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PLACEBO AND NOCEBO EFFECTS IN PAIN AND MOTOR PERFORMANCE:

Neurophysiological correlates and individual differences.

Coordinator: Prof. Leonardo Chelazzi

Supervisor: Prof. Mirta Fiorio

Ph.D Student: Dr. Nicole Corsi

To my parents,

my greatest strength

Invictus

Out of the night that covers me,
Black as the pit from pole to pole,
I thank whatever gods may be
For my unconquerable soul.

In the fell clutch of circumstance
I have not winced nor cried aloud.
Under the bludgeonings of chance
My head is bloody, but unbowed.

Beyond this place of wrath and tears
Looms but the Horror of the shade,
And yet the menace of the years
Finds and shall find me unafraid.

It matters not how strait the gate,
How charged with punishments the scroll,
I am the master of my fate:
I am the captain of my soul.

William Ernest Henley

CONTENTS

PREFACE

The present thesis is based on the researches conducted by Nicole Corsi during her Ph.D. program between January 2014 and December 2016.

Study 1, Study 2 and Study 5 presented in the Part II and Part IV were performed at the University of Verona (Italy), Department of Neurosciences, Biomedicine and Movement Sciences under the supervision of Prof. Mirta Fiorio and the collaboration with Prof. Michele Tinazzi and Dr Mehran Emadi Andani.

Study 3 and Study 4 presented in the Part II and Part III were performed at the University of Maryland Baltimore (United States of America), Pain and Translational Symptom Science Department under the supervision of Prof. Luana Colloca and the collaboration of Taylor Ludman, Dr. Cynthia Renn and Dr.George Wittenberg.

More precisely, the presented studies are included in the following publications:

Study 1:

Corsi N, Emadi Andani M, Tinazzi M, Fiorio M. Placebo and nocebo effects: a study on the congruency between conditioning and verbal suggestion. In preparation.

Study 2:

Corsi N, Emadi Andani M, Tinazzi M, Fiorio F. (2016). Changes in perception of treatment efficacy modulate the magnitude of the nocebo effect and are related to personality traits. Sci. Rep. 6, 30671; doi: 10.1038/srep30671

Study 3:

Corsi N and Colloca L (2017). Placebo and nocebo effects: The advantage of measuring expectations and psychological factors. Front. Psychol. 8:308. doi: 10.3389/fpsyg.2017.00308.

Study 4:

Corsi N, Ludman T, Renn C, Wittenberg G, Fiorio M, Colloca L. How motor-induced analgesia shapes placebo and nocebo effects in a heat model of human pain. In preparation.

Study 5:

Emadi Andani M, Tinazzi M, Corsi N, Fiorio F (2015). Modulation of Inhibitory Corticospinal Circuits Induced by a Nocebo Procedure in Motor Performance. PlosONE 10(4): e0125223.

ABSTRACT

The placebo effect is a fascinating psychobiological phenomenon that refers to an improvement led by an inert treatment. It has primarily and extensively been studied in the field of pain. Many researchers have underlined the importance of expectancy, prior experience and social learning as cognitive mechanisms of the placebo effect. The first consists of all the words (verbal suggestions) used to describe the effect of a treatment, thus creating positive or negative expectancy and influencing the real effect. To support the given verbal information, it is possible to make the patient acquire prior experience of benefit through the exposure to the effect of a treatment (learning). For instance, after repeated association between the ingestion of a drug and its effect, it is possible to obtain the same outcome by administering an inert drug that looks like the active one. Finally, recent evidences demonstrated that the placebo effect can be induced by observing other people undergoing a treatment and obtaining a positive effect (social learning). The placebo effect is also characterized by the activation of specific neural networks, namely the top-down activation of the endogenous analgesic activity via the descending pain modulatory pathway. Specifically, placebo analgesia has been shown to be associated to activity changes of the dorsolateral prefrontal cortex (dlPFC), the anterior cingulate cortex (ACC), and distinct subcortical structures such as the hypothalamus, amygdalae, and the periaqueductal gray (PAG).

