trials (CS50), a CS+ paired with the US on 100% of trials (CS100), and one cue not paired with the US (CS-). Skin conductance responses to unreinforced cues were entered into a computational model of reinforcement learning, allowing the estimation of expected US probabilities and cue associability at each trial. These fear learning parameters were then entered in multi-level regression analyses with spontaneous HPA activity (assessed using salivary cortisol levels) as a between-subjects moderator of the relationship between fear parameters and pain. Results showed overall lower pain reports during Pavlovian learning in individuals with higher cortisol levels. However, pain also increased with higher expected US probability during learning, independent from cortisol levels. In contrast, the NFR decreased with higher US expectations but this modulatory effect was significantly reduced or reversed (i.e. facilitation) in participants with higher cortisol levels. The results of this study show

distinct effects of cortisol on higher vs lower order pain processes: whereas cortisol is related to a general higher order hypoalgesia, its effects on descending inhibitory controls appear to operate via fear learning mechanisms, more precisely, learned expectations of probabilities of receiving pain. These results demonstrate that HPA axis activity promotes nocifensive responses in the context of inescapable and repeated pain exposure, and may have implications for the vulnerability to developing pain/stress-related pathologies.

Keywords: cortisol, pain, RIII-reflex, fear conditioning, computational modeling, classical conditioning, learning, expectation, uncertainty, reinforcement learning models

Introduction

In the face of perceived threat, the hypothalamic-pituitary-adrenal (HPA) axis is activated, triggering a cascade of endocrine responses at the end of which cortisol is secreted by the adrenal cortex (LeDoux & Phelps, 2008). It has also recently been shown that activity in the HPA axis, as indexed by salivary levels of the stress hormone cortisol, has pain modulatory effects (Vachon-Presseau et al., 2013). Given the partly shared neuroanatomical circuits, behavioral and physiological processes between the stress, fear, and pain systems (LeDoux & Phelps, 2008; Lupien & McEwen, 1997; Price, 2000), it is possible that HPA axis activation, as indexed by salivary cortisol change during fear conditioning, moderates certain relationships involved in the pain modulating effects of fear conditioning at different levels of nociceptive signal processing.

Following the premise of stress-induced analgesia (Fanselow, 1986), elevated endogenous salivary cortisol was related to reduced acute pain perception in healthy individuals and chronic pain patients (Vachon-Presseau et al., 2013). However, the level of nociceptive processing at which HPA-axis activity modulates pain could not be determined in the latter study. It is possible that attenuated HPA-axis activity modulates pain by uniquely targeting higher-order centers affecting pain perception, by recruiting descending pain controls, or a combination of both mechanisms. This question, however, remains to be tested, and is physiologically relevant to determining the role of individual differences in HPA axis activity on the relationship between fear and pain.

In addition, it is possible that HPA axis activity interacts with pain processing systems directly, or indirectly by targeting separate systems involved in fear learning and affect regulation. Indeed, the amygdala, hippocampus, and prefrontal cortex are rich in glucocorticoid receptors (Lupien & McEwen, 1997). Given the partly shared neurocircuitry between stress, fear learning and pain systems (Lupien & McEwen, 1997; Price, 2000; Quirk, Garcia, & Gonzalez-Lima, 2006), the stress response may modulate pain by moderating the impact that fear learning mechanisms have on pain. In our recent study (Taylor et al., In Press), we applied computational modeling of fear learning to show that uncertainty and a higher expected probability of receiving a painful US increase pain responses. However, the facilitation of spinal nociceptive responses by latent variables governing fear learning responses was enhanced in participants reporting higher levels of harm vigilance and lower levels of emotional detachment. This indicates that the modulation of pain by aversive learning processes depends on individual factors reflecting affective regulation. Therefore, it is possible that inter-individual differences in HPA-axis activity operate to alter pain by affecting the pain modulating influence of aversive learning mechanisms in a similar fashion.

This psychophysiological study aimed to determine whether HPA activity in general and cortisol in particular are related to pain modulation at a higher-order perceptual level of pain processing, or whether they also recruit descending pain controls affecting spinal nociceptive responses. This study also aimed to determine the relation between cortisol levels and the effects of fear learning mechanisms on pain. To address these issues, we sampled salivary cortisol levels while assessing the effects of Pavlovian fear conditioning

on pain responses (subjective ratings and spinal nociceptive flexion reflex 'NFR', (Willer, 1977)) to noxious electrical stimulations (unconditioned stimulus 'US'). Expectations and uncertainty (associability) were estimated by fitting a computational reinforcement Rescorla-Wagner/Pearce-Hall learning model to anticipatory SCRs. These fear-learning parameters were then used to predict pain outcomes at each US using multi-level regression analyses. Finally, the impact of inter-individual differences in HPA axis activity (assessed using salivary cortisol levels) was examined on the relationship between fear learning parameters and pain. First, we expected to observe an overall cortisol increase during our fear conditioning protocol, as previously observed in an experiment administering painful stimuli (Vachon-Presseau et al., 2013). Finally, in addition to test the hypoalgesia previously associated with higher cortisol levels (Vachon-Presseau et al., 2013), we expected that pain modulation by fear learning would depend on cortisol levels.

Methods

Participants

The sample consisted of a group of 24 healthy adult participants between the age of 18 and 35 years (12 females, 12 males) recruited from poster advertisements in local University settings (Université de Montréal, Concordia, McGill). All experimental procedures conformed to the standards set by the latest revision of the Declaration of Helsinki and were approved by the Research Ethics Board of our institution ("Comité mixte d'éthique de la recherche du Regroupement Neuroimagerie Québec; CMER-RNQ #11-12-014). All participants gave written informed consent, acknowledging their right to withdraw from the experiment without prejudice, and received a monetary compensation equivalent to about 15$/hour for their transportation expenses, time, and commitment.

Potential participants were considered eligible to take part in the study upon meeting the following criteria: no pregnancy, no psychological/psychiatric condition (such as major depressive disorder and substance abuse), no medication intake (including oral contraceptives), no pain-related diseases (such as chronic pain or neuropathic pain), and no regular use of anti-inflammatory or analgesic medications. Potential participants were invited to visit the Laboratory of the Neuropsychophysiology of Pain (Centre de recherche de l'Institut universitaire de gériatrie de Montréal, Canada) for a screening and familiarization session involving the assessment of their pain thresholds and physiological responses (skin conductance and NFR). Selected participants were invited

to a second visit on a separate day to complete the experimental paradigm. Among the 29 volunteers invited to the screening session, 5 participants were not retained for one of the following reasons: extreme (high or low) pain thresholds (n=1), use of medication not disclosed at the time of recruitment (n=1), discomfort with the electrical stimulations (n=1), or absent/unstable skin conductance or NFRs to the painful stimuli (n=3). Twenty-four participants completed the experimental session, but one subject was excluded from data analysis due to poor electrodermal signal, yielding a remaining total of 23 participants included in the analyses (13 females, 10 males).

Testing procedure

In the screening session, participants provided informed consent and were asked a series of questions concerning demographic information. The first salivary cortisol sample was administered (S1) upon their arrival at the laboratory. They were then prepared for electrophysiological recordings after which they were submitted to the NFR thresholding procedure. Finally, they were given a battery of self-report questionnaires to fill out to examine potential relationships between cortisol levels, and negative affect or mindful trait dispositions.

Participants were invited to return for a second visit on a separate day within the following week to complete the experiment. The experimental session began after 12PM to minimize diurnal variations in endogenous cortisol levels. The first salivary cortisol (S1) sampled was collected upon participants' arrival. After being prepared for electrophysiological recordings, the procedure for NFR thresholding was conducted to determine the intensity of electrocutaneous stimulation to be administered during the task

(i.e. 135% of NFR threshold; see below). The second salivary sample (S2) was then collected. Then, a 'baseline' block of 10 electrical stimulations (US alone) was administered at the individually determined intensity, with an inter-stimulus interval jittered between 6 and 10 sec. Immediately after this baseline block, the fear conditioning task (Figure 1) was administered. The third salivary cortisol sample (S3) was collected at the end of the fear conditioning task. A final block of 10 electrical stimulations without any CS was then administered in order to account for possible non-specific changes in the NFR as a function of time. At the end of the experiment, electrodes were removed and participants completed a post-experimental interview assessing their awareness of CS-US pairings adapted from previous studies (Bechara et al., 1995; LaBar et al., 1995). They were then debriefed and the last salivary cortisol sample (S4) was collected just before leaving the laboratory.

Fear Conditioning Paradigm

Prior to completing the task, participants were instructed that they would see images appear on the screen, following which they may or may not receive a painful shock. They were also told that they may notice a relationship between a cue and the presentation of a shock, and that this relationship should remain the same across trials but may or may not change throughout the task. The fear conditioning paradigm began with CS habituation, i.e. the presentation of 3 trials of each CS (without US). The task then followed with phases of acquisition, reversal1, reversal2, and extinction (presentations of each cue alone). In the acquisition and reversal blocks, one image was paired and co-terminated with the US at a contingency rate of 50% (CS+50), one image was paired and co-

terminated with the US at a contingency rate of 100% (CS+100), and one image was never paired with the US (CS-). In the reversal phases, each cue was assigned to a different condition, such that the cue assigned as CS+100 became CS+50, the cue assigned as CS+50 became CS-, and the cue assigned as CS- became CS100. Each US was rated on a Visual Analog Scale of pain. The inter-trial intervals consisted of a white cross centered on a black background (duration jittered between 6, 7, 8, and 9 seconds). Acquisition and reversal blocks consisted of 48 trials (16 CS-, 8 CS+50 unpaired, 8 CS+50 paired, 16 CS+100 paired). Trials were presented in a randomized order, with the constraint that the first trial of acquisition and reversal phases always consisted of a paired CS+ (either 100 or 50), to instantiate learning contingencies at the onset of a new phase. The extinction block consisted of 24 trials of unreinforced CSs (8 trials for each image). The assignment of the CS+ in the acquisition phase (cue 1, 2, or 3) was counter-balanced across subjects. The entire task was subdivided into 4 runs of about 13 minutes separated by short breaks and including 43 trials in runs 1-3 and 48 trials in run 4. This distribution of trials insured that changes in CS-US contingencies did not occur at the beginning of a run. The fear conditioning protocol is illustrated in Figure1.

