When do placebo and nocebo work? The role of time on placebo analgesia and nocebo hyperalgesia.

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Doctoral Thesis Abstract

Abstract in English

Verbal suggestions are strong modulators of one's expectations and they can be used to induce placebo and nocebo responses. Research so far has investigated the magnitude (i.e. stronger or weaker) and the direction (i.e. increase or decrease of pain) of verbal suggestions, while no attention has been given to the dimension of time. Relying on three main experiments, which investigated the influence of temporal verbal suggestions in modulating the onset of action of placebo analgesia and nocebo hyperalgesia, this thesis seeks to address this shortcoming.

In Study 1, pain was induced experimentally on healthy participants via short-lasting, medium-to-low intensity electrical stimuli. After each noxious stimulus participants rated their pain from 0 (no pain) to 10 (unbearable pain). Participants were assigned to one of three placebo groups, three nocebo groups, a no expectancy (NE) group, or a natural history (NH) group. An inert cream was administered to all participants, except from those in the NH group, while different verbal suggestions were given according to group allocation. Participants in the placebo groups were told that the cream had analgesic properties setting in after 5 (Placebo Group 5, P5), 15 (Placebo Group 15, P15) and 30 (Placebo Group 30, P30) minutes from cream application. Participants in the nocebo groups were told that the cream had hyperalgesic properties setting in after 5 (Nocebo Group 5, N5), 15 (Nocebo Group 15, N15) and 30 (Nocebo Group 30, N30) minutes from cream application. Participants in the NE group were told that the cream only

had hydrating properties and that would not influence pain perception, while those in the NH group did not receive the cream and served to control for pain natural fluctuations over time. Participants repeated the pain test at baseline, after 10, 20 and 35 minutes after the cream application. Mixed-method analysis of variance showed a significant interaction between group and time, indicating that pain ratings varied between time-points and between groups. As expected, post hoc comparisons revealed that placebo and nocebo groups began to show a significant change in pain ratings than the NE group at the expected time point but not earlier. Interestingly, once triggered, the analgesic effect remained stable over time, while the hyperalgesic effect increased over time.

In Study 2 and 3, the influence of temporal suggestions on placebo analgesia (Study 2) and nocebo hyperalgesia (Study 3) onset was investigated using a long lasting, high-intensity, tonic pain model, induced with the Cold Pressor Test (CPT). Heart Rate (HR) was measured to assess whether it correlated with placebo analgesia and nocebo hyperalgesia. In Study 2, participants were assigned to one of two placebo groups, or to the No Expectations (NE) group. In Study 3, participants were allocated to one of two nocebo groups, while the control group (NE) was taken from the previous study (Study 2). In this case participants also received an inert cream and those in the placebo groups were told that the cream had analgesic properties that would set in after 5 (placebo 5, P5) and 30 (placebo 30, P30) minutes from its application. Participants in the nocebo groups were told that the cream had hyperalgesic properties setting in after 5 (nocebo 5, P5) and 30 (nocebo 30, N30) minutes from application, while those in the NE group were told that the cream only had hydrating properties. All the participants repeated the CPT at baseline and after 10 and 35 minutes from cream application. Percentage change in exposure time (pain tolerance) from baseline to Test 10 ($\Delta 10$) and to test 35 ($\Delta 35$) and changes in HR during

CPT were compared between the three groups. In both studies, data were non-parametric and non-parametric statistics were used accordingly. In Study 2, $\Delta 10$ was greater in P5 than in NE and P30, indicating analgesia only in the group expecting cream early onset effect. $\Delta 35$ was greater in P5 and P30 compared to NE, showing a delayed onset of analgesia in P30 and maintained analgesia in P5. The same results, but in the opposite direction, were reported in Study 3, where hyperalgesia onset followed the temporal verbal suggestions that participants received. HR differences between groups were not significant in Study 2 nor 3.

In conclusion, the experiments demonstrated that both placebo analgesia and nocebo hyperalgesia follow the temporal information provided. In addition, it was shown that once triggered, both placebo analgesia and nocebo hyperalgesia endure over time (at least for the duration of the experimental session). These data apply to experimentally induced pain both of a phasic nature with medium-low intensity and of a tonic nature, reaching high intensities. The important role of verbal suggestions in modulating the onset of action of a given (inert-) intervention could not only aid the clinical use of placebo treatment (e.g., in open-label placebo), but also support the efficacy of active drugs. Indeed, further research is needed to extend these results from healthy participants to patients and from placebos to active interventions.

Abstract in Italian

I suggerimenti verbali sono forti modulatori delle aspettative e possono essere utilizzati per indurre risposte placebo e nocebo. Finora sono state indagate le dimensioni della grandezza (es. suggerimenti più o meno forti) e della direzione (es. aumento o diminuzione del sintomo clinico) delle suggestioni verbali, mentre

ancora non ha ricevuto attenzione la dimensione del tempo. Il dottorato è stato quindi mirato ad indagare l'influenza delle suggestioni verbali, caratterizzate temporalmente, nel modulare l'insorgenza dell'analgesia da placebo e dell'iperalgesia da nocebo. A questo fine sono stati condotti tre esperimenti.

Nello Studio 1, il dolore è stato indotto sperimentalmente su partecipanti sani tramite stimoli elettrici di breve durata e di intensità medio-bassa. Dopo ogni stimolo nocicettivo i partecipanti hanno valutato il loro dolore da 0 (nessun dolore) a 10 (dolore insopportabile). I partecipanti sono stati distribuiti in 8 gruppi: tre gruppi placebo, tre gruppi nocebo, un gruppo no aspettativa (NE) o un gruppo di storia naturale (NH). Una crema inerte è stata somministrata a tutti i partecipanti, ad eccezione di quelli del gruppo NH che aveva il solo scopo di controllare le fluttuazioni naturali del dolore nel tempo. A seconda del gruppo di appartenenza sono state date specifiche informazioni verbali . A coloro che sono stati assegnati ai gruppi placebo è stato detto che la crema aveva una azione analgesica che si sarebbe manifestata dopo 5 (Gruppo Placebo 5, P5), 15 (Gruppo Placebo 15, P15) e 30 (Gruppo Placebo 30, P30) minuti dall'applicazione. Ai partecipanti dei gruppi nocebo è stato detto che la crema aveva una azione iperalgesizzante che si sarebbe manifestata dopo 5 (Nocebo Gruppo 5, N5), 15 (Nocebo Gruppo 15, N15) e 30 (Nocebo Gruppo 30, N30) minuti. A coloro che sono stati assegnati al gruppo NE è stato detto che la crema aveva solo proprietà idratanti e che non avrebbe influenzato la percezione del dolore. Tutti i partecipanti hanno ripetuto il test del dolore prima dell'applicazione della crema (test basale) e dopo 10, 20 e 35 minuti. L'analisi della varianza con metodo misto ha mostrato un'interazione significativa tra gruppo e tempo, indicando che le valutazioni del dolore variavano tra i test in diversi punti temporali e tra i gruppi. Come ipotizzato, i confronti post hoc hanno rivelato che i gruppi placebo e nocebo hanno iniziato a mostrare un cambiamento significativo nelle valutazioni del dolore rispetto al gruppo NE

al momento coincidente con l'aspettativa temporale di ciascun gruppo ma non prima. È interessante notare che, una volta attivato l'effetto iperalgesico è progressivamente aumentato, mentre l'effetto analgesico è rimasto stabile nel tempo.

Nello Studio 2 e 3, è stata studiata l'influenza delle suggestioni temporali sull'insorgenza dell'analgesia da placebo (Studio 2) e dell'iperalgesia da nocebo (Studio 3) utilizzando un modello di dolore tonico di lunga durata, ad alta intensità, indotto con il Cold Pressor Test (CPT). La frequenza cardiaca (FC) è stata misurata per valutare se fosse correlata con le risposte placebo e nocebo. Nello Studio 2, i partecipanti sono stati assegnati a uno di due gruppi placebo o al gruppo No Aspettativa (NA). Nello Studio 3, i partecipanti sono stati assegnati a uno di due gruppi nocebo, mentre per il gruppo di controllo (NE) è stato utilizzato quello dello studio precedente (Studio 2). Anche in questo caso, i partecipanti hanno ricevuto una crema inerte. Ai partecipanti dei gruppi placebo è stato detto che la crema aveva proprietà analgesiche che si sarebbero manifestate dopo 5 (placebo 5, P5) e 30 (placebo 30, P30) minuti dalla sua applicazione. Ai partecipanti dei gruppi nocebo è stato detto che la crema aveva proprietà iperalgesiche che si sarebbero instaurate dopo 5 (nocebo 5, P5) e 30 (nocebo 30, N30) minuti dall'applicazione, mentre ai partecipanti del gruppo NE è stato detto che la crema aveva solo proprietà idratanti . Tutti i partecipanti hanno ripetuto il CPT prima dell'applicazione della crema (test basale) e dopo 10 e 35 minuti. La variazione percentuale del tempo di esposizione (tolleranza al dolore) dal test basale al test 10 (Δ10) e al test 35 (Δ35) e le variazioni della FC durante il CPT sono stati confrontati tra i tre gruppi. In entrambi gli studi, i dati sono risultati non parametrici e di conseguenza sono state adottate analisi statistiche non parametriche. Nello studio 2, il Δ10 era maggiore in P5 rispetto a NE e P30, indicando analgesia solo nel gruppo che si aspettava un effetto di insorgenza precoce della crema. Δ35 era maggiore in P5 e P30 rispetto a NE, mostrando

un'insorgenza ritardata dell'analgesia in P30 e un'analgesia mantenuta in P5. Gli stessi risultati, ma nella direzione opposta, sono stati conseguiti nello Studio 3, in cui l'insorgenza dell'iperalgesia appariva essere in linea con i suggerimenti temporali riferiti ai partecipanti. Non sono state riportate differenze significative in termini di FC, né nello Studio 2 né nello Studio 3.

In conclusione, è stato dimostrato che sia l'analgesia da placebo che l'iperalgesia da nocebo seguono le informazioni temporali fornite. È inoltre stato dimostrato che una volta innescate, sia l'analgesia da placebo che l'iperalgesia da nocebo persistono nel tempo (almeno per la durata della sessione sperimentale). Questo fenomeno sembra essere presente sia nel dolore indotto sperimentalmente di natura fasica con intensità medio-bassa che in quello tonico, con intensità elevata. L'importante ruolo dei suggerimenti verbali nel modulare l'inizio dell'efficacia del trattamento, in questo caso una sostanza inerte, potrebbe non solo potenziare l'uso clinico del trattamento con placebo (ad esempio, somministrazione *'open-label'* placebo), ma soprattutto supportare e ottimizzare l'efficacia dei farmaci attivi. Sarebbero a tal scopo necessarie ulteriori ricerche, per validare questi risultati su pazienti e su interventi attivi.

Abstract in Dutch

Het gebruik van suggestieve taal kan iemands verwachtingen sterk regelen. Suggestief taalgebruik wekt placebo- en nocebo reacties op. De omvang en richting van suggestief taalgebruik op deze reacties werd reeds onderzocht, terwijl er geen aandacht werd besteed aan het tijdsaspect. Deze tekortkoming wordt in dit proefschrift aangepakt. Het doel van dit proefschrift is het onderzoeken van de invloed van tijdsafhankelijk suggestief taalgebruik op de start van pijnstilling (placebo) en pijnopwekking (nocebo). Hiervoor zijn drie experimenten uitgevo-

erd.

In het eerste onderzoek werd pijn experimenteel opgewekt bij gezonde deelnemers via kortdurende elektrische prikkels van gemiddelde tot lage intensiteit. Na elke schadelijke prikkel beoordeelden de deelnemers hun pijn op een schaal van 0 (geen pijn) tot 10 (ondraaglijke pijn). Deelnemers werden toegewezen aan een van de drie placebogroepen, aan een van de drie nocebo-groepen, aan een groep zonder verwachting of een groep met natuurlijk beloop. Een niet-werkzame zalf werd toegediend aan alle deelnemers, behalve aan de deelnemers in de groep met natuurlijk beloop, terwijl verschillende verbale suggesties werden gegeven volgens de groepstoewijzing. Deelnemers in de placebogroepen werd verteld dat de zalf pijnstillende zou werken na 5, na 15 en na 30 minuten na het aanbrengen van de zalf. Deelnemers in de nocebo-groepen werd verteld dat de zalf pijn zou opwekken na 5, na 15 en na 30 minuten na het aanbrengen van de zalf. Deelnemers in de groep zonder verwachting werd verteld dat de zalf enkel hydrateerde en dat dit de pijnbeleving niet zou beïnvloeden. De deelnemers in de groep met natuurlijk beloop kregen de zalf niet en dienden om natuurlijke pijnschommelingen in de loop van de tijd te bestuderen. Deelnemers beoordeeelden hun pijn bij de start van het experiment, na 10, na 20 en na 35 minuten na het aanbrengen van de zalf. Variantieanalyse toonde een significante interactie aan tussen groep en tijd, wat aangeeft dat pijnscores veranderden in de tijd en tussen de groepen. Zoals verwacht lieten post-hoc vergelijkingen zien dat de placebo- en nocebo groepen een significante verandering in pijn vertoonden ten opzichte van de groep zonder verwachting op het verwachte tijdstip, maar niet eerder. Interessant is dat het pijnstillende effect, eenmaal geactiveerd, stabiel bleef in de tijd, terwijl het pijnopwekkende effect in de loop van de tijd toenam.

In het tweede en derde onderzoek werd de invloed van tijdsafhankelijke verbale suggesties op placebo-pijnstilling (onderzoek 2) en nocebo-pijnopwekking

(onderzoek 3) onderzocht met behulp van een langdurige, zeer intense koudwater proef. De hartslag werd gemeten om te beoordelen of deze samenhing met placebo-pijnstilling en nocebo-pijnopwekking. In het tweede onderzoek werden deelnemers toegewezen aan een van de twee placebogroepen of aan een groep zonder verwachtingen (controlegroep). In het derde onderzoek werden deelnemers toegewezen aan een van de twee nocebo-groepen, met als controlegroep de groep zonder verwachtingen uit het tweede onderzoek. Ook in dit geval kregen de deelnemers een niet-werkzame zalf. De proefpersonen in de placebogroepen vernamen dat de zalf de pijn zou verminderen na 5 en na 30 minuten na het aanbrengen. Degenen in de nocebo-groepen werd verteld dat de zalf pijn zou opwekken na 5 en na 30 minuten na het aanbrengen. Degenen in de controlegroep kregen te horen dat de zalf enkel hydrateerde. Alle deelnemers herhaalden de koudwaterproef bij aanvang, na 10 en na 35 minuten na het aanbrengen van de zalf. Procentuele veranderingen in pijntolerantie na 10 en na 35 minuten en veranderingen in de hartslag tijdens de koudwaterproef werden vergeleken tussen de drie groepen. In het tweede onderzoek was de pijnstilling na 10 minuten groter in vergelijking met de controlegroep en zij die pijnstilling verwachtten na 30 minuten. Dit wijst op pijnstilling die enkel optreedt in de groep die een vroeg begineffect van de zalf verwacht. Bij alle deelnemers was de pijnstilling na 35 minuten groter in vergelijking met de controlegroep. Dit wijst op een een vertraagd begin van pijnstilling bij wie pijnstilling na 30 minuten verwacht. Het laat ook zien dat pijnstilling zich handhaaft in de tijd bij wie vroege pijnstilling verwacht. Dezelfde resultaten, maar in de tegenovergestelde richting, werden gevonden in het derde onderzoek. De start van pijnopwekking volgde de tijdsafhankelijke verbale suggesties die de deelnemers ontvingen. In beide onderzoeken werden geen verschillen in hartslag tussen de groepen gevonden.

Samengevat werd er aangetoond dat bij gezonde deelnemers zowel placebo-

pijnstilling als nocebo-pijnopwekking de verstrekte tijdsafhankelijke informatie volgen. Dit gold zowel voor pijn van gemiddelde tot lage intensiteit als voor pijn met hoge intensiteit. Bovendien werd aangetoond dat eenmaal geactiveerd, zowel pijnstilling als pijnopwekking in de loop van de tijd aanhouden (tenminste voor de duur van de experimentele proef). De belangrijke rol van suggestief taalgebruik bij het regelen van het begin van de actie van een bepaalde (niet-actieve) interventie zou niet alleen het klinische gebruik van placebobehandelingen kunnen helpen, maar ook de werkzaamheid van actieve geneesmiddelen kunnen ondersteunen. Er is inderdaad meer onderzoek nodig om deze resultaten uit te breiden naar patiënten en actieve interventies.

Abbreviations

ALE: Activation Likelihood Estimation

AD: Alzheimer Disease

ACC: Anterior cingulate

AI: Anterior Insula

ACTH: Adrenocorticotropic hormone

ANOVA: Analysis of variance

BAI: Beck Anxiety Inventory

BD: Behavioral Domain

BIS/BAS: Behavioural Inhibition/Approach scales

BMI: Body mass index

BOLD: Blood OxygenLevel Dependent

CCK: Cholecystokinin

CONSORT: Consolidated Standards of Reporting Trials

CPT: Cold Pressor Test

ECG: Electrocardiography

F: Female

F: Functional Connectivity

fMRI: Functional magnetic resonance imaging

FPQ: Fear of Pain Questionnaire

HPA: Hypothalamic-pituitary-adrenal axis

HR: Heart rate

IC: Insular cortex

IQR: Interquartile range

L: Left

M: Male

mPFC: Medial prefrontal corte

NAc: Nucleus Accumbens

NE: No expectations

NH: Natural history

NRS: Numerical Rating Scale

N5: Nocebo 5

N15: Nocebo 15

N30: Nocebo 30

OFC: Orbitofrontal cortex

PAG: Periaqueductalgray

PET: Positronemission tomography

PFC: Prefrontal cortices

P5: Placebo 5

P15: Placebo 15

P30: Placebo 30

Q1: First quartile

Q3: Third quartile

R: Right

RLOT: Life-Orientation Test-Revisited

SD: Standard Deviation

SPSS: Statistical Package for Social Sciences

STAI I/ STAI II: State-Trait Anxiety Inventory (I-II)

SNK: Student-Newman Keuls

S1, S2: Secondary somatosensory areas

vmPFC: Ventro-medial prefrontal cortex

VTA: Ventral Tegmental Area

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Chapter 1

Introduction

1.1 Placebo and nocebo effects: A brief overview

The term *placebo* (Latin word for 'I shall please') refers to an intervention that resembles an active medical treatment but is not. The classical sugar pill without active agent, a sham surgical procedure or a sham injection, are all good examples of placebos [1]. The *placebo effect* is a phenomenon in which amelioration of symptoms follows the administration of the inert (placebo) treatment [2]. The term *nocebo* (Latin word for 'I shall harm') was introduced to describe the case in which negative effects follow the administration of these sham interventions. Accordingly, the *nocebo effect* is a phenomenon in which the administration of an inert substance is followed by negative side effects [3].

The positive and negative outcomes that may follow the administration of placebos and nocebos are not to be attributed to the substances themselves but to the psychosocial context in which these are given. Such psychosocial context includes verbal suggestions delivered to the patient, social cues, medical setting characteristics, the features of the treatment (e.g. whether the treatment is more or less invasive) and specific features of the patient (e.g. previous experience,

mindset, pre-existing expectations, age and cognitive impairments) [4; 5] and of the doctor (i.e., professionalism and appearance) (See Figure 1.1) [6]. A placebo effect is likely to occur if the active compound of the treatment is replaced by a sham intervention while the psychosocial factors remain the same influencing one's mind and body as if the active treatment had been given [7]. The contextual factors are internalised by the patient, eliciting specific emotions and contributing to the formation of either positive or negative expectations of therapeutic outcome which then result in placebo and nocebo responses, respectively [6].

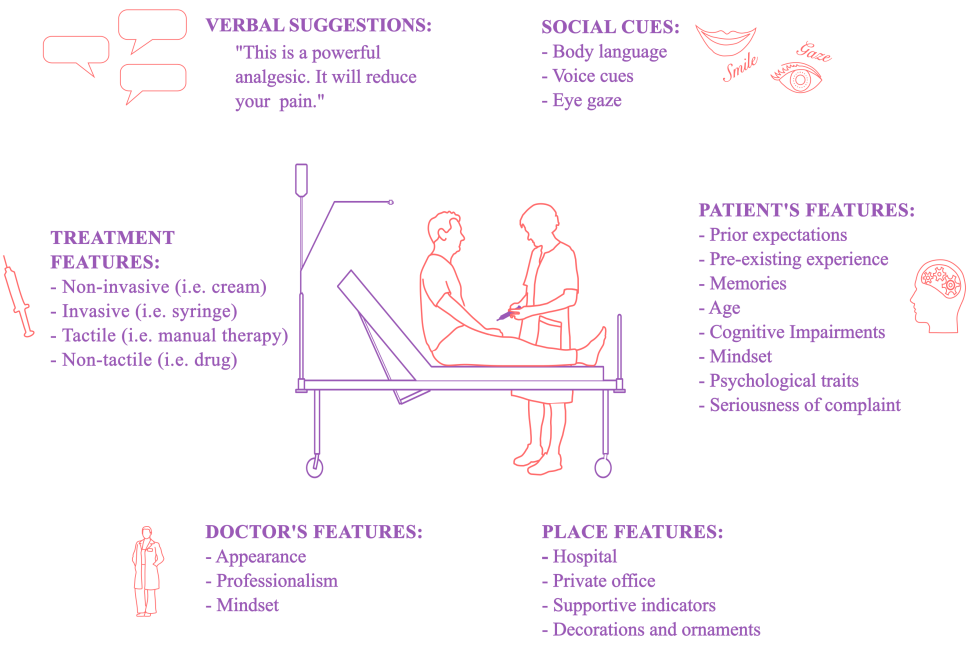

Figure 1.1: The psychosocial context in the clinical setting.

Expectations have been extensively studied and are, arguably, the main factor influencing placebo and nocebo phenomena, playing such a crucial role that the term 'expectation effect' is often used interchangeably with 'placebo effect' or 'placebo response' [7; 8]. Expectations are at the core of this doctoral thesis and will be discussed in more detail in Section 1.2.2. Factors within the psychoso-

cial context, such as verbal suggestions, directly trigger expectation formation, whereas learning mechanisms, namely classical conditioning [1] and social observational learning, are more complex processes and their effect can either trigger or bypass expectation formation [10; 11; 12; 13] (See Figure 1.2).

In 2003, Benedetti and colleagues demonstrated that placebo response following classical conditioning is mediated by expectation [11]. Here, participants were assessed on pain perception after heat pain stimulation and they were preconditioned for two consecutive days with ketorolac, an antiinflammatory drug that produces pain reduction. On the third day, participants received a placebo instead of ketorolac, along with suggestions of pain reduction (analgesia) in one group and of pain increase (hyperalgesia) in the other. Those who received suggestions of analgesia reported pain reduction as if they had received the active intervention. Interestingly, those who received suggestions of hyperalgesia reported pain increase, despite analgesia pharmacological preconditioning. The nocebo response after ketorolac preconditioning shows that both placebo and nocebo effects following conditioning are meditated by expectations rather than being the mere consequence of pharmacological preconditioning per se [11]. Further studies have shown that conditioning can be used as an 'expectation enhancer'. For instance, expectations of analgesia can be reinforced by the administration of an active painkiller in the conditioning phase, providing evidence for the truthfulness of verbal suggestions of analgesia. Such boosted expectations are maintained even when, in the post-conditioning phase, the analgesic intervention is replaced with a placebo, supporting the enhanced placebo response. Accordingly, placebo

[1] Classical conditioning, or Pavlovian conditioning, is a learning mechanism in which the repeated association between one stimulus, the *conditioned stimulus*, and another stimulus, the *unconditioned stimulus*, leads to a learnt *conditioned response* that occurs even in the absence of the *unconditioned stimulus* [9]. Accordingly, placebo and nocebo responses are the *conditioned responses* resulting from the learnt association between the*conditioned stimulus*(i.e. swallowing of the pill) and the *unconditioned stimulus* (i.e. active agent inside the pill) [2]

responses preceded by conditioning are stronger compared to placebo responses preceded by expectation modulation alone [14; 15]. It is noteworthy that, in the same aforementioned study, Benedetti et al. (2003) [11], showed a different role of expectations when investigating hormonal secretion, both growth hormone (GH) and cortisol. For two consecutive days, participants were preconditioned with sumatriptan, a 5-HT1B/1D agonist that stimulates GH and inhibits cortisol secretion. On the third day, placebo was administered instead of sumatriptan, and opposite verbal suggestions were given. Some participants were told they were receiving the same drug that would increase GH and reduce cortisol secretion, while others were told they were receiving a different compound that would lead to the opposite effect, decrease of GH and increase of cortisol. In both groups, GH and cortisol secretion occurred as if sumatripan was administered. Thus, hormonal secretion was in line with verbal suggestions in one case, and in opposition to verbal suggestions in the other, indicating that the occurring placebo response was the consequence of pharmacological preconditioning *per se* rather than being mediated by expectations [11]. In line with these findings it would appear that expectations play a greater role on cognitive mediated processes, like pain, while these become less influential on unconscious and automatic processes such as hormonal secretion [11].

Social observational learning has also been shown to directly influence expectations, acting as an 'expectations enhancer' [12]. Social learning refers to the learning process by which the observer modifies their behaviour or belief on the basis of the behaviour or belief of the demonstrator [16].

Although it has been demonstrated that social learning leads to stronger placebo responses compared to when expectation are triggered by verbal suggestions alone [13; 17], the extent to which expectations are responsible for such stronger responses is yet to be clarified [13]. In fact, social observational learning

is likely to have a unique role, interacting and working alongside other cognitive and emotional components such as empathy [18]. Accordingly, individual degree of empathy has been shown to positively correlate with the magnitude of the placebo response following social observational learning [13].

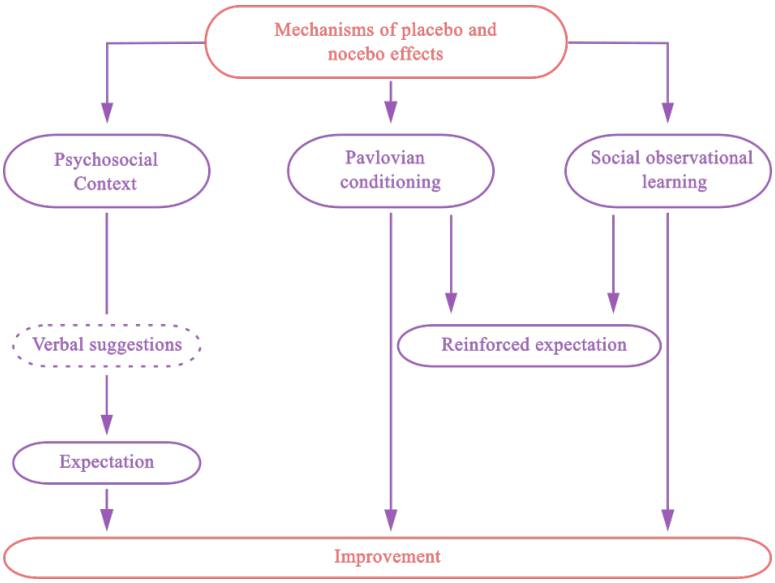

Figure 1.2: Mechanisms of placebo and nocebo effects

When we talk about placebo, we are referring to the administration of an inert intervention. However, research has shown that the influence of expectations on therapeutic outcome works similarly when delivering an active treatment. Indeed, shaping expectations can enhance or reduce treatment effectiveness above and below the biological effect that the treatment would have if delivered in isolation from the psychosocial context. Placebo-related effect refers to the positive therapeutic outcome that has to be attributed, not to the active compound, but to the positive psychosocial context in which the active treatment is delivered. Nocebo-related effect refers to the negative therapeutic outcome arising from a negative psychosocial context [2; 19]. Placebo and nocebo-related effects have been repeatedly demonstrated using the open-hidden paradigm [20; 21; 22]. In

the open condition, the patient is informed of being administered a painkiller (patient is aware of active treatment), whereas in the hidden condition the patient is not informed of being administered a painkiller (patient is unaware of active treatment). However, in both cases a constant dose of painkiller is delivered. Open-hidden paradigm has been used to test at least five different analgesics (morphine, buprenorphine, tramadol, ketorolac, metamizole) [20; 21; 22]. Findings are consistent across studies, showing that the analgesic effect of the drug is significantly greater in the open compared to the hidden condition. For example, in a study comparing the analgesic effectiveness of open administered ketorolac with hidden administered ketorolac, it was demonstrated that tolerance to the painful stimulus was significantly higher ($p < 0.05$) in the former case (tolerance, 21.5 +/- 5.11 min) than in the latter (tolerance 17.23 +/- 2.4 min) [20]. This shows that by simply informing the patient of drug administration we can significantly enhance treatment efficacy. Placebo-related effects are particularly important because the final goal of placebo research is not to treat patients with inert treatments, but instead to use what we know about the influence of the psychosocial context to maximise the effect of active treatments.

Placebo and nocebo effects have been identified and studied across a variety of conditions (See Table 1.1). However, pain remains the most studied and, arguably, the most informative model when investigating these phenomena [2]. Pain is a subjective experience which undergoes psychological modulation more than other conditions and is therefore particularly susceptible to the influence of placebo and nocebo interventions (See Section 1.2).

Table 1.1: Placebo and nocebo effects across diseases/systems

Condition	References
Parkinson's Disease	[23; 24; 25]
Depression	[26]
Anxiety	[27]
Hormonal Secretion	[21]
Cardiovascular	[28]
High Altitude	[29]
Pain	[30; 31; 32; 33; 34; 35]

Throughout my PhD I was actively involved in data collection, data analysis and manuscript writing for research projects investigating placebo-related effects in Parkinson's disease (see Section 6.1), Myastenia gravis (see Section 6.2) and high altitude headache (see Section 6.3). However, my core doctoral research focuses on the model of pain.

1.2 The model of pain: Placebo analgesia and nocebo hyperalgesia

1.2.1 Pain and nociception are not synonyms

Pain experience is a complex process that goes beyond mere nociception. Nociception can be defined as the system that encodes and processes noxious stimuli (i.e. tissue damage, excessive pressure, heat or cold)[36]. In nociception, sensory neurons, namely nociceptors, respond to damaging (or potentially damaging) stimuli, sending a 'warning signal' from the periphery to the spinal cord and the brain; this is referred to as the ascending pathway [36] (See Figure 1.3b). Peripheral nociceptors need to reach a specific threshold to activate. If the stimulus is strong enough, the threshold is reached and a signal is delivered through neurons' axons into the dorsal horn in the spinal cord and to the central nervous system

[37]. Peripheral nociceptors include both phasic and tonic receptors, the former responding to short lasting noxious stimulation (i.e. receptors are activated by changes in stimulus intensity), and the latter to longer lasting stimulation (i.e. receptors continuously respond to the lengthy stimulation) [38; 39]. Activation of phasic receptors results in a sharp, short-lasting and often very localized sensation, referred to as phasic pain [7; 40], whereas the activation of tonic receptors leads to an enduring sensation of pain which extends over a wider area and engages both superficial skin as well as deeper tissues, referred to as tonic pain [7; 40; 41].