The placebo effect is known to have a negative counterpart, the so-called nocebo effect. It is defined as a worsening induced by an inert treatment, and it appears to be characterized by the same cognitive mechanisms (expectancy, conditioning and social learning) as the placebo effect. Some authors have questioned whether the neural mechanisms underlying the nocebo effect in the field of pain are the same of placebo analgesia. Different studies showed that the brain regions involved in the hyperalgesic nocebo effect include the bilateral dorsal ACC, insula, left frontal and parietal operculum, orbital prefrontal cortex, and hippocampus. Basically, they found evidence that the nocebo hyperalgesic effect may be produced through the medial system of the central pain matrix responsible for affective/emotional and cognitive aspects of pain perception.

Whilst placebo effect has been widely studied in pain, the motor domain is still unappreciated. In the last decades, authors deepened the role of the placebo effect in the motor domain by studying pain endurance during a simulated sport competition, or the role of ergogenic placebos on the level of fatigue. In all the cases, an improvement of the performance was recorded following the administration of an active substance as conditioning and/or the verbally induced expectancies.

A still under-debate topic regards the factors that most likely influence the magnitude of the placebo and nocebo effects. Thus, we decided to deepen this topic in motor performance by analyzing the two main cognitive mechanisms that modulate these effects: expectancy and learning. We found that participants are more prone to rely on verbal suggestion than on conditioning, as it is demonstrated by both behavioral and subjective results (*Study 1*).

Despite the nocebo effect is definitely less studied than the placebo, we decided to focus our research efforts especially on it because the nocebo effect is characterized by fascinating neurobiological, psychological and cognitive aspects that influence not only a motor task execution but also the modulation of pain.

By drawing our attention on the nocebo effect, we decided to investigate which personality traits play a major role in inducing a nocebo response during a motor performance. Our findings disclosed that individual differences play an important role in modulating nocebo response. Additionally, personality traits such as optimism, anxiety, persistence and harm avoidance are related to participants' tendency to strongly believe in the negative effect of the applied treatment (*Study 2*).

Despite a large number of studies on placebo and nocebo effects have been performed in the field of pain, the reason why some people are more prone to show a placebo and/or a nocebo response is an open topic not only in motor performance but also in pain. Thus, we extended the study of the personality traits to the field of pain, by investigating the potential role of specific personality traits in inducing placebo and nocebo responses. Interestingly, we found that by aggregating personality traits in different clusters, it is possible to account for a wider percentage of variability in placebo and nocebo responses (*Study 3*).

Since most of the placebo and nocebo studies are performed in either pain or motor field, we were interested in pointing out whether is there a link connecting pain

and motor field. We know from the literature that it is possible to induce a temporary analgesic effect by stimulating the motor cortex. However, the existing studies involved passive stimulations of the cerebral motor area. Thus, we investigated whether an active movement modulates pain perception and whether it is possible to evoke an analgesic effect even with voluntary movement executions. Our findings showed a strong modulation of pain due to the motor movement execution and an enhancement of this analgesic effect when placebo response is induced (*Study 4*).

Finally, we know from the literature that the neurophysiological correlates of the placebo effect consist on a modulation of the corticospinal excitability. Namely, an increase of the motor evoked potential amplitude and a decrease of the cortical silent period duration, highlighting not only a cortical but also a corticospinal activation due to this effect. However, the neurophysiological correlates of the nocebo effect in motor performance are still unknown. Thus, we developed a protocol to investigate whether the neurophysiological correlates of the nocebo effect in the motor field are the opposite of those of placebo, or if these two effects share some common neurophysiological mechanisms. By applying transcranial magnetic stimulation over the primary motor cortex, we found that expectation of worsening in motor performance diminishes the inhibitory activation of the primary motor cortex and does not involve the excitatory circuits (*Study 5*).

PART I: PLACEBO AND NOCEBO EFFECTS

1.1. Definition and pioneering studies

The term *placebo* means "I shall please" (Jacobs, 2000) and derived from the Latin verb *placere*, “I please”. It was used in mediaeval prayer in the context of the phrase *Placebo Domino* ("I shall please the Lord") and originated from a biblical translation of the 5th century AD (Gensini, Conti, & Conti, 2005). Following this meaning, the Roman Catholic Church's Office of the Dead developed an obsolete usage of this term, to indicate someone who dishonestly claimed a connection to the dead to get the chance to access to the funeral meal and so a deceptive act to please (Shapiro, 1968).