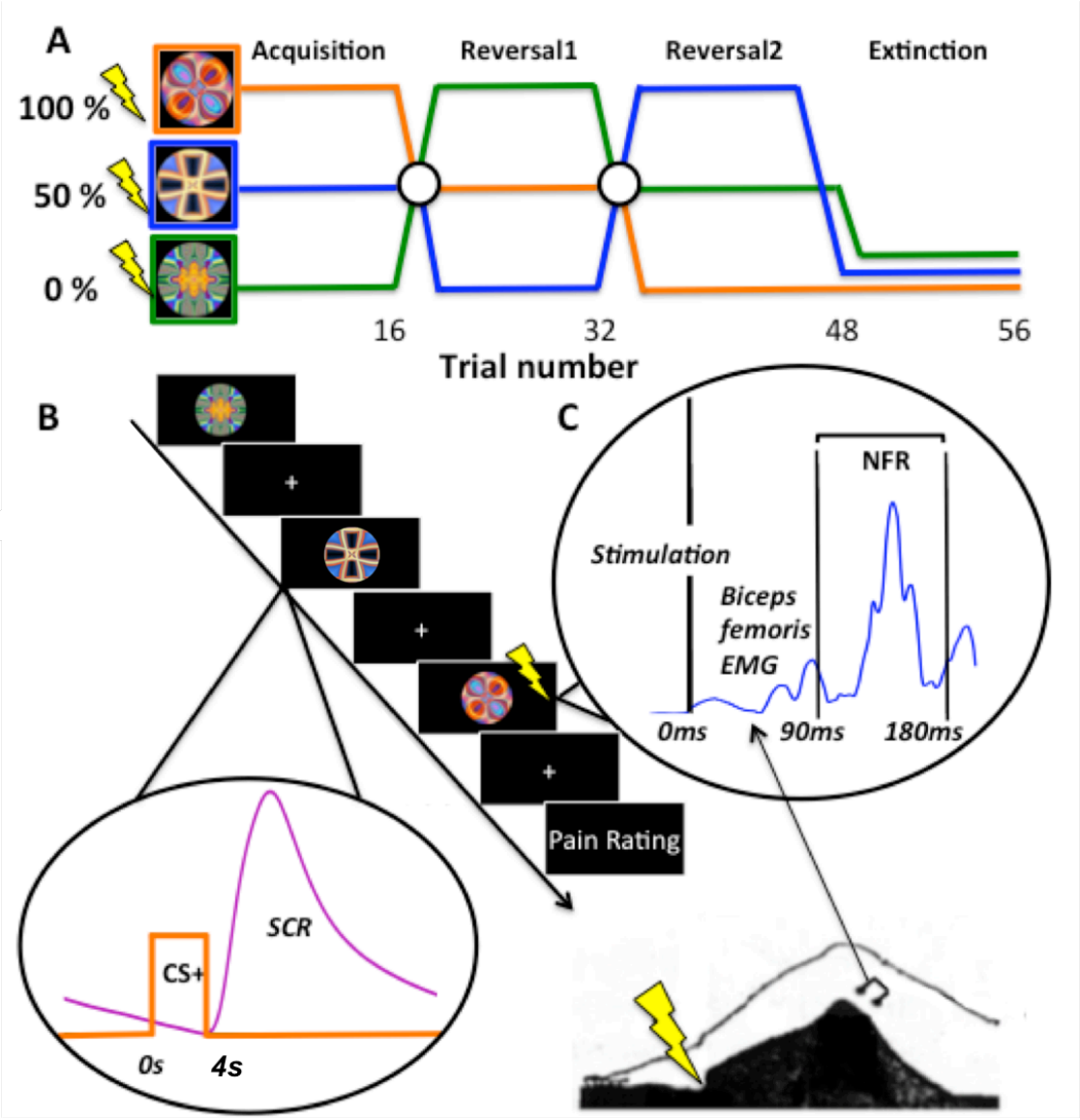

Figure 1. Experimental paradigm. A) In the initial acquisition stage (trials 1- 40), one cue was associated with a 100% chance of being followed by an electric shock (CS100), another cue was associated with a 50% chance of being followed by an electric shock (CS50), while the other cue was associated with a 0% chance of shock (CS-). In the reversal stages, the reinforcement contingencies between cues were changed, such that the previous CS100 became CS50, the previous CS- became the new CS100, and the previous CS50 became CS-. In the extinction phase, all cues were associated with a 0%

chance of shock. B) Example of each type of trial (CS-, CS50, and CS100). Each trial began with the presentation of one of the three cues. On reinforced trials, the presentation of the cue co-terminated with an electric shock (30 ms) to the right sural nerve and participants were asked to rate their pain after a jittered interval of 4-8s. Then, after another jittered inter-trial interval (ITI) of 9-12s, the following cue was presented. During unreinforced (CS- or CS50unpaired) trials, there were no pain ratings, and fear conditioned responses to visual cues were assessed by examining skin conductance responses (SCR; with a typical latency between 0.5 and 2s) from electrodermal activity recordings. C) Electromyographic (EMG) activity was recorded using electrodes placed on the biceps femoris. The NFR was observable at a latency of 90-180 ms post-stimulation onset.

Stimuli

Visual stimuli were shown on a computer screen using E-Prime2 Professional (Psychology Software Tools, Sharpsburg, PA). The CSs (cue1, cue2, and cue3) were shown for 4s on a black background colored circles (fractal images filled with randomly colored and shaped). The US consisted of transcutaneous electrical stimulation of 30 ms composed of a train of ten pulses (1ms) delivered at a 333 Hz frequency, and co-terminated with the CS. US delivery was conducted using an isolated DS7A constant current stimulator (Digitimer Ltd, Welwyn Garden City, United Kingdom) and was initiated by a train generator (Grass Medical Instruments, Quincy, MA). US delivery was controlled by the computer on which E-Prime2 Professional was operated. US administration was performed by $1cm^2$ stimulation electrodes placed on cleaned skin at the level of the right sural nerve behind the ankle. In the initial familiarization session, NFR thresholds were evaluated as well as at the start of the second session, using the NFR staircase thresholding method previously elaborated (Willer, 1977). The intensity determined for US administration during fear conditioning was set at 135% of the intensity of the NFR threshold.

Measures and Dependent Variables

AcqKnowledge data acquisition software (version 4.2; BIOPAC Systems Inc.; Goleta, CA, USA) was used to record physiological measurements.

Pain Ratings. Pain levels induced from electrical stimulations were evaluated using a visual analog scale (VAS) (0: no pain to 100: extremely painful). The scale was a horizontal bar shown on the screen, and participants moved a cursor using a response pad to indicate their pain rating. The position of the cursor was shown on the screen using visual numeric feedback.

Electromyographic (EMG) Recording. Spinal nociceptive responses were assessed using the RIII-reflex. The EMG signal was recorded with two disposable pre-gelled electrodes (EL508) on cleaned and shaved (if necessary) skin placed on the right biceps femoris. In addition, a ground electrode was positioned at the level of the right tibial bone. The EMG signal was sampled at 1000 Hz, amplified (1000 times), and filtered online (bandpass filtering: 100 -500 Hz). The root mean square transform ('RMS', over 20 consecutive samples) was applied to the EMG signal online. The integral (between 90-180 ms after shock administration) of the EMG RMS was computed offline, and consisted in raw NFR indices. NFR scores were normalized across trials of the fear learning task within each subject.

Electrodermal Recording. Two disposable Ag-AgCl electrodes placed on the palm of the left hand were used to record electrodermal activity. The signal was amplified (5 μs/volt) and filtered online (bandpass: 1-5 Hz), and was temporally smoothed offline (at 500 ms). The magnitude of the skin conductance response (SCR) to CS-, CS+50 unpaired, and CS+50/100 paired was assessed using SCRalyze (Bach et al.,

2010).

Self-Report Questionnaires. The following self-report questionnaires were administered: the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983), the Pain Catastrophizing Scale (PCS) (Sullivan et al., 1995), the Beck Depression Inventory (Beck et al., 1961), and the Behavioral Inhibition/Activation Scale (Carver & White, 1994). In addition, the Five Factor Mindfulness Questionnaire (Baer et al., 2006) and the Mindful Attention Awareness Scale (MAAS) (Brown & Ryan, 2003) were administered due to the inverse relationship between mindfulness trait and pain catastrophizing (Schutze et al., 2010), and due to the role of mindfulness meditation in attenuating pain perception and developing resilience in the management of chronic pain (Grant & Rainville, 2009; Kabat-Zinn et al., 1985; Zeidan et al., 2010). These self-report questionnaires were administered to examine potential relationships between cortisol levels, and negative affect or mindful trait dispositions.

Saliva Sampling. Participants were always tested after 12PM to limit variance in salivary cortisol due to circadian fluctuations. One h prior to their appointment, they were instructed to avoid eating, drinking (except for water), and exercising heavily, and to avoid consuming alcohol at least 12 hours before their testing appointment. Saliva was directly expressed with a straw into 10ml plastic vials. Samples were frozen at minus 80°C until assayed for cortisol using an Enzyme Immunoassay kit from Salimetrics at the Institut universitaire en santé mentale de Montréal. Saliva was sampled at the following

time points during the experiment: S1) immediately upon arrival (Mean time = 13.5h), S2) after pain thresholding procedure (Mean time = 14.25h), S3) at the end of the fear conditioning task (Mean time = 15.5h), S4) approximately 20 min after S3 upon departure from the laboratory (Mean time = 16h).

Given that the experiment was conducted in the afternoon, we expected a general decrease in cortisol concentration from time 1 to 4. However, we expected that the experimental task and the exposure to painful electrical stimulation might induce a cortisol response, the amplitude of which would vary between subjects as a function of the reactivity of the HPA axis.

To obtain reactive cortisol indices throughout the fear conditioning task, the area under the curve with respect to increase (AUCi) using S2-S3-S4 was computed using the following formula (Equations 1 and 2) previously described (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003), in which *m* denotes the measurement for the cortisol concentration obtained at sample *i*, and n denotes the number of samples used to calculate the area under curve.

$$\text{AUCi} = \left(\sum_{i=1}^{n-1} (m_{i+1} + m_i)/2 \right) - (n - 1) * m_1 \quad \text{Equation 1}$$

Because our objective was to obtain a measure of cortisol changes during the fear conditioning paradigm, samples S2 (collected just before the task) S3 (immediately after the task), and S4 (approximately 20 minutes after the task), were used to calculate the AUCi index according to Equation 2.

AUCi = (S3 + S2)/2 -2*S2 + (S4 + S3)/2 -2*S3 _____Equation 2

The magnitude of the cortisol change in each subject was taken as a reflection of the magnitude of the cortisol reactivity to the conditioning experiment.

Moreover, in order to ensure that relationships observed between cortisol, pain, and fear-conditioned pain modulation were specific to individual differences in cortisol change during fear conditioning and were not due to individual differences in basal cortisol levels on the day participants were tested. To do this, the area under the curve with respect to ground (AUCg) using S1-S2-S3-S4, with *t1-t2-t3* denoting the average time interval between the collection of samples, was computed using the following formula (Equation 3) (Pruessner et al., 2003):

AUCg = (S2 + S1)*t1/2 + (S3 + S2)*t2/2 + (S4 + S3)*t3/2 _____Equation 3

Data Analyses

Skin Conductance Response Analyses

The skin conductance response (SCR) was assessed to the conditioned stimuli using SCRalyze (Bach et al., 2010). A general linear model-based approach was used to determine trial-by-trial SCR amplitude estimates. This approach involves the convolution of a standard canonical SCR function onto each event onset. This function was then regressed onto the electrodermal activity data, yielding beta values estimated for each event of the task. One model per trial was computed to obtain an SCR for each CS trial, a recommended procedure shown to be effective to estimate trial-by-trial responses in timeseries data using event-related designs (Mumford et al., 2012). In each model, a regressor was entered representing the event onset for each trial of interest. Another regressor for all other CS onsets was entered, and regressors of non-interest were included for shock onsets and pain rating periods. An estimate, henceforth referred to as SCRs for conciseness, was obtained for each unreinforced CS trial. SCRs to reinforced CS+paired trials could not be appropriately estimated due to overlap between SCRs to CS and US. Thus, SCRs to unreinforced trials were used in the computational learning model analyses to estimate fear learning parameters of expected US probabilities and associability on the reinforced trials.