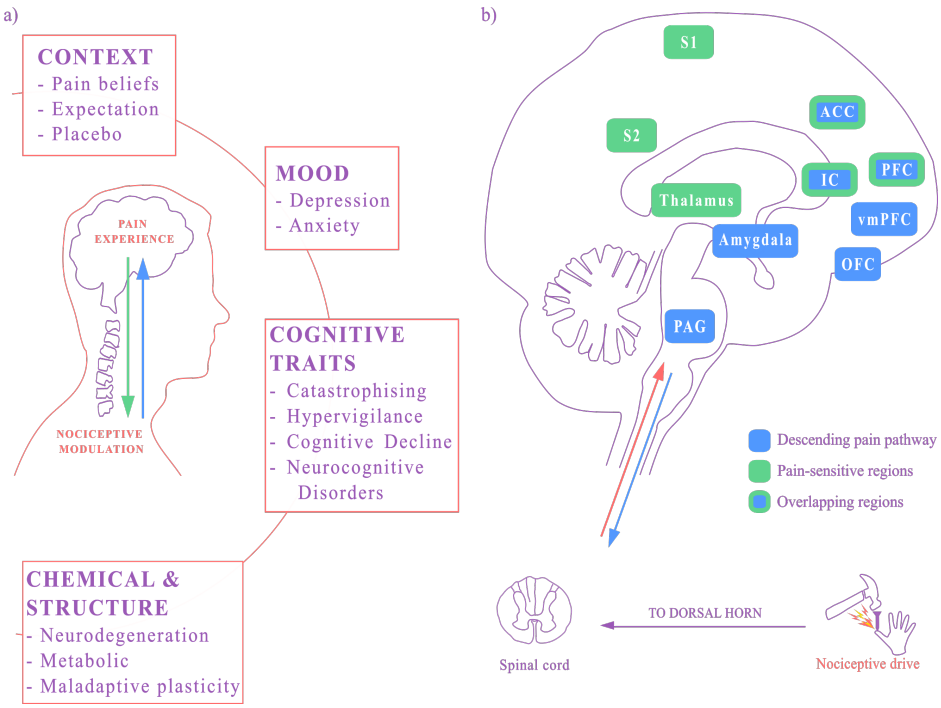

Figure 1.3: From nociception to pain. a) Schematic representation of the biological, psychological and social factors that modulate pain perception. b) Brain regions involved in pain processing, from pain-sensitive regions to the descending pain pathway. The red arrow going up, represents the nociceptive input coming from the periphery

Regulation of nociceptive processing occurs via the descending pathway, a

brainstem subcortical-cortical network that either facilitates or inhibits nociceptive signalling [42]. This network includes higher level brain areas - i.e. ventromedial prefrontal cortex (vmPFC), anterior cingulate (ACC), insular cortex (IC), orbitofrontal cortex (OFC) and the amygdala - that project to the periaqueductal gray (PAG) [43] from which the signal travels downwards to the dorsal horn of the spinal cord [43]. Precisely, three inhibitory neurotransmitters are released from the PAG (i.e. serotonin, noradrenaline and enkephalins), which are responsible for the inhibition of the nociceptive signalling [44; 45]. In the case of serotonin, depending on the receptor subtype, this can also have a facilitatory effect on the transmission of the nociceptive signalling [46]. Note that enkephalins are one of the three families of opioid peptides of the endogenous opioid system, which is fundamental in the mechanisms of analgesia and which will be discussed later on in this thesis [47]. Once adjusted, the nociceptive signal is sent back up via the ascending pathway (See Figure 1.3b). The loop continues and regulates the experience of pain [43; 48].

Although nociception is likely to lead to the sensation of pain, this relationship is not linear and the resulting pain may not correspond to nociception intensity. Indeed, nociception can occur without pain, and pain without nociception [43]. The International Association of the Study of Pain defines pain as 'an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage' [49]. Pain is in fact a subjective and conscious experience that is modulated by biological, psychological and social factors [43] (See Figure 1.3a). The discrepancy between nociception and pain is understood in light of the interplay between ascending and descending pathways, where the descending pathway is influenced by higher order regions involved in cognitive and emotional processing and is responsible for the down or up regulation of nociceptive input. Expectations leading to placebo and nocebo responses have

been shown to down-and-up regulate pain via the engagement of the descending pathway (See Section 1.2.2.2).

Functional magnetic resonance imaging (fMRI) is an indirect measure of brain activity. By looking at the changes in oxygenation concentration (Blood Oxygen Level Dependent, or BOLD contrast) that occur as a consequence of increased neural activity it is possible to infer which areas are activated or deactivated in that given moment [50]. Accordingly, pain research has used this technique to identify the regions that are the most active during pain, these include the primary and secondary somatosensory areas (S1, S2), IC, ACC, prefrontal cortices (PFC) and the thalamus [43; 51; 52]. Activation of these pain-sensitive regions was also demonstrated during pharmacologically induced analgesia [53; 54; 55]. In addition, Wager and colleagues [52] were able to use an algorithm to predict the intensity of participants' subjective pain experience on the basis of the activation of these same areas (S1, S2, IC, ACC) (See Figure 1.3b). These areas are a mixture of somatosensory, limbic and associative regions, attesting for the complexity of pain experience. While S1 and S2 are involved in sensory encoding, higher order areas, both limbic and associative, are responsible for cognitive and emotional appraisal of the nociceptive signal [43]. Other regions such as basal ganglia, cerebellum, amygdala, hippocampus and others within the parietal and temporal cortices have also been shown to be involved in pain processing [43]. The engagement of different limbic and affective brain regions is likely to vary depending on the factors involved in characterising each specific and unique pain experience (i.e. mood, cognition, context, nociception) [43].

Noteworthy is the influence that age and cognitive impairments can have on pain processing [56; 57; 58; 59; 60; 61; 62; 63] and on placebo analgesia responsiveness [4; 5]. Regarding pain processing, pain tolerance has been shown to decrease with age [56; 61]. Accordingly, Cole et al., observed a decrease in striatal activity

during pain in older individuals and the authors suggest this may indicate a dysfunction in inhibitory circuits rendering older individuals more vulnerable to pain suffering [57]. As for neurocognitive disorders, patients suffering from Alzheimer Disease (AD) have been shown to have augmented responses to pain compared to other patients suffering from milder forms of dementia [58; 59; 60; 61]. Accordingly, a systematic review looking at the insurgence of adverse events in the placebo arm of donepezil trials, reported that AD patients experienced greater adverse reactions than patients with mild cognitive impairments [62]. A subsequent review confirmed higher level of adverse events in placebo -treated AD patients who participated in RCTs, and extended these findings to other neurodegenerative disorders, including Parkinson's disease [63]. Concerning the influence of cognitive dysfunction on placebo processes, it has been observed that patients with neurocognitive disorders have a reduced or absent response to placebo analgesia [4]. In another study, the neural correlates of placebo analgesia in patients with neurocognitive disorders were elucidated in subjects complaining of various cognitive deficits (from mild cognitive impairment probably due to Alzheimer's disease to mild AD). In particular, the study aimed to assess how and to what extent executive (dys)functions of the medial prefrontal cortex (mPFC) may be related to placebo analgesia. Placebo analgesia was studied with the experimental venipuncture pain paradigm (open versus hidden [O-H] application of lidocaine). Patients also underwent a comprehensive neuropsychological assessment and a GO/No-GO fMRI task to elicit selective activation of the mPFC. The results showed a relationship between lower PA and mPFC dysfunction by neuropsychological assessment and fMRI. A separate voxel-based morphometry analysis also controlled for the possible influence of reduced grey matter volume on both fMRI results and placebo analgesia. fMRI results were not directly influenced by, and thus independent of, disease-specific grey matter atrophy, which was indeed

located more anteriorly within the rostral anterior cingulate and inversely correlated with placebo analgesia [5]. Altogether, these data indicates that age and cognitive impairments are important modulators of pain processing, and these may be associated with a dysfunctionality in the pain inhibitory system. According with the literature, minimal cognitive decline may already start from 25 years old, but a steeper decline is likely to occur from the sixth decade of life [64]. Therefore, age-related aspects become particularly important when studying pain processing, placebo analgesia and nocebo hyperalgesia in participants older than 60 years old and over, in which case cognitive deterioration and neurocognitive dysfunctions must be controlled for [63]. Paraphs most importantly, these findings must be considered when designing the placebo group of RCTs [5; 62; 63].

1.2.2 The influence of expectations on pain

1.2.2.1 Expectancy effect on pain-related brain regions

Positive and negative expectations change both subjective experience and objective activity of pain areas in the brain [65]. In a functional magnetic resonance imaging study conducted by Wager and colleagues in 2004 [32], pain was experimentally induced on healthy participants via electrical stimulation and an inert cream was applied along with suggestions of analgesia. This resulted in a reduction in the subjective perception of pain (Visual Analogue Scale, VAS) and was mirrored by reduced activity in pain-sensitive regions, including thalamus, IC and ACC.

The first attempt to summarize the brain areas involved in placebo analgesia in human experimental pain was done in 2011 by Amanzio and colleagues [66]. Here, the activation likelihood estimation (ALE) meta-analysis, a quantitative voxel-based method, was used to investigate placebo-related cortical activity as

a function of time, meaning that the authors divided between anticipatory phase (i.e., while anticipating pain decrease/increase), noxious stimulation phase (i.e., while receiving noxious inputs) and post-stimulation phase (i.e., after having received noxious stimulation). During the expectation phase, increased activity was observed in areas implicated in the descending inhibitory pain pathway, such as the ACC, the PAG along with the rostral PFC, further attesting for the down regulatory effects that expectations have on pain perception [67]. During the noxious stimulation phase, increased activity was revealed in regions involved in the descending inhibitory pain pathway (i.e., ACC, IC, the thalamus, the hypothalamus, the PAG and the pons) [43; 68], while decreased activity was shown in pain-sensitive regions (i.e., posterior and mid-cingulate cortex, the IC and the basal ganglia) [43; 69]. Reduced activity in pain-sensitive regions is likely to reflect an antinociceptive effect associated with placebo analgesia, along with cognitive modulation of pain as suggested by the increased activity of the descending pain pathway [66]. For what concerns the post-stimulation phase, it was not possible to perform the analysis due to the lack of sufficient data in the literature. A subsequent meta-analysis investigating placebo analgesia during noxious stimulation reported similar patterns of activation and deactivation, confirming the influence of placebo-induced changes in pain sensitive brain regions and in the descending inhibitory pain pathway [70]. Precisely, increased activity was observed in the OFC, ACC, dorsolateral PFC, ventral striatum, left thalamus and in the midbrain surrounding the PAG during noxious stimulation, and decreased activity was shown in IC, ACC, the thalamus, amygdala and right lateral PFC [70].

Although generally less attention has been given to the nocebo phenomenon, a recent meta-analysis reported nocebo-induced increases during noxious stimulation in pain-modulating areas, including the dACC, the parietal operculum and the posterior IC [71]. Similarly to placebo analgesia, but in the opposite direc-

tion, such increased activity in pain-sensitive region during the noxious stimulation phase suggests a pro-nociceptive effect associated with nocebo hyperalgesia. Importantly, two pivotal positronemission tomography (PET) studies have further demonstrated the strong neurophysiological responses associated with both placebo analgesia and nocebo hyperalgesia [31; 72]. The first PET study showed the engagement of the endogenous opioid system in placebo analgesia [31]. In this study, neurological correlates of placebo-induced and opioid-induced (i.e. remifentalin administration) analgesia were compared. In both cases, subjective pain ratings decreased and opioid neurotransmission increased in the OFC and ACC. Opioid-induced analgesia also activated the vmPFC and IC. It was therefore demonstrated for the first time that placebo analgesia uses a similar, but reduced, pathway of opioid-induced analgesia. The second PET study replicated and expanded these findings, suggesting opposite opioid responses between placebo and nocebo effects [72]. While placebo analgesia led to increased opioid neurotransmission in pain-related regions, nocebo hyperalgesia decreased opioid release in these same regions (ACC, OFC, IC, nucleus accumbens, thalamus, amygdala). Higher opioid neurotransmission corresponded to lower pain ratings, whereby lower opioid neurotransmission was associated with higher pain ratings [72].

As mentioned earlier, expectations of pain increase are known to play an important role in enhancing pain experience and evidence is accumulating that demonstrates the influence of negative expectations on the activity of pain areas in the brain [65; 67] (For further details on the neurophysiology of expectations See Section 1.2.2.2). The first attempt to identify brain regions that consistently engage in pain anticipation, by looking at multiple studies with pain anticipation paradigms in a single analysis, was done by Palermo and colleagues [73] using ALE meta-analysis. Here, increased activation was observed in the dorso-

lateral PFC, IC, medial and inferior frontal gyri, inferior parietal lobule, middle and superior temporal gyrus, thalamus, and caudate, while deactivation was reported in the ACC, superior frontal gyrus, parahippocampal gyrus and in the claustrum. Since the anticipation of a painful event is likely to be underpinned by multiple processes, Palermo et al. investigated the behavioral domains (BD) (i.e., interoception, emotion, perception, action, cognition) associated with functional connectivity networks, instead of limiting their analysis to single areas of activation/deactivation. Network analysis was therefore used to assess FC of preselected ROIs, the anterior insula (AI) and cingulate cortices, separately. AI showed higher coactivation with the claustrum, thalamus, inferior/middle and superior frontal gyri, inferior parietal lobule, precuneus, lentiform nucleus (putamen), parahippocampal gyrus, pre-/postcentral gyri, and middle temporal gyrus. A second cluster of coactivation involved the medial frontal gyrus, anterior and posterior cingulate. Coactivation with the ACC was observed in the insula, thalamus, inferior/middle and superior frontal gyri, inferior parietal lobule, lentiform nucleus (putamen), pre-postcentral gyri, and transverse temporal gyrus. Such functional connectivity meta-analytic analysis provides, for the first time, an overview of the interconnected brain responses triggered by the anticipation of pain. Importantly, AI and ACC and related clusters were highly correlated with BD of action (imagination, inhibition, and execution), emotion and perception (pain and interoception). Accordingly, the authors suggest that pain anticipation may engage a supramodal system in which AI and ACC regulate emotional, attentional and sensory (pain/interoception) responses, accounting for the complexity of pain perceptual processes, including pain anticipation.

Overall, this in-depth section outlining the brain areas involved in placebo analgesia and nocebo hyperalgesia during the anticipation and the noxious stimulation phases suggests that these phenomena engage brain regions involved in the

pain downregulation pathway and influence pain-sensitive brain areas, decreasing and increasing their activity for placebo analgesia and nocebo hyperalgesia, respectively. Overall, this neuroimaging evidence adds scientific strength to these phenomena, indicating that it is unlikely that these are simply the result of a response bias and of social pressure to comply to instructions.

1.2.2.2 Neurophysiology of expectations

From a neuroscientific perspective, expectations of a future outcome have an important role in preparing the body for the future event. Positive expectations indicate the body should prepare for a positive event and trigger biological reactions such as activating the reward system and reducing anxiety. Conversely, negative expectations prepare the body for a negative event by increasing anxiety, which is essential for reacting to threat [74; 75].

Positive expectations may induce placebo responses through the engagement of the reward system. The reward circuit is a network that drives our behaviour towards pleasurable stimuli (i.e.food) and pleasurable emotions (i.e joy), and drives our behaviour away from unpleasant stimuli (i.e. pain) and unpleasant emotions (i.e. sadness). The anatomical structures of the reward system are located within the cortico-basal ganglia-thalamo-cortical loop [76] and when reward is experienced, dopamine is released from two main dopaminergic pathways: the mesolimbic and the mesocortical pathways. The mesolimbic pathway transmits dopamine from the Ventral Tegmental Area (VTA) in the midbrain to the Nucleus Accumbens (NAc) in the ventral striatum (which also includes the olfactory tubercle) [77]. Dopamine release from the VTA to the NAc is a core component of the reward circuit.

Scott et al. (2007) used receptor-binding PET to show that placebo responsiveness was related to dopamine activation in the NAc. Here, pain was exper-

imentally induced and placebo responsiveness was evaluated according to pain reduction following the administration of a placebo intervention. Additionally, they compared activation of the dopaminergic pathway during a monetary reward task, a well studied paradigm known to activate the reward system, and during a placebo analgesia task. It was demonstrated that the larger the NAc activation for monetary reward, the stronger the NAc activation during placebo response, suggesting that placebo responsiveness depends on the functioning of the reward system [78]. In a similar study, dopaminergic activation in the NAc was observed during the anticipatory phase of placebo analgesia, while opioid neurotransmission in pain-related brain regions (ACC, OFC, IC, NAc, amygdala, and periaqueductal gray matter) was shown during placebo analgesia. Greater placebo analgesia was associated with greater dopaminergic and opioid activity. Oppositely, nocebo responses were associated with decreased dopaminergic and opioid activity [72]. This evidence suggests that expectations of therapeutic amelioration following placebo intake activate the reward system, which in turn interacts with endogenous opioid release resulting in placebo analgesia.

Placebo analgesia has been also associated with a decrease in perceived anxiety [79] and reduced activation of anxiety-related areas (i.e., emotional network, including amygdala) [27; 80]. Indeed, the larger the placebo response, the greater the deactivation of the emotional network [27]. Along with other physiological parameters of stress, heart rate (HR) has been used as a physiological indicator of anxiety reduction in placebo research. Since HR has been used in the research conducted during this Doctoral Degree, greater attention is given to this physiological parameter throughout this chapter, compared to others (i.e galvanic skin response, heart rate variability). Yet, the reliability of HR is still under debate. For instance, a recent meta-analysis reported six placebo studies showing HR reduction in association with analgesia, while eight showed that placebo effects

did not impact HR [81].

Negative expectations leading to nocebo hyperalgesia have been associated with two biochemical pathways strongly linked to stress reactions: cholecystokinin (CCK) secretion and hypothalamic-pituitary-adrenal axis (HPA). CCK is a neuropeptide in the central nervous system, which is secreted in response to anxiogenic and panicogenic responses [82]. In 1997 [83], it was shown that nocebo hyperalgesia was blocked by pre-treatment with proglumide, a non- specific CCK-A/CCK-B receptor antagonist. While not being a painkiller, proglumide prevented nocebo hyperalgesia in a dose dependent manner, attesting for the critical role of CCK secretion in nocebo. The HPA axis is a system formed by the feedback interactions of the hypothalamus, pituitary gland and adrenal glands. This neuroendocrine system is responsible for the regulation of multiple body processes including regulation of stress via cortisol secretion [84]. In 2006, Benedetti and colleagues [85] demonstrated that nocebo hyperalgesia induced by verbal suggestions of pain worsening led to an increase in plasma concentration of adrenocorticotropic hormone (ACTH) and cortisol, attesting for HPA axis hyperactivity. Interestingly, it was shown that administration of diazepam, an anti-anxiety benzodiazepine, blocked both nocebo hyperalgesia and the HPA axis hyperactivity, demonstrating the crucial role of anxiety in nocebo responses. Also HR is a physiological parameter that has been used as an indicator of anxiety during the anticipatory phase of threat in nocebo hyperalgesia. Ploghaus et al. (2001) [86] showed decreased HR during high anxiety anticipatory phase and increased HR during low anxiety anticipatory phase. HR deceleration in response to high anxiety may seem peculiar, as greater anxiety is usually associated with HR acceleration [87]. Contrasting evidence is provided by Colloca and Benedetti (2009) which reported HR increase in high anxiety anticipatory phase and HR decrease in low anxiety anticipatory phase [13]. Although Ploghaus et al.'s (2001)

findings are in line with the defence cascade model [88], which suggests that during anticipation of pain HR first decreases and then increases, further research is needed to better understand HR variations in the anticipatory phase of nocebo hyperalgesia.

Finally yet importantly, neuroimaging evidence has demonstrated that during the anticipatory phase prior to noxious stimulation, expectations of high and low pain engage key cortical regions of the descending pain modulatory circuit (rACC, mPFC, and PAG), and result in enhanced pain perception in the case of expectations of high pain and reduced pain in the case of expectations of low pain, attesting for the critical role of expectancy in up-and-down regulating pain experience [31; 32; 67; 89].

1.2.2.3 Understanding expectations: A predictive coding framework

From an evolutionary perspective, our brain can be seen as a 'prediction machine' whose goal is to make sense of our surroundings as efficiently as possible. This brain function is crucial for survival because we need to predict potential risks to avoid them and foresee potential rewards to approach them [75]. Perceptual accuracy depends on the amount of information we gather about the incoming stimulus yet gathering information takes time [90]. From a survival perspective, speed is crucial. It is safer to mistake a wood-stick for a snake than to take time accurately examining the shape of the object and discover it is in fact a deadly snake. It follows that in perception, there is always a trade-off between accuracy and speed [75]. Consequently, when we are presented with a clear stimulus, we easily gather sufficient information to accurately interpret it in a short time. However, when presented with an ambiguous stimulus we attempt to exploit all available information to fill in the gaps; taking advantage of sensory inputs on one side and resourcing from our previous experience and expectations on the

other [90]. Thus, the more ambiguous the stimulus, the more room there is for expectancy to influence perception [90; 91].

The key role of expectancy in perception is in line with the Bayesian predictive coding framework in which computational tools are used to explain how expectations shape perception. Predictive coding has been applied to some areas of perception, vision being the most studied [92]. In 2016, Wiech [93] applied the predictive coding framework to explain pain encoding. Sensory cues trigger pain related expectations and the model of expected pain is compared with sensory experience; if there is a match between the expected and experienced pain, the expectations are confirmed and reinforced. Otherwise, if the mismatch between the predicted model and the sensory experience is large enough, expectations are updated accordingly with the newly accumulated evidence; a 'prediction error message' is generated in the brain, which updates the pre-existing model (See Figure 1.4). This mechanism of learning and updating is essential to maintain a functional and accurate perception of reality. It has been suggested that disruption of updating mechanisms, leading to change-resistant mental representation, is involved in the development and maintenance of chronic pain [94].

The moment of comparison between sensory input and our predicted model is crucial for the resulting experience. The predictive coding framework acknowledges the bias towards higher weighting of elements that are in agreement with expectations and down weighting of elements that contradict expectations. This predisposition of favouring evidence in line with our expectations results in a perceptual experience that is more coherent with the predicted model. Placebo analgesia and nocebo hyperagesia are clear examples of this predictive framework in which pain is either down or up regulated accordingly with one's expectations. In line with this, Koyama et al. (2005) and Keltner et al. (2006) [67; 89] investigated the influence of cues on perception of noxious stimuli and showed that

noxious stimuli are perceived as more or less intense according to whether these are preceded by visual cues indicating high or low pain, respectively. Importantly, not only participants self-reported lower or higher pain according to the cues that preceded the noxious stimulation, but pain-related regions activity during the pain phase increased if preceded by high-pain cues, and decreased if preceded by low-pain cues. Moreover, engagement of descending pain modulatory circuit regions during the anticipatory phase of placebo and nocebo responses attests for the role of expectancy in down and up regulation of pain [31; 32; 67; 89]. As previously explained, in domains of perception such as vision, the influence of expectancy increases with more ambiguous stimuli [92; 95; 96; 97]. Yet, influence of the ambiguity of noxious stimuli on pain perception has not been systematically investigated. However, it is likely that expectations have stronger influence on more noisy and ambiguous incoming noxious stimuli.

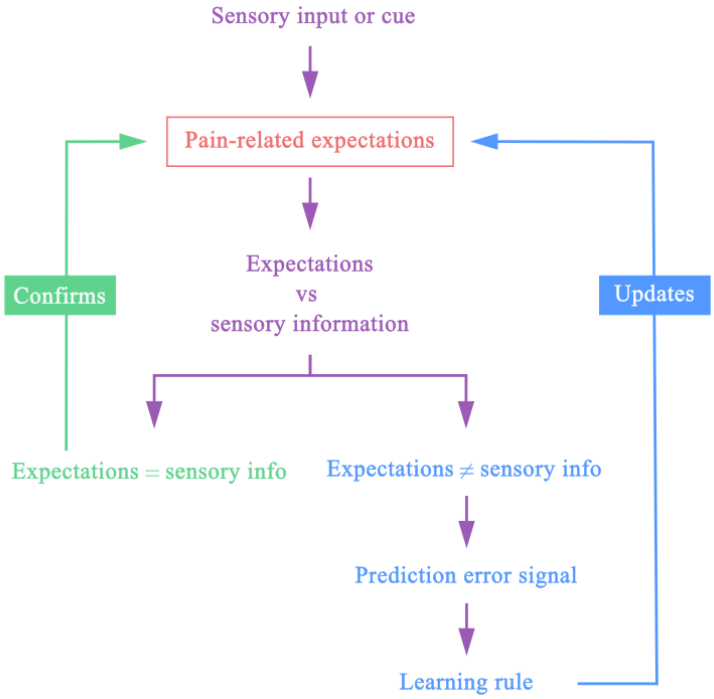

Figure 1.4: Predictive coding framework of pain

1.2.3 Verbal suggestions trigger analgesia and hyperalgesia

Verbal suggestions are, arguably, the stronger factor directly influencing expectation formulation. In the clinical setting, verbal suggestions are constantly present. These range from the doctor's specific instructions to the nurse's reassuring words and the advice of family members expressing their concerns and beliefs. Therefore, the patient is exposed to multiple, and at times contrasting, suggestions that are likely to have a strong influence on the patient's recovery (See Figure 1.5).

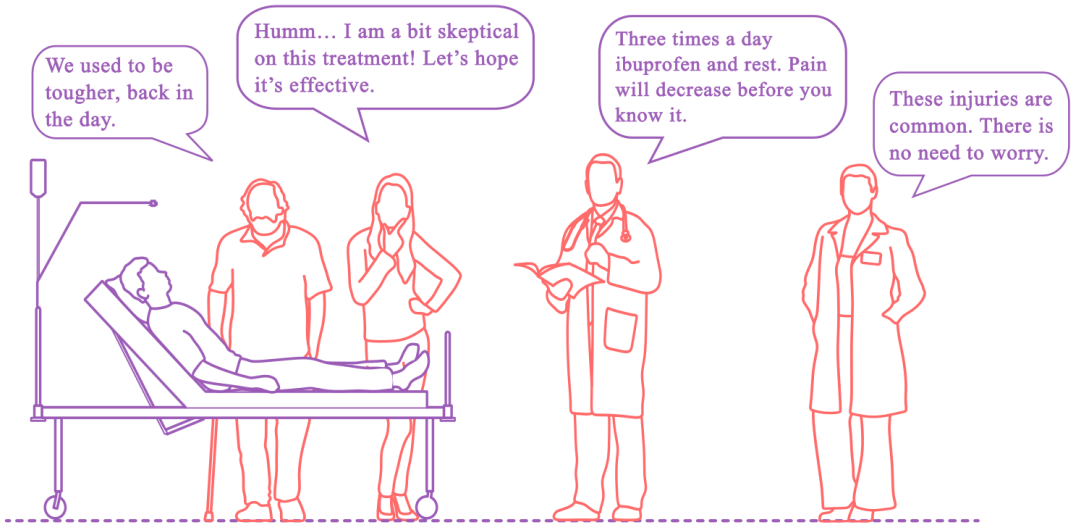

Figure 1.5: Verbal suggestions of various nature characterise the clinical setting

1.2.3.1 Verbal suggestions vary in 'direction' and in 'magnitude'

Generally, research has studied how verbal suggestions shape expectations in terms of their 'direction', either positive (placebo) or negative (nocebo), and of their 'magnitude' , either strong or weak. Let us now consider some examples.

In 2011, Van Laarhoven et al. [34] demonstrated that the same noxious stim-

uli, induced experimentally via electrical stimulation, were perceived as more or less painful according to the direction of verbal suggestions. Specifically, when an inert cream was applied along with suggestions of analgesia, the stimuli were perceived as less painful, whereas when the cream was given with suggestions of hyperalgesia, the stimuli were perceived as more painful. Similar effects were reported by other studies modulating the direction of verbal instructions while delivering placebo and nocebo interventions [14; 98].

Furthermore, verbal suggestions are potent to the extent of reversing the action of active treatments. Bingel and colleagues in 2011 [35] used an open-hidden paradigm while inducing pain to healthy participants via heat stimulation. Opioid treatment, remifentanil, was administered intravenously throughout the experiment, at a constant dosage. Positive and negative expectations were induced by first telling participants that the anaesthetist would *'start opioid injection'* (positive expectations of pain reduction) and then by telling them that the anaesthetist would *'stop opioid injection to investigate the possible increase in pain after ceasing the opioid infusion'* (negative expectations of pain increase). Here, it was demonstrated that positive verbal suggestions doubled the analgesic effect of remifentanil, whereas negative suggestions abolished remifentanil analgesia [35]. In a different study, administration of an active analgesic cream associated with positive suggestions led to enhanced analgesics effects, while administration of the same cream with negative suggestions led to pain increase (nocebo response), despite the analgesic properties of the treatment. This is in line with a previous study in which the analgesic effect of nitrous oxide, was reversed into a hyperalgesic response, simply by telling participants that pain might increase as a consequence of the treatment administration [99]. Taken altogether, these findings attest for the power of verbal suggestions not only in enhancing, but also in reversing active treatments effects.

Suggestions can also vary in terms of their magnitude. Changing the certainty of delivered information shapes the magnitude of the suggestion. More certain information increases the magnitude, whilst less certain information decreases the magnitude. Pollo et al. [100] assessed post-operative pain after informing some patients they would receive a powerful painkiller (certain information) while informing others they would receive either a placebo or a painkiller (uncertain). Although the information varied across groups, every patient received the same dose of painkiller. It was demonstrated that post-operative pain was lower (greater analgesia) in the certain information group compared to the uncertain group. Interestingly, both certain and uncertain groups reported significant analgesia compared to the control group, in which patients were unaware that they were receiving a painkiller. Furthermore, the effects of delivering certain or uncertain verbal suggestions on analgesia induced by both placebo intervention and active treatment were investigated in two studies with Irritable Bowel Syndrome (IBS) patients. The two studies were almost identical, with the main difference lying within the information delivered. In one case, verbal suggestions were certain: *'The agent you have just been given is known to significantly reduce pain in some patients'* [101]. In the other case, patients were told they would receive either lidocaine or inert treatment (uncertain condition) [102]. Comparison between the studies demonstrated that certain suggestions [101], compared to uncertain ones [102], led to stronger analgesic effects, both for the placebo and for the active treatment. Similar findings were reported in conditions other than pain, including physical performance in healthy participants [103] and motor performance in Parkinson's patients [25].

1.2.3.2 The time component of verbal suggestions

Time is an important aspect in the clinical context both to make informed decisions on the basis of patient's history (i.e. how long has the patient been experiencing symptoms) [104; 105] and in terms of the prognosis (i.e. how long before amelioration of symptoms) [106; 107]. Indeed, when a treatment is administered, temporal features such as the duration required by the treatment before setting into action, the longevity of the treatment effect and the temporal window within which side effects may arise, are important for patients, giving them a better sense of what is ahead, in other words, of what to expect [107].

Temporal expectations refer to one's ability to extract temporal information from the environment [108]. On the basis of temporal expectations it is possible to predict when an event is going to occur, allocating the attention towards relevant sensory inputs at the correct time, leading to behavioural benefits including faster reaction times, increased movement accuracy and improved perception [109; 110; 111; 112]. In addition, temporal preparation has been associated with the contingent negative variation (CNV), a cortical potential of negative polarity that builds up in central cortical regions and peaks just before the onset of the temporally predicted stimulus [112; 113; 114].

Given the centrality of time when starting a treatment, and given the important role of temporal expectations in preparing ourselves to the incoming event, gaining a better understanding of whether modulating temporal expectations can influence the onset of action of a given intervention becomes particularly important. For example, is it possible to anticipate the onset of action of a treatment by delivering temporal expectations of anticipated onset of action? If so, does this effect last over time? On the opposite hand, is it possible to delay the onset of action of the treatment by giving temporal instructions of delayed effect? Before this Doctoral project, no research had been conducted to address these questions.

Placebo and nocebo expectancy modulation procedures are particularly suited to start addressing these questions because they allow to isolate the effect of temporal expectations on the onset of a given effect, without biases due to the effectiveness of an active treatment. The most efficient way to modulate one's temporal expectations of treatment onset of action is by delivering temporal suggestions. Although placebo and nocebo research has largely focused on the modulation of verbal suggestions, no study has so far investigated the consequences of modulating temporal verbal suggestions on the onset of placebo and nocebo effects.

In a few placebo experiments temporal details were explicitly given while delivering placebo verbal instructions [28; 115; 116], however, temporal indications were there to give credibility to the placebo treatment, rather than to test the influence of the temporal information on the onset and duration of the placebo response. For example, in a study looking at whether placebo coffee would boost physical performance the following suggestions were given: *'Caffeine would become effective within 8-10 minutes.'* [115]. In another study, assessing placebo analgesic cream effectiveness, participants were told it would take 30 seconds for the cream to begin numbing their hand [116].

Pollo et al. [28] is, to my knowledge, the only exception in which verbal information on time was given both in terms of when the treatment would start to be effective (placebo onset: *'It takes a couple of minutes to work'*), and for how long it would last (placebo extinction: *'The anesthetic effect takes a couple of minutes to vanish'*). In line with the temporal indications, placebo analgesia was present after few minutes from cream application and vanished soon after. Although the scope of this study was not to investigate the role of temporal suggestions, this remains the first experiment showing that the analgesic effect associated with placebo administration sets in and fades away accordingly with the temporal

suggestions that were given. Yet, the study lacks the methodological structure to rigorously evaluate the influence of temporal information on placebo onset and duration, therefore it is important to consider the results as preliminary, rather than drawing strong conclusions from them. First, the study does not compare multiple groups receiving different temporal information. Consequently, it is not possible to conclude that the onset varies according with temporal instructions. Second, the time frame is extremely small (i.e. few minutes overall), and this is problematic for at least two reasons. One is that the occurrence of both onset and extinction of the effect within minutes is not an accurate representation of the real time-frame in which painkillers work. Consider that the time of action of popular painkillers such as paracetamol (acetaminophen) is approximately 10-20 minutes and their effect last for hours [117; 118]. An additional reason why the short time window is problematic is the reduced likelihood to encounter cognitive fluctuations such as expectation re-evaluation and update that may arise by leaving a longer interval for the participant to experience and familiarise themselves with the treatment effect and incoming sensory input [90]. This last point leads to an important final consideration. Pollo et al. [28] assessed analgesia on one single fast stimulus, instead of delivering multiple stimuli one after the other and then calculating the average, as commonly done in other pain studies [14; 34]. A fast stimulus gives us less time to gather information [90], therefore one could argue that the short-lasting stimulus used by Pollo et al. has high ambiguity. In addition, participants do not get the opportunity to further explore the noxious sensation by receiving multiple stimuli one after the other, further increasing the level of ambiguity of the stimulus. In line with the predictive coding framework (see Section 1.2.2), delivering one highly ambiguous stimulus limits the possibility for our brain to collect data to eventually update expectations, while inflating the influence of expectations [90].

In conclusion, only few placebo studies have included temporal details in their instructions, and although Pollo et al. [28] gave some preliminary insight into the role of temporal suggestions, no study had been purposely designed to investigate the influence of temporal suggestions on placebo and nocebo onset modulation. Indeed, a systematic investigation is required to empirically test if and how temporal information influences the onset and maintenance of placebo and placebo-related effects. Accordingly, the goal of the present Thesis is to address this gap in the literature, conducting purposely designed experiments to investigate the effects of temporal suggestions in modulating the onset and duration of placebo analgesia and nocebo hyperalgesia.

1.3 General organization of the research project

The main goal of the research conducted during this Doctoral Degree is to *investigate whether temporal suggestions modulate the onset and the duration of placebo analgesia and nocebo hyperalgesia both on phasic and tonic pain models.* A secondary aim, is to offer a preliminary reflection on *whether the degree of ambiguity of the incoming painful stimulus influences the extent to which temporal suggestions modulate these effects.* Overall, three studies were conducted during the 3-year doctoral training programme (2017-2020).

The introductory chapter that just ended (Chapter 1) is followed by three chapters (Chapter 2, 3, 4), each one covering one research project. The fifth chapter (Chapter 5) summarises the primary findings across the studies, particularly focusing on their clinical implications and future directions. The last chapter (Chapter 6), briefly outlines the collateral projects, aside from my primary investigation, in which I was involved during my PhD.

1.3.1 Chapter 2 - The effect of temporal information on placebo analgesia and nocebo hyperalgesia.

This chapter contains the first of three studies conducted during the 3-years of doctoral training, which will be henceforth referred to as Study 1. Study 1 has been published in 2021 in *The Psychosomatic Medicine Journal* [119]. Here, it was investigated whether the onset of placebo analgesia and nocebo hyperalgesia can be modulated by varying the information participants received about the onset of the expected treatment effect. Pain was induced experimentally on healthy participants via short-lasting, medium-to-low intensity, phasic electrical stimuli. No physiological measures were taken. *It was hypothesised that both placebo analgesia and nocebo hyperalgesia onsets would follow the temporal information that participants received along with the inert cream, setting in at the expected time point, and not earlier, in a phasic pain model.* In line with our predictions, participants who were told that the (inert-)treatment had a fast time of action reported the (inert-)treatment effect to occur early on, while those who were instructed that the (inert-)treatment required a longer time of action, reported a delayed (inert-)treatment response. This was true for both placebo and nocebo, indicating that the onset of both placebo analgesia and nocebo hyperalgesia can be modulated by the given temporal suggestions.

1.3.2 Chapter 3 - 'External timing' of placebo analgesia in an experimental model of sustained pain.

This chapter contains the second of the three studies, which will be henceforth referred to as Study 2. Study 2 has been published in 2021 in *The European Journal of Pain* [120]. Here, the influence of temporal suggestions on placebo analgesia onset was investigated using a long lasting, high-intensity, tonic pain

model, induced with the Cold Pressor Test (CPT). Heart Rate (HR) was measured to assess whether it correlated with placebo analgesia. *It was hypothesised that placebo analgesia onset would follow temporal suggestions, setting in at the specified time point, in a tonic pain model.* In agreement with our predictions and accordingly with the results from Study 1, placebo analgesia onset shifted accordingly with the temporal suggestions that participants received. Precisely, analgesia occurred early when participants were told that the (inert-)treatment had a fast time of action, while analgesia was delayed when participants were told that it would have a slow time of action.

1.3.3 Chapter 4 - The temporal modulation of nocebo hyperalgesia in a model of sustained pain.

This chapter contains the third of the three studies, which will be henceforth referred to as Study 3. Study 3 is currently in preparation and will be shortly submitted for consideration to a peer-reviewed journal. This project relies on the same experimental design of Study 2, but investigates the influence of temporal suggestions on nocebo hyperalgesia. *It was hypothesised that nocebo analgesia onset would follow temporal suggestions, setting it at the specified time point, in a tonic pain model.* As expected, hyperalgesia onset followed the temporal verbal suggestions that participants received.

1.3.4 Chapter 5 - General Discussion

This chapter pulls together the strands of research conducted during this Doctoral Programme. By considering the primary findings of the three studies altogether, and comparing them one with the other, it offers a comprehensive discussion of their clinical implications and future perspectives.

1.3.5 Chapter 6 - PhD Collateral Projects

This chapter contains the abstracts of the collateral projects in which I was involved during my PhD. Although these were not part of my main research investigation, I was involved in data collection, data analysis and manuscript writing. These projects investigated placebo and nocebo effects in conditions other than pain, including Parkinson's disorder, Myasthenia Gravis and High Altitude headache.

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Chapter 2

The effect of temporal information on placebo analgesia and nocebo hyperalgesia

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2.1 Abstract

Objective. Expectations are known to be key determinants of placebo and nocebo phenomena. In previous studies, verbal suggestions to induce such expectations have mainly focused on the direction and magnitude of the effect while little is known about the influence of temporal information. [121]

Methods. Using an experimental placebo and nocebo design, we investigated whether information about the expected onset of a treatment effect modulates the start and time-course of analgesic and hyperalgesic responses. Healthy volunteers (N=166) in three placebo and three nocebo groups were informed that the application of an (inert) cream would reduce (placebo groups) or amplify pain (nocebo groups) after 5, 15 or 30 minutes. Two control groups were also included (Natural History and No Expectations). Participants' pain intensity rating of electrical stimuli administered before and 10, 20 and 35 minutes after cream application were obtained.

Results. Mixed-method analysis of variance showed a significant interaction between group and time $F(12, 262) = 18.172$, $p < 0.001$, $p\eta^2 = 0.454$, suggesting that pain variations differed across time points and between groups. Post-hoc comparisons revealed that placebo and nocebo groups began to show a significantly larger change in perceived pain intensity than a no-expectancy control group at the expected time-point ($p < 0.05$) but not earlier ($p > 0.05$). Once triggered, the analgesic effect remained constant over the course of the experiment whereas the hyperalgesic effect increased over time.

Conclusions. Our results indicate that temporal suggestions can shape expectancy-related treatment effects which – if used systematically - could open up new ways to optimise treatment outcome.

2.2 Introduction

The outcome of analgesic treatment has been shown to considerably depend on an individual's expectations. The anticipation of pain relief can boost the treatment effect whereas expectations of increased pain can aggravate pain [122; 123; 124]. Studies using brain imaging techniques have begun to unravel the neural basis of expectancy effects on pain perception. They identified a network of brain regions including the dorsolateral prefrontal cortex, rostral anterior cingulate cortex, periaqueductal gray and amygdala which modify pain-related brain activity in a top-down fashion depending on the expectation of the individual [125; 126; 127]. In experimental contexts, expectancy effects on the perception of pain have mainly been studied using placebo or nocebo paradigms where participants are led to believe that an inert substance or procedure will have an analgesic effect (placebo) or a hyperalgesic effect (nocebo) [122; 123; 124; 128]. However, the modulatory influence of expectations has also been demonstrated in clinical studies on pain [129; 130; 131] and other health conditions [132]. These observations have recently inspired a new wave of research aiming to harness the potential of positive expectations and avoid the detrimental effect of negative expectations in clinical populations [133].

So far, research in this field has mainly focused on two characteristics of expectation, namely its direction (i.e., whether the treatment is expected to improve or aggravate symptoms) and magnitude (i.e., how strong the expected effect will be). However, any expectation will also be linked to aspects of time – for instance, when the treatment is going to take effect and for how long it is going to last. Although some studies include information about the expected onset and duration of the effect [134; 135; 136; 137], the influence of temporal information on treatment outcome has not been studied systematically.

Here, we investigated in healthy volunteers whether the onset of placebo analgesia and nocebo hyperalgesia can be modulated by varying the information participants receive about the onset of the expected treatment effect. We hypothesise that the onset of the treatment effect (i.e., analgesia in placebo groups and hyperalgesia in nocebo groups) depends on the information provided and coincides with the expected onset time.

2.3 Methods

2.3.1 Participants

166 healthy volunteers were recruited from the student population of the University of Turin. Sample size calculation has been calculated using G*Power (see Sample size calculation in Supplemental Digital Content Section 2.6.0.1. Students were recruited during classes and were informed about the possibility to participate in a study investigating the onset of action of an analgesic or hyperalgesic cream. Those interested in participating in the study were contacted by phone. Participants provided written informed consent before starting the experiment. Signing the consent, all participants agreed that some details of the experimental procedure would have been omitted during the experiment and that they would be debriefed via email about the details of the study at the end of the experiment. Both in the informed consent and in the debriefing email, participants were told that they could decide to withdraw their consent and data; no one decided to do so. Participants received neither payment nor credits to participate in the study.

Data from nine participants were excluded from the analysis because they met our outlier criteria (See Section 2.3.2.12), leading to a total sample size of 157 participants (for participants' characteristics see Table 2.1). Data collection

started in February 2019 and ended in June 2019. Participants had no history of neurological, psychiatric, or other medical conditions and were instructed to refrain from consuming alcohol or taking analgesic medication for at least 12 hours prior to the experiment. Experimental procedures were conducted according to the policies and ethical principles of the Declaration of Helsinki. The study was approved by the Ethics Committee of the University of Turin (registration number: 138875).

	Age		Sex	BMI		STAI-I		STAI-II		Pain baseline	
	Mean	SEM	Females/Males	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
NH	25.37	0.83	F=9; M=10	21.13	0.47	32.58	1.69	38.74	1.64	2.59	0.27
NE	24.7	0.63	F=9; M=12	22.14	0.58	30.57	1.21	39.33	1.57	3.01	0.25
P5	22	0.36	F=10; M=11	21.55	0.49	31.19	1.42	37.52	1.84	2.43	0.22
P15	22.1	0.23	F=10; M=10	20.95	0.58	31.7	1.13	38.05	2.27	2.52	0.21
P30	22.7	0.25	F=12; M=8	21.82	0.58	33.55	1.56	38.75	1.42	2.59	0.24
N5	22.16	0.22	F=12; M=7	20.95	0.55	32.32	1.3	41	2.45	2.46	0.22
N15	22.74	0.4	F=10; M=9	21.1	0.46	32.32	1.27	39.84	2.12	2.78	0.23
N30	22.78	0.34	F=12; M=6	21.53	0.53	32.17	1.32	39.44	1.56	3.23	0.27

Table 2.1: Mean and standard deviation (SEM) of age, sex, BMI, STAI-I/II and pain intensity at baseline.

2.3.2 Experimental design

Participants were assigned to one of three placebo groups, three nocebo groups, a no expectancy (NE) group or a natural history (NH) group (see below for details; Figure 2.1). The NH group was filled first, as a form of pilot study. For this reason, this group has been excluded from the primary analysis and only serves as control for pain perception fluctuations over time. The remaining participants were divided into the seven experimental groups (i.e. NE group, Placebo groups, Nocebo groups) using stratified randomization in order to control for demographic variables (e.g. age and sex) [138].

2.3.2.1 Placebo groups

Participants in the placebo groups were informed that an analgesic cream would be applied to reduce the painful sensation induced by an electrical stim-

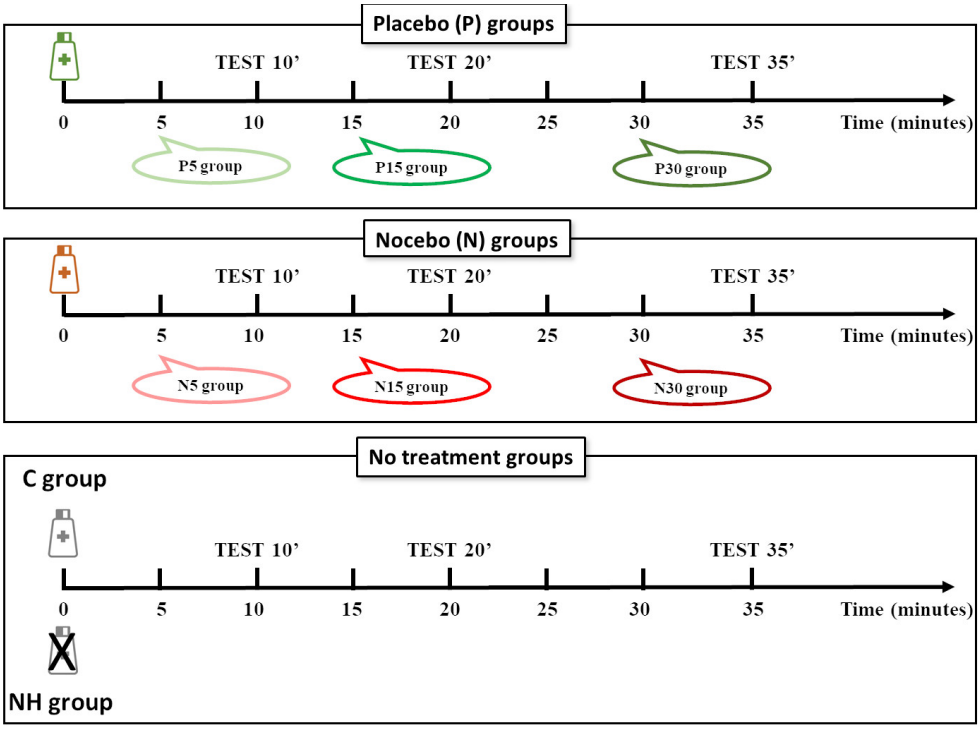

Figure 2.1: Experimental Groups: Placebo and nocebo groups received different temporal information. P5 and N5 expected the effect of the treatment to set in 5 minutes after cream application, P15 and N15 after 15 minutes and P30 and N30 after 30 minutes. The no expectancy and natural history do not receive temporal instructions.

ulation (See Section 2.6.0.2). They were led to believe that the analgesic would become effective after 5 minutes (Positive Verbal Suggestion 5 Group, P5 group, N=21), 15 minutes (Positive Verbal Suggestion 15 Group, P15 group, N=20) or 30 minutes (Positive Verbal suggestion 30 Group, P30group, N=20). The 5 minutes interval was chosen to mimic the effect of a fast-acting analgesic, whereas the 15 and 30 minute interval were intended to resemble the effect of analgesics with a delayed onset time.

2.3.2.2 Nocebo groups

Participants in the nocebo groups were informed that a hyperalgesic cream would be applied to increase the painful sensation induced by the electrical stimulation. Participants were informed that the hyperalgesic effect would set in after 5 minutes (Negative Verbal Suggestion 5 Group, N5 group, N=19), after 15 minutes (Negative Verbal Suggestion 15 Group, N15 group, N=19) or after 30 minutes (Negative Verbal Suggestion 30 Group, N30 group, N=18). The time intervals were identical to those used in the placebo groups.

2.3.2.3 No expectancy group

Participants assigned to the No Expectancy Group (NE group, N=21) were informed that an inert cream would be applied that would have no effect on their pain perception.

2.3.2.4 Natural history group

A Natural History Group (NH group, N=19) was added to control for the natural course of pain. In this group, no cream was applied and no verbal suggestions concerning treatment effectiveness were given.

2.3.2.5 Noxious stimulation

Pain was induced using electrical stimuli delivered by a somatosensory stimulator (Neuroscan, Compumedics, Charlotte, NC, USA). See Noxious stimulation in the Supplemental Digital Content Section 2.6.0.2 for more details.

2.3.2.6 Pain intensity ratings

All participants were instructed to rate the perceived intensity of the ten noxious electrical stimuli 10 minutes (Test10'), 20 minutes (Test 20') and 35

minutes (Test 35') after the cream had been applied. Ratings were provided verbally using the same NRS as during the calibration phase (i.e., 0 representing no pain, 1 the beginning of a painful sensation, 5 moderate and 10 unbearable pain) and were recorded by the experimenter.

2.3.2.7 Assessment of expectations and anxiety

Participants completed the State-Trait Anxiety Inventory (I-II) [139] prior to the actual experiment, to assess potential baseline differences in anxiety level between groups. Moreover, after the experiment, they were asked to rate their expectations about the efficacy of the cream. The question asked was: *"Before the experiment, what was your expectation about the analgesic action of the cream?"*. Five possible answers were proposed: *1) the cream will completely reduce my pain; 2) the cream will partially reduce my pain; 3) the cream will slightly reduce my pain; 4) the cream will not alter my pain; 4) the cream will increase my pain.* Questions were reversed for the nocebo groups, in which the hyperalgesic action of the nocebo cream was investigated.

2.3.2.8 Experimental procedure

The experimental procedure is summarized in Figure 2.2. Participants sat on a chair with the right arm placed on a desk in front of them. A customised wall clock with 5-minute intervals (i.e., 5 to 55) was positioned in front of them. The clock face also showed an icon of a cream tube at the 12 o'clock position to indicate the time-point at which the cream had been applied. Participants were informed that the clock was used to help them keep track of time and to know when the next test session was imminent. Following calibration of the individual stimulation intensity, participants were familiarised with the experimental setup in a practice run. After a 5 minutes break, the actual experiment commenced with the baseline

session. Subsequently, participants were informed about the group they had been assigned to and the cream was applied in all groups except the no treatment group along with the verbal instructions (See Section 2.3.2.11 for details). Immediately after the cream had been applied, the experimenter adjusted the clock so that the minute hand pointed at the 12 o'clock position, indicating the time of cream application ('Time 0'). Although no cream was applied in the no treatment group, the same procedure was followed and participants were told that 'Time 0' referred to the time at which the cream would have been applied if they had been allocated to the active condition. Ten minutes after cream administration, the first test session was run (Test 10') which was followed by a 10 minute rest period. The second test session was completed 20 minutes after cream application (Test 20') and followed by a 15 minute rest period. Participants underwent the third and final test session 35 minutes after cream application (Test 35'). Note that the test was performed 10, 20 and 35 minutes after cream application and not after 5, 15 and 30 minutes, which were the specific time points at which participants expected the cream to set in, depending on group allocation. We allowed a 5-minute leeway to avoid participants doubting that the effect of a cream could be so precisely timed (i.e., setting in exactly after 5, 15 and 30 minutes). During the rest periods between test sessions participants were instructed to relax and were given the opportunity to read, study or use their phone. Although participants were able to track time by looking at the watch in front of them, the experimenter communicated verbally that the next test session was about to start by saying *"Okay, now we will repeat the test again"*.

2.3.2.9 Verbal suggestions

Participants in the placebo groups expected to receive a cream that would

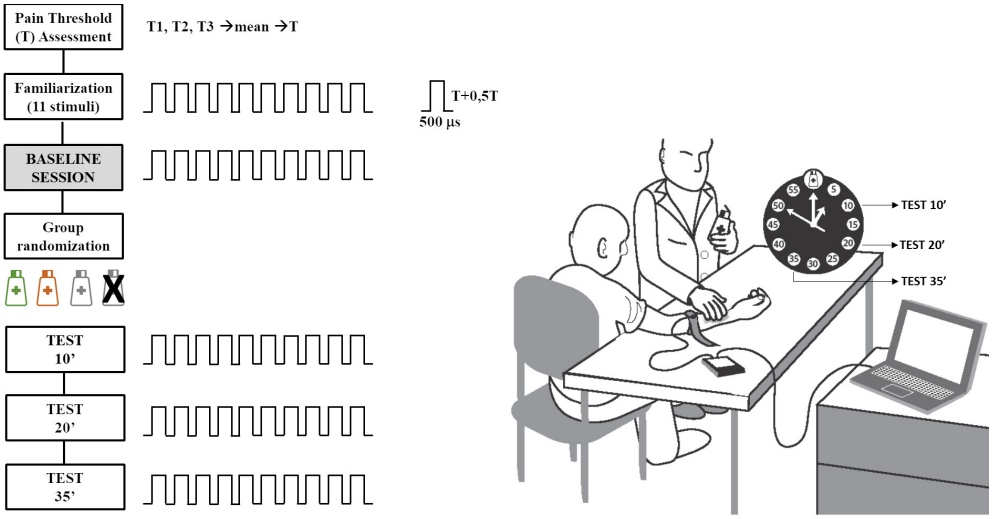

Figure 2.2: Experimental protocol: Diagram showing each experimental step (A). Firstly, pain threshold assessment. Secondly, familiarization trial followed, after a 5 minutes break, by the Baseline Session. Thirdly, cream administration along with verbal instructions which varied accordingly with group allocation. Lastly, the three Test Sessions occurring after 10, 20 and 35 minutes from cream application. Stimuli intensity remains stable within and across pain trials. To be noted that the NH group was not included in the randomization and was part of a pilot study. The image next to the diagram (B) shows experiment set up, including the customised wall clock facing the participant.

decrease their pain (analgesic cream), whereas participants in the nocebo groups expected to receive a cream that would increase their pain (hyperalgesic cream). The instructions were provided at the time-point of cream application. Below, an example of the instructions provided to the P5/N5 group is reported. *"Studies have shown that this is an effective analgesic/hyperalgesic cream. Specifically, they have shown that this cream already takes effect after 5 minutes from its application. Therefore, we expect that all the tests we will do after this moment are under the effect of the cream, for this reason you will feel less/more pain (compared to the first test) in all three test sessions after 10, 20 and 35 minutes [experimenter points at time 10, 20 and 35 minute marks on the clock]".* Participants in the NE group were told that an inert cream would be applied:

"The agent you will receive is an inert cream that only has hydrating properties but no effect on pain perception. The pain at the three test sessions after 10, 20 and 35 minutes [experimenter points at time 10, 20 and 35 minute marks on the clock] will therefore be similar to the first baseline". Participants in the NH group were told that they would receive no treatment and the following instructions were given: *"Your group serves to assess natural variations of pain over time. Your pain may vary or may remain the same. Simply report your perceived pain intensity when you are prompted to do so".*

2.3.2.10 Cream

A sham cream was administered in the placebo, nocebo and NE groups. It consisted of AquaGel Solution (2g) and water (17ml) and was presented to participants in a clear plastic tube. The experimenter applied the cream within a radius of 2cm around the electrode and massaged it into the skin to ensure that it was fully absorbed.

2.3.2.11 Debriefing

Participants were debriefed via email, giving details on the real aim of the study, a list of readings on the topic, as well as the possibility of discussing doubts or concerns with their data being used.

2.3.2.12 Statistical analyses

Analyses were performed using Statistica Software (StatSoft, version 9 for Windows). First, a mean pain intensity rating was calculated for each time-point (i.e., baseline and T10', T20' and T35') by averaging across the 10 ratings per time-point, resulting in four mean pain intensity ratings for each participant. Second, these average measures were tested for normal distribution using the

Shapiro-Wilk test. The test did not report significance for any of the variables. Before running further statistical analyses, the data were screened for outliers. Participants were defined as outliers and discarded from further analysis if any of their average pain intensity ratings were 2.5 times the standard deviation higher or lower than their group mean value. Two outliers were removed from the NH group (N=19), the N15 group (N= 19) and the N30 group (N=19). One outlier was excluded from the NE group (N=21), the P30 group (N=20), and the N5 group (N=19). The final sample size was therefore 157 participants. To test for baseline differences in demographic variables, baseline pain intensity scores and STAI I-II scores, we first compared the eight groups using an analysis of variance (ANOVA) for continuous variables, or Chi-square test for categorial variables. Turning to pain intensity ratings, to test whether pain perception changed over time irrespective of treatment and treatment onset expectations, changes in mean pain ratings of the NH group for the four different time-points were analyzed separately using repeated measure ANOVA. Similarly, to investigate whether the application of the cream in itself (e.g. moisture, sensation of freshness) interacts with pain perception, repeated measure ANOVA was used to assess pain rating changes the four different time-points in the NE group. To test whether placebo and nocebo effects occurred, we conducted 6 repeated measure ANOVAs, one for each experimental group (P5, P15, P30, N5, N15, N30), to evaluate changes in mean pain ratings (raw scores) within each group for the four different time-points.

Subsequentially, we calculated the percentage change in NRS scores from the Baseline Session to each Test Session (delta%, $\Delta\%$) in the placebo and nocebo groups. Thus, three Δs were calculated for each group, corresponding to the percentage change in pain perception at T10', T20' and T35' (Δ10, Δ20 and Δ35, respectively). To compare the effect of information regarding the onset of

the expected treatment effect between groups, Δ10, Δ20 and Δ35 of the placebo, nocebo and no expectancy group were entered into a 3 x 7 mixed ANOVA with the within-group factor TIME (3 levels: T10', T20' and T35') and between-group factor GROUP (7 levels: P5, P15, P30, N5, N15, N30 and NE). Significant effects were followed up using pairwise Student-Newman-Keuls (SNK) post hoc tests. To investigate the significant results of the factor GROUP and the interaction between GROUP and TIME (see Section 4.4), the placebo and the nocebo groups were compared against the NE group, which served as a reference. To explore the duration of the induced effects in each group, changes in pain intensity relative to baseline (i.e., Δ10, Δ20 and Δ35) were compared separately for P5, P15, N5 and N15.

Furthermore, as done in other studies [140], sex, age and BMI were included as covariates. Since we found no significant effects of these covariates on pain perception nor interactions between these predictors and results were identical to the 3 x 7 mixed ANOVA, we have only included the more parsimonious analysis. To investigate the possible influence of anxiety (scores of STAI I and STAI II) on pain perception, two correlation analysis were performed: 1) a correlation analysis between anxiety scores and mean pain intensity ratings at Baseline and 2) a correlation analysis between anxiety scores and the percentage (Δ) of pain decrease (in the placebo groups) and increase (in the nocebo groups) after the application of the cream (i.e. Δ10 in P5 and N5 groups, Δ20 in P15 and N15 groups, Δ35 in P30 and N30 groups). To investigate the possible influence of expectations of treatment efficacy on pain perception, a correlation analysis was performed between expectancy scores and the percentage (Δ) of pain decrease (in the placebo groups) and increase (in the nocebo groups) after the application of the cream. Note that data from the NH group were not included in further analyses as participants in this group did not show a significant change in pain

perception over time (Section 2.4.0.1). Data are presented as mean ± standard error of the mean (SEM), and the level of significance was set at $p < 0.05$.