Computational Modeling

As in our previous study (Taylor et al., submitted), different computational learning models (Rescorla-Wagner, Rescorla-Wagner/Pearce-Hall hybrid (LePelley & McLaren, 2004)) were fitted to trial-by-trial SCR data to the different cues of the unreinforced trials (CS- and CS+50 unpaired). A Rescorla-Wagner model (RW model; in which learning occurs solely as a function of prediction errors), a RW/Pearce-Hall hybrid model (RW/PH hybrid), in which the expected probability of the US is estimated at each trial as a function of prediction errors with a learning rate that is dynamically modulated on a trial basis by an associability term. In this hybrid model, associability increases following trials with high prediction error and is therefore an index of uncertainty.

Learning Model Selection. Model fit to SCR data was assessed for each subject using Akaike Information Criteria (AIC), and Bayesian Information Criteria (BIC). Paired samples comparisons (Wilcoxon test, non-parametric) were performed on AIC and BIC, in order to compare fit indices between models. Model fits were superior for the RW/PH hybrid model compared to the other (p's < .05, AIC and BIC indices were smaller for the RW/PH hybrid model vs the RW model).

Model Description. In the model selected - Rescorla-Wagner/Pearce Hall hybrid model (Equations 1-3) - ***expected shock probabilities*** (‘*V’)* at each trial ‘*t*’ were updated as a function of the ***prediction error*** (δ) estimated on the trial preceding it. A constant

learning rate (***α***) modulated the rate at which prediction errors – (difference between the administered outcome (λ) on a trial, i.e. shock or absence of shock, and the expected outcome) – updated expected shock probabilities. Shock administration was coded as 1 and absence of US as 0. In addition, learning rates were dynamically modulated by an associability term (*a*). The associability term was modulated by a constant term (**γ**) and updated as a function of the prediction error's absolute value, referring to the outcome's surprising aspect (whether it be unexpected pain or unexpected pain omissions).

$$V_{t+1} = V_t + a_t * \alpha * \delta_t \quad \text{(Equation 1)}$$

$$\delta_t = \lambda_t - V_t \quad \text{(Equation 2)}$$

$$a_{t+1} = \gamma * |\delta_t| + (1 - \gamma) * a_t \quad \text{(Equation 3)}$$

Trial-by-trial expected shock probabilities/associability for each subject were computed from the following fixed parameters, i.e. the model's free parameters averaged across subjects, as previously recommended (Daw, 2011): $\alpha = 0.19$, $\gamma = 0.21$, $V_{0=} 0.35$, $a_{0=} 0.49$. Expected shock probabilities and associability related to each cue are shown in Figure 2.

Prediction of Pain Responses by Fear Conditioning Parameters and Moderation by Cortisol

The relation between AUCi and pain responses across all conditions was first assessed using a bivariate Pearson correlation. A one-tail $p < .05$ was used based on our previous observation of reduced pain in individuals with higher cortisol levels (Vachon-Presseau et al., 2013).

Expected probability of receiving a shock-US and associability at each CS+paired trial were entered into multi-level regression analyses to predict the magnitude of the unconditioned pain responses to the shock-US. The AUCi of cortisol output during fear conditioning was entered as a between subjects moderator of the relationship between fear learning parameters and pain. Two separate regression models were tested to predict changes in pain ratings and in the NFRs. These exploratory models were thresholded at $p < .05$ two-tailed.

Results

All of the analyses including the AUCi of the cortisol response were also conducted using the AUCg, to assess whether effects of cortisol on pain, fear conditioning, and fear-conditioned pain modulation were related to cortisol change during the experiment as opposed to basal cortisol levels. All of the tests conducted using the AUCg yielded non-significant results; thus, these are not discussed further and only results with respect to the AUCi are reported.

Cortisol and Pain Responses during Fear Conditioning

First, a one-way ANOVA on salivary cortisol levels sampled before and after the learning task (S2,S3,S4) did not reveal any significant increases in cortisol during the fear conditioning paradigm at the group level ($F_{(2,42)} = 1.01$, $p = 0.342$). This suggests that the task did not constitute a significant inducer of a stress response in this group.

Nevertheless, inter-individual variability was present among the salivary cortisol concentration sampled during the experiment. Thus, to index inter-individual differences in cortisol change during the experiment, the AUCi of the cortisol response was then computed as described in Equations 1-2, and was used to investigate interactions with anticipatory responses and pain during fear conditioning.

Pain ratings and NFRs measured across all trials during the task are shown in Figure 2. Mean pain ratings across trials were negatively correlated with cortisol AUCi during the fear conditioning task (r = -0.402, p = 0.003 Figure 3). No significant relation was found between cortisol and averaged RIII responses (r = 0.28, p = 0.206).

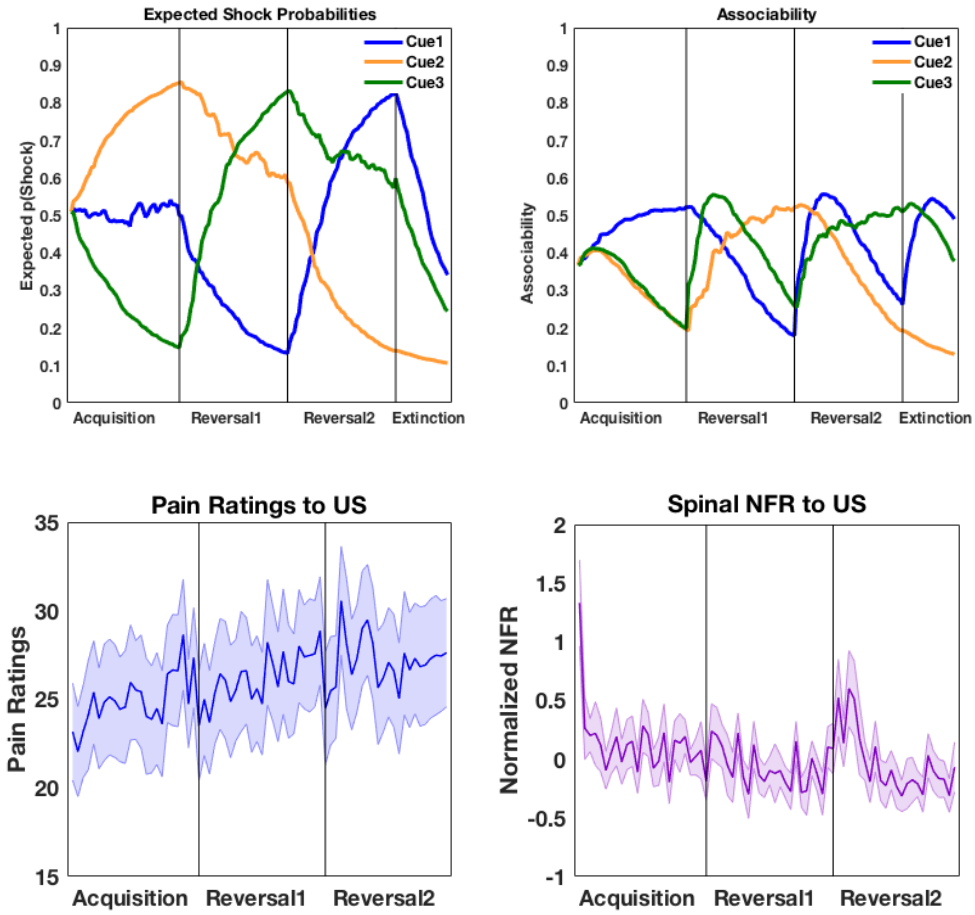

Figure 2. Expected probability of shock (expected p(shock)) and associability estimates obtained from the RW/Pearce-Hall hybrid computational reinforcement learning modeling throughout the acquisition, the two reversal, and extinction phases of the Pavlovian fear learning task. Cue1 (blue) corresponds to the CS50 in the acquisition phase, the CS- in reversal1, and CS100 in reversal2. Cue2 (orange) corresponds to the CS100 in the acquisition phase, the CS50 in reversal1, and the CS- in reversal2. Cue3 (green) corresponds to the CS- in the acquisition phase, the CS100 in reversal1, and CS50 in reversal2. Pain ratings (lower left panel) and NFRs (lower right panel) averaged across participants during the fear conditioning task.

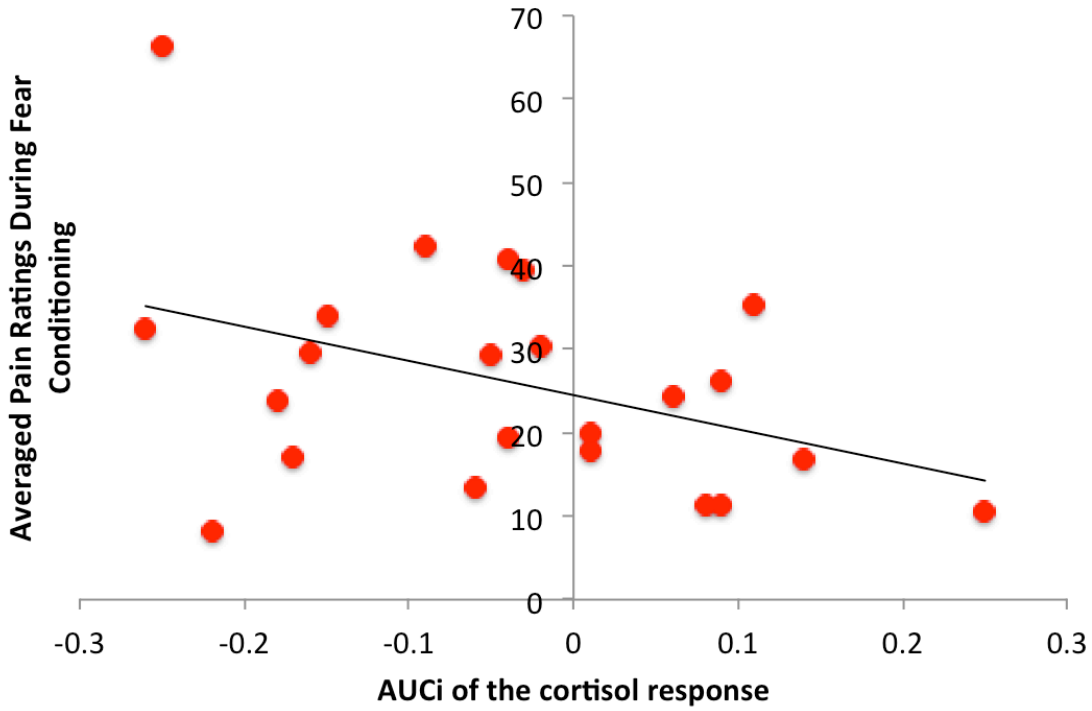

Figure 3. Correlation between cortisol levels (AUCi) and averaged pain ratings during the entire fear conditioning task.

Cortisol and Fear Conditioning

Multi-level analyses examining the difference in anticipatory SCRs between unreinforced CS50 and CS- revealed that SCRs to unreinforced CS50 were enhanced compared to CS- (*Beta* = 0.12, *STE* = 0.06, *t* = 2.14, *p* = 0.002). AUCi was entered as a between-subjects moderator, and did not reveal any significant relationship on the differential effects between CS50 and CS- (*Beta* = -0.11, *STE* = 0.31, *t* = -0.33, *p* = 0.789).

Pain responses and Contingency Type during Fear Conditioning

No main effect of cue contingency or interaction between learning phase and cue condition were found, as revealed by a repeated-measures Cue (CS50/CS100) X Learning phase (Acquisition Early/Acquisition Late/Reversal1 Early/Reversal1 Late/ Reversal2 Early/Reversal2 Late) ANOVA: main effect of cue: $F = 1.36$, $p = 0.26$, $F = 0.44$, $p = 0.52$ for pain ratings and NFRs respectively, cue x phase interaction: $F = 0.84$, $p = 0.52$, $F = 0.61$, $p = 0.69$ for pain ratings and NFRs respectively). Therefore, effects of fear learning on pain outcomes were further analyzed irrespective of cue-shock contingency type.