2.4 Results

The groups did not differ with respect to age, Sex, BMI, state (STAI-I) and trait (STAI-II) anxiety scores nor pain intensity at baseline ($p > 0.05$ for all comparisons) (See Table 2.1 in Section 2.6)

2.4.0.1 Effects of no treatment

Repeated measure ANOVA showed no significant changes in mean pain ratings of the NH group for the four different time points [F(3,54)=0.664, $p = 0.578, p\eta^2 = 0.181$]. This indicates that without cream administration and verbal instruction regarding expected changes in pain perception, pain remained stable over the entire course of the experiment. Repeated measure ANOVA showed a significant main of time on mean pain ratings in the NE group [$F(3, 60) = 2.88, p = 0.043, p\eta^2 = 0.660$]. SNK post hoc tests reported a tendency to significance when comparing baseline with T10 ($p = 0.051$) and T20 ($p = 0.057$), and significant pain increase between baseline and T35 ($p = 0.044$). This suggests that pain perception tends to increase with time when the inert cream is applied with no expectation modulation.

2.4.0.2 Placebo and Nocebo Raw Data Analysis

Three repeated measure ANOVAs for the three placebo groups showed significant changes in mean pain rating for P5 [$F(3, 60) = 9.77, p < 0.001, p\eta^2 = 0.997$],

P15 $[F(3,57) = 22.86, p < 0.001, p\eta^2 = 1.000]$, P30 $[F(3,57) = 15.01, p < 0.001, p\eta^2 = 1.000]$, for the four different time points. In P5 group, SNK post hoc tests showed a significant reduction in mean pain ratings from baseline to T10, T20 and T35 ($p < 0.001$), suggesting that placebo analgesia occurs in all the Test Sessions occurring 10 minutes after cream application. However, no significant difference was shown between T10 and T20 ($p = 0.254$), T10 and T35 ($p = 0.334$) and T20 and T35 ($p = 0.786$), indicating that placebo analgesia remains stable once triggered. In P15 group, SNK post hoc tests indicated a significant decrease in pain ratings from baseline to T20 ($p < 0.001$) and to T35 ($p < 0.001$). Similarly, a significant decrease was reported from T10 to T20 and from T10 to T35 ($p < 0.001$). These comparisons show that placebo analgesia arise in the two test sessions occurring after 20 minutes from cream application. No significant difference was shown between T20 and T35 ($p = 0.839$) showing, similarly to P5, that once analgesia is triggered it remains stable over time. In P30 group, SNK post hoc tests reported a significant decrease in pain ratings from baseline to T35 (p¡0.001), from T10 ($p = 0.001$) to T35 and from T20 to T35 ($p < 0.001$) showing placebo analgesia to arise in the final test session, after 35 minutes from cream application.

Three repeated measure ANOVAs for the three nocebo groups showed significant changes in mean pain rating for N5 $[F(3,54) = 26.27, p < 0.001, p\eta^2 = 1.000]$, N15 $[F(3,54) = 33.40, p < 0.001, p\eta^2 = 0.997]$, N30 $[F(3,51) = 26.44, p < 0.001, p\eta^2 = 1.000]$, for the four different time points. In N5 group, SNK post hoc tests showed a significant increase in mean pain ratings scores from baseline to T10 ($p < 0.001$), T20 ($p < 0.001$), and T35 ($p < 0.001$), suggesting that nocebo hyperalgesia occurs in all the test sessions occurring after 10 minutes from cream application. Additionally, significant increase in pain ratings was reported between T10 and T20 ($p = 0.045$) and between T10 and T35 ($p = 0.006$), suggest-

ing that once triggered, nocebo hyperalgesia increases over time. In N15 group, SNK post hoc tests indicated a significant increase in pain rating from baseline to T20 ($p < 0.001$) and to T35 ($p < 0.001$) as well as from T10 to T20 and to T35 ($p < 0.001$). These comparisons indicate that nocebo hyperalgesia occurs during the two test sessions after 20 minutes from cream application. In this case also, a significant increase was shown between T20 and T35 (p=0.011). Therefore, the N5 and N15 groups agree that once nocebo hyperalgesia is triggered, it increases over time in this study. In N30 group, SNK post hoc tests displayed a significant increase in pain ratings from baseline to T35 ($p < 0.001$), from T10 ($p < 0.001$) to T35 and from T20 to T35 ($p < 0.001$) showing nocebo hyperalgesia to arise in the final test session, after 35 minutes from cream application.

2.4.0.3 Placebo and Nocebo Percentage Change Analysis

The 3x7 mixed ANOVA revealed a significant main effect of TIME ($F(2, 262) = 7.363), p = 0.001, p\eta^2 = 0.053$) and a significant main effect of GROUP ($F(6, 131) = 31.701, p < 0.001, p\eta^2 = 0.069$). See Section 2.6.0.3 for more details. Most importantly, we found a significant interaction between TIME and GROUP ($F(12, 262) = 18.172, p < 0.001, p\eta^2 = 0.454$) (Figure 2.3).

Comparisons between placebo groups (P5, P15, P30) and the NE group showed significantly stronger pain reduction in the P5 group after 10 ($p = 0.003$), 20 ($p < 0.001$) and 35 ($p < 0.001$) minutes. In the P15 group, the analgesic effect was stronger after 20 ($p < 0.001$) and 35 minutes ($p < 0.001$) whereas the P30 group only showed a significantly stronger decrease in pain after 35 minutes ($p < 0.001$). These results indicate that the analgesic effect strictly followed the verbal information about the expected onset of the analgesic effect. As previously reported in the raw data analysis, P5 and P15 did not show differences

in perceived pain after analgesia onset, confirming that once triggered, placebo analgesia is stable over time.

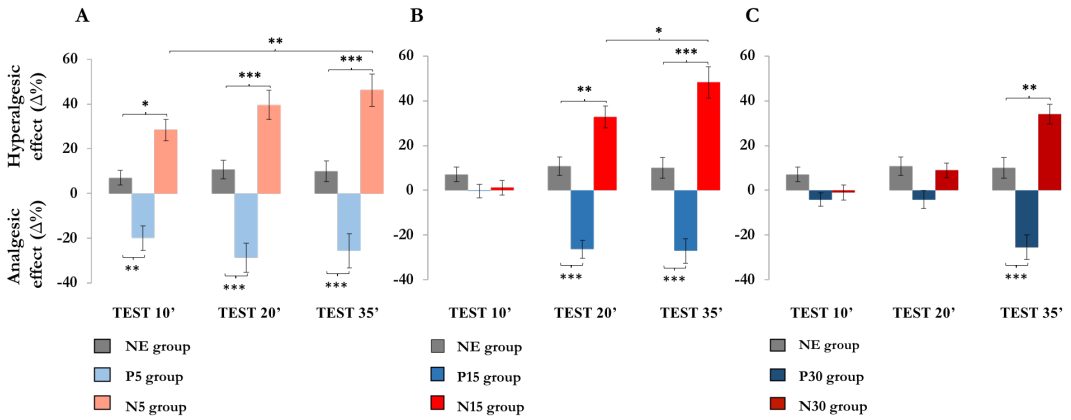

Figure 2.3: Results are presented as percentage of change ($\Delta\%$) from Baseline in each test (Test 10, Test 20, Test 35). Left side: groups where the expected onset of action of the treatment was 5 minutes (plus no expectancy group). Middle: groups where the expected onset of action of the treatment was 15 minutes (plus no expectancy group). Right side: groups where the expected onset of action of the treatment was 30 minutes (plus no expectancy group). Gray bars depict the NE group, green bars (light, middle and dark) depict placebo groups and red bars (light, middle and dark) depict nocebo groups. Asterisks represent significant differences ($* = p < 0.05; ** = p < 0.01; *** = p < 0.001$). Error bars represent standard errors of the mean (SEMs).

Turning to the nocebo groups (N5, N15, N30), the N5 group showed a significantly stronger increase in pain ratings than the NE group after 10 ($p = 0.02$), 20 ($p < 0.001$) and 35 ($p < 0.001$) minutes. In the N15 group, a significantly stronger pain increase compared to the NE group was found only after 20 ($p = 0.006$) and 35 ($p < 0.001$) minutes. In the N30 group, a significant pain increase was only detected after 35 minutes ($p = 0.007$).Again, these results suggest that the effect on pain perception strictly follows the verbal information provided. In contrast to the placebo effect which remained stable over time, the hyperalgesic effect became stronger over the course of the experiment. In the N5 group, $\Delta 35$ was significantly larger than $\Delta 10$ ($p = 0.002$). Similarly, an increasing hyperalgesic

effect was found in the N15 group with a larger $\Delta 35$ than $\Delta 20$ ($p = 0.013$). These results replicate what previously shown in the raw scores analysis, and indicate that, once the hyperalgesic effect has set in it continues to increase over time. To further explore this effect, compared to stable placebo analgesic effects, a series of t-test was performed in order to specifically compare Δs between the N5 and P5 groups, as well as between the N15 and P15 groups. Results showed significant differences only between $\Delta 35$ of N5 compared to P5 ($p = 0.028$) and $\Delta 35$ of N15 compared to P15 ($p = 0.011$).

Finally, considering the effect of anxiety scores on pain perception, we found a positive correlation between STAI II and the percentage (Δ) of pain increase after the application of the cream in the nocebo groups ($r(56) = 0.329, p = 0.013$), but not in the placebo group. No significant correlation between expectancy scores and the percentage (Δ) of pain decrease (in the placebo groups) and increase (in the nocebo groups) after the application of the cream was found.

2.5 Discussion

This study demonstrates for the first time that verbal information regarding the expected onset of a treatment effect can influence the time-course of placebo and nocebo effects. Participants who had been informed that the cream would take effect promptly following application showed an early analgesic (placebo group) or hyperalgesic effect (nocebo group). Similarly, both effects only set in later in those who expected their treatment effect to unfold after a longer delay.

Previous studies have shown that verbal information can stir placebo and nocebo effects [124; 141; 142]. However, the majority of these studies focused on information about the direction (e.g., increase or decrease of pain) [131; 143; 144] or the magnitude of the effect (e.g., strong or weak) [145; 146]. Results of the

present study demonstrate that expectations also contain a temporal aspect that determines the onset of placebo analgesia and nocebo hyperalgesia and that can be modulated through information provided to the individual. Our observation of a delayed analgesic response could therefore be of interest in clinical contexts where - due to the pharmacological properties of the treatment - the effect of an intervention only becomes noticable to the patient after days or even weeks. For instance, the delayed mechanism of action of certain antidepressants requires patients to maintain their positive treatment expectations over an extended period of time until the drug has reached the required blood concentration to take effect. Informing patients about the delayed onset could prevent (premature) abandonment of positive treatment expectations during the period when no treatment effect is detectable (yet) and preserve the supportive effect of these expectations for the time-point when the pharmacological effect sets in. Our findings show that the positive influence of expectations on pain perception could be withheld for at least 30 minutes. Whether these encouraging findings translate to longer delays requires further investigation. Regarding the magnitude of the effects found, we reported a 25.6% pain decrease in the placebo groups (that is mean pain reduction from the baseline after the sham analgesic cream took effect) and a 37.5% pain increase in the nocebo groups (mean pain increase from the baseline after the sham hyperalgesic cream took effect). Considering the baseline ratings of the different groups, this result equals an overall mean decrease of 0.6 points in the NRS in the placebo groups and an overall mean increase of 1 point in the NRS in the nocebo groups. While these are small changes from the baseline values, similar ranges of pain ratings have been observed in recent experimental studies using behavioral paradigms [147; 148] as well as in classic neuroimaging studies [149]. Here we demonstrated the effect of verbal information following the application of an inert substance, but verbal information can also affect the efficacy of

active treatments. Information leading to expectations of pain relief doubled the analgesic outcome of an opioid treatment whereas information inducing negative expectations abolished its beneficial effect [125].It follows that the small effects of placebo and nocebos interventions become larger when delivered in association with active treatments, rendering such phenomena valuable strategies in the clinical context. However, the impact that temporal information can have on the clinical setting is yet to be explored. Future studies are needed to investigate clinical conditions, such as low back pain or neuropathic pain, in order to achieve more ecological results. The effect of temporal information on analgesia should be explored when delivered in association with active treatments.

A second important finding of this study relates to the changes in placebo and nocebo effects once they have been triggered. As shown in Figure 2.3, the hyperalgesic effect becomes stronger over the course of the experiment while the analgesic effect remained stable. Given that the stimulation was calibrated to the same perceived level in all groups, and neither the placebo nor the no treatment groups showed an increase in pain ratings, it seems unlikely that the hyperalgesic effect was the result of peripheral sensitization. Contemporary models of perception, such as the predictive coding model [150], have offered explanations for such changes in perception which are rooted in the understanding that any type of perception is based on an inferential process. In this framework, incoming sensory information is compared to expectations which the individual holds. If sensory input is as expected, the expectation is confirmed. However, if expectations and incoming information are incongruent, the expectation will be updated following a learning rule that determines the translation of expectancy violation (formalized as prediction error) into expectation updating. Within this framework, two scenarios could explain the difference between the placebo and nocebo group we observed. First, the intensity of the noxious input at T10 confirmed

expectations in the placebo group but was stronger than expected in the nocebo group. As a result, expectations would be updated (towards a higher intensity) only in the nocebo group. Alternatively, a similar discrepancy between expectation and incoming information might have been detected in the placebo and nocebo groups but a different learning rule was applied that led to an upwards correction of expectations (and subsequently of the perceived stimulus intensity in subsequent test sessions) in the nocebo group but not the placebo group. A recent study using a cue probability paradigm suggested a similar asymmetry in expectation updating [126] which might be driven by the higher biological relevance of an aggravation of aversive sensory experience (as in the nocebo condition) compared to a turn for the better (as in the placebo condition). Because we did not acquire trial-by-trial expectancy ratings and are therefore unable to verify whether expectancy ratings were adapted between test sessions, further studies are needed to explore the link between the changes in pain perception over time and expectation updating. This study also confirms the crucial role of anxiety on nocebo effects and is in line with previous data that highlight how anxiety affect hyperalgesia [151; 152].

Some limitations of the present study need to be considered. The first source of limitation to be discussed here is the number of participants. Even though more than 160 participants were recruited, different shortcomings of the current experiment could be derived from the smaller number of participants included in each experimental group (i.e. NE, P5, P15, P30, N5, N15, N30). Firstly, the use of the SNK post hoc test instead of more strict corrections for multiple comparisons, such as the Bonferroni correction, could be questioned. However, we performed a planned-comparison Bonferroni correction which resulted in the same significant results highlighted by the SNK test, leaving out only the difference between $\Delta 10$ and $\Delta 35$ in the N5 group, thus confirming our main results

and conclusions. In addition, this study is the first to directly investigate the temporal aspects of placebo/nocebo effects. Therefore, the usage of a less strict post hoc test can be justified because it allows to discover important albeit small differences that are present in new and poorly understood phenomena [153; 154]. The second limitation stemming from the low number of participants is the lack of a full randomization assignment in favor of a stratified randomization due to the need to balance specific characteristics, such as sex and age between groups. However, adding sex, age and BMI characteristics as covariates to the main analysis did not show any significant impact of these factors on pain perception nor significant differences between groups regarding these variables. Still, future studies, possibly focusing on one single experimental question (e.g. focusing only on placebo analgesia or nocebo hyperalgesia), should reach a higher number of participants per group to avoid these limitations. The second source of limitation is that our study focused on rather low pain intensities (NRS below 5) which may have induced a "floor effect" such that nocebo effects (i.e. changes toward the higher part of the scale) could have been overestimated, while placebo effects (i.e. changes toward the lower part of the scale) could have been underestimated. Nonetheless, the investigation of the magnitude of these effects goes beyond the purpose of the present study. Further research is needed to explore whether our findings persist with the use of more intense pain stimuli as well as in clinical contexts. The third source of limitation is that verbal instructions used in this study were directive as they not only described the drug effect (i.e., increases/decreases pain) but anticipated what the participant is going to feel. Although it could be argued that these suggestions make it difficult to discriminate between placebo and nocebo responses and a simple 'experimental demand' effect, other studies have used similar instructions [155; 156]. Future studies should focus on the description of the drug effect and should involve more objective measure of pain

perception (neuroimaging or electrophysiological measure) to confirm that participants are actually reporting their pain changes rather than following experimental demand. Indeed, our study results are based on participants' pain intensity ratings only. Although these ratings have been shown to correlate with peripheral (e.g., skin conductance and heart rate [138; 157] and central measures including activity in brain regions associated with pain processing [126; 127], follow-up studies should consider simultaneous recording of these measures to investigate accompanying changes in objective parameters; examples include neuroimaging studies and electrophysiological measures of pain expectations [124; 136]. Given the multi facet nature of pain perception and the importance of expectation of pain, future studies on the temporal aspects of placebo analgesia or nocebo hyperalgesia should also collect behavioral measures before the experimental sessions such as "a priori" questionnaires on expectations but also subjective reports on the unpleasantness of the pain stimuli during the different time frames.

To conclude, our data suggest that the delivery of temporal information influences the onset of placebo analgesia and of nocebo hyperalgesia. Even if these findings have been collected in an a strictly experimental context, their potential implications in a clinical context are remarkable and additional work is required to explore how our findings relate to the effect of active drugs. Strategic timing of treatment effects through targeted temporal information may have the potential to substantially enhance desired therapeutic effects and delay or abolish unwanted side effects.

2.6 Supplemental Digital Content

2.6.0.1 Sample size calculation

Sample size calculation was performed on the basis of Van Laarhoven et al. [156] because the design of that study was the most similar to ours. Specifically, placebo analgesia and nocebo hyperalgesia were induced with verbal suggestions alone, as well as cream being used to induce deception. Our sample size calculation was conducted using G*Power and it was based on Van Laarhoven et al.'s (2011) [156] effect size ($\eta^2 = 0.372$). Based on this, our recommended sample size per group is of 21 subjects (level of significance set at 5% and a statistical power set at 90%).

2.6.0.2 Noxious stimulation

Two silver chloride electrodes were positioned on the ventral side of the right forearm. The electrodes were placed on the forearm, between the ventral side of the wrist and of the elbow; at a distance from the wrist equal to half the distance between the tip of the middle finger and the wrist line. The stimulation intensity was calibrated using an ascending staircase method [158]. Three series of electrical stimuli were delivered, starting at an intensity of 0.5 mA, and increasing in steps of 0.5 mA. Each stimulus was 1 second long and was comprised of a square pulse of 500 µs duration being delivered at a frequency of 1Hz. Participants had to rate the perceived intensity of each stimulus on a Numerical Ratings Scale (NRS) ranging from 0 to 10, where 0 indicated no pain, 1 represented the beginning of a painful feeling, 5 moderate pain and 10 unbearable pain [122; 141]. The calibration run was stopped when participants' ratings reached pain threshold (i.e., when the intensity of the stimulus was rated as 1). The threshold was calculated as the average of the three pain threshold intensities. During the

actual experiment, a total of ten stimuli, with 1.5 times the current used to set the initial pain threshold, were delivered in the practice run, baseline and each of the three test time-points.

2.6.0.3 Placebo and Nocebo Percentage Change Analysis

The main effect of TIME indicated that changes in pain rating (relative to baseline) differed between test time-points across groups. Pairwise SNK tests found a significant difference between Δ10 and Δ35 (p=0.002), a trend towards a significant difference between Δ20 and Δ35 (p=0.056), but no significant difference between Δ10 and Δ20. The significant main effect of GROUP indicated that changes in pain ratings differed between groups irrespective of the time-point. SNK post hocs were computed, comparing groups across time-points. The three placebo groups showed a significantly stronger pain reduction (lower overall Δs) compared to the NE group (P5, $p < 0.001$; P15, $p < 0.001$; P30, $p < 0.001$). However, changes in pain did not differ significantly between P5, P15 and P30 which indicates that changes in pain intensity were comparable between the placebo groups if time is not taken into account. For the nocebo groups we found a significantly greater increase in pain in N5 and N15 relative to the NE group (N5, $p < 0.001$; N15, $p = 0.007$), whereas no significant difference was found between N30 and NE. Comparison between nocebo groups showed no difference between N5 and N15, however significantly higher increase in pain occurred between N5 and N30 ($p < 0.001$) and between N15 and N30 ($p = 0.024$).

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Chapter 3

'External timing' of placebo analgesia in an experimental model of sustained pain

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3.1 Abstract

Background. Research on placebo analgesia commonly focuses on the impact of information about direction (i.e., increase or decrease of pain) and magnitude of the expected analgesic effect, whereas temporal aspects of expectations have received little attention so far. In a recent study, using short-lasting, low-intensity stimuli, we demonstrated that placebo analgesia onset is influenced by temporal information. Here, we investigate whether the same effect of temporal suggestions can be found in longer lasting, high-intensity pain in a Cold Pressor Test (CPT).

Methods. Fifty-three healthy volunteers were allocated to one of three groups. Participants were informed that the application of an (inert-) cream would reduce pain after 5 min (P5) or 30 min (P30). The third group was informed that the cream only had hydrating properties (NE). All participants completed the CPT at baseline and 10 (Test 10) and 35 min (Test 35) following cream application. Percentage change in exposure time (pain tolerance) from baseline to Test 10 ($\Delta 10$) and to Test 35 ($\Delta 35$) and changes in heart rate (HR) during CPT were compared between the three groups.

Results. $\Delta 10$ was greater in P5 than in NE and P30, indicating that analgesia was only present in the group that was expecting an early onset of analgesia. $\Delta 35$ was greater in P5 and P30 compared to NE, reflecting a delayed onset of analgesia in P30 and maintained analgesia in P5. HR differences between groups were not significant.

Conclusions. Our data suggest that 'externally timing' of placebo analgesia may be possible for prolonged types of pain.

Significance. Research on placebo effects mainly focuses on the influence of information about direction (i.e., increase or decrease of pain) and magnitude

(i.e., strong or weak) of the expected effect but ignores temporal aspects of expectations. In our study in healthy volunteers, the reported onset of placebo analgesia followed the temporal information provided. Such 'external timing' effects could not only aid the clinical use of placebo treatment (e.g., in open-label placebos) but also support the efficacy of active drugs.

3.2 Introduction

Pain is understood to not only be determined by noxious afferent input but also by the individual's expectations [159]. Although this influence has been demonstrated in various experimental paradigms, placebo analgesia remains the most intriguing of them. The mere information that one has received a potent painkiller can lead to substantial pain reduction – even though the 'painkiller' contains no analgesic properties. Experimental and clinical studies aiming to harness the power of expectations have focused on providing information about the direction (i.e., whether treatment is expected to ameliorate or aggravate symptoms) [160; 161; 162] and magnitude of the expected effect [163] but have so far ignored that expectations also include a temporal aspect that determines when the desired effect is expected to set in and how long it lasts. In a recent study we demonstrated that temporal information about the expected onset of the placebo effect determines the reported start of pain reduction suggesting that 'external timing' of placebo effects is possible [164]. By 'external timing' we mean the unfolding of the placebo response in line with externally provided temporal information. While participants who were told that the placebo effect would commence after five minutes reported early pain reduction, those who were

instructed that the analgesic effect would only set in thirty minutes after cream application showed a delayed onset in their analgesic response. Like many experimental pain studies, we used short-lasting electrical stimuli to probe the influence of temporal information. While this phasic pain model offers several advantages in an experimental setting (e.g., more repetitions for a higher number of trials), it has been criticized for its limited ecological validity [165; 166]. Furthermore, the short duration and low stimulus intensity might have made it easy for verbal suggestions to bias perception. According to contemporary models of perception, verbal suggestions shape expectations which serve as a prior in an inferential process that interprets incoming sensory information [167]. Importantly, the relative impact of sensory information in this process critically depends on its precision. Expectations are more likely to impact perception when stimuli are weak, noisy and ambiguous [168; 169], such as the short-lasting, low-intensity electrical stimuli we used in the previous study. The temporal shift in the onset of the placebo response we found might therefore at least partly be explained by these stimulus features. Here, we investigated whether the modulatory effect of temporal information on the onset and duration of placebo analgesia extends to more intense, longer-lasting (tonic) pain in a Cold Pressor Test (CPT). The test assesses pain tolerance operationalised as the time participants are able to keep their hand in ice-cold water before the pain becomes unbearable. This paradigm allowed us not only to investigate whether tonic pain is equally susceptible to temporal information as phasic pain, but also to use a behavioural outcome measure (i.e., time until the hand is withdrawn from the water) instead of verbal pain reports only.

3.3 Methods

3.3.1 Participants

Seventy-seven healthy volunteers were recruited from the student population of the Vrije Universiteit Brussel (VUB), Belgium. Participants had no history of neurological, psychiatric, or other chronic medical conditions and were instructed not to consume alcohol or analgesic medication twelve hours prior to the experiment. 29 participants had to be excluded: one participant developed muscle cramps in her arm during the experiment, whilst 28 participants did not withdraw their hand from the ice water within the maximum exposure time that we set for safety reasons - i.e. maximum exposure time was set at 10 min during the familiarisation trial and at 15 min during the test sessions [170; 171]. Of these 28, 26 exceeded the maximum exposure time during the familiarisation trial, i.e., before participants were assigned to the different groups. The remaining 2 out of these 28 participants which were ultimately excluded reached maximum exposure time during one of the test sessions (baseline, test10, test35). The final sample therefore comprised 48 participants. Participants were informed they would take part in a study investigating the onset of the effect of a newly developed analgesic cream. All participants provided written informed consent in which they also agreed to be debriefed about the details of the study at the end of the experiment. All experimental procedures were conducted in accordance with the policies and ethical principles of the Declaration of Helsinki. The study was approved by the Ethics Committee of the Vrije Universiteit Brussel (B.U.N. 143201940102).

3.3.2 Group allocation

Participants were randomised by blind extraction to one of two placebo groups or a control group.

3.3.2.1 Placebo groups

In the two placebo groups, participants were informed that the inert cream (see details below) that was applied contained an analgesic substance that would reduce the painful sensation induced during the CPT. The two groups differed in the information they received about the expected onset of the analgesic effect. The first placebo group was led to believe that the analgesic would become effective after 5 min (Positive Verbal Suggestion 5 Group, P5 group, N=16), mimicking a fast-acting analgesic (*'The agent you will receive is known to have a strong analgesic effect which sets in about 5 min after application. You will therefore become less sensitive to pain and be able to keep your hand in the cold water for a longer period of time in the two test sessions after 10 and 35 min [experimenter points at time 10 and 35 minute marks on a clock] compared to the first test [CPT baseline].'*). The second placebo group was informed that the analgesic would become effective after 30 min (Positive Verbal suggestion 30 Group, P30 group, N=16), resembling the effect of analgesics with a delayed onset time (*'The agent you will receive is known to have a strong analgesic effect which sets in about 30 min after application. You will therefore become less sensitive to pain and be able to keep your hand in the cold water for a longer period of time in the test session after 35 min [experimenter points at time 35 min marks on a clock] compared to the first test [CPT baseline] and a second test after 10 min.'*).

3.3.2.2 Control groups

Participants assigned to the control group were informed that an inert cream would be applied that would have no effect on their pain perception (No Expectancy, NE group, N=16; *'The agent you will receive is an inert cream that only has hydrating properties but no effect on pain perception. Because the cream has no analgesic properties, your test performance after 10 and 35 min [experimenter points at time 10 and 35 minute marks on a clock] may be similar to the performance in the first test [CPT baseline] but it can also be longer or shorter than before'.*).

3.3.3 Experimental protocol

After providing written informed consent, participants were seated in a comfortable chair positioned next to the CPT device (Figure 3.1). A stopwatch displayed on a computer screen in front of the participants and a customised wall clock were used for participants' temporal orientation. The wall clock with 5-minute intervals (i.e., 5 to 55) showed an icon of a cream tube at the 12 o'clock position to indicate the time-point of cream application. A poster depicting the pain intensity rating scale was positioned on the desk. The experiment started with a 4-minute heart rate measurement at rest during which the participant was asked to breath naturally and relax. Participants were then introduced to the CPT, completed the familiarization run and filled in the psychological questionnaires. Subsequently, all participants underwent the CPT baseline test before they were allocated to one of the three groups, the cream was applied and participants were provided with information about the nature of the cream and the expected onset of the analgesic effect (placebo groups only). Immediately after

the cream had been applied, the experimenter adjusted the clock so that the minute hand pointed at the 12 o'clock position, indicating the time of cream application ('Time 0'). Afterwards, the CPT was repeated 10 min ('Test 10') and 35 min ('Test 35') after cream application. Note that the test was performed 10 and 35 minutes after cream application and not after 5 and 30 minutes, which were the specific time points at which participants expected the cream to set in, depending on group allocation. We allowed a 5-minute leeway to avoid participants doubting that the effect of a cream could be so precisely timed (i.e., setting in exactly after 5 and 30 minutes)(Figure 3.2).

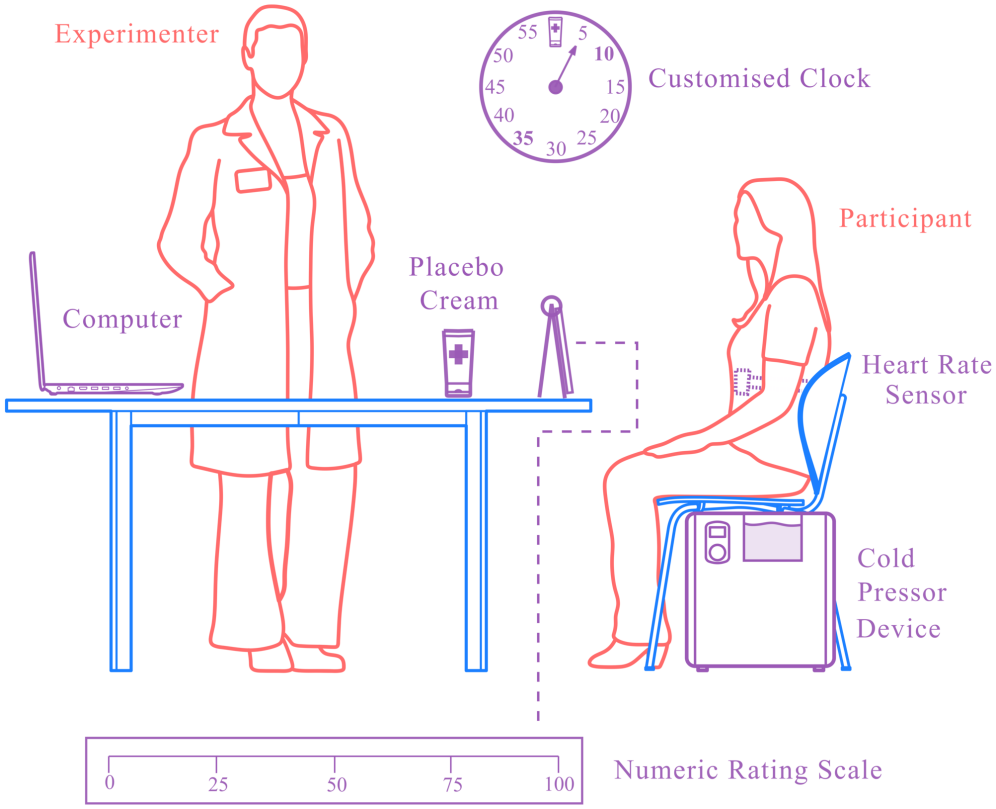

Figure 3.1: Experimental Set-up.