Effects of expected shock probabilities and associability on pain response

Multi-level analyses revealed a significant positive effect of EShock and associability, on reported pain (see Table 1 and Figure 4). In contrast, a significant negative effect of EShock and associability was found on spinal responses during fear conditioning.

Moderation of fear-induced pain modulation by AUCi

AUCi cortisol during fear conditioning was included as a 2nd-level moderator in the multi-level analyses conducted separately using pain ratings and NFRs as dependent variables (with expectations and associability as first-level predictors). These analyses revealed that higher cortisol AUCi was associated with a significant *reduction* in the inhibition of the NFR by EShock. Cortisol AUCi did not affect NFR modulation by associability and did not change effects of EShock or associability on pain ratings (Table1).

Table 1 Multi-level regression analysis on pain ratings and NFR scores predicted by fear learning parameters, and moderated by salivary cortisol levels (AUCi)

	Beta	*SE*	*t*	*p*
Dependent Variable: Pain Ratings to US				
LEVEL-1 Predictors				
Expected Shock(US) Probabilities	0.89	0.17	4.30	<0.001*
Associability	0.72	0.30	2.65	0.01*
LEVEL-2 Predictors				
Cortisol (AUCi): Moderation of effect of EShock on Pain Ratings	-1.36	1.44	-0.92	0.40
Cortisol (AUCi): Moderation of effect of Associability on Pain Ratings	1.12	2.07	0.55	0.58
Dependent Variable: NFR scores to US				
LEVEL-1 Predictors				
Expected Shock(US) Probabilities	-0.71	0.19	-3.91	<0.001*
Associability	-0.37	0.20	-1.57	0.04*
LEVEL-2 Predictors				
Cortisol (AUCi): Moderation of effect of EShock on NFRs	3.80	1.58	2.84	0.01*
Cortisol (AUCi): Moderation of effect of Associability on NFRs	0.57	1.70	-0.33	0.72

Notes. Significant effects of predictors are indicated on the graph with asterisks (*p<.05)

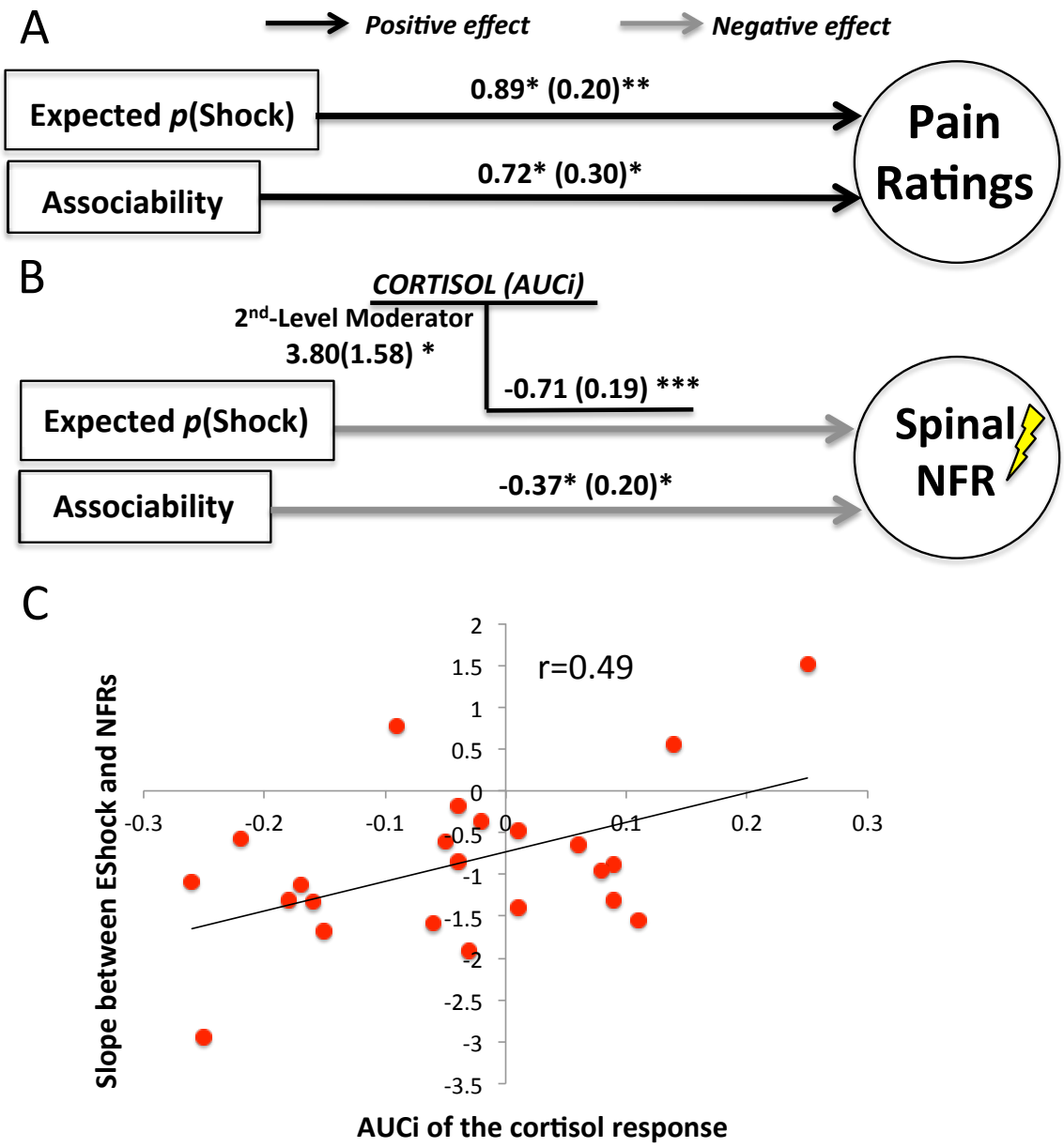

Figure 4. Multi-level models of the effects of expected probability of shock (expected p(shock)) and associability on pain outcomes. Path coefficients are shown with standard errors in parentheses. A) Expected probability of shock (expected p(shock)) had a positive effect on pain ratings. B) Expected probability of shock (expected p(shock)) had a negative effect on NFRs. C) Individual differences in cortisol levels (AUCi) decreased the negative impact of expected *p*(shock) on NFRs. *p<0.05

Relationship between AUCi and Self-reported Questionnaire Data Variables

Exploratory correlation analyses examining the relation between cortisol AUCi and personality traits were conducted. The only significant relationship found was a negative correlation with self-reported 'Non-reactivity' to experiences (r = -0.33, p = 0.01), a subscale of the FFMQ assessing dimensions of dispositional mindfulness. This finding indicates that individuals displaying higher HPA- axis reactivity during the experiment reported higher dispositional personality traits related to an emotionally reactive temperament.

Discussion

The results of the present study demonstrate that individual differences in cortisol change during fear conditioning have dissociable effects on higher-order pain responses and lower order spinal responses to nociceptive stimuli. However, the present study did not demonstrate a significant reactive cortisol response on average during the task; thus exposure to a fear conditioning paradigm involving painful stimuli may not constitute a substantial stressor for a detectable stress-response at the group level. Nevertheless, individual variability in task-concurrent cortisol change during fear conditioning was related to pain modulation and moderation of fear conditioning effects on pain. Thus, more positive (and/or less negative) changes in individual cortisol change during the task was globally associated with lower pain ratings but did not affect spinal responses to nociceptive stimuli. In contrast, individual differences in HPA reactivity moderated the effects of fear-conditioning on the NFR but not on pain.

First, the fact that an overall cortisol increase during fear conditioning was not observed does not replicate previous findings that an experiment using noxious stimulus administration elicits a cortisol stress response (Vachon-Presseau et al., 2013). In the study by Vachon-Presseau and colleagues (2013), it is possible that the functional magnetic resonance imagery scanner environment, in combination with pain administration, have consisted in a significant source of stress for participants. Other psychophysiological studies also failed to observe significant cortisol increase during fear conditioning protocols (Corbo, 2011; Zorawski et al., 2005), albeit using non-noxious stimuli as the US. Nevertheless, the inter-individual variability in cortisol change during

fear learning present among the present study's participants was related to pain modulation during fear conditioning.

The fact that individuals with greater cortisol output during fear learning reported decreased pain during fear conditioning directly replicates that of a previous study from our laboratory using thermal nociceptive stimuli (Vachon-Presseau et al., 2013). Therefore, this effect is likely reflected by cortico-cortical interactions between higher-order brain centres and cerebral targets of spino-thalamic pain transmission (Vachon-Presseau et al., 2013). On the other hand, the effects of individual changes in cortisol concentration on descending pain modulation occurred by altering the relationship between learning processes and pain transmission signals at the spinal cord. Indeed, for individuals with enhanced HPA axis reactivity, the inhibiting effect of learned expectations on defensive responses was *reduced.* A potential neurobiological mechanism of this effect is through HPA-induced activation of the hypothalamus and/or amygdala, with descending projections to brainstem nuclei inhibiting pain transmission at the dorsal horn of the spinal cord (Vachon-Presseau et al., 2013). It is possible that, in a context in which numerous inescapable threatening stimuli are administered, the organism 'conserves' defensive resources. Under this perspective, participants with enhanced cortisol output exhibited enhanced defensive responses to threat despite its inescapable nature.

Moreover, the results of this study demonstrating that expected probabilities of receiving pain positively predict perceived pain and negatively predict NFRs during fear learning are precisely in line with those from Martins and collaborators (2015). In this study, predictable noxious stimuli were rated as more painful consistent with a previous independent study from our laboratory (Taylor et al., In Press), but elicited decreased nocifensive spinal responses, compared to unpredictable noxious stimuli (Quelhas Martins, McIntyre, & Ring, 2015). This result is and may reflect underlying affective processes, i.e. that pain is more aversive when it is predictable but cannot be escaped.

By contrast, the gradual inhibition of defensive NFRs to US as stimuli became more predictable may reflect 'conservation' mechanisms of defensive resources in the face of inescapable pain. This latter effect of fear learning parameters on defensive NFRs is in the opposite direction as that previously reported: Taylor and collaborators (In Press) had found that fear learning parameters during Pavlovian fear learning predicted an *enhancement* of defensive NFRs. The discrepancy with the results observed here may reflect the different contexts between the two studies: approximately twice as many US were administered in the present task compared with that previously reported (Taylor et al., In Press). This is consistent with the notion of inverted-U shaped relationships between stress/glucocorticoids and several cognitive/affective functions (Lupien & McEwen, 1997). In other words, the Yerkes-Dodson law predicts that there is an inverted-U shaped function between arousal and performance on several cognitive functions: poor performance is observed under low levels of arousal, while optimal performance occurs at moderate arousal levels, and poor performance is observed under

very high levels of arousal (Yerkes & Dodson, 1908). The effect of fear learning parameters on NFRs may also present a comparable inverted U-shape relationship with context 'aversiveness': fear learning would predict *enhanced* defensive responding under moderately aversive contexts, while under higher threat, expected probabilities of receiving pain and associability would *diminish* the recruitment of defensive processes to preserve these resources.

In conclusion, the results of the present study demonstrate that inter-individual differences in HPA axis reactivity are related to an overall hypoalgesia at a higher-order level of pain processing. On the other hand, individual differences in HPA axis reactivity have a moderating influence over the impact of fear learning on spinal defensive responses to pain. These results contribute a novel piece of information to the understanding of the effects of fear learning on pain, and have clinically relevant implications concerning the moderating impact of individual predispositions to stress/fear related pathologies.

References

Bach, D. R., Flandin, G., Friston, K. J., & Dolan, R. J. (2010). Modelling event-related skin conductance responses. *Int J Psychophysiol, 75*(3), 349-356.

Baer, R. A., Smith, G. T., Hopkins, J., Krietemeyer, J., & Toney, L. (2006). Using self-report assessment methods to explore facets of mindfulness. *Assessment, 13*(1), 27-45.

Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A. R. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science, 269*(5227), 1115-1118.

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., & Erbaugh, J. (1961). An inventory for measuring depression. *Arch Gen Psychiatry, 4*, 561-571.

Brown, K. W., & Ryan, R. M. (2003). The benefits of being present: mindfulness and its role in psychological well-being. *J Pers Soc Psychol, 84*(4), 822-848.

Carver, C. S., & White, T. L. (1994). Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: The BIS/BAS Scales. *Journal of Personality and Social Psychology, 67*, 319-333.

Corbo, V. (2011). *Interaction of fear and stress: from healthy population samples to post-traumatic stress disorder.* (PhD), Université de Montréal, Montreal.

Daw, N. D. (2011). Trial-by-trial data analysis using computational models. In M. R. Delgado, E. A. Phelps, & T. W. Robbins (Eds.), *Decision Making, Affect, and Learning*

Attention & Performance XXIII (pp. 3-38). New York: Oxford University Press.

Fanselow, M. S. (1986). Conditioned fear-induced opiate analgesia: a competing motivational state theory of stress analgesia. *Ann N Y Acad Sci, 467*, 40-54.

Grant, J. A., & Rainville, P. (2009). Pain sensitivity and analgesic effects of mindful states in Zen meditators: a cross-sectional study. *Psychosom Med, 71*(1), 106-114.

Kabat-Zinn, J., Lipworth, L., & Burney, R. (1985). The clinical use of mindfulness meditation for the self-regulation of chronic pain. *J Behav Med, 8*(2), 163-190.

Kirschbaum, C., Kudielka, B. M., Gaab, J., Schommer, N. C., & Hellhammer, D. H. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosom Med, 61*(2), 154-162.

LaBar, K. S., LeDoux, J. E., Spencer, D. D., & Phelps, E. A. (1995). Impaired fear conditioning following unilateral temporal lobectomy in humans. *J Neurosci, 15*(10), 6846-6855.

LeDoux, J. E., & Phelps, E. A. (2008). Emotional Networks in the Brain. In M. Lewis, J. M. Haviland-Jones, & L. Feldman Barrett (Eds.), *Handbook of Emotions*. New York: The Guilford Press.

LePelley, M. E., & McLaren, I. P. (2004). Associative history affects the associative change undergone by both presented and absent cues in human causal learning. *J Exp Psychol Anim Behav Process, 30*(1), 67-73.

Lupien, S. J., & McEwen, B. S. (1997). The acute effects of corticosteroids on cognition: integration of animal and human model studies. *Brain Res Brain Res Rev, 24*(1), 1-27.

Mumford, J. A., Turner, B. O., Ashby, F. G., & Poldrack, R. A. (2012). Deconvolving BOLD activation in event-related designs for multivoxel pattern classification analyses. *Neuroimage, 59*(3), 2636-2643.

Price, D. D. (2000). Psychological and neural mechanisms of the affective dimension of pain. *Science, 288*(5472), 1769-1772.

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology, 28*(7), 916-931.

Quelhas Martins, A., McIntyre, D., & Ring, C. (2015). Aversive event unpredictability causes stress-induced hypoalgesia. *Psychophysiology, 52*(8), 1066-1070.

Quirk, G. J., Garcia, R., & Gonzalez-Lima, F. (2006). Prefrontal mechanisms in extinction of conditioned fear. *Biol Psychiatry, 60*(4), 337-343.

Schutze, R., Rees, C., Preece, M., & Schutze, M. (2010). Low mindfulness predicts pain catastrophizing in a fear-avoidance model of chronic pain. *Pain, 148*(1), 120-127.

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Sullivan, M. J. L., Bishop, S. R., & Pivik, J. (1995). The Pain Catastrophizing Scale: Development and Validation. *Psychological assessment, 7*(4), 524-532.

Taylor, V. A., Chang, L., Rainville, P., & Roy, M. (In Press). Learned Expectations and Uncertainty Facilitate Pain Processing During Classical Conditioning. *Pain*.

Vachon-Presseau, E., Martel, M. O., Roy, M., Caron, E., Albouy, G., Marin, M. F., et al. (2013). Acute stress contributes to individual differences in pain and pain-related brain activity in healthy and chronic pain patients. *J Neurosci, 33*(16), 6826-6833.

Willer, J. C. (1977). Comparative study of perceived pain and nociceptive flexion reflex in man. *Pain, 3*(1), 69-80.

Yerkes, R. M., & Dodson, J. D. (1908). The relation of strength of stimulus to rapidity of habit-formation. *Journal of Comparative Neurology and Psychology, 18*, 459-482.

Zeidan, F., Gordon, N. S., Merchant, J., & Goolkasian, P. (2010). The effects of brief mindfulness meditation training on experimentally induced pain. *J Pain, 11*(3), 199-209.

General Discussion

In this section, we will integrate the findings obtained in the empirical studies of this thesis, and discuss their meaning in terms of the current state of knowledge on fear conditioning and pain modulation systems (at a behavioral and neuroanatomical level), as well as variables moderating these relationships. We will also discuss the relevance of these findings in terms of clinical implications with respect to the fear avoidance model of chronic pain.

Modulation of Pain by fear learning parameters

The results of these studies reveal that parameters governing fear conditioning processes at each trial of learning predict pain outcome measures. Indeed, the results of Study 1 reveal that associability and expected shock probabilities positively predict pain ratings and defensive spinal responses to noxious electrical shocks. The effects of fear learning parameters on pain at a higher-order level of processing are in part direct, and in part mediated by facilitation at a spinal level. This reveals two important mechanisms involved in the modulation of pain by fear learning processes: 1- direct effects on pain perception likely reflect interactions between higher order centres and targets of the spinothalamic tract. These interactions are potentially reflected by signals encoding prediction errors coming from the amygdala to the dorsal MPFC (McNally et al., 2011), and then projected onto pain-processing areas, such as the ACC (Price, 2000). Second, the partial mediation of higher-order pain perceptual responses by spinal responses likely

reflects descending controls from the amygdala to brainstem sites, modulating nociceptive transmission at the spinal cord (Flor & Turk, 1999). These changes are then relayed through the ascending spinothalamic tract to brain centers involved in pain perception (Price, 2000).

Importantly, the results of study 1 reveal that the modulation of pain by fear learning processes is an effect that can be observed at a very fine timescale, i.e. at the level of a single trial. These results, therefore, provide a novel approach to analyze pain outcomes for future studies investigating the modulatory effects of learning on pain.

Evidence for fear-pain cycle involved in the central maintenance of pain

The results of study1 also emphasize the fact that the modulation of pain by fear learning is a cyclic process that can contribute to the central maintenance of pain, and may explain pathological manifestations (eg. chronic pain, anxiety, depression) resulting from repeated pain exposure. Indeed, following the *first* trial of a learning phase, we observed a sharp increase in pain and spinal NFRs, likely due to the fact that both expected shock probabilities and associability were elevated. Indeed, the first cue-shock pairing likely causes the most 'surprising' effect that largely contributes to the learning process at the beginning of the acquisition phase. Following this sharp pain enhancement it is therefore quite difficult for pain ratings to return to a baseline level, and to break from the initial effects of fear on pain. The data from this study show that breaking the fear-pain cycle may constitute an effective way to prevent central 'escalation' and maintenance of pain

resulting from repeated exposure to noxious stimuli. These results support the efficacy of exposure therapies targeting the fear-pain cycle to achieve significant treatment improvement in pathologies related to fear and pain (anxiety disorders, chronic pain) (Bailey, Carleton, Vlaeyen, & Asmundson, 2010).

Inter-individual variability in the effects of fear learning on pain

The results of Study1 also reveal that there is inter-individual variability in the effects of fear learning on pain. Indeed, participants with greater anxious tendencies, such as those reporting more pain catastrophizing, trait anxiety, punishment sensitivity, harm avoidance, and less trait mindfulness (a principal component analysis factor which we named 'harm vigilance'), showed an enhanced relationship between associability and defensive reflex responses to pain. This shows that, for these individuals, pain enhancement from vigilance to the CS (associability) is more strongly mediated by spinal responses. This effect may explain why certain individuals, i.e. those who tend to interpret pain as more threatening and who are less mindful of present-moment awareness, are more at risk for developing pathological manifestations from repeated pain exposure (Bailey et al., 2010).

The predictive relationship between associability and cerebro-spinal processing of nociceptive input is stronger in these individuals, and the cycle may be more difficult to break. Indeed, participants with the opposite tendencies, i.e. emotional detachment, show the opposite effect, i.e. that of a reduced predictive effect of associability on NFRs. This

trait may therefore constitute a protective factor against the instilment of central pain maintenance from repeated exposure. This finding indicates that the development of this trait, notably from the practice of mindfulness meditation, could constitute a means of breaking the fear-pain cycle and prevent or attenuate pathological manifestations from fear learning effects on pain. Our finding validates and encourages clinical interventions integrating aspects of contemplative practices to improve mental health in the context of pain.

The results of Study2 also support the notion that the practice of mindfulness meditation contributes to braking the fear-pain cycle. Indeed, experienced mindfulness meditators not only showed a general hypoalgesia at a higher-order level of processing (pain ratings), but showed that perceived pain was not under the influence of fear learning. Contrary to individuals reporting enhanced emotional detachment in study1, meditators did not show a decreased predictive relationship between associability and spinal NFRs. It is important to note that controls in Study 1 were meditation-naïve participants, and that the mere trait associated with states cultivated via mindfulness meditation could have distinct effects than the long-term practice of meditation.

Figure 1 illustrates a hypothetical model through which mindfulness meditation practice influences pain perception through effects of fear learning systems. This figure shows that nociception leads to pain perception and fear learning. In turn, fear learning modulates pain directly and indirectly through facilitation of spinal nociception.

Mindfulness would have a moderating role in this fear-pain cycle by reducing the effects of fear learning on pain directly, at a higher-order level.