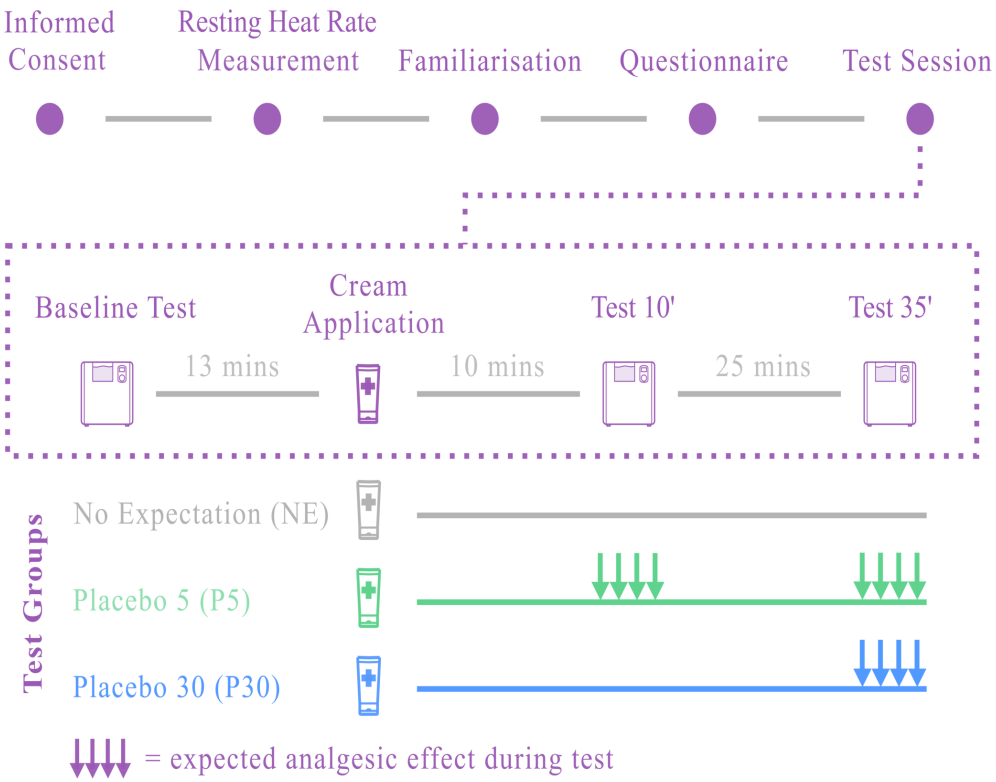

Figure 3.2: Experimental Protocol: After signing the consent form, participants' heart rate at rest was measured before participants completed the CPT familiarisation run and the psychological questionnaires. Thirteen minutes after the CPT baseline test, the cream was applied and verbal suggestions were provided (P5, P30, NE) (approx. 2 min). Placebo groups P5 (green) and P30 (blue) were informed that the cream has a strong analgesic effect that sets in 5 min (group P5) or 30 min (group P30) after application. The No Expectation group (NE, grey) was informed that the cream only had hydrating properties. The CPT was then repeated after 10 and 35 min after cream application. Placebo analgesia was expected both at Test 10' and at Test 35' for P5, and only at Test 35' for P30. No effect was expected for NE.

3.3.4 Cold Pressor Test

During the CPT, participants had to immerse their right hand in seven litres of circulating cold water (7C°, ±0.2C°; CPT device: Thermo Scientific™ VersaCool™ Refrigerated Circulating Bath, procedure adapted from Mitchell, Macdonald and

Brodie [172]. To indicate the level to which participants had to lower their hand, the experimenter drew a red line from the participant's ulnar styloid process to the radial styloid process (wrist level). The CPT was repeated a total of four times (familiarisation, baseline, Test 10', Test 35') with approximately 25 min breaks between tests to restore the baseline hand temperature (Figure 3.2). During the breaks between each CPT session, participants were allowed to read a book. Each CPT block started with one minute of HR recording at rest, followed by the actual CPT. Ten seconds before participants had to place their hand into the CPT device, they were alerted by the experimenter to get ready to immerse their hand into the water. Upon a verbal prompt from the experimenter ("Go"), the participant lowered their hand into the CPT device and the experimenter started the stopwatch to record the time between beginning of exposure and hand withdrawal. The stopwatch was displayed on a computer screen located in front of the participant for temporal orientation. Participants were instructed not to move their fingers or hand while they were immersed in the water and to keep their fingers spread with the palm parallel to the ground, but without touching it. The experimenter prompted the participant every 15 seconds to provide a verbal rating of their current pain intensity (See Section 3.3.5) which the experimenter recorded manually on a spreadsheet. The participants' task was to keep their hand in the water basin until the pain reached tolerance level. Participants then removed their hand from the water basin and rested it on a towel placed on their knees. The time elapsed between immersion and withdrawal of the hand was recorded as CPT tolerance.

3.3.5 Pain Intensity Ratings

A poster depicting the rating scale including verbal and numerical anchors

(0= *not painful at all*, 25= *somewhat painful*, 50= *moderately painful*, 75= *very painful*, 100= *unbearable pain*) was positioned in front of the participant (Figure 3.1). Participants provided numerical pain intensity ratings every 15 seconds during the CPT. Note that pain intensity ratings are not considered primary outcome measures because all participants were instructed to maintain their hand in the water until the pain reached an intensity of 100.

3.3.6 Heart Rate Recording

A decrease in heart rate (HR) has previously been shown to accompany placebo-induced analgesia [173; 174], thus HR was chosen as potential physiological correlate of this effect. Exposure to cold water triggers vasoconstriction which leads to changes in blood pressure and HR [175; 176]. However, this effect which occurs irrespective of pain should on average have been comparable in all three groups because the same time window of observation was chosen in all groups (See Section 3.3.10 for details). We therefore expected pain to have an additional effect on HR recordings which was hypothesized to vary between groups depending on the placebo effect induced by the different temporal information. The ECG signal was measured using a heart rate monitor (Polar V800) which was connected to two standard surface electrodes positioned on the participant's lower end of the sternum. Data was collected at a sampling rate of 700 Hz. The HR was first recorded for 4 min during a rest period in which the participant was asked to sit comfortably and breath normally. Subsequently, HR recording started one minute prior to each CPT and continued until two min after completion of the test. To limit HR artefacts, participants were instructed to maintain a constant and relaxed breath during each test session and avoid hyperventilating when in pain.

Table 3.1: Psychological questionnaires.

Questionnaire	Abbreviation	Construct/Process	Reference
Beck Anxiety Inventory	BAI	Anxiety	[181]
Behavioural Inhibition /Approach Scales	BIS/BAS	Disposition for behavioural inhibition/approach	[182]
Fear of Pain Questionnaire	FPQ	Fear of pain	[183]
Revised Life Oriented Test	RLOT	Degree of optimism	[184]

3.3.7 Assessment of pain-related psychological traits

Participants completed a set of questionnaires (Table 3.1) to assess psychological traits that have previously been linked to placebo responsiveness [177; 178; 179; 180]. Questionnaires were completed between the familiarization with the CPT and the test sessions. At the end of the experiment, the two placebo groups were asked to retrospectively rate how they had expected the cream to affect (i) their pain during the experiment ('When the cream was applied on your hand, did you expect it to make you feel less pain during the task?') and (ii) their ability to keep their hand in the cold water ('When the cream was applied to your hand, did you expect it to make you keep your hand in the water for longer?'). Furthermore, participants had to retrospectively rate between 0 (= *not at all*) to 7 (= *very much*) to which extent they had believed the information regarding the onset of the analgesic effect ('When the cream was applied on your hand, how much did you agree with the following statement: The cream will start to become effective after 5 min (P5); The cream will start to become effective after 30 min (P30)').

3.3.8 Cream

A sham cream was administered in all three groups. It consisted of a water-

based gel (KY-gel, Johnson & Johnson) which was presented to participants in a transparent plastic tube. The experimenter applied the cream on the volar and dorsal side of the hand up to the red line which had previously been drawn onto the participant's wrist to indicate how deep the hand had to be submerged into the water. The cream was massaged into the skin for approximately one minute to ensure that it was fully absorbed.

3.3.9 Debriefing

To debrief participants they were sent an email that explained in detail the actual purpose of the study and why deception that had been used. Participants were offered to contact the experimenter in case they felt the need to discuss their participation and any concerns related to it. They were also given the opportunity to withdraw their data. None of the participants decided to do so.

3.3.10 Statistical Analysis

Analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 9.6). To test for baseline differences in demographic variables and questionnaire scores, we compared the three groups using an analysis of variance (ANOVA) for continuous variables, or Chi-square test for categorial variables. Pain ratings (NRS from 0 to 100) were not included as an outcome measure but served to check whether participants had kept their hand in the cold water until tolerance level had been reached. Note that these ratings do not necessarily represent the level of pain at the moment of hand withdrawal, but the last rating participants provided before they removed their hand (e.g. if the hand

was removed after 59 second, the last pain intensity rating was obtained after 45 seconds). Median and interquartile range (IQR) are reported in Section 3.4.

As data for CPT tolerance at baseline, after 10 (Test 10') and 30 (Test 35') minutes did not follow a normal distribution (Shapiro-Wilk tests $p < .05$), non-parametric tests were used throughout. First, Kruskal-Wallis H-Test was performed to test for baseline differences in CPT tolerance between groups. Second, Friedman Tests were performed to detect differences in CPT tolerance between the three different time points (Baseline, Test 10' and Test 35') separately for each group. Data are presented as median ±interquartile range (IQR) and level of significance was set at $p < .05$. Significant results were followed up using Wilcoxon Signed Rank Tests, Bonferroni-corrected for multiple comparisons. Effect sizes were calculated as $r = z/\sqrt{N}$ [185]. Third, we compared changes in CPT tolerance between groups using a Kruskal-Wallis H-Test. To this end, we calculated the percentage change in CPT tolerance from baseline to CPT10' (Δ 10) and baseline to CPT35' (Δ 35) for each participant as follow:

Δ 10 = (CPT Test 10'*100)/ Baseline CPT-100
Δ 35 = (CPT Test 35'*100)/ Baseline CPT-100.

Because baseline tolerance level varied considerably across participants (see IQR in Table 3.3; Section 3.4), percentage changes were used as a way to scale the results with respect to each participant's baseline tolerance, thus making the increase or decrease more comparable between participants. Significant results were followed up using pairwise Mann Whitney U Tests, Bonferroni-corrected for multiple comparisons. Effect sizes were calculated as $r = z/\sqrt{N}$ [185].

To investigate the possible influence of expectations of treatment efficacy on CPT tolerance, Spearman rank-order correlation analyses were performed in the placebo groups between retrospective expectancy measures and Δ 10 and Δ 35.

Expectancy measures assessed how participants expected the cream to affect a) their *pain*, b) *pain tolerance* as well as their expectations regarding the *onset of the analgesic effect*.

For the analysis of HR data, ECG recordings were first truncated at the shortest tolerance time recorded (i.e., 15 seconds after hand immersion) to ensure comparability across participants. The mean HR for each of the three CPTs (i.e., baseline, Test 10' and Test 35') was calculated for each participant by averaging the number of heartbeats within this time window, resulting in three mean HR indices for each participant. As HR data were normally distributed (Shapiro-Wilk tests $p > .05$), parametric analysis was used. To compare the HR between groups and time-points, an ANOVA with the within-subject factor TIME (Baseline, Test 10' and Test 35') and between-subject factor GROUP (NE, P5, P30) was used. Significant results were followed up using Bonferroni-corrected t-tests.

3.4 Results

The groups did not differ with respect to age, sex, BMI and psychological variables (anxiety, disposition for behavioural inhibition/approach, fear of pain and degree of optimism) ($p > .05$ for all comparisons). For participants' characteristics, see Table 3.2 (demographics) and Table 3.3 (psychological traits). Participants' pain intensity ratings served to check whether participants indeed only removed their hand from the ice water when the pain had become unbearable. As shown in Table 3.4, ratings reached on average (median) NRS of 90 or higher in all test sessions.

Table 3.2: Participants' demographics.

Demographics			
Groups	**No Expectations**	**Placebo 5**	**Placebo 30**
N	16	16	16
Age in years (Mean/SD)	28.14 ±2.13	24.78 ±3.17	26.08 ±5.99
BMI (Mean/SD)	24.3 ±2.6	22.2 ±4.0	22.1 ±3.0
Sex	9 M ; 7 F	6 M; 10 F	9 M; 7 F
Handedness	12 R ; 4 L	15 R; 1 L	16 R

Note: M, Male; F, Female; R, Right; L, Left.

Table 3.3: Psychological traits

Psychological Traits			
Groups	**No Expectations**	**Placebo 5**	**Placebo 30**
BAI	10.25 ±5.05	9.25 ±6.84	10.31 ±5.62
BAS-Drive	8.81 ±1.84	9.69 ±2.02	8.69 ±2.33
BAS -Fun-Seeking	8.25 ±1.84	8.25 ±2.18	7.88 ±1.67
BAS-Reward	8.50 ±2.1	7.56 ±1.82	8 ±1.67
BIS	14.75 ±2.08	15.13 ±2.63	15.38 ±2.73
FPQ	72.75 ±13.23	78.38 ±14.26	77 ±13.97
RLoT	13.94 ±3.99	14.38 ±6.28	14.75 ±5

Abbreviations: BAI, Beck Anxiety Inventory; BAS, Behavioural Approach Scale; BIS, Behavioural Inhibition Scale; FPQ, Fear of Pain Questionnaire; RLoT, Life-Orientation Test-Revisited.

3.4.1 Placebo Effects: Within group comparison

Friedman Tests for within-group comparisons showed that CPT tolerance changed significantly in both placebo groups (P5, $\chi^2(2) = 18.95, p < .001$; P30, $\chi^2(2) = 21.37, p < .001$) but not in the NE group, $\chi^2(2) = 3.124$, $p = .210$ (Table 3.5). Post hoc Wilcoxon Signed Rank tests showed that in the P5 group, CPT tolerance significantly increased from baseline to Test 10' (z=-3.47, p=.002, r=.613) and was still higher than at baseline when assessed at Test 35' (z=-3.34,

Table 3.4: Median and first (Q1) and third (Q3) quartiles of maximum numerical rating of pain intensity (0-100).

	NE			P5			P30		
	Median	**Q1**	**Q3**	**Median**	**Q1**	**Q3**	**Median**	**Q1**	**Q3**
Max Baseline	90	83	99	96	90	100	92	82.5	99.75
Max T10	90	75	99	96	90	99.75	95	90	99.75
Max T35	90	80	98	97	91.25	99.75	96.5	90.5	99.75

Abbreviations: Q1, First quartile ; Q3, Third quartile

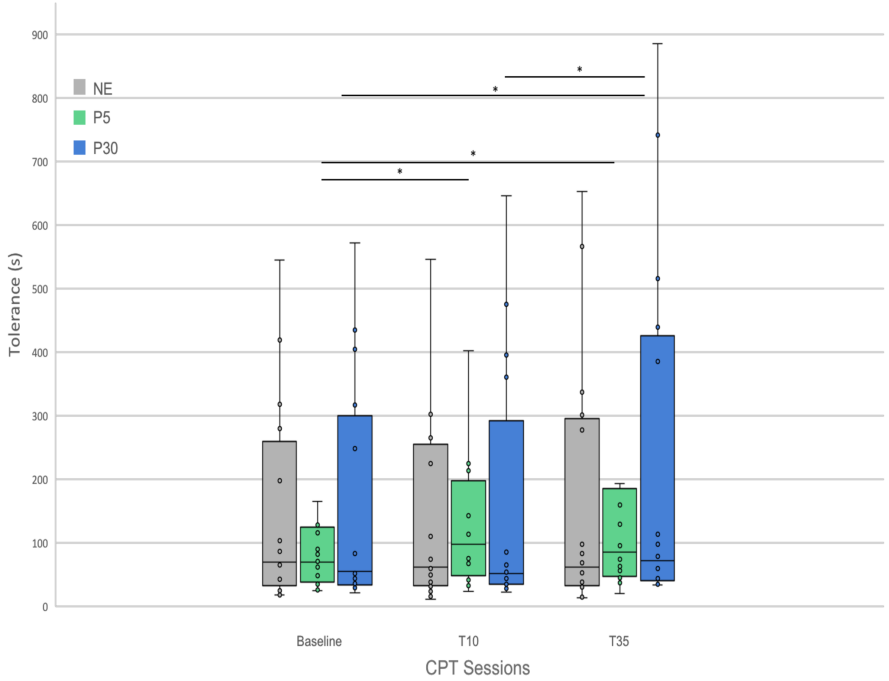

Figure 3.3: Within-group Comparison: CPT tolerance at Baseline, Test 10' and Test 35' for each group (NE, P5, P30). Asterisks indicate significant differences in tolerance time within each group ($p < .05$). In the P5 group, tolerance was significantly higher at Test 10' and at Test 35' compared to Baseline. In the P30 group, tolerance was significantly higher at Test 35' compared to both Baseline and Test 10'. The lowest and highest boundaries of the boxes indicate the 25th and the 75th percentiles, respectively. The black line within each box indicates the median. Whiskers above and below the boxes indicate the largest and the lowest data points (excluding any outliers), respectively.

p=.002, r=.590). No significant difference was found between Test 10' and Test 35' (z=-.710, p=1.434, r=.125). These results suggest that placebo analgesia

occurred at the expected time-point and once analgesia had been triggered, it remained stable over time, at least up until 35 minutes after cream application (Figure 3.3). In contrast, the P30 group showed no significant difference in CPT tolerance between baseline and Test 10' (z=-.828, p=.224, r=.146). Only at the later test time-point (Test 35'), CPT tolerance was significantly higher than baseline (z=-3.46, p=.002, r=.612) and in Test 10' (z=-3.52, p=.001, r=.622), indicating that the analgesic effect only set in late in accordance with the instructions provided (Figure 3.3).

Table 3.5: Median and IQR of CPT pain tolerance (in seconds).

	Tolerance Baseline		**Tolerance Test 10**		**Tolerance Test 35**	
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**
NE	69.50	226	62.50	223	62.50	263
P5	69.50	87	98	149	85.50	138
P35	55	266	51.59	257	72.50	386

Abbreviations: IQR, Interquartile Range.

3.4.2 Placebo Effects: Between group comparison

No significant difference in baseline CPT tolerance level between the three groups was reported by Kruskal-Wallis H-Tests (p = .988). Kruskal-Wallis H-Tests showed a significant group difference in Δ 10, $\chi^2(2) = 23.05, p < .001$, with a mean rank Δ 10 of 37.81 in P5, 20.72 in P30 and 14.97 in NE. Post hoc Mann-Whitney U-tests revealed that Δ 10 was significantly higher in P5 than in both NE (U=16.5, $p < .001$, r = .743) and P30 (U=26.5, $p < .001$, r=-.676) but did not differ significantly between the NE group and P30 (U=87, p=.266, r=.274). This indicates that 10 minutes after cream application, pain reduction was stronger in P5 than in NE and P30 (Figure 3.4). For Δ 35, Kruskal-Wallis H-Test also showed a statistically significant difference between groups, $\chi^2(2) = 18.06, p < .001$, with

a mean rank Δ 35 of 29.31 in P5, 31.75 in P30 and 12.44 in NE. Post hoc Mann-Whitney U-tests revealed that Δ 35 was significantly higher in both P5 (U=38, p=.002, r= .600) and P30 (U=25, p¡.001, r= .686) compared to the NE group, indicating that pain reduction after 35 minutes was stronger in the two placebo groups than in the NE (Figure 3.4). No significant difference in Δ35 was found between P5 and P30 (U=155, p=1.872, r=.179). Median and IQR of percent change in CPT pain tolerance (Δ10 ,Δ35) in the three experimental groups are reported in Table 3.6.

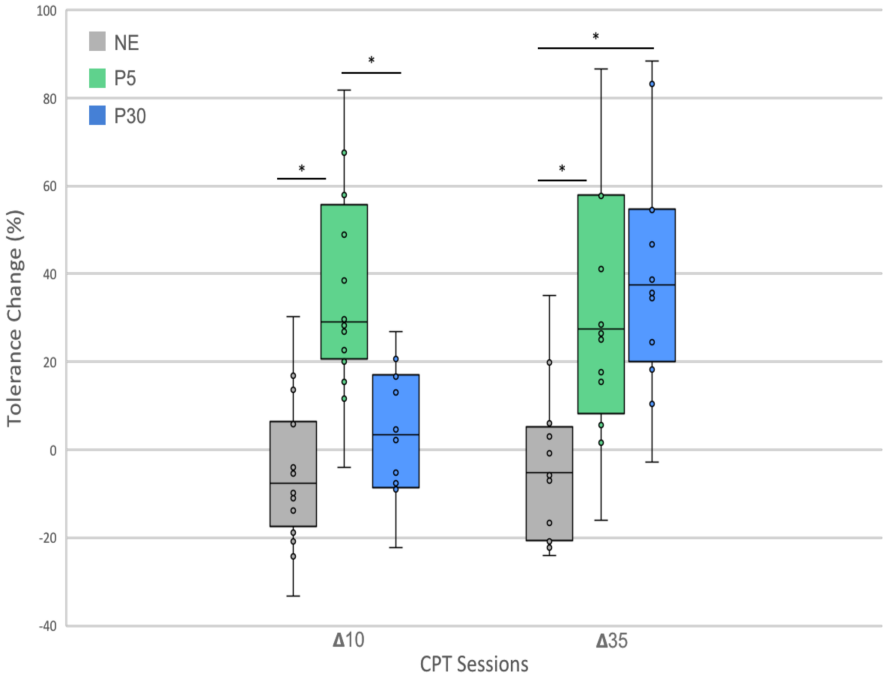

Figure 3.4: Between-group Comparison: Percent change in CPT pain tolerance from Baseline to Test 10' (Δ10) and to Test 35' (Δ 35) for each group (NE, P5, P30). Asterisks indicate significant differences in Δ s between groups ($p < .05$). Δ 10 was significantly higher in P5 than in both NE and P30. Δ 35 was significantly higher in both P5 and P30 compared to the NE group. The lowest and highest boundaries of the boxes indicate the 25th and the 75th percentiles, respectively. The black line within each box indicates the median. Whiskers above and below the boxes indicate the largest and the lowest data points (excluding any outliers), respectively.

Table 3.6: Median and IQR of percent change in CPT pain tolerance (Δ 10 ,Δ 35).

	Δ 10		**Δ 35**	
	Median	**IQR**	**Median**	**IQR**
NE	-7.54	24	-5.18	26
P5	29	35	27.55	50
P35	3.46	26	37.48	35

Abbreviations: IQR, Interquartile Range

3.4.3 Retrospective expectancy

Median and IQR of retrospective expectancy measures for both P5 and P30 are reported in Table 3.7. Spearman rank-order correlations between retrospective expectations of i) pain, ii) CPT resistance and iii) onset of analgesic effect and Δ 10 and Δ 35 did not reach significance in either of the two placebo groups.

Table 3.7: Median and IQR of retrospective expectancy ratings for pain (1 to 7), CPT resistance (1 to 7) and onset of analgesic effect (1 to 7).

	P5		**P30**	
	Median	**IQR**	**Median**	**IQR**
Retrospective Expectancy				
Pain	5.50	1.00	5.50	1.00
Resistance	6.00	1.00	6.00	1.00
Onset of analgesic effect	5.00	2.00	5.00	3.00

Abbreviations: IQR, Interquartile Range.

3.4.4 Heart Rate

HR data showed a significant main effect of TIME ($F(2,90)=19.39$, $p < .001$) but no main effect of GROUP or interaction between both factors (both $p > 0.05$). Bonferroni-corrected post hoc comparisons between the different time-

Table 3.8: HR mean and standard deviation (SD).

	Baseline		**Test 10'**		**Test 35'**	
Heart Rate	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Overall	82.60	11.74	79.04	11.28	77.75	12.33
Control	81.26	8.21	76.53	8.94	75.73	8.44
P5	81.95	11.38	80.80	11.00	77.76	13.28
P30	84.59	15.11	79.80	13.68	79.76	14.85

points revealed that the HR decreased significantly between baseline and Test 10' ($p < 0.001$) and between baseline and Test 35' ($p < 0.001$). Changes in HR from Test 10' to Test 35' did not reach significance ($p > 0.05$). Overall means and standard deviations across the three groups as well as for each group separately are reported in Table 3.8 below.

3.5 Discussion

Previous experimental placebo studies have focused on the effect of information about the direction or magnitude of the expected effect on 'treatment' outcome. In a recent study, we demonstrated that the outcome of a placebo manipulation is also influenced by information about the expected time-course of the effect[164]. Using low-intensity and short-lasting electrical stimuli, we showed that those who had been informed that the 'analgesic' would become effective shortly after administration displayed immediate (and sustained) pain reduction. In contrast, those who expected analgesia to set in after 30 minutes reported a delayed decrease in pain. Here, we extend these findings by demonstrating a similar effect in an experimental model of sustained pain (CPT) with pain tolerance as an independent behavioural outcome measure.

Our results confirm two key findings of our previous study. First, the onset of analgesia was determined by the temporal information that participants

received at the beginning of the experiment (Figure 3.4). Only those who had been instructed that the analgesic effect would commence shortly after cream application showed increased pain tolerance at the first test after baseline. The group that was informed that the pain alleviating effect would only set in later showed no analgesic effect at this early test time-point but at the expected time after 30 minutes. Such 'external timing' of placebo effect is noteworthy for several reasons. Neuroimaging studies have shown that placebo effects are mediated by top-down regulatory processes in the brain which alter responses to noxious stimuli at various stages of the neuraxis including the spinal cord [186]. However, very little is known about factors triggering this cascade. Our findings of an 'external timing' effect suggest that information reaching this top-down modulatory circuit do not necessarily prompt an immediate response but also provide a 'time tag' that determines when the effect is to be set in motion. Where and how temporal aspects of treatment expectations interface with the pain system in the brain needs to be explored using brain imaging technology. The timing effect is also noteworthy from a clinical perspective as it could open up new ways to enhance placebo effects (e.g., open-label placebo treatments [187]) but even more importantly also the efficacy of active drug treatment [188]. Expectancy effects have been shown to contribute substantially to the overall treatment outcome of active drugs [189; 190]. Although most drugs develop their maximum effect shortly after administration, some require days or weeks to become effective. For example, the desired effect of some tricyclic antidepressants often only sets in several weeks after start of treatment. Medication discontinuation is therefore a frequent problem in the early weeks of such treatment [191; 192]. Importantly, the lack of noticeable symptoms improvement can cause patients to abandon their initially positive treatment expectations. This means that once the drug has reached its critical concentration and the pharmacological effect unfolds, it

may no longer be supported by positive expectations and even be counteracted by the impression of 'treatment failure' which has been demonstrated to squelch also unrelated subsequent treatment attempts [193]. Our observation of 'external timing' of placebo effects suggests that explicitly informing patients about the delayed onset could prevent the abandoning of treatment expectations and instead trigger the supporting placebo effect when the pharmacological drug effect sets in. Because our paradigm only tested whether the onset can be shifted by thirty minutes, further studies are needed to explore more substantial delays.

We also confirmed that once placebo analgesia had been triggered, it was maintained for the duration of the experiment (Figure 3.3). In the P5 group, which expected and showed an early reduction in pain, analgesia was still present after 30 minutes without a decrease in strength. Findings from experimental studies indicate that placebo analgesia can at least be maintained for the duration of a single experimental session [161; 194] and observations from a randomised controlled trial suggest that placebo effects can even increase over time [195]. However, more systematic investigations are needed to explore the longevity of placebo effects.

The current study extends our previous findings in one very important aspect. While we previously showed an effect of temporal information on placebo analgesia using short-lasting, low-intensity stimuli, we demonstrate here that similar results can be achieved in an experimental model of high-intensity tonic pain. Phasic pain models have been criticised for their lack of ecological validity as their stimuli have little resemblance with chronic pain with respect to duration and aversiveness [166]. In contrast, CPT-induced pain increases over time until it reaches tolerance level and participants withdraw their hand. Although this type of pain is still different from clinical pain, it is undoubtedly the better proxy. Stimulus duration and intensity also play a key role for the degree to which ex-

pectations can influence perception. Modern concepts of perception posit that any sensation is determined not only by incoming sensory information but also by the individual's expectations. In this framework, expectations are assumed to have a stronger effect if the afferent input is weak, noisy or ambiguous [168; 169] leaving more room for expectations to "fill the gap" and bias the interpretation of sensory information in the expected direction. It could therefore be speculated that temporal expectations induced by verbal suggestions are more likely to impact the onset of placebo analgesia in a model using short-lasting, low-intensity stimuli (as in our previous study, [164]), than in a high-intensity and long-lasting pain model (CPT). However, a direct comparison of the strength of placebo effects suggests the opposite. While an average placebo effect of r=0.47 was found in our previous study, it was considerably stronger in the current trial (r=.71)(see Section 3.6 for details).Of note, a similar result was found in a meta-analysis by Vase et al (2009), who reported larger placebo effects for longer ($> 20s$, d=0.96) than for shorter pain stimuli ($< 20s$, d=0.81). In addition to physical stimulus features, differences in perceived controllability of the stimulation which is known to dampen the perception and neural processing of pain [196; 197] might explain the stronger placebo effects in the current study. In our CPT study, participants had to decide for how long they could keep their hand in cold water. Exposure to noxious input was therefore entirely controllable. In contrast, our previous study used a passive stimulation with no element of control.

Using (self-determined) exposure time as the key outcome also allowed us to quantify the effect of the temporal information in a way that is less prone to report bias than the commonly used pain intensity ratings. Because participants were instructed to reach tolerance level and analgesia was defined as increased exposure time, (deliberate) overreporting, for instance due to social desirability is unlikely because this would have required significantly longer exposure beyond

the previous maximum tolerance level.

In pursuit of further changes in objective parameters, we also tested whether the 'external timing' effect would be reflected in HR variations. However, HR decreased over the course of the experiment in all three groups. So far, studies exploring HR changes related to placebo analgesia have yielded inconsistent results. Studies using CPT-induced pain [194; 198] and electrical stimulation model [199; 200] found no changes in HR associated with placebo analgesia. However, other studies using ischemic arm pain [174] and thermal pain[173] reported a reduction in HR during placebo analgesia.

Another aspect to take into account is that participants were provided with a clock to help them keep track of time. It would be interesting to investigate whether temporal suggestions have the same influence in shifting treatment onset of action if participants were asked to internally monitor time passing, without the help of an external tracker, like in our case the clock. The ability to perceive time is a critical adaptive skill for individuals' survival; from predicting the time an object takes to reach us, either to catch it or to avoid it, to estimating the time to perform tasks in our everyday life [201]. Since humans are good at perceiving and predicting time, we could hypothesise that similar findings to ours would be shown in the absence of the clock. Yet, time perception is a complex phenomenon and further investigation of this aspect could give interesting outputs, especially if looking into the clinical population, in which deficits in temporal processing have been reported [202; 203].

A limitation of this study is that pain-related expectations were only assessed retrospectively (instead of repeatedly during the experiment) to avoid drawing attention to this variable and potentially disclosing the actual purpose of the experiment. Our data do therefore not allow for any conclusions regarding changes of temporal expectations over the course of the experiment. As expectations not

only impact perception but are in turn also continuously updated to reflect past (sensory) experiences, future studies could use trial-by-trial assessments of expectations to explore the dynamics of analgesic experience and expectation updating in more detail. Another aspect that warrants further investigation is the precision of temporal expectations. While in the present study, information about the expected onset of the analgesic effect were very precise and a clock helped participants to keep track of time such exact timing might be less feasible in clinical practice. Insights into the robustness of 'external timing' effects against delayed or premature onsets of treatment effects would therefore be desirable.

Taken together, our data confirm previous findings of 'external timing' of a placebo analgesic effect and extend it to an experimental model of sustained pain using pain tolerance as an observable outcome parameter. While these findings hold promise for a systematic use of this effect in therapeutic contexts, further research is required to investigate if and how it translates to clinical pain, active drug effects and different treatment approaches.

3.6 Supporting Information

3.6.1 Comparison of effect sizes of placebo responses

The present study and our previous one [164], both included two placebo groups in which participants expected the analgesic effect to start after five (P5 group) or thirty minutes (P30 group) from cream application. Both studies also included a No Expectancy (NE) group that did not expect an analgesic effect.

	Effect Size(r)
Test 10 : P5 vs NE	.468
Test 35: P30 vs NE	.465
Average r:	**.47**

Table 3.9: Effect sizes (r) of the placebo responses in Study 1, [164]

	Effect Size(r)
Test 10 : P5 vs NE	.743
Test 35: P30 vs NE	.686
Average r:	**.71**

Table 3.10: Effect sizes (r) of the placebo responses in Study 1 [204]

We calculated effect sizes (r) of the placebo responses for our previous study (Study 1, [164]) by comparing NRS scores at Test 10 between P5 and NE and at Test 35 between P30 and NE. We then computed the average effect size (Table 3.9. We calculated the effect sizes (r) of the placebo response of the current study (Study 2, [204]) by comparing tolerance change at Test 10 between P5 and NE and at Test 35 between P30 and NE. We then computed the average effect size (Table 3.10).

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3.8 References

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Chapter 4

The temporal modulation of nocebo hyperalgesia in a model of sustained pain

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4.1 Abstract

Background. Negative verbal suggestions enhance pain perception. However, the role of their temporal content remains largely unexplored. Here, we investigate whether temporal suggestions modulate the timing of nocebo hyperalgesia.

Methods. Fifty-one healthy participants were randomised across three groups. Participants received an inert cream and were instructed that the agent had either hyperalgesic properties that would manifest themselves in 5 (Nocebo 5, N5) and 30 (Nocebo 30, N30) minutes from cream application, or hydrating properties (No Expectation Group, NE). Pain was induced by the Cold Pressure Test (CPT) which was repeated before cream application (baseline) and after 10 (Test10) and 35 (Test35) minutes.

Results. Changes in pain tolerance and in HR at each test point in respect to baseline were compared between the three groups. Tolerance change at Test10 was greater in N5 (MED=-36.8;IQR=20.9) compared to NE (MED=-5.3;IQR=22.4;$p < .001$) and N30 (MED=0.0;IQR=23.1; $p < .001$), showing that hyperalgesia was only present in the group that expected the effect of the cream to set in early. Tolerance change at Test35 was greater in N5 (MED=-36.3;IQR=35.3; $p = .002$) and in N30 (MED=-33.3;IQR=34.8; $p = .009$) compared to NE, indicating delayed onset of hyperalgesia in N30, and sustained hyperalgesia in N5. No group differences were found for HR.

Conclusions. Our study demonstrated that temporal expectations can shift nocebo response onset in a tonic-pain model.

Trial registration. The trial has been registered at the ISRCTN register (21/07/20; ISRCTN96623027).

Perspective. This study demonstrates that temporal suggestions modulate

the timing of nocebo hyperalgesia on a model of sustained pain. Expectations of pain increase can be anticipated or delayed and, once triggered, remain stable over time. These findings could inform us on how to minimise the negative side effects that are associated with patients' negative expectations.

4.2 Introduction

Expectations of pain worsening can significantly increase one's perception of pain [205]. The impact of expectations on pain is evident in nocebo hyperalgesia, where pain worsens following the administration of an inert treatment delivered in association with negative verbal suggestions (i.e. suggestions of pain rise) [206; 207; 208]. Even though the nocebo effect is a widely studied phenomenon, little is known about the influence of temporal expectations on nocebo hyperalgesia, that is, when the effect is expected to set in and for how long it is meant to last.

In a recent experiment, we demonstrated that it is possible to shift the onset of nocebo effects by modulating one's temporal expectations in a phasic-pain model, induced with short-lasting electrical pulses of medium-to-low intensity [209]. Here, after the first pain test, an inert cream was administered along with suggestions of pain increase. Yet, some participants were told that the cream had a fast time of action (i.e. that the effect would set in five minutes after its application), while others that the effect would be slower to set in (i.e. after fifteen and thirty minutes from application). As expected, those who believed the cream to have a fast time of action reported early onset of nocebo hyperalgesia, while those that were instructed it would take longer showed delayed hyperalgesia onsets.

From a clinical perspective, the use of a short-lasting, medium-to-low inten-

sity pain model, induced with electrical pulses, restricts the results to a type of painful experience that has limited resemblance with clinical pain, which is rarely brief and precisely timed [210]. Furthermore, within the framework of contemporary perception theories, the use of such pain model is significant to the outcome of the study. According to these theories, pain perception is conceptualised as the result of the interaction between one's prior expectations and the incoming noxious input [211]. Besides, there is evidence stemming from research on perceptual domains other than pain which demonstrates that prior expectations have a greater influence on perception when the sensory stimulus is ambiguous (i.e. low intensity, low precision) compared to when this is non-ambiguous (i.e. high intensity, high precision) [212; 213; 214]. Although the greater influence played by expectancy on ambiguous stimuli has not been tested in the context of pain perception, a similar rule is likely to apply [211]. Accordingly, the ambiguous nature of the noxious stimuli (i.e. short-lasting, medium-to-low intensity electrical pulses) that we used in our previous experiment [209] might have made it easier for verbal suggestions to bias pain perception.

If in our earlier experiment [209] we looked at the influence of temporal suggestions on nocebo hyperalgesia on a phasic pain model with a high level of ambiguity (i.e. short duration, medium-to-low intensity), here we aim to extend the investigation to a tonic pain model characterised by a lower level of ambiguity (i.e. long duration, high intensity). In this experiment, tonic pain was induced by means of the Cold Pressor Test (CPT), which offers a good approximation of clinical pain [215]. Pain tolerance, operationalised as the maximum time participants could resist with their hand dipped in freezing-cold water (7C°), was the primary outcome measure of this experiment. This design allowed us both to explore whether unambiguous tonic pain is equally affected by temporal suggestions as ambiguous phasic pain and to use a behavioural outcome (i.e. maximum

pain tolerance) instead of relying solely on verbal pain reports (as done in [209]).

4.3 Methods

4.3.1 Trial Design

A randomised controlled trial with three parallel groups (No Expectations, Nocebo 5 and Nocebo 30) is reported. This experiment is one of two studies examining the temporal onset of placebo and nocebo effects. The first experiment investigated the placebo effect [216], while the second one, here reported, studied the nocebo phenomenon. These two studies were conducted from June 2019 to July 2020 and shared a common randomised control group (N=17). This decision was made in line with an ethical approach that sought to avoid the induction of pain in a larger sample size, since the two studies followed the same protocol for the control group. Specifically, recruitment and testing for the two nocebo groups took place between April and July, 2020, while for the control group this occurred between June and July 2019. Here, the influence of temporal information on the onset and duration of nocebo effects was tested using an established nocebo manipulation [209; 217] combined with the Cold Pressure Test (CPT). All experimental procedures followed the policies and ethical principles of the Declaration of Helsinki. The study was reported in line with the Consolidated Standards of Reporting Trials (CONSORT) [218]. The Ethics Committee of the Vrije Universiteit Brussel approved this study (18/03/20; BUN1432020000002/I/U). The trial has been registered at the ISRCTN register (21/07/20; ISRCTN96623027).

4.3.2 Participants

The study took place at the Experimental Anatomy Research Department at the Vrije Universiteit Brussel (VUB), Belgium. Forty-four healthy volunteers were recruited from the student population of the VUB, and from the general population through different social media outlets. Participants between 18 and 45 years of age were considered eligible to join the study. Participants that were in cure with antidepressants or anxiolytics, had a history of cardiovascular disease, and that suffered from psychiatric, neurological, chronic musculoskeletal and pain-related disorders were not considered eligible to participate in the study. Moreover, we instructed the participants not to consume alcohol, caffeine-based drinks, supplements, and/or analgesic medications twelve hours before the experiment. We informed participants that they would take part in a study investigating the time of action of a newly developed hyperalgesic cream. We disclosed the actual purpose of the study only after full data collection was completed (see Section 4.3.6 for details on debriefing). Participants provided written informed consent agreeing to be debriefed with all the study details at the end of the experiment. These participants were recruited and randomised between two nocebo groups. For the control group, on the other hand, we relied on data that was previously collected (See Camerone et al., [216] for findings regarding placebo analgesia).

4.3.3 Intervention

An inert cream was applied to the participants' dorsal and volar left hand. The cream consisted of a water-based gel (KY-gel Johnson&Johnson) which was presented to participants in a transparent plastic tube. Participants received different information regarding the given cream:

- **No Expectation (NE):** experimenter explained that the cream is inert,

without any effects on pain perception;

- **Nocebo 5 (N5):** experimenter explained that the cream is a powerful hyperalgesic that would have an effect after 5 minutes;
- **Nocebo 30 (N30):** experimenter explained that the cream is a powerful hyperalgesic that would have an effect after 30 minutes.

After providing written informed consent, participants were asked to sit on a chair positioned next to the CPT device. The investigator used a stopwatch displayed on a computer screen in front of the participants as well as a customised wall clock for participants' temporal orientation. The wall clock with 5-minute intervals (i.e., 5 to 55) showed an icon of a cream tube at the 12 o'clock position to indicate the time-point of application of the cream (Figure 4.1).

The experiment started with a 4-minute heart rate measurement at rest, during which participants were asked to relax and breathe naturally. After instructing participants on how to perform the CPT task, they completed a familiarisation trial. During the CPT, participants were asked to immerse their left hand in seven litres of circulating cold water (7C°, ±.2C°; CPT device: Thermo Scientific model Haake A 10B, Haake SC 100; Thermo Fisher Scientific, Waltham, MA; procedure adapted from Mitchell et al., [219]). The experimenter drew a red line from the participant's ulnar to the radial styloid process (wrist level) to indicate the level to which participants had to lower their hand. Before starting the CPT, one minute of HR at rest was recorded. Ten seconds before the beginning of the test, participants were prompted by the experimenter to get ready (i.e. experimenter said 'Get ready!') and to place their hand above the CPT device, showing readiness to immersion. Upon a verbal prompt from the experimenter ("Go"), the participant lowered their hand into the CPT device. The experimenter started the stopwatch to record the time between the beginning

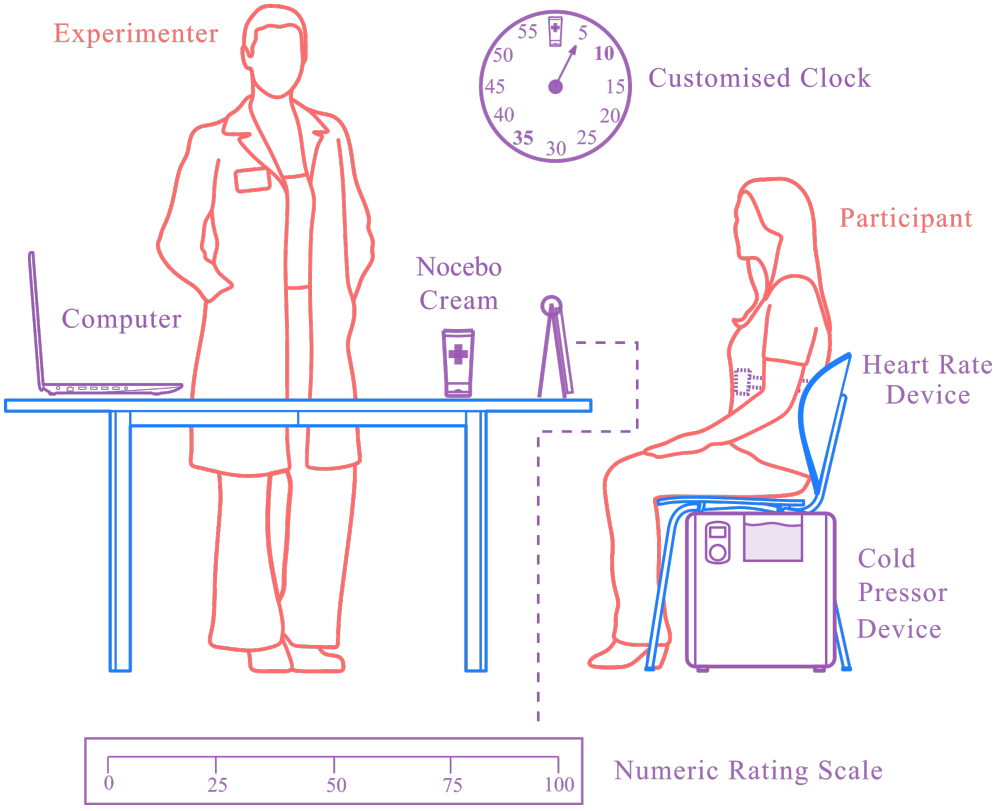

Figure 4.1: Experimental Setting.

of exposure and hand withdrawal. The stopwatch was displayed on a computer screen located in front of the participant for temporal orientation. Participants were instructed not to move their fingers or hand while in the water and to keep their fingers spread with the palm parallel to the bottom of the device without touching it. For safety reasons, 10 minutes were set as the maximum time participants were allowed to spend with their hand in the water [220; 221], after which the test was discontinued, and the experiment ended.

During CPT, subjective pain ratings were recorded every 15 seconds. The experimenter asked participants to quantify the pain they were experiencing on a scale from 0 (no pain) to 100 (unbearable pain). In order to facilitate participants' self-reporting of pain, a poster depicting the rating scale was placed in front of

them, which included verbal and numerical anchors (0= *not painful at all*, 25= *somewhat painful*, 50= *moderately painful*, 75= *very painful*, 100= *unbearable pain*) (Figure 4.1). Once pain became unbearable, participants' removed their hand from the water basin and rested it on a towel placed on their knees. The time elapsed between hand immersion and withdrawal was recorded as CPT tolerance. Despite verbal pain ratings were recorded every 15 seconds, the last pain score was taken at the moment of hand withdrawal to ensure that the maximum tolerance level was reached (i.e. this was the case for the two nocebo groups, but not for the control group, in which the last pain rating was recorded at the last 15s interval prior hand withdrawal).

After the CPT familiarisation trial, all participants underwent the CPT baseline test, followed by participants' randomisation to groups and cream application. The cream was applied on the palmar and dorsal side of participants' hand, and it was massaged into the skin for approximately one minute to ensure full absorption. Along with cream administration, the experimenter provided participants with information about the nature of the cream (hyperalgesic cream in both nocebo groups and inert cream in the control group), as well as about the expected onset of the hyperalgesic effect (nocebo groups only). Simultaneously with the application of the cream, the experimenter adjusted the customised wall-clock so that the minute hand pointed at the noon position, indicating the time of cream application ('Time 0'). CPT was then repeated 10 (Test 10) and 35 minutes (Test 35) from the cream application ('Time 0') (Figure 4.2). Overall, the CPT was repeated a total of four times (familiarisation, baseline, Test 10, Test 35) with a break of 20 minutes between tests to restore the baseline hand temperature (Figure 4.2). Note that the test was performed 10 and 35 minutes after cream application and not after 5 and 30 minutes, which were the specific time points at which participants expected the cream to set in, depending on group allocation.

We allowed a 5-minute leeway to avoid participants doubting that the effect of a cream could be so precisely timed (i.e., setting in exactly after 5 and 30 minutes). During the breaks between the test sessions, participants completed the psychological questionnaires (see Section 4.3.4). Once finished, they were allowed to read or study, but were asked not to use their phones.

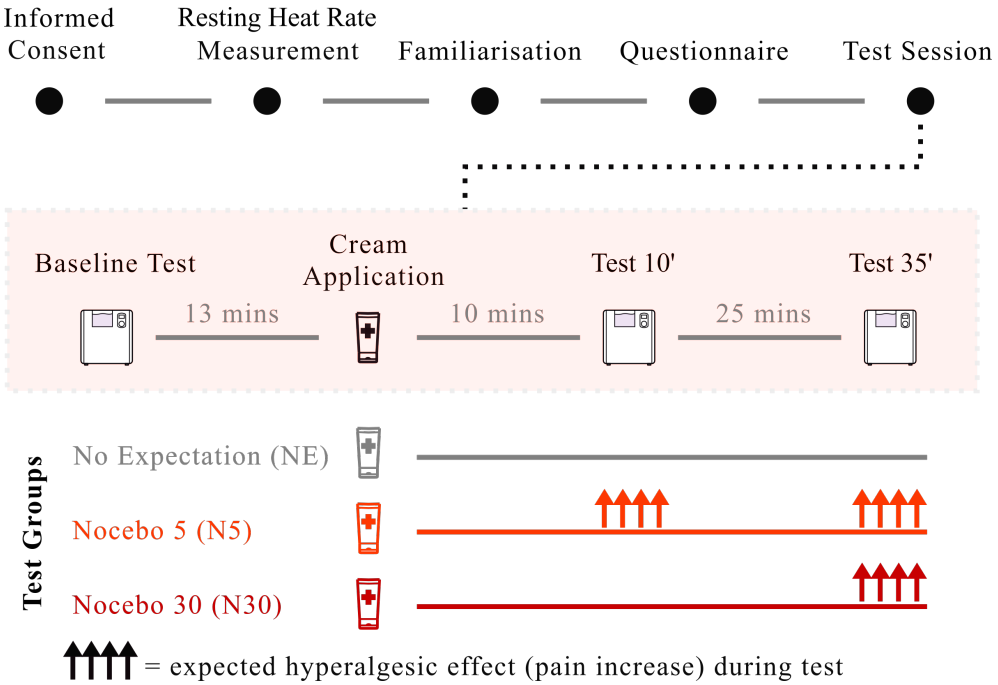

Figure 4.2: Study Paradigm. After giving consent, participants' heart rate at rest was measured for 4 minutes. Participants completed the CPT familiarisation run and filled in the psychological questionnaires. After the CPT Baseline test, the cream was applied along with suggestions of hyperalgesia (N5, Bright red; N30, Dark red) and neutral suggestions (NE), depending on group randomisation. Application of the cream and the delivery of suggestions took approximately 2 minutes. The CPT was then repeated after 10 and 35 minutes from cream application. Nocebo hyperalgesia, visualised as upper-facing arrows in the image, was expected both at Test 10 and at Test 35 for N5, and only at Test 35 for N30. No effect was expected for NE.

4.3.3.1 Control group

Participants that were assigned to the control group were informed that they would receive an inert cream (No Expectation, NE): *"The agent you will receive is an inert cream that only has hydrating properties but no effect on pain perception. Therefore, your test performance after 10 and 35 minutes* [experimenter points at time 10 and 35 minute marks on a clock] *may be similar to the performance in the first test* [CPT baseline], *but it can also be longer or shorter than before".*

4.3.3.2 Nocebo groups

Participants in the two nocebo groups were instructed that the cream had hyperalgesic properties that would augment the painful sensation induced during the CPT. We provided both groups with specific details about the onset of action of the hyperalgesic cream.

Participants allocated to the Nocebo 5 group (N5) were told that the hyperalgesic effect would arise after 5 minutes from cream application, mimicking a fast-acting drug. They received the following instructions: *"The agent you will receive is known to have a strong hyperalgesic effect which sets in after 5 minutes from its application. You will, therefore, become more sensitive to pain and be able to keep your hand in the cold water for a shorter time in the two test sessions after 10 and 35 minutes* [experimenter points at time 10 and 35 minute marks on a clock] *compared to the first test* [CPT baseline]*."*

Participants allocated to the Nocebo 30 group (N30) were told that the hyperalgesic effect would set in 30 minutes from cream application. Specifically, the following instructions were given: *"The agent you will receive is known to have a strong hyperalgesic effect which sets in after 30 minutes from its application. You will, therefore, become more sensitive to pain and be able to keep your hand in the cold water for a shorter time in the test session after 35 minutes* [experimenter

points at time 35 minute marks on a clock] *compared to the first test* [points at CPT baseline] *and the second test after 10 minutes* [points at Test 10]."

4.3.4 Assessment of Retrospective Expectancy and Psychological Traits

Participants were asked to complete multiple questionnaires that had previously been shown to link nocebo responsiveness with given psychological traits [222; 223; 224; 225]. Specifically, participants completed the Beck Anxiety Inventory (BAI) to test the level of trait anxiety [226], the Behavioural avoidance/inhibition scale (BIS/BAS) to test individuals' motivational systems [227], the Fear of Pain Questionnaire (FPQ) to test fear of pain [228] and the Revised Life Oriented Test (R-LOT) to test the degree of optimism [229]. Participants completed the questionnaires during the breaks between CPT trials.

At the end of the experiment, participants were asked to rate retrospectively, on a scale from 0 (= not at all) to 7 (= very much), how much they had expected the cream to affect (i) their pain during the experiment (*"When the cream was applied on your hand, did you expect it to make you feel more pain during the water task?"*), and (ii) their ability to keep their hand in cold water (*"When the cream was applied to your hand, did you expect it to make you last less with your hand in the water?"*). Participants were also asked to rate the extent to which they had believed the given information regarding the onset of the hyperalgesic effect (*"When the cream was applied on your hand, how much did you agree with the following statement: The cream will start to become effective after 5 minutes"* (P5)/ *The cream will start to become effective after 30 minutes (P30)"*).

4.3.5 Heart Rate Recording

In 2009, Colloca and Benedetti [230] showed that heart rate (HR) increases during the anticipatory phase, before the onset of nocebo hyperalgesia. Accordingly, we decided to include this physiological parameter to detect nocebo-related anticipatory anxiety responses. Additionally, we investigated whether HR changes characterised nocebo-modulated CPT trials compared to CPT trials without nocebo modulation.

The electrocardiogram (ECG) signal was measured using an HR monitor (Polar V800, Polar Electro Oy, Kempele, Finland), connected to two standard surface electrodes positioned on the participant's sternum with a band. Data were collected at a sampling rate of 700 Hz/sec. HR was recorded for four minutes during a rest period in which participants were asked to sit comfortably and breathe normally. HR recording started one minute before each CPT and continued through the test until two minutes after its completion. In order to limit the HR artefacts that might arise from hyperventilation related to pain-response, participants were instructed to maintain a regular and relaxed breath during each test session.

4.3.6 Debriefing

Participants were debriefed through an email sent once full data collection was completed. Here, we explained the actual purpose of the study, and clarified why deception had been necessary. Participants were invited to contact the experimenter if they felt the need to discuss their participation in the study or any other concerns. They were also reminded that they could withdraw their data if they wished. However, none of the participants decided to do so.

4.3.7 Outcome

4.3.7.1 Primary Outcomes

Tolerance time during each CPT test is the primary outcome (Baseline, Test 10, Test 35), along with the percentage of tolerance change from baseline to Test 10 ($\Delta 10$) and Test 35 ($\Delta 35$) as described below (see Section 4.3.10).

4.3.7.2 Secondary Outcomes

The secondary outcomes included participants' HR, subjective pain ratings during test sessions, psychological traits and retrospective nocebo expectations. See specific sections above for further details on the secondary outcomes (see Sections 4.3.5, 4.3.4).

4.3.8 Sample Size

A *priori* analysis was run with G*Power 3.1 to calculate the sample size needed. Based on ANOVA for repeated measure test, a sample of 42 participants was determined to accept a power of 80%, a significant level of 0.05 and an effect size of 0.41 [231]. By assuming a dropout rate of 20%, 50 participants were required to run the study.

4.3.9 Randomisation

Participants eligible for the study were randomly assigned to the two nocebo groups (allocation ratio 1:1) using computer-generated random numbers lists with simple randomisation (www.random.org). The same experimenter was responsible for participants' enrolment, randomisation (by means of computer-generated sequence) and testing.

4.3.10 Statistical Methods

A one-way ANOVA was run to test for baseline differences between the three groups in demographic parameters, and psychological constructs were assessed via the questionnaires. Data for CPT tolerance at baseline, after 10 (Test 10) and 35 (Test 35) minutes did not follow a normal distribution (Shapiro-Wilk tests $p < .05$), therefore non-parametric tests were used. Worth of mention is that, since the noxious stimulus was not calibrated accordingly with each individual pain threshold (water temperature was 7C° for everyone), individuals' raw scores were not directly comparable between participants. Therefore, the between-group analysis required standardised tolerance scores. This was not necessary for the within-group analysis, in which there was no need to account for differences in individuals' pain thresholds and tolerance raw scores were directly comparable within the same individual.

4.3.10.1 *Within-group analysis*

Friedman Tests were performed to detect differences in tolerance time across CPT trials at the three different time points (Baseline, Test 10 and Test 35) within each group. Data are presented as median ±interquartile range and the significance level was set at $p < .05$. Significant results were followed up using Wilcoxon Signed-Rank Tests. Significance acceptance level for pairwise comparison was adjusted for the number of comparisons (k) using the Dunn-Sidak Correction ($\alpha_{new} = 1 - (1 - \alpha_o)^{1/k}$), resulting in a p=.017 [232].

4.3.10.2 *Between-group analysis*

Percentage change in pain tolerance from baseline to Test 10 (Δ10) and Test 35 (Δ35) was calculated as follow:

$\Delta 10$ = (Test 10*100)/Baseline - 100

$\Delta 35$ = (Test 35*100)/Baseline - 100.

Percentage change ($\Delta 10, \Delta 35$) scores were used instead of raw scores in the between-group analysis to rely on more standardised values. Kruskal-Wallis H-Tests were used to compare percentage changes ($\Delta 10, \Delta 35$) in pain tolerance between groups, allowing to check nocebo response magnitude differences directly. Data are presented as median ±interquartile range and the significance level was set at $p < .05$. Significant results were followed up using pairwise Mann-Whitney U-Tests. Significance acceptance level for pairwise comparison was adjusted for the number of comparisons (k) using the Dunn-Sidak Correction, ($\alpha_{new} = 1 - (1-\alpha_o)^{1/k}$) resulting in a p=.017. Effect sizes were calculated as $r = z/\sqrt{N}$ [233]. The effect size measures between the groups were used to assess the actual power of the study in percentage, based on the data of the trial. A threshold > 80% was set as satisfactory.

Pain rating analysis was performed only for the two nocebo groups. We calculated the slope of pain ratings as a function of time; the steeper the slope, the faster maximum pain tolerance was reached. Friedman Tests were performed to detect differences in the slope across CPT trials at the three different time points (Baseline, Test 10 and Test 35) within each nocebo group. We could not perform pain rating analysis in the NE group because we did not record the pain rating at the moment in which the participant removed their hand from the water. Therefore, we could not safely assume any value at this point in time; instead, the last pain rating for this group was recorded at the 15 seconds interval that preceded hand-withdrawal. To address this limitation, we updated our protocol when collecting the nocebo groups, adding pain rating recording at the time of hand-withdrawal.

Correlation analysis was conducted to investigate the relationship between

retrospective expectancy in nocebo groups and $\Delta 10$ and $\Delta 35$. Retrospective expectations included participants' expectations of (i) pain, (ii) tolerance, and (iii) cream onset of action.

Further correlation analyses were performed to explore the relationship between participants' psychological traits and nocebo effects. Specifically, correlations between psychological traits in nocebo groups and $\Delta 10$ and $\Delta 35$ were investigated.

Heart rate data followed a normal distribution (Shapiro-Wilk tests $p > .05$). Therefore, parametric analysis was used. Firstly, mean HR was computed for the 10 seconds that preceded the beginning of the CPT, allowing us to assess HR during the anticipatory phase before the test session (Anticipatory HR). Anticipatory HR was calculated for each test, resulting in three mean indices for each participant (Anticipatory HR Baseline; Anticipatory HR Test 10; Anticipatory HR Test 35). A three-way mixed ANOVA was run, with the within factor TIME (Anticipatory HR Baseline; Anticipatory HR Test 10; Anticipatory HR Test 35) and the between factor GROUP (N5, N30, NE). Secondly, for each test session, the mean HR value was calculated by averaging HR measurements over the first 10 seconds, resulting in three mean indices (HR Baseline; HR Test 10; HR Test 35). We selected the first 10 seconds because this was the shorter tolerance score across participants, allowing us to have a parameter for all participants. A three-way mixed ANOVA was run, with the within factor TIME (HR Baseline; HR Test 10; HR Test 35) and the between factor GROUP (N5, N30, NE). Significant results were followed up using Bonferroni-corrected t-tests.