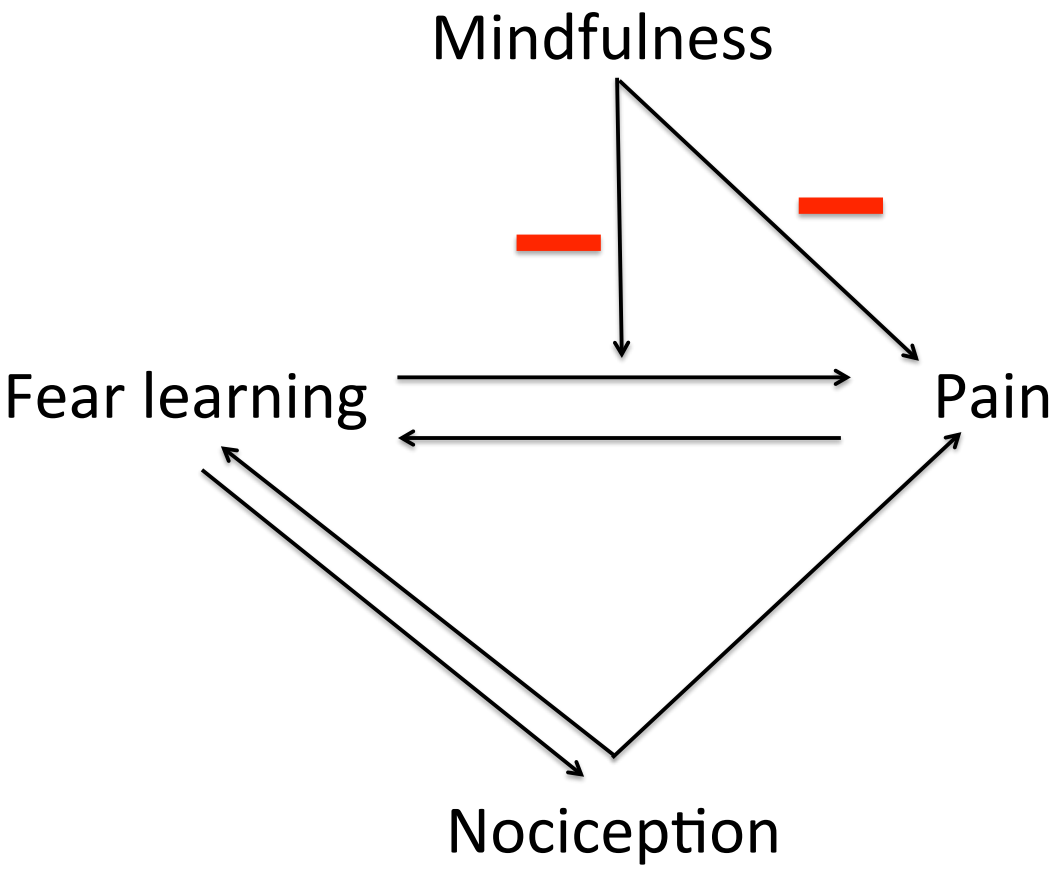

Figure 1. Hypothetical model of interactions between fear learning and pain systems through which mindfulness meditation modulates pain perception. Our results show that mindfulness meditation experience is associated with reduced pain perception and reduced impact of fear learning on pain.

The effects we observed in Study 2 were specific to higher-order pain processing, and consistent with previous studies on the effects of long-term meditation experience on pain or emotionally aversive experiences (Grant et al., 2011; Grant & Rainville, 2009; Taylor et al., 2011). Indeed, and consistent with the philosophy of mindfulness meditation, brain imaging studies support the notion that long-term experience is not associated with a voluntary inhibition of aversive emotions. Rather, mindfulness would induce a *disinhibition* of control systems (eg. prefrontal cortex) regulating aversive experiences, supported by a decrease in prefrontal activity associated with executive control (Gard et al., 2012; Grant et al., 2011). During a mindful state of awareness, aversive experiences are voluntarily acknowledged as opposed to voluntarily changed, reflected by enhanced brain activity in sensory/affective pain processing (Grant et al., 2011; Grant & Rainville, 2009; Taylor et al., 2011). Paradoxically, this stance of welcoming and openness to the experience results in dissipation or dampening of the aversive sensation along with any other event occupying the field of awareness (Schutze et al., 2010). This is potentially due to the reduction of catastrophizing cognitions/feelings about pain (Schutze et al., 2010), in the sense that individuals higher in trait mindfulness interpret pain as less threatening, which in turn reduces their pain evaluations.

In our study, we show that the practice of mindfulness meditation is quite adaptive in the processing of repeated pain: experienced meditators preserved fear learning mechanisms to detect threat signals from the environment and adaptive defense mechanisms to pain. However, the aversive quality and higher-order 'suffering' resulting from noxious events

was attenuated. This attenuation could have positive impacts in terms of 'liberating' cognitive/affective resources to deal with threat more effectively.

The results of Study 2, therefore, provide scientific support for interventions using mindfulness meditation to treat clinical pathologies related to fear and pain. These results support the hypothesis that the effects of mindfulness alter the connections between higher-order brain centres and the amygdala, which would then activate descending pain controls at the spinal cord. Our results also suggest that meditation would reduce any supplementary cortico-cortical interactions between higher order brain centres involved in secondary pain affect (eg. fear of meaning of pain in terms of the future) and targets of spinothalamic pain transmission (eg.: ACC).

Finally, the results of Study 3 indicate that HPA-axis activity modulates pain differently at higher-order vs lower-order levels of processing. At a higher-order level of processing, pain attenuation related to stress hormone output is directly in line with previous results (Vachon-Presseau et al., 2013) and does not depend or interact with fear learning processes. By contrast, modulation at a spinal level of nociceptive transmission is facilitatory and operates via fear learning systems. Individuals with enhanced cortisol output during fear learning showed a more positive relationship between learned expected pain and defensive responses.

In terms of fear learning effects on pain, the results of Study 3 replicated the results of Study 1 in terms of learning effects on pain perception (positive predictive relationship

between fear learning parameters and pain ratings). By contrast with the results from Study 1, participants exhibited a negative relationship between fear learning parameters and NFRs. This contrasting result between studies may result from the different paradigms between the two studies, and in a context in which there is enhanced exposure to inescapable threat (approximately twice as many noxious stimuli were administered in Study 3), the organism *preserves* defensive response resources. This explanation could be akin to the Yerkes-Dodson theory which purports that several behaviors are optimally performed under moderate levels of arousal (Yerkes & Dodson, 1908). Levels of arousal under, or over this optimal level, would result in reduced behavioral performance (Yerkes & Dodson, 1908). Thus, the number of US presented in the context of Study1 may have induced moderate levels of arousal, and a positive relationship between fear learning and pain. In contrast, the enhanced number of US administrated in Study3 may have induced increased arousal for participants, and yielded a relationship in the opposite direction. This negative relationship between expected shock probabilities and associability in a context of high inescapable threat may reflect the organism's attempt to preserve defensive resources.

Thus, individuals with enhanced HPA-axis reactivity would show enhanced defensive responding in a context of inescapable threat. Pain modulation related to stress hormone output would therefore yield global reduced pain perception and an enhanced defensive response, potentially to more effectively cope with threat (Fanselow, 1986). Though beneficial on an 'acute' short-term basis - hypoalgesia and enhanced defensive responding - induced from stress systems could 'deplete' the organism's defensive

resources in contexts in which fight/flight responses are not adaptive due to the inescapable nature of threat. Figure 2 shows a hypothetical functional model through which HPA-axis activation would be related to modulation of pain and nociception with the involvement of fear learning mechanisms.

Nonetheless, in Study3 there was a lack of an overall cortisol stress response as was previously observed in a study from our laboratory (Vachon-Presseau et al., 2013). In this study we have noticed that the overall cortisol increase was not observed in all subjects, but the inter-individual variability in cortisol change during fear conditioning was related to pain and effects of fear on pain. Therefore, we cannot conclude that these effects are due to a reactive stress response, but conclusions can be drawn to the fact that there is an involvement of the HPA-axis in the effects of fear conditioning on pain. Future studies should therefore examine the role of stress hormones following an experimental stressor on the effects of fear learning pain. In addition, studies administering exogenous glucocorticoids should be conducted to delineate whether a causal relationship between HPA-axis activation exists with the effects of fear learning on pain.

Due to the divergent effects of fear learning on spinal NFRs obtained between Studies 1 and 3, it is important to note aspects differing between studies as well as rationales underlying the implementation of such differences. Indeed, a different experimental paradigm was used in Study 3 compared to Study 1: Study 3 included a stimulus conditioned with the US on 100% of trials, in addition to the cue paired with the shock on 50% of trials. The task also involved two reversals as opposed to one reversal in Study 1.

The decision to apply these changes to the paradigm in Study 3 was taken to obtain an increased range of expected shock probabilities as that of Study 1 by including the CS100 condition. In addition, since pain outcomes in Study 1 were importantly modulated at reversal onsets, a second reversal was included in Study 3's paradigm.

Finally, another discrepancy between Studies 1 and 3 were the instructions given to participants prior to completing the learning task: participants in Study1 had received no prior specific knowledge of task structure/contingencies (they were simply told that they would see images, and may or may not receive stimulations), while participants in Study3 were told that they would see images (which may or may not be followed by a stimulation), and that they may notice a relationship between a cue and the presentation of a shock. They were also told that the relationship between images and the shock should remain the same across trials but may (or may not) be subject to change throughout the task. This change in the explicit nature of instructions between Studies 1 and 3 was applied due to the fact that not all participants of Study 1 reported being aware of task contingencies (assessed using a post-experiment interview performed after the fear conditioning task). In order to maximize learning in Study3 to study its effects on pain modulation, and to ensure that subjects paid sufficient attention to conditioned cues, we opted to make instructions more explicit before the learning task in Study3.

While previous reports have found that instructed learning enhances learning performance relative to uninstructed learning (Atlas, Doll, Li, Daw, & Phelps, 2016; Li, Delgado, & Phelps, 2011), discriminatory SCRs were found for both Study1 and Study3,

and the trial-by-trial changes in parameters confirmed robust learning processes in both experiments. Nonetheless, it remains a possibility that instructions have altered fear learning processes and parameter estimation, although previous work shows that this would have only *optimized* the learning process as well as the estimation of fear learning parameters (Atlas et al., 2016). It is also possible that the discrepant instructions have accounted for discrepant effects of fear on NFRs between studies. For instance, enhanced readiness or explicit knowledge about the task could explain the negative relationship between NFRs and learning parameters, consistent with evidence that fear rather than apprehensive anxiety decreases spinal nociception (Rhudy et al 2000). This hypothesis is speculative until the effects of instructions on fear learning effects on pain are compared between groups having received explicit instructions and those not having received explicit prior knowledge of task structure using identical fear conditioning tasks.

Limitations and Future Directions

The studies presented within this thesis are not without limitations. First, the cross-sectional and quasi-experimental design used in Study 2 does not allow for inferences to be made as to causal effects of mindfulness meditation practice. Thus, other factors characteristic to the meditation practitioners may have played a role in the effects observed (eg. lifestyle, individual traits). Future prospective studies are needed using mindfulness meditation interventions to examine effects of fear conditioning on pain.

Another limitation is with respect to the computational analyses used in our studies. The computational models of behavior provide estimations of hidden states (expectations, associability) governing behavior. The advantage of these methods is that they permit an understanding of explaining behavior using quantifiable variables; nonetheless, these remain estimations from calculations using mathematical laws and the subject's associative learning history at each trial (shock / absence of shock). Though these latent variables have been shown to reflect neural activity in key brain regions underlying reward and fear (Boll et al., 2013; Li, Schiller, et al., 2011; McNally et al., 2011), they still remain an indirect reflection of hidden cognitive and neural processes. Future studies should examine brain function underlying pain modulation during fear conditioning using brain imaging techniques such as functional magnetic resonance imaging (fMRI) or electroencephalography (EEG) to examine whether our fear learning parameters obtained in our studies would be associated with striatal and amygdala activity as preceding reports have found (Boll et al., 2013; Li, Schiller, et al., 2011; McNally et al., 2011; Zhang et al., 2016). It would also be interesting to examine whether the latent fear learning parameters obtained in our studies correlate with corresponding self-reported expectations (at each trial) and attention (eg. by incorporating a dot-probe attentional paradigm (Posner, 1980) during cue presentation of the conditioning task to assess attentional engagement). Although adding behavioral measurements to a classical task may alter the natural process of acquiring and updating fear learning parameters from classical conditioning, this would reflect the best compromise from available methods to investigate hidden underlying cognitive processes during learning.