4.4 Results

We recruited 44 participants, 10 of which had to be excluded since they exceeded the maximum exposure time allowed with their hand into freezing-cold water (Figure 4.3). We relied on the same control group (N=17) recruited beforehand for our study on placebo, resulting in a final sample size of 51 participants. One-way ANOVA and Chi-Square tests showed no baseline groups differences ($p > .05$) with respect to age, BMI, gender and key psychological traits (Table 4.1 - 4.2). Kruskal-Wallis H-Test showed no significant baseline differences between groups in CPT tolerance (p=.237).

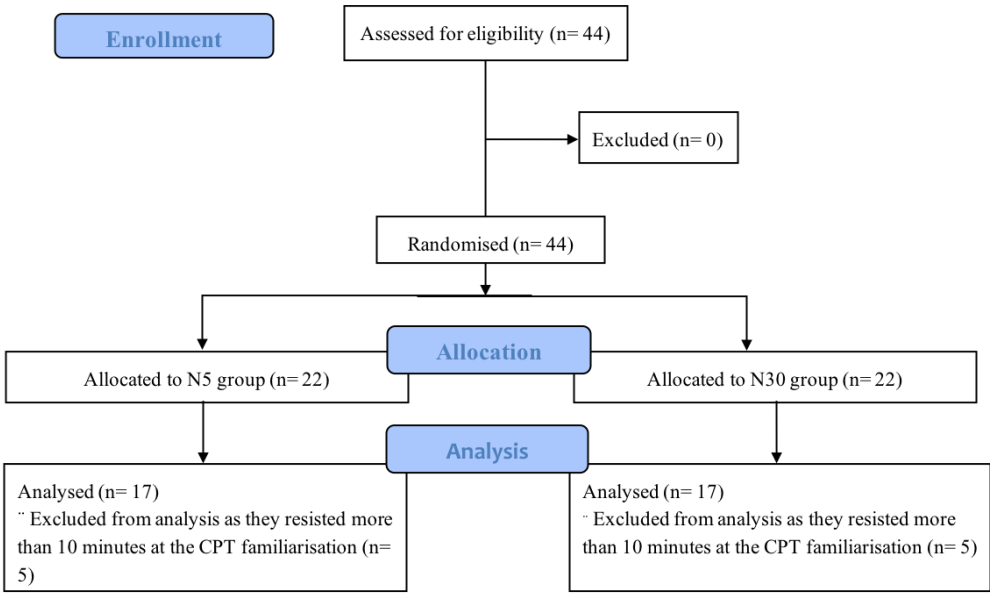

Figure 4.3: CONSORT Flow-Diagram.

Table 4.1: Participants' descriptive analysis.

Demographics			
Groups	**No Expectations**	**Nocebo 5**	**Nocebo 30**
N	17	17	17
Age in years (Mean/SD)	28.3 ±3.4	24.3 ±3.9	27.2 ±4.6
BMI (Mean/SD)	24.4 ±2.5	24.1 ±3.7	24.0 ±2.3
Sex (F(%);M(%))	7(41.2); 10(58.8)	9(52.9); 8(47.1)	11(64.7); 6(35.3)
Handedness (R(%))	13 (76.5)	17 (100.0)	17 (100.0)

Note: SD, Standard Deviation; BMI, Body Mass Index; M, Male; F, Female; R, Right.

Table 4.2: . Participants' psychological traits.

Psychological Traits			
Groups	**No Expectations**	**Nocebo 5**	**Nocebo 30**
BAI	10.4 ±4.9	14.8 ±11.9	14.0 ±9.2
BAS-Drive	8.8 ±2.3	8.8 ±2.1	9.0 ±1.7
BAS -Fun-Seeking	8.1 ±1.9	8.2 ±2.1	8.8 ±1.8
BAS-Reward	8.3 ±2.1	7.5 ±2.1	8.0 ±1.8
BIS	14.6 ±2.1	13.7 ±3.3	13.2 ±3.9
FPQ	72.4 ±12.9	71.3 ±18.1	78.9 ±14.2
RLoT	14.3 ±4.1	13.8 ±5.6	15.1 ±3.5

Abbreviations: BAI, Beck Anxiety Inventory; BAS, Behavioural Approach Scale; BIS, Behavioural Inhibition Scale; FPQ, Fear of Pain Questionnaire; RLoT, Life-Orientation Test-Revisited.

4.4.1 Nocebo Effects

Within-group analyses using Friedman Tests revealed, in both nocebo groups, a statistically significant difference in CPT tolerance depending on the temporal execution of the CPT test, either at baseline, after 10 (Test 10) or 35 (Test 35) minutes [Nocebo 5, $\chi^2(2) = 15.394, p < .001$; Nocebo 30, $\chi^2(2) = 10.836, p = .004$] from cream application. Contrarily, no significant difference in CPT tolerance across time-points was shown in the NE group, $\chi^2(2) = 2.471, p = .291$. Post-hoc analyses were run using the Wilcoxon Signed Ranks tests (Table 4.3 - 4.4). N5 group showed a significant decrease in CPT tolerance at Test 10 (p = .001) and

Table 4.3: Median and interquartile range of CPT pain tolerance of all groups at the three tests.

	Baseline		**Test 10**		**Test 35**	
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**
NE	72.0	262.5	65.0	250.5	69.0	284.5
N5	57.0	112.5	38.0	91.5	50.0	85
N30	53.0	37	50.0	64	38.0	49.5

Abbreviations: IQR, Interquartile Range.

Table 4.4: Within-group comparisons of CPT tolerance.

Groups	**Comparisons**	**Wilcoxon Signed rank test**	**Effect Size**	**Power Analysis**
NE	No Post-hoc	/	/	/
N5	T10 vs Baseline	Z = -3.315, p=.001	r = .568	>80%
	T35 vs Baseline	Z = -2.912, p=.004	r = .499	>80%
	T10 vs T35	Z =-.398, p=.691	r = .068	>80%
N30	T10 vs Baseline	Z = -.700, p=.484	r = .120	>80%
	T35 vs Baseline	Z = -2.392, p=.017	r = .410	>80%
	T10 vs T35	Z = 2.864, p=.004	r = .491	>80%

at Test 35 ($p = .004$) compared to baseline. No significant difference was shown in CPT tolerance between Test 10 and Test 35 ($p > .05$). N30 group showed no significant difference in CPT tolerance between Test 10 and baseline ($p > .05$). However, CPT tolerance significantly decreased at Test 35 compared to both baseline ($p = .017$) and Test 10 ($p = .004$).

Between-group analysis using Kruskal-Wallis H-Tests showed a statistically significant difference in $\Delta 10$ between the different groups, $\chi^2(2) = 18.1, p < .001$, Post-hoc Mann-Whitney U-tests (Table 4.5 - 4.6) showed that $\Delta 10$ did not differ significantly between the NE group and N30 ($p > .05$). However, $\Delta 10$ was significantly higher in N5 than in both NE ($p < .001$) and N30 ($p < .001$). For $\Delta 35$, Kruskal-Wallis H-Test showed a statistically significant difference between groups, $\chi^2(2) = 12.0, p = .002$ (Table 4.6). Post-hoc Mann-Whitney U-tests (Table 6) revealed that $\Delta 35$ was significantly higher in both N5 ($p < .002$) and N30 ($p < .009$) compared to the NE group. No significant difference in $\Delta 35$

was found between N5 and N30 ($p > .05$) (Table 4.6). Figure 4.4 summarises between-group results employing bar graph representation.

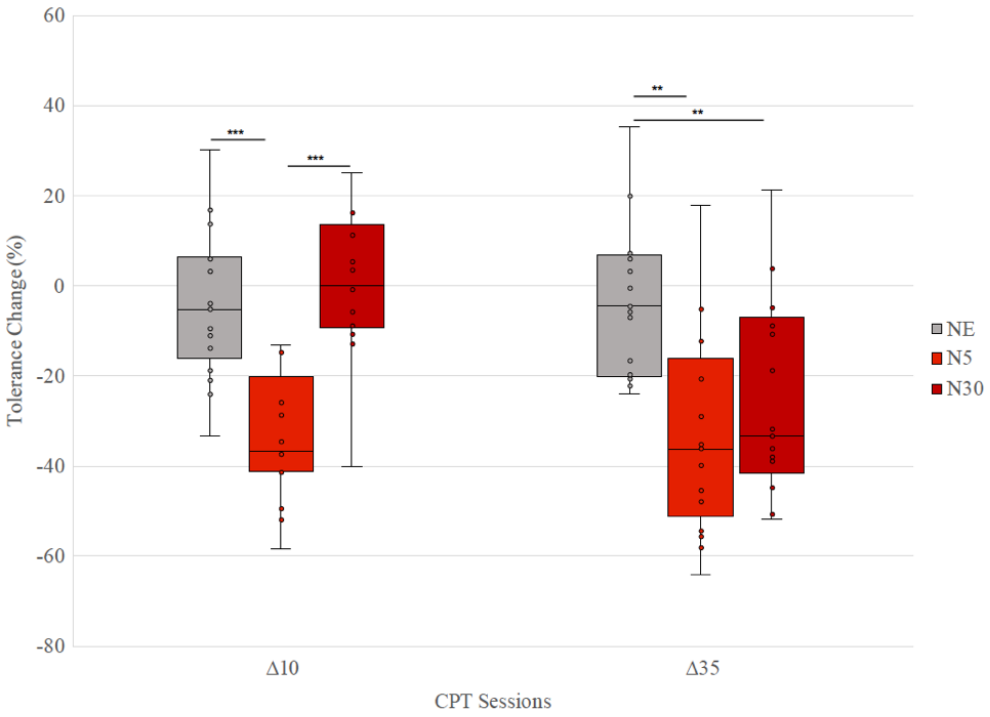

Figure 4.4: Between-group Comparison: Percent change in CPT tolerance from Baseline to Test 10 ($\Delta 10$) and to Test 35 ($\Delta 35$) for each group (NE, N5, N30). Asterisks indicate significant differences in Δs between groups ($* \; p < .05; ** p < .01; *** p < .001$). $\Delta 10$ was significantly lower in N5 than in both NE and N30. $\Delta 35$ was significantly lower in both N5 and N30 compared to the NE group. The lowest and highest boundaries of the boxes indicate the 25th and the 75th percentiles, respectively. The black line within each box indicates the median. Whiskers above and below the boxes indicate the largest and the lowest data points (excluding any outliers), respectively.

Table 4.5: Median and interquartile range of percent change in CPT pain tolerance (Δ10, Δ35) in the three experimental groups.

	Δ10		**Δ35**	
	Median	**IQR**	**Median**	**IQR**
NE	-5.3	22.4	-4.6	26.8
N5	-36.8	20.9	-36.3	35.3
N30	0.0	23.1	-33.3	34.8

Abbreviations: IQR, Interquartile Range.

Table 4.6: Study 3: Between-group comparisons of CPT percental tolerance change.

Group Comparisons	**Dependent variable**	**Mann-Whitney U-Test**	**Effect Size**	**Power Analysis**
	Δ10			
NE vs N5		U=43.0, p<.001	r = .599	>80%
NE vs N30		U=107.0, p=.196	r = .221	>80%
N5 vs N30		U=38.0, p<.001	r = .629	>80%
	Δ35			
NE vs N5		U=53.0, p=.002	r = .541	>80%
NE vs N30		U=69.0, p=.009	r = .446	>80%
N5 vs N30		U=112, p=.263	r = .192	>80%

4.4.2 NRS Ratings

Within-group analyses using Friedman Tests did not show significant differences in pain slope over time; this was the case for both the N5 $[\chi^2(2) = 5.158, p = .076]$ and the N30 $[\chi^2(2) = 5.792, p = .055]$ groups. Yet, p-values show a tendency towards significance.

4.4.3 Retrospective Expectancy and Psychological Tests

No significant correlations were shown, in either of the two nocebo groups, between retrospective expectations of (i) pain, (ii) tolerance, and (iii) cream onset of action and Δ10 and Δ35. Also, none of the psychological measures for either nocebo groups correlated with Δ10 and Δ35.

4.4.4 Heart Rate

Mixed-methods ANOVA showed no significant main effect of TIME, GROUP, interaction between factors (p values> 0.05) on anticipatory HR measures. Instead, a significant main effect of TIME on HR test measures (HR Baseline; HR Test 10; HR Test 35) was shown ($F(2,96)=6.601$, $p = 0.002$), indicating that mean HR differed significantly across the three-time points (BSL, Test 10, Test 35). Yet, no significant main effect of GROUP nor interaction between both factors were observed (both p values > 0.05). Post hoc pairwise comparison using the Bonferroni correction revealed that HR decreased significantly between baseline (M =79.68, SD=13.53) and Test 35 (M =75.84, SD=10.79) (p=0.006). Despite HR decreasing at Test 10 (M =77.44, SD=11.62) compared to baseline (M =79.68, SD=13.53), this difference did not reach significance ($p > 0.05$). Similarly, also the decrease from Test 10 (M =77.44, SD=11.62) to Test 35 (M =75.84, SD=10.79) did not reach significance ($p > 0.05$).

4.5 Discussion

Our previous study demonstrated that temporal suggestions modulate the onset of nocebo hyperalgesia on a phasic pain model, induced by short-lasting, medium-to-low intensity electrical pulses [209]. Here, these findings are extended to a longer-lasting, high-intensity, tonic pain model while relying on a behavioural outcome measure (i.e. maximum tolerance) instead of subjective pain ratings, as done in Camerone et al., [216]. Experimental pain induced with mild and short-lasting electrical pulses has limited resemblance with clinical pain, both in terms of stimuli duration and their level of aversiveness [210; 215]. In the present study, we induced tonic pain using the CPT, which, despite still being far from clinical pain, has a longer duration and reaches higher intensity (i.e. maximum

tolerance), leading to a sensation that is a better proxy to real-life pain [215].

Here, we replicated the main findings of our previous work, showing that the onset of nocebo hyperalgesia is dependent on the temporal suggestions that participants receive at the moment (inert-)treatment administration. Participants that were told that the cream had a fast time of action (N5) showed a decrease in tolerance level at the test session that took place soon after cream application (Test 10), demonstrating that suggestions of a fast-acting cream lead to early nocebo hyperalgesia onset. Differently, participants who were told that the cream would require a longer time before setting in (i.e. 30 minutes from application, N30) did not show a reduction in tolerance level at the early test session (Test 10), instead tolerance reduction set in at the delayed test trial (Test 35), showing that suggestions of delayed cream onset were responsible for postponing the hyperalgesic effect. These results demonstrate that negative verbal suggestions increase pain perception, and that they can modulate the timing of hyperalgesia.

The use of verbal cues to delay nocebo onsets can be particularly important from a clinical standpoint. The acute phase of pain in several musculoskeletal conditions (e.g. in acute low back pain) is characterised by the interaction between pain-intensity, the level of threat attributed to the pain, and pain-related anxiety [234]. Following an injury, individuals with high anxiety sensitivity are more likely to attribute a high level of threat to the traumatic event than those with lower anxiety sensitivity (i.e. fear of not regaining full function after back injury) [234; 235]. An increase in perceived pain threat level enhances pain-related anxiety, which in turn increases pain perception (i.e. high anxiety increases pain sensitivity, [236; 237; 238]), often resulting in pain avoidance behaviours (Turk and Wilson [234] for a full review of a fear-avoidance model of pain). Pain increase and pain avoidance strategies feed back into this negative, self-reinforcing loop, which is often responsible for the passage from acute to chronic pain [239].

Relieving the acute phase of pain, for instance by postponing the detrimental effects of negative expectations, can represent a functional strategy that could help preventing acute pain from turning into chronic.

Given what has been said, it is worth pointing out that although we measured psychological factors that are central to pain modulation in the clinical setting (i.e. pain catastrophising and anxiety sensitivity) [234], our analysis showed no correlations between these traits and enhanced pain sensitivity. This can be explained by the fact that, unlike patients, the participants knew that the pain was limited to the duration of the experiment and that such pain would not have consequences for their health. In further studies, it would be interesting to explore temporal expectations while modulating the experimental-pain threat-level, as done by Cimpean and David[240] and, most importantly, by extending these results to a clinical sample.

A second important finding of this study is that, once triggered, nocebo hyperalgesia remains stable over time (i.e. no difference was shown between Test 10 and Test 30 in the N30 group). This result is partially in line with our previous study which shows that once the nocebo response sets in, it increases over time [209]. In both studies, the effect did not wear off over time. However, in one case (present study) it remained stable, while in the other it continued to increase [209]. This discrepancy could be due to the different nature of pain, phasic in one case and tonic in the other, as well as to the different method of measuring pain, with subjective ratings on the one hand and with maximum tolerance threshold in the other. In line with the present findings, Rodriguez-Raecke et al.[241] have shown that negative expectations induced by verbal suggestions at day one, not only lead to pain worsening on that day, but also that this negative effect remains stable over the next eight days. Accordingly, studies monitoring patients' recovery expectations from back pain onset during a 3-month [242] and

a 2-week [243] period, have reported that expectations remained stable over time for most of the patients, and that the direction of expectations (i.e. positive, neutral, negative) was positively correlated with the therapeutic outcome. The consistency across these studies in showing that negative expectations are likely to endure over time underscores the importance of preventing the development of negative expectations in clinical routine when patients start new therapies, given that such expectations are likely to accompany the patient throughout the intervention, thus limiting, or in the worse cases abolishing, its positive effects [242; 243; 244].

A third outcome of this study is the comparison that can be made between the magnitudes of the nocebo effects when using an ambiguous stimulus, as done in our previous work [209], compared to when using an unambiguous one, as done in this study. Research on perceptual domains other than pain has demonstrated that one's expectations have a greater influence upon perception when the incoming stimulus is ambiguous compared to when this is unambiguous [245; 246]. If the same principle applied to pain perception, then we might anticipate that expectations would have a greater influence over the ambiguous pain model (i.e. short-lasting and medium-to-low intensity) used in Camerone et al.,[209], than over the unambiguous one (i.e. longer-lasting and high-intensity) used in the present experiment, resulting in greater nocebo effects in the former, than in the latter. Instead, the magnitude of nocebo effects was comparable between the two experiments, with an average effect of r=.446 in Camerone et al., [209], and r=.522 in this study (see Section 4.6 for details), suggesting that the level of ambiguity of the incoming noxious stimulus does not interact with the extent to which expectations influence perception. Yet, confounding factors could have influenced the magnitude of such effect sizes, including specific characteristics of each pain model (i.e. familiarity with the sensation of cold, as opposed to

unfamiliarity with the sensation of electric shocks), differences in the pain test modality (i.e. maximum tolerance versus non-maximum tolerance test), and participants' perceived controllability over the incoming noxious stimuli (i.e. participants controlled when to end the pain in the CPT, while they had no control over electrical pulses onsets). In order to isolate the influence of stimulus ambiguity on perception, future research should deliver the same type of pain (i.e. electrical pulses) while modulating stimuli level of ambiguity, adjusting their duration and intensity. Though this was not the purpose of the study, Van Laarhoven et al., [208] have done the first step in this direction by investigating nocebo effects on electrical stimuli of either low (itch) or high intensity (pain), inducing more or less ambiguous stimuli, respectively. In this case as well, the magnitude of nocebo effects on itch (r=0.39) and pain (r=0.36) was comparable, suggesting that expectations have a similar degree of influence on sensory information, independently from the ambiguines of the incoming sensory input. Further investigation is required to properly assess this hypothesis, and it should rely on experimental designs created specifically to address this issue.

To introduce an objective outcome that reflects the influence of temporal expectations on pain, we looked at heart rate data. No differences in HR were shown between groups, suggesting that nocebo hyperalgesia is not associated with HR changes. However, in line with our previous data, HR during the pain test decreased over time in all three groups, suggesting a physiological habituation response to the cold pressor test [216]. Lack of HR sensitivity as a physiological correlate of nocebo effects is in line with Daniali and Flaten [247] meta-analysis, in which heart rate variability, but not HR, was demonstrated to be a good physiological correlate of nocebo hyperalgesi. Also, anticipatory HR (i.e. HR during the ten seconds that preceded hand immersion) did not differ between groups, and it remained stable over time, failing to pick up on anticipatory anxiety

responses that are associated with nocebo hyperalgesia onsets [248]. Our results contrast with Colloca and Benedetti's whose reported HR acceleration during the anticipatory phase before nocebo-cued noxious stimulations. Yet, the different type of noxious stimuli (electrical pulses in Colloca and Benedetti [248]) could account for the diverse anticipatory anxiety reactions, as well as for the associated HR responses.

The main limitation of this study is that participants' expectations were only measured retrospectively, instead of being recorded throughout the experiment. On one hand, measuring expectancy retrospectively prevented participants' from questioning the true nature of the study. On the other hand, the lack of trial-by-trial expectations recording prevents our data from giving us information on the variation of temporal expectations over the course of the experiment. Since expectations update accordingly with (sensory) experiences, further research is needed to investigate the interplay between expectations updating and nocebo hyperalgesia temporal modulation.

To conclude, we demonstrated that temporal suggestions modulate the onset of nocebo hyperalgesia, extending our previous findings to a model of tonic pain, relying on maximum pain tolerance as a behavioural outcome measure. Sometimes pain cannot be avoided but has to be tolerated (i.e. some chronic pain cases). Therefore, understanding how to modulate one's tolerance levels can be particularly relevant in the clinical context [249]. These results are promising, and further studies must build upon this evidence to better understand the influence of temporal expectations in the clinical setting and across diverse therapeutic interventions.

4.6 Supporting Information

4.6.1 Comparison of effect sizes of nocebo responses

The present study and our previous one [209], both included two nocebo groups in which participants expected the hyperalgesic effect to start after five (N5 group) or thirty minutes (N30 group) from cream application. Both studies also included a No Expectancy (NE) group that did not expect the cream to influence their pain.

We calculated effect sizes (r) of the nocebo responses for our previous study [209] by comparing NRS scores at Test 10 between N5 and NE and at Test 35 between N30 and NE. We then computed the average effect size (see Table 4.7).

We calculated the effect sizes (r) of the placebo response of the current study by comparing tolerance change at Test 10 between N5 and NE and at Test 35 between N30 and NE. We then computed the average effect size (see Table 4.8).

	Effect Size(r)
Test 10 : P5 vs NE	.417
Test 35: P30 vs NE	.475
Average r:	**.446**

Table 4.7: Effect sizes (r) of the nocebo responses in Study [209].

	Effect Size(r)
Test 10 : P5 vs NE	.599
Test 35: P30 vs NE	.446
Average r:	**522**

Table 4.8: Effect sizes (r) of the nocebo responses in the present study.

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4.8 References

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Chapter 5

General Discussion

The first chapter of this thesis is an introduction to placebo and nocebo effects, outlining the rationale behind the experiments conducted during this PhD. Specifically, three experiments were conducted, each of which has a dedicated chapter in this thesis (Chapter 2, 3 and 4). These studies investigated the influence of temporal suggestions in placebo analgesia and nocebo hyperalgesia, both in phasic and tonic pain models. In this last Chapter, the primary findings of this research are summarised and discussed in relation to their clinical implications, and their significance from a predictive coding standpoint. In addition, their strengths and limitations are outlined, along with suggestions for future directions. Throughout this discussion, I will refer to Study 1, 2 and 3 when mentioning the three studies conducted during this PhD Doctoral Programme. In the interests of clarity:

- **Study 1:** Experiment described in Chapter 2, investigating both placebo analgesia and nocebo hyperalgesia on a short-lasting , medium-to-low intensity phasic pain model [250].
- **Study 2:** Experiment described in Chapter 3, investigating placebo analgesia on a longer-lasting, high-intensity tonic pain model [251].
- **Study 3:** Experiment described in Chapter 4, investigating nocebo hyper-

algesia on a longer-lasting, high-intensity tonic pain model [252].

5.1 Main Findings

The research projects presented in this Doctoral Thesis systematically investigated if and how temporal expectancy directly influences the onset of action of a given (inert-)intervention, exploring for the first time the causal relationship between expected and perceived onset of action. In these studies, an inert cream was administered along with suggestions of either analgesia (i.e. pain reduction) or hyperalgesia (i.e. pain increase). To investigate the temporal component of expectations, some participants were told that the effect of the given (inert-)treatment would set in straight away, while others that it would take a longer time. As expected, the onset of the effect was either anticipated or delayed accordingly with the temporal information that participants received. This demonstrates that *placebo and nocebo onsets strictly follow temporal suggestions*, indicating that temporal expectancy influences the onset of action of the given (inert-)intervention. More specifically, it was shown that once placebo analgesia and nocebo hyperalgesia had set in they lasted for the duration of the experiment, indicating that *once triggered these effects are maintained over time.* Furthermore, it was demonstrated that when participants expected a slower time of action, the onset of both placebo analgesia and nocebo hyperalgesia was delayed in time without these effects occurring before the expected time point. This shows that expectations can be withheld over time, until it is their anticipated time to set in. In other words, *expectations do not expire over time.* These findings were consistent in both short-lasting phasic pain [250] (Study 1, see Chapter 2) and long-lasting tonic pain [251; 252] (Study 2 and 3, see Chapter 3 and 4, respectively).

Before moving on to discussing the clinical implications of these results, it is

useful to reflect on the magnitude of the effects that were shown in the present research, giving a better sense of the impact that placebo and nocebo effects could have on patients.

In the first experiment (Study 1), just by delivering positive verbal suggestions, it was possible to decrease participants' perception of pain of half a point (mean=-0.6; sd=0.5; average across placebo groups at the time point of expected onset of action) over a scale going from 0, no pain, to 10, unbearable pain. Importantly, once the effect was triggered, it remained stable over time (i.e. within group comparison over time was not significant, $p > .05$). Precisely, participants expecting the analgesic effect to set in after 5 minutes from its application, reported pain reduction of 0.5 points at the test session after 10 minutes (mean=-0.5, sd=0.6), and such decrease remained stable after 20 (mean=-0.6, sd=0.7) and 35 (mean=-0.7, sd=0.8) minutes. Similarly, those expecting the effect to set in after 15 minutes, reported approximately half a point pain reduction after 15 (mean=-0.7, sd=0.5) and 35 (mean=-0.7, sd=0.6) minutes from cream application, but not earlier.

On the contrary, the delivery of negative verbal suggestions was sufficient to enhance participants' perception of pain of 1 point (mean: 0.9; sd =0.6; average across nocebo groups at the time point of expected onset of action). Interestingly, nocebo hyperalgesia significantly increased over time (i.e. within group comparison over time reached significance, $p > 0.5$). Those participants expecting the hyperalgesic effects to set in after 5 minutes from cream application reported a gradual increase of pain ratings over time (i.e. Test 10: mean= 0.7, sd=0.5; Test 20: mean=0.9, sd=0.6; Test 35: mean=1.1, sd=0.7). The same was reported by participants expecting hyperalgesia to set in after 15 minutes (i.e. Test 20: mean=0.9, sd=0.6; Test 35: mean=1.3, sd=0.9). Noteworthy is that these data indicate a difference of almost 1.5 points in pain ratings between positive and neg-

ative verbal suggestions, i.e. +0.9 in the case of negative verbal suggestions and -0.6 in the case of positive verbal suggestions. The magnitudes of these effects, both positive and negative, are in line with previous behavioural [253; 254; 255] and neuroimaging studies [256].

The magnitude of these changes may not seem clinically relevant at first glance, but on closer inspection their relevance becomes evident. First, such effects arise from verbal suggestions in the absence of any treatment, which means that positive effects can easily be induced and negative effects avoided, simply by modulating the information that the clinician gives to the patient. Second, the magnitude of the effects of recognized treatments for musculoskeletal pain are not on a much larger scale than the effects arising from placebo modulations. For example, a recent RCT showed that after a week of manual therapy, lateral epicondylitis pain decreased from an initial level of 4.47 to 4.03, over a scale from 0 to 10, resulting in an overall pain reduction of 0.44 [257]. In the same study, acupuncture was shown to reduce pain of 1 point, from 4.00 to 3.00 [257]. In a different study, manual therapy intervention was shown to reduce non-specific neck pain of 1.3 points (i.e. 5.5 at baseline to 4.2, after three weeks of treatment), while physical therapy reduced pain of 1.2 points (i.e. 5.8 at baseline to 4.6 after three weeks) [258]. This observation does not aim to undermine nor question the effectiveness of the aforementioned interventions for musculoskeletal pain, which were shown to significantly ameliorate pain compared to the control group, but wants to highlight that gaining half a point of pain reduction due to positive verbal suggestions is a clinically relevant change, given that recognised interventions often lead to changes on the scale of 1 point. Indeed, the purpose of positive verbal suggestions is not to be used in substitution to active therapies, but instead, these are to accompany the treatment, boosting its effectiveness (i.e. effect of the treatment + effect of verbal suggestions) [259; 260].

In the second (Study 2) and third (Study 3) experiments of this Doctoral Degree, maximum pain tolerance was the primary outcome measure, instead of pain ratings as in the case of the first study. Unravelling strategies to maximise patients' tolerance to pain can be particularly important in those clinical cases in which pain cannot be avoided, but has to be tolerated (i.e. some chronic pain cases) [261]. Positive verbal suggestions (i.e. Study 2) led, on average, to a 33% increase in maximum pain tolerance (i.e. at the time point of expected onset of action), whereas negative verbal suggestions (i.e. Study 3) led, on average, to a 35% decrease in maximum pain tolerance (i.e. at the time point of expected onset of action). Also in this case, the positive effects of verbal suggestions were maintained over time, as shown by those participants that expected early analgesia onset and reported an increase of 29% in maximum tolerance after 10 minutes and of 27% after 35 minutes. The magnitude of these effects is in line with data from Pollo et al [262] looking at the effect of positive expectations on thoracotomized patients' painkiller requests in the three consecutive days after surgery. Despite all patients were receiving buprenorphine, those who were told that they were receiving a potent painkiller requested 34% less buprenorphine compared to those patients who were not informed of the analgesic effects of the treatment (natural history). Interestingly, both groups reported similar pain ratings over time, indicating that administering different doses of painkiller led to a comparable analgesic effect. In line with Study 2 which reports an average increase of 33% in pain tolerance due to positive verbal suggestions, patients became more tolerant to pain (i.e. 34% decrease in painkiller requests) due to positive expectations induced with positive verbal suggestions.

In Study 3, the negative effects of verbal suggestions were also maintained over time, as demonstrated by a reduced maximum tolerance both after 10 (i.e. 37% decrease) and 35 (i.e. 36% decrease) minutes in those participants that

expected early onset hyperalgesia. Once again, these data suggest that clinicians could increase and decrease patients' tolerance to pain of a third simply by delivering positive and negative verbal suggestions, respectively. Moreover, the consequences of the verbal suggestions can be carried over time, highlighting the importance of promoting positive effects, while avoiding negative ones.

5.1.1 Expectations are maintained over time: Clinical Considerations

The finding that once triggered, placebo analgesia remains stable over time could have important implications in the clinical setting. Symptoms improvement arising from patients' positive expectations have been repeatedly demonstrated, both in clinical trials in which expectations were directly manipulated [262; 263; 264; 265] and in observational studies of correlational nature [266; 267]. Indeed, the results of correlational studies showing the association between positive expectations at the beginning of the treatment and positive clinical outcomes at its completion [266; 267] are supported by clinical trials data [262; 263; 264; 265], attesting for the causal link between positive expectations and therapeutic benefit. For instance, the previously mentioned study conducted by Pollo et al. [262] and investigating the effect of positive expectations on thoracotomized patients' painkiller requests showed that despite all patients had been given buprenorphine, those who were told that they would certainly be receiving a painkiller (i.e. certain and strong positive expectations) showed a 16% reduction in drug requests compared to those patients who were told that they would be receiving either a painkiller or a placebo (i.e. uncertain and weaker positive expectations). Patients' pain ratings between the two groups were similar over the course of the three days, indicating that the same analgesic effect was obtained, in spite of the different doses of buprenorphine administered [262]. A different clinical trial

showed that when patients were told (i.e. open administration) they were receiving a potent painkiller (i.e. morphine), post-operative pain decreased of 3.6 points (on a scale from 0 to 10), compared to a decrease of 1.9 points when patients were not aware of receiving the painkiller (i.e. hidden administration), indicating that verbal suggestions of analgesia account for 1.7 points of pain decrease [263]. Interestingly, in the same study, on a different cohort of thoracotomized patients, anxiety scores (STAI-I, [268]) variations before and after diazepam administration were also investigated. Note that STAI-I scores go from a minimum of 20 to a maximum of 80 and they are commonly classified as *'no or low anxiety'* (20-37), *'moderate anxiety'* (38-44), and *'high anxiety'*(45-80) [269]. Patients that were aware of receiving an anxiolytic drug reported a significant decrease of 12 points in anxiety scores, going from high anxiety (pre=49.7) to medium to low anxiety (post=37.7). Differently, those patients who were not informed of receiving such drug reported no significant changes in their anxiety level (i.e. anxiety scores post anxiolytic administration increased of 2.1 points; pre=51.0; post=53.1), maintaining a high anxiety score before and after anxiolytic administrations. These data demonstrate that expectations accounted for the full effect of the anxiolytic drug. Considering the important role of anxiety in musculoskeletal pain sufferers, these findings are particularly relevant to this discussion [270; 271]. On these grounds, if we combine existing evidence demonstrating the influence of positive expectations on clinical outcomes with evidence that placebo analgesia can remain stable over time (i.e. as shown by the data of this Thesis), the importance of inducing positive expectations at the beginning of the therapeutic treatment becomes evident. Indeed, if positive expectations induced at session one are maintained over time, these are likely to maximise treatment effectiveness throughout. Note that according to the research presented in this Thesis, positive suggestions can decrease pain of approximately half a point throughout (over a scale from 0 to

10).

On the opposite hand, maintaining negative expectations over time is likely to obstacle clinical amelioration. Observational clinical studies showed a correlation between initial patients' negative expectations and negative clinical outcomes [266; 267]. Studies that experimentally induced pain also demonstrated that administering inert treatments alongside negative verbal suggestions, worsens pain [255; 272; 273; 274]. Pain changes due to negative suggestions tend to be of 1 point on a scale from 0 (no pain) to 10 (unbearable pain). This was the case for Van Laarhoven et al.[255] study in which pain ratings varied of 1.2 points between those who received suggestions of pain increase (mean = 03.09; sd = 2.39) and those who did not (mean = 1.89; sd = 1.34). Similarly, Colloca et al. [274] reported a difference of 1.2 points in perceived pain between noxious stimulation preceded by expectations of low pain *versus* expectations of high pain. Alike, negative verbal suggestions induced in Study 2 also accounted for, on average, 1 point increase in pain ratings during nocebo trials compared to baseline. Furthermore, in a study investigating the influence of expectations on drug efficacy it was demonstrated that the analgesic effect of remifentalin was completely abolished once negative verbal suggestions were given. Precisely, participants were informed that remifentalin administration was discontinued, even if in reality drug infusion continued, and that this could lead to pain increase [259]. Therefore, considering the strong impact that negative expectations have on therapeutic outcome, the longevity of negative expectations over time becomes particularly concerning since these have the potential to reduce treatment effectiveness from the start, limiting or even abolishing its overall effectiveness. Consequently, preventing the development of negative expectations is a fundamental step that can have a significant positive impact upon the overall treatment success rate.

The findings provided by this PhD research indicating that both placebo anal-

gesia and nocebo hyperalgesia, once triggered, are maintained over time must be considered carefully before generalising them to real-life scenarios. If on one hand, the experimental nature of these studies allowed a controlled environment in which the only factor that could have influenced one's expectations of the received (inert-)treatment effectiveness was one's own experience of pain at each test. On the other hand, this controlled environment prevents us from inferring whether expectations would remain stable over time in real life, where multiple uncontrolled factors interact with one's expectations, potentially leading to their update. In the clinical setting, for example, patients are likely to encounter situations that have the potential to change their expectations of a given treatment, and of their chance of recovery. These include attending educational programs about their pathology and about the intervention they are receiving, meeting patients suffering from a similar condition, meeting those who are undertaking the same treatment and report positive/negative experiences and having a good/bad quality relationship with their physician [275; 276]. In nowadays society the internet is also an important source of information which is likely to influence patients' expectations regarding their condition and regarding the treatment they are undertaking [277].

Importantly, the data that emerged from this PhD suggesting that expectations remain stable over time, is supported by correlational research that monitored patients' expectations over time in real-life settings [266; 267]. For instance, over a sample of 874 back pain sufferers, expectations were shown to remain stable over the course of three months for 80% of the patients. Notably, their expectations, either negative, neutral or positive, correlated with the therapeutic outcome [267]. Carstens et al., [266] also reported that the majority of the patients with low back pain (n=281) did not vary the direction of their expectations over time (i.e. 2 weeks). However, both Kamper et al., [267] and Carstens et al.,

[266] found a decrease in positive expectations in a subgroup of patients whom did not experience positive treatment outcomes. This finding is in line with the predictive coding framework of expectations [278], suggesting that expectations update if there is a large enough mismatch between what one is expecting and what one is receiving. Accordingly, a study looking at weight loss expectations in obese patients reported that those patients with overly optimistic expectations at the beginning of the treatment, were also the ones discontinuing the treatment [279]. The correlation between excessive positive expectations at the beginning of the treatment and treatment abandonment, is, arguably, a strong indicator that such positive expectations were abandoned throughout the course of the medical program, suggesting that expectation do not remain stable over time, if violated. Yet, since these patients were not present at the follow up (i.e. after 12 months), during which expectations were measured, it is not possible to draw conclusions on if and how their expectations updated in light of the newly acquired incoming data. It is important to notice that the aforementioned evidence is of correlational nature, thus caution is encouraged before drawing definite conclusions from these data.

Although sparse, there is explanatory research showing that once triggered, placebo and nocebo effects remain stable over time. For instance, a review of several randomised clinical trials in neuropathic pain reported that when present, placebo response lasted over a period of > 12 weeks [280]. In addition, the findings from Study 1 and Study 3, showing that once triggered nocebo hyperalgesia can be maintained for 35 minutes, are supported by the findings of Rodriguez-Raecke et al., [281] which showed that nocebo negative effects can endure for much longer, specifically up to 8 days. However, the lack of direct measures of expectations prevents us from concluding that the stability of these effects is to be attributed to expectations stability. An additional interesting study in this context is the

one from Benedetti et al. [263], in which the effects of patients' knowing that morphine administration was interrupted were investigated. Those patients who were not told that morphine was discontinued, maintained analgesic effects in the following hours (after 2h =2.2; after 4h = 2.6; after 6h=3.4), while those patients who were informed of drug discontinuation reported an increase in pain ratings (after 2h =3.5; after 4h = 4.1; after 6h=4.5). Pain scores between the two groups were significantly different after 2h and 4h, but not after 6h. At first glance, these results could be interpreted as suggesting that once triggered, placebo-related analgesia can be maintained over time (i.e. maintained for 4 hours). However, it is not possible to infer from these data whether the differences in pain scores between the two groups are to be attributed to an increase in pain ratings due to a nocebo-related response associated with the information of drug discontinuation, or to the maintenance of expectations of analgesia over time. Further investigation directly measuring patients' expectations is required to better understand the nature of the pain changes.

In conclusion, the findings of this PhD research indicating that both placebo analgesia and nocebo hyperalgesia, once triggered, are maintained over time, find partial support in the available literature. Specifically, observation of patients' expectations in real life settings, indicates that positive and negative expectations are likely to remain stable over time, supporting positive and negative clinical outcomes, respectively [266; 267; 280]. However, if evidence that contradicts these expectations is accumulated, expectations seem to update accordingly [266; 267; 279]. Yet, further studies are required to systematically investigate patients' expectations fluctuations and update over time.

5.1.2 Expectations do not expire over time: Clinical Considerations

One's ability to withholding positive expectations until it is their time to set in can be particularly interesting for those clinical conditions in which the treatment requires a longer time before setting into action. A good example are some tricyclic antidepressants which often need a few weeks to set in [282]. Over the course of these weeks, after the patient began the treatment and before they start perceiving any clinical benefit, they may feel discouraged and demotivated, and may doubt the effectiveness of the intervention. This often results in medication discontinuation during the early weeks of the treatment [283; 284]. Results from this PhD research demonstrate that if one is made aware of when to expect the treatment effect, expectations can be withheld over time. Therefore, informing patients of delayed onset of treatment action, such as weeks in the case of tricyclic antidepressants, may be sufficient to support the maintenance of positive expectations over time. This could prevent the development of "treatment failure" beliefs, so that when the treatment sets in, its effectiveness is supported by positive expectations. Another example is that of patients suffering from musculoskeletal pain. Here, the treatment is rarely straight-forward and often requires a combination of pharmacological, physiotherapeutic and psychological approaches [285; 286]. Although it is not possible to provide a precise time course for when different approaches will start to deliver their clinical benefits, it is often the case that it does take time, from weeks to several months [287; 288; 289]. In those patients in which acute pain has become chronic, expectations may be particularly fragile due to the likelihood of having attempted previous treatments which failed to alleviate their pain [290]. In this context, informing patients that clinical amelioration might require time is particularly important to prevent them from abandoning their positive expectations.

One's ability to withholding negative expectations until it is their time to set in can be a valuable tool during one's recovery path. Let us consider musculoskeletal conditions such as back pain in which, the coping strategies that the patient uses during the initial phase after a back injury are crucial in determining pain recovery or transition to chronic pain [291]. During this acute phase, those patients that are more susceptible to anxiety and more likely to feel overwhelmed by catastrophising thoughts, are also more likely to engage in pain avoidance behaviours, which are known to worsen pain and increase recovery time [270; 271]. Since psychological traits such as anxiety sensitivity [292], pain catastrophising [293] and pessimism [294] have been associated with enhanced nocebo responsiveness, and since these are common traits in patients with poor coping strategies, these patients are likely to be greatly influenced by negative expectations (i.e. greater nocebo responsiveness). Therefore, if it is the case that negative expectations find a fertile ground in those patients with poor coping strategies, these are likely to have a significant negative impact on the physical and mental state of the patient, feeding into the pre-existing anxiogenic and fear-characterised response to pain, and finally increasing the likelihood of acute pain transitioning to chronic pain [291]. Therefore, using temporal suggestions to delay the onset of patients' negative expectations could allow us to lighten this initial acute phase, facilitating recovery. For example, if a patient is about to receive a treatment which is known to sometimes have side effects it may be possible to delay the onset of such negative effects until the patient has more resources to face them. However, it is important to point out that the research covered by this PhD focused on a reduced time window, investigating a maximum delay of thirty-five minutes. Hence, it is not possible to draw conclusions on whether expectations can be withheld over a longer period of time, such as weeks or months. Future research is required to directly test the maximum time that expectations can be

maintained for (i.e. weeks or months) on a clinical sample.

5.1.3 A Predictive Coding Perspective

The three studies of this PhD relied on a similar experimental design with the main difference being the type of experimentally-induced pain. This allows us to raise some interesting, but preliminary, considerations from a predictive coding perspective. Contemporary perception theories posit that perception arises from the integration between incoming sensory information and one's expectations about the incoming input [295]. The influence of expectations over visual [296] and auditory [297] perception was found to be greater for more ambiguous incoming stimuli. Indeed, if the stimulus is unclear, expectations are needed to fill in the gaps and make sense of our sensory experience. Despite this never being directly demonstrated on pain, it is likely that the same rule applies [298].

In this Doctoral Thesis, the two pain models that were used varied in terms of their duration and intensity. Since these two features determine the level of ambiguity of an incoming noxious stimulus, these two pain models differed from each other in respect to their level of ambiguity. Precisely, gathering information takes time, and therefore, a short-lasting stimulus (i.e. short duration) allows less time to collect cues and decode the signal compared to a longer lasting one (i.e. long duration) , making the former more ambiguous than the latter [299; 300]. Concerning stimulus intensity, a highly painful (i.e. high intensity) stimulus triggers our alarm system and is recognised as a threat straight away, thus making it unlikely for this stimulus to be perceived as ambiguous. Differently, the milder the painful stimulus, the more ambiguous this becomes, to the point that it might be difficult to classify the sensation as either pain, itch or touch. Accordingly, the pain model used in Study 1, consisting of short-lasting electrical pulses with a medium-to-low intensity, can be classified as having high ambiguity, while the

pain model used in Study 2 and 3, consisting of longer lasting and high intensity stimuli, as having low ambiguity. Perception theories (see Section 1.2.2.3) postulate that one's expectations are more influential when the incoming stimulus is ambiguous rather than non-ambiguous. Therefore, one would predict expectations to be more influential in the high-ambiguity study (i.e. Study 1 using a short-lasting, medium-to-low intensity pain model), than in the low-ambiguity studies (i.e. Study 2 and 3 using a longer-lasting, high intensity pain model). This would result in greater placebo and nocebo effects in Study 1 than in Studies 2 and 3. Comparison of effect sizes did not confirm such prediction. The model with lower ambiguity (i.e. Study 2 and 3), reported greater placebo analgesia than the model with greater ambiguity (i.e. Study 1). Differently, for nocebo hyperalgesia the magnitude of the effect was similar across the two pain models and the two levels of stimulus ambiguity. Such discrepancy between placebo and nocebo could indicate that the influence of expectations on perception may vary depending on whether such expectations predict pain increase, thus triggering alertness, or pain decrease, thus downregulating anxiety. The magnitude of these effects is not in line with what would have been predicted according to perception theories. This could be due to confounding factors that were not controlled for including participants' control over pain, which has been previously shown to dampen pain perception [301; 302]. In fact, in Study 1 the experimenter, and not participants themselves, decided when and for how long to deliver the noxious stimuli, while in Studies 2 and 3, participants were able to control how long they kept their hand in the water, deciding exactly when the pain was going to end by withdrawing their hand. In the former case, participants had no control over the incoming pain, while in the latter they did. Most importantly, comparison between the effect sizes of the three studies may be difficult due to fact that the experiments were not purposely designed to systematically investigate

the influence of expectations on stimuli that varied in their level of ambiguity. For instance populations were different; Study 1 was conduced in Italy, recruiting mostly Italian participants, while Study 2 and 3 were conducted in Belgium, mainly recruiting Belgian participants. In addition, Study 3 was conducted after the first Belgian national lockdown due to COVID-19 pandemic, whereas Study 1 and 2 were conducted before the pandemic outbreak. In the light of these considerations, it is important not to draw strong conclusions from the comparison between these studies. Indeed, the objective of such comparisons was not to provide with solid answers on the influence of stimulus ambiguity on pain perception, but to provide preliminary data that could trigger the curiosity of the scientific community to address these unanswered questions in future, purposely designed experiments.

5.2 Strengths and Limitations

Novelty is the primary strength of this research project. Here, the influence of temporal suggestions on placebo analgesia and nocebo hyperalgesia onsets was investigated for the first time, paving the way for future studies. A second important strength is the generalisability of the results to both phasic and tonic pain, which was achieved by testing the same research question (i.e. the influence of temporal suggestion on placebo analgesia and nocebo hyperalgesia) using the two different pain models. Thirdly, the initial results that relied on subjective pain reports (i.e. Study 1), were confirmed by follow up studies (i.e. Study 2 and 3) using maximum pain tolerance as a behavioural objective measure, showing that it is unlikely that these effects are due to participants' wanting to comply with the experimenter's demands. Overall, the consistency of the findings across three experiments with a similar but not identical design attests for the strength and reproducibility of the effects.

Yet, these studies are not without limitations. First, the lack of neurophysiological outcomes prevents us from associating our behavioural findings with neurophysiological responses. As outlined in section 1.2.2.1, neuroimaging evidence has reported activity changes in pain-sensitive brain regions during placebo analgesia and nocebo hyperalgesia [303; 304; 305; 306]. Therefore, placebo and nocebo effects that arose in the studies included in this Doctoral Thesis are likely to also be mirrored in such neurophysiological correlates, yet this requires direct testing in future research. During the anticipation phase of placebo analgesia and nocebo hyperalgesia, neuroimaging evidence has shown activation of cortical regions of the descending pain modulatory system (see Section 1.2.2.2). Differently from previous research, the studies presented here added temporal details to one's expectations. Since there is compelling evidence for the involvement of specific brain regions in formulating representations of time, the main candidate being the basal ganglia [307; 308], there may be some differences in the brain regions that are activated during the anticipatory phase of placebo and nocebo effects when expectations have a specific time tag (as in the case of the studies presented in this Thesis) and when this is not the case, as done in previous research [303; 304; 305; 306; 309]. Further research is required to explore the neurophysiological correlates that underpin the temporal component of placebo and nocebo effects.

A second limitation to consider is the lack of trial-by-trial expectations recording. Expectations were only measured retrospectively to avoid making the participants suspicious about the true purpose of the study. As shown by Jepma et al., [310] the relationship between expectations and perception is bidirectional, whereby one's expectations influence the painful experience, and in turn, the pain experience feeds back, updating expectations. Unfortunately, the lack of trial-by-trial expectations recording prevents us from drawing any sort of con-

clusion regarding the interplay between expectations updating mechanisms and placebo and nocebo effects, and their modulation over time.

5.3 Future Directions

The findings of this Doctoral Thesis are the starting point of an exciting stream of research that could have great implications, both clinical and non-clinical.

For instance, *a first line of investigation* is to explore whether these findings persist with patients suffering from acute and chronic pain. This line of research is particularly pressing considering the numerous clinical implications that could arise from modulating patients' temporal expectations (See previous sections 5.1.2 5.1.1 for further discussion).

A second line of research must investigate the neurophysiological correlates of placebo analgesia and nocebo hyperalgesia when shifted in time by temporal expectations. On one hand, this would suggest once more that the shifts in time of placebo and nocebo responses are not to be attributed to participants wanting to comply with the experimenter, but to a physiological response to time-specific expectancy. On the other hand, as mentioned in the previous section, this line of investigation could provide valuable insight into the neurophysiological correlates of time-specific expectancy. Altogether, supporting the behavioural results presented in this Thesis with neuroimaging evidence, would give the appropriate scientific credibility to the temporal phenomenon, so this knowledge can be implemented in the clinical context to maximise clinical positive outcomes.

*A third line of investigatio*n is needed to extend these findings to active drugs. It has been previously demonstrated that positive and negative expectations influence the analgesic effectiveness of remifentanil, boosting its effect in the first case, and abolishing it in the second case [259]. Accordingly, a solid amount of research using the open-hidden design (see Section 1.1) has demonstrated that

treatment effect greatly depends on whether the patient is aware (i.e. open) or not (i.e. hidden) of being administered the intervention, whereby the effectiveness is significantly stronger in the first case, compared to the second one [260; 263; 311]. Therefore, considering the impact of expectations upon active treatment effectiveness, temporal expectations are likely to modulate the onset of action for active treatments as they do with placebo and nocebo interventions. Demonstrating that the offset of treatments can be shifted in time by temporal suggestions would unlock multiple research paths investigating how to maximise treatment outcomes, while minimising its negative side effects, therefore having a strong clinical impact.

A fourth line of research is required to extend these findings to domains beyond pain, where expectations are known to be influential. Placebo responses have been reported in patients suffering from Parkinson's disorder [312; 313], depression [314; 315], anxiety [316] and high-altitude headache [317]. In addition, placebo responses are also present outside of the clinical context, for instance in physical performance [318] and breath holding [319]. Exploring the influence of temporal expectations in those domains known to be susceptible to expectancy modulation, could give us insights into how to deliver time-specific expectations to maximize intervention effectiveness in the clinical context and to boost performance outside the clinical context.

5.4 Conclusions

The experiments within this Doctoral Thesis have shown that by delivering different timing information about the onset of action for a given (inert-)treatment, it is possible to modulate the onset of its effect. This was the case both when administering a placebo (i.e. inert treatment along with suggestions of pain decrease) and a nocebo (i.e. inert treatment along with suggestions of pain increase)

treatment. Specifically, it has been shown that, once triggered, the effect lasts at least for the period covered by the experiment (i.e. up to 35 minutes), suggesting that, once induced, expectations persist over time. In addition, demonstrating that the effect can be delayed indicates that expectations can be withheld over time until it is their anticipated time-point of action.

Purposely modulating temporal expectations could have large implications in the clinical setting. Yet, further research is needed to extend these beyond placebo and nocebo interventions to patients and active treatments. Furthermore, these behavioral findings need to be corroborated with objective neurophysiological parameters. Deepening our knowledge in this unexplored area could lead to important discoveries that could boost the effectiveness of interventions and maximize patients therapeutic benefits.

5.5 References

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Chapter 6

PhD Collateral Projects

Here, the abstracts of the collateral projects in which I was involved during my PhD are reported.

6.1 Collateral Project 1

Title:

Effectiveness of a dance-physiotherapy combined intervention in Parkinson's disease: a randomized controlled pilot trial.

Published as:

Frisaldi, E., Bottino, P., Fabbri, M., Trucco, M., De Ceglia, A., Esposito, N., Barbiani, D., **Camerone, E.M.,** Costa, F., Destefanis, C., Milano, E., Massazza, G., Zibetti, M., Lopiano, L. & Benedetti, F. (2021). Effectiveness of a dance-physiotherapy combined intervention in Parkinson's disease: a randomized controlled pilot trial. *Neurological Sciences*, 1-9.

Abstract:

Background: Physical therapies have been recommended as crucial components in Parkinson's disease (PD) rehabilitation.

Objective: The study aims to examine the effectiveness of a new dance - physiotherapy combined intervention, called DArT method, in mild PD patients.

Methods: A prospective, randomized, single-blind, controlled pilot trial was conducted on 38 mild PD patients under dopaminergic therapy. The intervention consisted in an add-on protocol: the control group received 1 h of conventional physiotherapy followed by 1 h of conventional physiotherapy each day, 3 times a week, for 5 weeks. The experimental group received 1 h of conventional physiotherapy followed by 1 h of dance class each day, 3 times a week, for 5 weeks. The week before and after the training period, patients were assessed for motor, cognitive, emotional, and sensory components of PD, with MDS-UPDRS-III as primary outcome measure

Results: DArT method was associated with a 2.72-point reduction in the post-treatment MDS-UPDRS-III total score compared to control group (95% CI 5.28, 0.16, $p = 0.038$, $d = 0.71$), and with a 2.16-point reduction in the post-treatment MDS-UPDRSIII upper body subscore (95% CI 3.56, 0.76, $p = 0.003$, $d = 1.02$). Conversely, conventional physiotherapy program was associated with a 2.95-point reduction in the post-treatment trait anxiety compared to the experimental group (95%CI 0.19, 5.71, $p = 0.037$, $d = 0.70$). Withdrawal and fall rates were equal to 0% in both groups.

Conclusion: DArT method showed to be safe, well accepted, and more effective than an intensive program of conventional physiotherapy in improving motor impairment in mild PD.

6.2 Collateral Project 2

Title:

The impact of treatment expectations on clinical, physiological and fatigue responses: a preliminary investigation in myasthenia gravis.

Submitted as:

Frisaldi, E., Ferrero, B., Di Liberto, A., Barbiani, D., **Camerone, E.M.,** Piedimonte, A., Cavallo, R., Lopiano, L., Shaibani, A. & Benedetti, F.

Abstract:

Background: Expectations influence health outcomes in various conditions and represent a major determinant of the placebo and nocebo effects.

Objective: Investigate treatment expectations in myasthenia gravis (MG) within routine medical practice.

Methods: In this preliminary phase of the EMPAThy-EU (Expectations and Myasthenic PATients- Europe) project, 17 patients were neutrally assessed for their treatment expectations - Stanford Expectations of Treatment Scale and Credibility Expectancy Questionnaire - before starting azathioprine, and after 3 and 6 months, and then clinically monitored over one-year follow-up (experimental group). Quantitative Myasthenia Gravis (QMG) score and pyridostigmine daily dose were the primary outcomes used to compare treatment response between this group and a retrospective standard medical care (SMC) group of 17 patients. Disease-specific parameters and fatigue were used to assess the role of expectation in the experimental group.

*Results:*The experimental group showed significantly higher improvement in QMG score at 6 months compared with the retrospective SMC group (95% CI,

-4.52 -0.77, p = 0.007, d = 1.02). Correlations of strong effect sizes (from r s = 0.608 to r s = 0.764) were found between treatment expectations and: clinical (QMG $p = 0.002$, pyridostigmine $p = 0.005$), physiological (forced vital capacity $p = 0.002$, single-fiber electromyography $p = 0.0004$), fatigue (Chalder Fatigue Scale $p = 0.009$) responses.

Conclusion: These findings, to be confirmed on a larger sample size, revealed that treatment expectations in MG finely modulate treatment response and are able to predict clinical, physiological and fatigue responses, with important implications for medical practice and clinical trials.

6.3 Collateral Project 3

Title:

What is the relative contribution of biological and psychosocial factors to the generation of hypoxia headache?

Published as:

Barbiani, D., **Camerone, E.M.,** & Benedetti, F. (2018).

Abstract:

Background: The biopsychosocial model claims that illness is generated by both biological and psychosocial factors. Accordingly, several studies have shown that both factors contribute to the generation of pain.

Aims: The aim of the present study is to manipulate biological, psychological, and social factors in hypobaric hypoxia headache in order to understand their relative contribution to the generation of headache pain.

Methods: Healthy subjects were subdivided into three groups and brought to our high-altitude labs for the assessment of hypoxia-induced headache, blood oxygen saturation (SO2), prostaglandins, and cortisol during the first 24 h after arrival. The first group did not undergo any manipulation. The second group (negative expectation) was told that severe headache would occur if SO2 dropped to less than 80% and their oximeters were set to display a saturation of 75%, even though real SO2 was much higher. The third group (negative expectation and social interaction) underwent the same manipulation as the second group, but these subjects spent the night together with people experiencing headache and insomnia.

Results: Although none of the three groups differed significantly for SO2, the second group, compared to the first, experienced more severe headache and showed an increase in prostaglandins and cortisol. The third group, compared to the second group, showed a further increase of headache as well as of prostaglandin (PG) E2 and cortisol.

Conclusions: These findings indicate that biological, psychological, and social factors are additive not only in the generation of headache but also for the biochemical changes related to hypoxia.

6.4 My PhD Publications

Camerone, E. M*., Battista, S*., Benedetti, F., Carlino, E., Sansone, L.G., Buzzati, L., Scafoglieri, A., & Testa, M. (*in preparation*).

Camerone, E. M., Wiech, K., Benedetti, F., Carlino, E., Job, M., Scafoglieri, A., & Testa, M. (2021a). 'External timing'of placebo analgesia in an experimental model of sustained pain. European Journal of Pain.

Camerone, E.M.*, Piedimonte, A*., Testa, M., Wiech, K., Vase, L., Zamfira, D., Benedetti, F. and Carlino, E. (2021b). The effect of temporal information

on placebo analgesia and nocebo hyperalgesia. Psychosomatic Medicine.

Frisaldi, E., Bottino, P., Fabbri, M., Trucco, M., De Ceglia, A., Esposito, N., Barbiani, D., **Camerone, E. M.,** ...& Benedetti, F. (2021). Effectiveness of a dance-physiotherapy combined intervention in Parkinson's disease: a randomized controlled pilot trial. Neurological Sciences,1-9.

Dottor, A*., **Camerone, E.M*,** Job, M., Barbiani, D., Frisaldi, E., & Testa, M. (2021). A new visual feedback-based system for the assessment of pinch force, endurance, accuracy and precision. A test-retest reliability study. Hand Therapy.

Rossettini, G., **Camerone, E. M.,** Carlino, E, Benedetti, F., & Testa, M. (2020) Context matters: the psychoneurobiological determinants of placebo, nocebo and context-related effects in physiotherapy. Archives of Physiotherapy, 10(11), 1-12.

Viceconti, A., **Camerone, E. M.,** Luzzi, D., Pentassuglia, D., Pardini, M., Ristori, D., Rossettini, G., Gallace, A., Longo M.R., & Testa, M. (2020). Explicit and implicit own's body and space perception in painful musculoskeletal disorders and rheumatic diseases: a systematic scoping review. Frontiers in Human Neuroscience, 14, 83.

Benedetti, F., Frisaldi, E., Barbiani, D., **Camerone, E.M.,** & Shaibani, A. (2019). Nocebo and the contribution of psychosocial factors to the generation of pain. Journal of Neural Transmission, 1-10.

Barbiani, D., **Camerone, E.M.,** & Benedetti, F. (2019). The Special Case of High-Altitude Headache. In Placebos and Nocebos in Headaches (pp. 57-63). Springer, Cham.

Barbiani, D*., **Camerone, E.M*.,** & Benedetti, F. (2018). What is the relative contribution of biological and psychosocial factors to the generation of hypoxia headache?. Canadian Journal of Pain, (2018).

Beedie, C., Benedetti, F., Barbiani, D., **Camerone, E.M.,** Cohen, E., Cole-

man, D., ... & Harvey, S. (2018). Consensus statement on placebo effects in sports and exercise: The need for conceptual clarity, methodological rigour, and the elucidation of neurobiological mechanisms. European journal of sport science, 1-7.

Benedetti, F., Barbiani, D., & **Camerone, E.M.** (2018). Critical Life Functions: Can Placebo Replace Oxygen?. In International review of neurobiology (Vol. 138, pp. 201-218). Academic Press

* *Denotes equal first author contribution*