Moreover, while computational models are informative of human behavior, the complexity and specificity of a model to explain variance in a given dataset may counteract its generalizability. Therefore, it is also necessary to replicate our results in other participant samples to assess the generalizability of the computational models used here in explaining the fear learning effects we observed on pain. The possibility also exists that other models than those used in the present studies explain fear learning effects on pain. Therefore, future studies should also examine different computational approaches, such as Bayesian models of decision-making (Daunizeau et al., 2010), to estimate latent variables acquired during classical conditioning and assess their effects on pain.

Future studies should also investigate the effect of context aversiveness on pain modulation during fear conditioning. This would allow to test whether the different results obtained in Study 3 with respect to effects of expected shock probabilities on spinal nociception are due to context aversiveness. For example, testing pain modulation during fear conditioning in contexts of different aversive levels could consist in either administering both conditioning tasks (one task with CS- and CS50, another with CS100/CS50/CS-) to the same subjects.

Study 2 was also limited with respect to the sample size for the group of experienced meditators. However, the reduced power due to the small number of meditator subjects would not have been problematic to accurately detect significant group differences in the absence of such effects, but would have rather impeded the ability to detect significant

group differences when real effects may have been present. Thus, in instances in which we observed non-significant group differences, Bayesian analyses were conducted to support the null hypothesis, yielding enhanced odds in support for the null hypothesis of no differences between groups. Our results should remain tentative, however, until replicated in larger sample sizes.

Finally, our results put forth neuropsychophysiological evidence that latent variables governing fear learning dynamically modulate pain, and validate clinical models explaining maintenance of pain/suffering in disorders involving classical conditioning. Therefore, future studies should assess the effects of fear learning on pain in clinical populations (anxiety disorders, chronic pain) to examine whether effects of fear learning on pain are heightened in these individuals. In addition, studies examining fear learning effects on pain using mindfulness interventions are needed in such clinical populations to determine the efficacy of acceptance-based treatments in breaking the cycle between fear and pain/suffering.

Conclusion

In conclusion, the results presented in this doctoral thesis support the notion that fear learning has considerable modulatory effects on lower-order and higher-order pain processing. In addition, the present results also showed that fear learning modulatory effects on pain are observable at a trial-level timescale. These effects appear to operate in part directly on pain, thus potentially reflecting cortico-cortical interactions between higher-order brain centres and targets of spinothalamic pain transmission. The present

findings also show that fear learning effects also occur in part indirectly on pain by facilitating spinal nociceptive transmission.

Moreover, the results of these studies put forth a potential model explaining the central chronicization of pain as a result of repeated exposure. We also showed that certain individual factors moderate the impact of fear learning on pain: personality traits related to harm vigilance and mindfulness meditation, enhanced HPA activation, and long-term mindfulness meditation experience. Thus, the results of this thesis contribute to the understanding of disorders involving classical conditioning. Moreover, the present studies provide scientific evidence for the therapeutic use of practices cultivating mindful dispositions/attitudes in breaking the fear-suffering cycle and preventing or treating maladaptive consequences (pain chronicization, anxiety) resulting from repeated threat exposure.

References

Armfield, J. M., Stewart, J. F., & Spencer, A. J. (2007). The vicious cycle of dental fear: exploring the interplay between oral health, service utilization and dental fear. *BMC Oral Health, 7*, 1.

Atlas, L. Y., Doll, B. B., Li, J., Daw, N. D., & Phelps, E. A. (2016). Instructed knowledge shapes feedback-driven aversive learning in striatum and orbitofrontal cortex, but not the amygdala. *Elife, 5*.

Bach, D. R., Flandin, G., Friston, K. J., & Dolan, R. J. (2010). Modelling event-related skin conductance responses. *Int J Psychophysiol, 75*(3), 349-356.

Baer, R. A. (2003). Mindfulness training as a clinical intervention: a conceptual andempirical review. *Clin. Psychol. Sci. Pract., 10*(2), 125-143.

Baer, R. A., Smith, G. T., Hopkins, J., Krietemeyer, J., & Toney, L. (2006). Using self-report assessment methods to explore facets of mindfulness. *Assessment, 13*(1), 27-45.

Bailey, K. M., Carleton, R. N., Vlaeyen, J. W., & Asmundson, G. J. (2010). Treatments addressing pain-related fear and anxiety in patients with chronic musculoskeletal pain: a preliminary review. *Cogn Behav Ther, 39*(1), 46-63.

Baliki, M. N., Geha, P. Y., Fields, H. L., & Apkarian, A. V. (2010). Predicting value of pain and analgesia: nucleus accumbens response to noxious stimuli changes in the presence of chronic pain. *Neuron, 66*(1), 149-160.

Beaulieu, P. (2013). *La Douleur - Guide pharmacologique et thérapeutique*. Montréal: Les Presses de l'Université de Montréal.

Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A. R. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science, 269*(5227), 1115-1118.

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., & Erbaugh, J. (1961). An inventory for measuring depression. *Arch Gen Psychiatry, 4*, 561-571.

Bishop, R. S. (2004). Mindfulness: a proposed operational definition. . *Clinical Psychological Science, 11*(3), 230-241.

Bodhi, B. (2005). *In the Buddha's Words*. Boston: Wisdom Publications.

Boll, S., Gamer, M., Gluth, S., Finsterbusch, J., & Buchel, C. (2013). Separate amygdala subregions signal surprise and predictiveness during associative fear learning in humans. *Eur J Neurosci, 37*(5), 758-767.

Borsook, D., Maleki, N., Becerra, L., & McEwen, B. (2012). Understanding migraine through the lens of maladaptive stress responses: a model disease of allostatic load. *Neuron, 73*(2), 219-234.

Boucsein, W. (2012). *Electrodermal Activity*. New York: Springer.

Brown, C. A., & Jones, A. K. (2010). Meditation experience predicts less negative appraisal of pain: electrophysiological evidence for the involvement of anticipatory neural responses. *Pain, 150*(3), 428-438.

Brown, K. W., & Ryan, R. M. (2003). The benefits of being present: mindfulness and its role in psychological well-being. *J Pers Soc Psychol, 84*(4), 822-848.

Cacioppo, J., Tassinary, L. G., & Berntson, G. G. (2007). *Handbook of Psychophysiology*. New York: Cambridge University Press.

Carver, C. S., & White, T. L. (1994). Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: The BIS/BAS Scales. *Journal of Personality and Social Psychology, 67*, 319-333.

Cloninger, C. R. (1994). Temperament and personality. *Curr Opin Neurobiol, 4*(2), 266-273.

Corbo, V. (2011). *Interaction of fear and stress: from healthy population samples to post-traumatic stress disorder.* (PhD), Université de Montréal, Montreal.

Crombez, G., Eccleston, C., Van Damme, S., Vlaeyen, J. W., & Karoly, P. (2012). Fear-avoidance model of chronic pain: the next generation. *Clin J Pain, 28*(6), 475-483.

Daunizeau, J., den Ouden, H. E., Pessiglione, M., Kiebel, S. J., Stephan, K. E., & Friston, K. J. (2010). Observing the observer (I): meta-bayesian models of learning and decision-making. *PLoS One, 5*(12), e15554.

Daw, N. D. (2011). Trial-by-trial data analysis using computational models. In M. R. Delgado, E. A. Phelps, & T. W. Robbins (Eds.), *Decision Making, Affect, and Learning*

Attention & Performance XXIII (pp. 3-38). New York: Oxford University Press.

Fanselow, M. S. (1986). Conditioned fear-induced opiate analgesia: a competing motivational state theory of stress analgesia. *Ann N Y Acad Sci, 467*, 40-54.

Fields, H. L., & Basbaum, A. I. (2006). Central nervous system mechanisms of pain modulation. In S. McMahon & M. Koltzenburg (Eds.), *Textbook of Pain*. Edinburgh, UK: Elsevier Churchill Livingstone.

Flor, H., & Turk, D. (1999). Cognitive and Learning Aspects. In P. D. Wall & R. Melzack (Eds.), *Textbook of Pain* (pp. 256-272). Edinburgh: Churchill Livingstone.

Gallistel, C. R. (2009). The importance of proving the null. *Psychol Rev, 116*(2), 439-453.

Gard, T., Holzel, B. K., Sack, A. T., Hempel, H., Lazar, S. W., Vaitl, D., et al. (2012). Pain attenuation through mindfulness is associated with decreased cognitive control and increased sensory processing in the brain. *Cereb Cortex, 22*(11), 2692-2702.

Grant, J. A., Courtemanche, J., & Rainville, P. (2011). A non-elaborative mental stance and decoupling of executive and pain-related cortices predicts low pain sensitivity in Zen meditators. *Pain, 152*(1), 150-156.

Grant, J. A., & Rainville, P. (2009). Pain sensitivity and analgesic effects of mindful states in Zen meditators: a cross-sectional study. *Psychosom Med, 71*(1), 106-114.

Grossman, P., Tiefenthaler-Gilmer, U., Raysz, A., & Kesper, U. (2007). Mindfulness training as an intervention for fibromyalgia: evidence of postintervention and 3-year follow-up benefits in well-being. *Psychother Psychosom, 76*(4), 226-233.

Hebb, D. O. (1949). *The Organization of Behavior*. New York: Wiley & Sons.

Holzel, B. K., Brunsch, V., Gard, T., Greve, D. N., Koch, K., Sorg, C., et al. (2016). Mindfulness-Based Stress Reduction, Fear Conditioning, and The Uncinate Fasciculus: A Pilot Study. *Front Behav Neurosci, 10*, 124.

Jackson, E. D., Payne, J. D., Nadel, L., & Jacobs, W. J. (2006). Stress differentially modulates fear conditioning in healthy men and women. *Biol Psychiatry, 59*(6), 516-522.

Jeffreys, H. (1961). *Theory of probability, 3rd ed.* Oxford, England: Oxford University Press.

Jensen, K., Kirsch, I., Odmalm, S., Kaptchuk, T. J., & Ingvar, M. (2015). Classical conditioning of analgesic and hyperalgesic pain responses without conscious awareness. *Proc Natl Acad Sci U S A, 112*(25), 7863-7867.

Kabat-Zinn, J., Lipworth, L., & Burney, R. (1985). The clinical use of mindfulness meditation for the self-regulation of chronic pain. *J Behav Med, 8*(2), 163-190.

Keng, S. L., Smoski, M. J., & Robins, C. J. (2011). Effects of mindfulness on psychological health: a review of empirical studies. *Clin Psychol Rev, 31*(6), 1041-1056.

Kim, J. J., & Jung, M. W. (2006). Neural circuits and mechanisms involved in Pavlovian fear conditioning: a critical review. *Neurosci Biobehav Rev, 30*(2), 188-202.

LaBar, K. S., LeDoux, J. E., Spencer, D. D., & Phelps, E. A. (1995). Impaired fear conditioning following unilateral temporal lobectomy in humans. *J Neurosci, 15*(10), 6846-6855.

LeDoux, J. E., & Phelps, E. A. (2008). Emotional Networks in the Brain. In M. Lewis, J. M. Haviland-Jones, & L. Feldman Barrett (Eds.), *Handbook of Emotions*. New York: The Guilford Press.

LePelley, M. E., & McLaren, I. P. (2004). Associative history affects the associative change undergone by both presented and absent cues in human causal learning. *J Exp Psychol Anim Behav Process, 30*(1), 67-73.

Li, J., Delgado, M. R., & Phelps, E. A. (2011). How instructed knowledge modulates the neural systems of reward learning. *Proc Natl Acad Sci U S A, 108*(1), 55-60.

Li, J., Schiller, D., Schoenbaum, G., Phelps, E. A., & Daw, N. D. (2011). Differential roles of human striatum and amygdala in associative learning. *Nat Neurosci, 14*(10), 1250-1252.

Lupien, S. J., & McEwen, B. S. (1997). The acute effects of corticosteroids on cognition: integration of animal and human model studies. *Brain Res Brain Res Rev, 24*(1), 1-27.

Lutz, A., McFarlin, D. R., Perlman, D. M., Salomons, T. V., & Davidson, R. J. (2013). Altered anterior insula activation during anticipation and experience of painful stimuli in expert meditators. *Neuroimage, 64*, 538-546.

Lutz, A., Slagter, H. A., Dunne, J. D., & Davidson, R. J. (2008). Attention regulation and monitoring in meditation. *Trends Cogn Sci, 12*(4), 163-169.

McCracken, L. M., & Eccleston, C. (2005). A prospective study of acceptance of pain and patient functioning with chronic pain. *Pain, 118*(1-2), 164-169.

McCracken, L. M., Gauntlett-Gilbert, J., & Vowles, K. E. (2007). The role of mindfulness in a contextual cognitive-behavioral analysis of chronic pain-related suffering and disability. *Pain, 131*(1-2), 63-69.

McNally, G. P., Johansen, J. P., & Blair, H. T. (2011). Placing prediction into the fear circuit. *Trends Neurosci, 34*(6), 283-292.

Merz, C. J., & Wolf, O. T. (2017). Sex differences in stress effects on emotional learning. *J Neurosci Res, 95*(1-2), 93-105.

Montgomery, G. H., & Kirsch, I. (1997). Classical conditioning and the placebo effect. *Pain, 72*(1-2), 107-113.

Morone, N. E., Greco, C. M., & Weiner, D. K. (2008). Mindfulness meditation for the treatment of chronic low back pain in older adults: a randomized controlled pilot study. *Pain, 134*(3), 310-319.

Mumford, J. A., Turner, B. O., Ashby, F. G., & Poldrack, R. A. (2012). Deconvolving BOLD activation in event-related designs for multivoxel pattern classification analyses. *Neuroimage, 59*(3), 2636-2643.

Park, H. J., & Friston, K. (2013). Structural and functional brain networks: from connections to cognition. *Science, 342*(6158), 1238411.

Pavlov, I., & Anrep, G. V. (1927). *Conditioned reflexes; an investigation of the physiological activity of the cerebral cortex*. London: Oxford University Press.

Pavlov, I., & Anrep, G. V. (1927). *Conditioned reflexes; an investigation of the physiological activity of the cerebral cortex.* . London: Oxford University Press.

Pearce, J. M., & Hall, G. (1980). A model for Pavlovian learning: variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychol Rev, 87*(6), 532-552.

Perlman, D. M., Salomons, T. V., Davidson, R. J., & Lutz, A. (2010). Differential effects on pain intensity and unpleasantness of two meditation practices. *Emotion, 10*(1), 65-71.

Posner, M. I. (1980). Orienting of attention. *Q J Exp Psychol, 32*(1), 3-25.

Price, D. D. (2000). Psychological and neural mechanisms of the affective dimension of pain. *Science, 288*(5472), 1769-1772.

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology, 28*(7), 916-931.

Quelhas Martins, A., McIntyre, D., & Ring, C. (2015). Aversive event unpredictability causes stress-induced hypoalgesia. *Psychophysiology, 52*(8), 1066-1070.

Quirk, G. J., & Beer, J. S. (2006). Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. *Curr Opin Neurobiol, 16*(6), 723-727.

Quirk, G. J., Garcia, R., & Gonzalez-Lima, F. (2006). Prefrontal mechanisms in extinction of conditioned fear. *Biol Psychiatry, 60*(4), 337-343.

Rainville, P. (2011). *La Lettre de l'Institut USPA de la douleur*. Paper presented at the 10è conférence internationale François Boureau de l'Institut USPA de la douleur dans le cadre du congrès annuel de la SFETD, Marseille, Parc Chanot.

Rainville, P. (2013). Pain and the Emotional Responses to Noxious Stimuli *The Cambridge Handbook of Human Affective Neuroscience*. New York, USA: Cambridge University Press.

Rescorla, R. A., & Wagner, A. R. (1972). A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement. In A. H. Black & W. F. Prokasy (Eds.), *Classical Conditioning II: Current Research and Theory* (pp. 64-99). New York, NY: Appleton Century Crofts.

Rhudy, J. L., & France, C. R. (2007). Defining the nociceptive flexion reflex (NFR) threshold in human participants: a comparison of different scoring criteria. *Pain, 128*(3), 244-253.

Rhudy, J. L., Williams, A. E., McCabe, K. M., Rambo, P. L., & Russell, J. L. (2006). Emotional modulation of spinal nociception and pain: the impact of predictable noxious stimulation. *Pain, 126*(1-3), 221-233.

Roy, M., Piche, M., Chen, J. I., Peretz, I., & Rainville, P. (2009). Cerebral and spinal modulation of pain by emotions. *Proc Natl Acad Sci U S A, 106*(49), 20900-20905.

Roy, M., Shohamy, D., Daw, N., Jepma, M., Wimmer, G. E., & Wager, T. D. (2014). Representation of aversive prediction errors in the human periaqueductal gray. *Nat Neurosci, 17*(11), 1607-1612.

Ruttledge, R. B., Skandali, N., Dayan, P., & Dolan, R. J. (2015a). A computational and neural model of momentary subjective well-being. *Proc Natl Acad Sci U S A, 111*(33), 12252-12257.

Ruttledge, R. B., Skandali, N., Dayan, P., & Dolan, R. J. (2015b). Dopaminergic Modulationo of Decision Making and Subjective Well-Being. *The Journal of Neuroscience, 35*(27), 9811-9822.

Sandrini, G., Serrao, M., Rossi, P., Romaniello, A., Cruccu, G., & Willer, J. C. (2005). The lower limb flexion reflex in humans. *Prog Neurobiol, 77*(6), 353-395.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev, 21*(1), 55-89.

Schiller, D., Levy, I., Niv, Y., LeDoux, J. E., & Phelps, E. A. (2008). From fear to safety and back: reversal of fear in the human brain. *J Neurosci, 28*(45), 11517-11525.

Schutze, R., Rees, C., Preece, M., & Schutze, M. (2010). Low mindfulness predicts pain catastrophizing in a fear-avoidance model of chronic pain. *Pain, 148*(1), 120-127.

Seymour, B., O'Doherty, J. P., Dayan, P., Koltzenburg, M., Jones, A. K., Dolan, R. J., et al. (2004). Temporal difference models describe higher-order learning in humans. *Nature, 429*(6992), 664-667.

Seymour, B., O'Doherty, J. P., Koltzenburg, M., Wiech, K., Frackowiak, R., Friston, K., et al. (2005). Opponent appetitive-aversive neural processes underlie predictive learning of pain relief. *Nat Neurosci, 8*(9), 1234-1240.

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Sullivan, M. J., & D'Eon, J. L. (1990). Relation between catastrophizing and depression in chronic pain patients. *J Abnorm Psychol, 99*(3), 260-263.

Sullivan, M. J. L., Bishop, S. R., & Pivik, J. (1995). The Pain Catastrophizing Scale: Development and Validation. *Psychological assessment, 7*(4), 524-532.

Tabachnick, B. G., & Fidell, L. S. (2007). *Using Multivariate Statistics, 6th Edition*. Boston: Pearson.

Taylor, V. A., Chang, L., Rainville, P., & Roy, M. (In Press). Learned Expectations and Uncertainty Facilitate Pain During Classical Conditioning. *Pain*.

Taylor, V. A., Grant, J., Daneault, V., Scavone, G., Breton, E., Roffe-Vidal, S., et al. (2011). Impact of mindfulness on the neural responses to emotional pictures in experienced and beginner meditators. *Neuroimage, 57*(4), 1524-1533.

Tracey, I., & Mantyh, P. W. (2007). The cerebral signature for pain perception and its modulation. *Neuron, 55*(3), 377-391.

Tracey, I., Ploghaus, A., Gati, J. S., Clare, S., Smith, S., Menon, R. S., et al. (2002). Imaging attentional modulation of pain in the periaqueductal gray in humans. *J Neurosci, 22*(7), 2748-2752.

Vachon-Presseau, E., Martel, M. O., Roy, M., Caron, E., Albouy, G., Marin, M. F., et al. (2013). Acute stress contributes to individual differences in pain and pain-related brain activity in healthy and chronic pain patients. *J Neurosci, 33*(16), 6826-6833.

Viane, I., Crombez, G., Eccleston, C., Poppe, C., Devulder, J., Van Houdenhove, B., et al. (2003). Acceptance of pain is an independent predictor of mental well-being in patients with chronic pain: empirical evidence and reappraisal. *Pain, 106*(1-2), 65-72.

Vlaeyen, J. W., & Linton, S. J. (2000). Fear-avoidance and its consequences in chronic musculoskeletal pain: a state of the art. *Pain, 85*(3), 317-332.

Wager, T. D., Rilling, J. K., Smith, E. E., Sokolik, A., Casey, K. L., Davidson, R. J., et al. (2004). Placebo-induced changes in FMRI in the anticipation and experience of pain. *Science, 303*(5661), 1162-1167.

Wall, P., & Melzack, R. (2006). *Textbook of Pain*. Philadelphia, USA: Elsevier Limited.

Weber, B., Jermann, F., Lutz, A., Bizzini, L., & Bondolfi, G. (2012). [Mindfulness-based therapeutic approaches: benefits for individuals suffering from pain]. *Rev Med Suisse, 8*(347), 1395-1398.

Willer, J. C. (1977). Comparative study of perceived pain and nociceptive flexion reflex in man. *Pain, 3*(1), 69-80.

Yerkes, R. M., & Dodson, J. D. (1908). The relation of strength of stimulus to rapidity of habit-formation. *Journal of Comparative Neurology and Psychology, 18*, 459-482.

Zeidan, F., Gordon, N. S., Merchant, J., & Goolkasian, P. (2010). The effects of brief mindfulness meditation training on experimentally induced pain. *J Pain, 11*(3), 199-209.

Zeidan, F., Martucci, K. T., Kraft, R. A., Gordon, N. S., McHaffie, J. G., & Coghill, R. C. (2011). Brain mechanisms supporting the modulation of pain by mindfulness meditation. *J Neurosci, 31*(14), 5540-5548.

Zhang, S., Mano, H., Ganesh, G., Robbins, T., & Seymour, B. (2016). Dissociable Learning Processes Underlie Human Pain Conditioning. *Curr Biol, 26*(1), 52-58.

Zorawski, M., Blanding, N. Q., Kuhn, C. M., & LaBar, K. S. (2006). Effects of stress and sex on acquisition and consolidation of human fear conditioning. *Learn Mem, 13*(4), 441-450.

Zorawski, M., Cook, C. A., Kuhn, C. M., & LaBar, K. S. (2005). Sex, stress, and fear: individual differences in conditioned learning. *Cogn Affect Behav Neurosci, 5*(2), 191-201.