In Chaucer’s *Canterbury Tales* (14th century), Placebo is the name of a servile character that is counter posed to a fairer man called Justinus, whose name means “the just one”. During the 18th century, the term was adopted in medicine and was used to indicate inert substances that were administered to patients as alternative drugs.

The term began to transform in 1920 (Graves, 1920), and went through various intermediate stages (Jellinek, 1946) (Evans and Hoyle, 1933; Gold, Kwit and Otto, 1937). Many studies tried to disclose the nature of this phenomenon. For example, in the 40s, a manufacturer asked to Elvin M. Jellinek to test whether the absence of a particular chemical would affect the drug’s efficacy in any way (Jellinek, 1946). He conducted an 8 weeks-trial including four groups for a total of 199 patients suffering from headache. There were three experimental groups, involving various permutations of the three drug constituents, and a placebo group as control. He found that 120 of them had a placebo response, while 79 did not. Jellinek discovered that there was a significant difference in responses to the active principles between the 120 who had responded to the placebo and the 79 who did not. He described the first group as being "*reactors to placebo*", and this seems to be the first time that anyone had spoken of either "*placebo reactions*" or "*placebo responses*". The concept of placebo was fully transformed in 1955 when it has finally been considered as an important portion of the therapeutic effect in general. Henry K. Beecher, in his 1955 paper, considered the placebo as a tool *“to distinguish pharmacological effects of suggestion, and (…) to obtain an unbiased assessment of the*

result of experiment" (Beecher, 1955). In his meta-analysis, the author proved an average significant effectiveness of 35.2 ± 2.2% for the placebo effect, suggesting that its power consists on adding subjective components to the effects evoked by the active drugs. In conclusion, Beecher affirmed that these findings have a practical and useful value, encouraging researchers to find out the neuroanatomical correlates and the neurochemical, psychological, subjective and behavioral mechanisms that modify (and are modified by) these responses.

It is worth noting that there are two terms usually used in literature: placebo effect and placebo response. These terms are considered interchangeable, but actually the difference is clear: *"The placebo effect refers to the multiple changes occurring in the body that are produced by a placebo administration or treatment simulation. (..) A placebo response would occur when 'the meaning of the illness experience for the patient is altered in a positive direction, and the factors that contribute to that positive change in the mind–body unit are essentially the patient's awareness of being listened to and attended by the caregiver"* (Colloca & Miller, 2011a).

The placebo effect is also known to have a counterpart, the so-called nocebo effect. It means "I shall harm" and it leads to the worsening of a condition (Kennedy, 1961; Kissel et al, 1964). Much less is known about the nocebo effect, since it is considered a procedure that induces stress and anxiety (Benedetti & Amanzio, 1997; Benedetti, Lanotte, Lopiano, & Colloca, 2007). Hahn (1997) distinguished between two nocebo forms, both highly influenced by expectancies specific one, that refers to a real situation that is going on; a generic one, that refers to the results of the negative state of the subjects (Hahn, 1997). The nocebo effect is common in clinical practice: after a negative diagnosis, the presence of negative symptoms may increase because of the negative expectancies evoked by the situation. Additionally, the doctor-patient's relationship is of main importance, since it is carried out with information about a treatment and its effects, and within a context (Balint, 1955; Benedetti, 2002). Different studies demonstrated that the doctor-patient's relationship in the general practice has a tremendous impact on patients that may compromise the outcome of the illness (Bass et al., 1986; Gracely, Dubner, Deeter, & Wolskee, 1985; Greenfield, Kaplan, & Ware, 1985; Kaplan, Greenfield, & Ware, 1989; Starfield et al., 1981; Stewart, 1995). Warnings about

the potential side effects of a treatment can increase the development of real side effects (Colagiuri, McGuinness, Boakes, & Butow, 2012; Neukirch & Colagiuri, 2015).

All these aspects lead to the need of minimizing negative information and avoiding the development of negative therapeutic context (Colloca & Finniss, 2012). As it is for the placebo effect, it is important to distinguish the nocebo effect from the nocebo response. The nocebo effect refers to the negative psychosocial context around the patient and the treatment, whereas the nocebo response refers to the mechanisms that can produce worsening of symptoms (Colloca & Miller, 2011b).

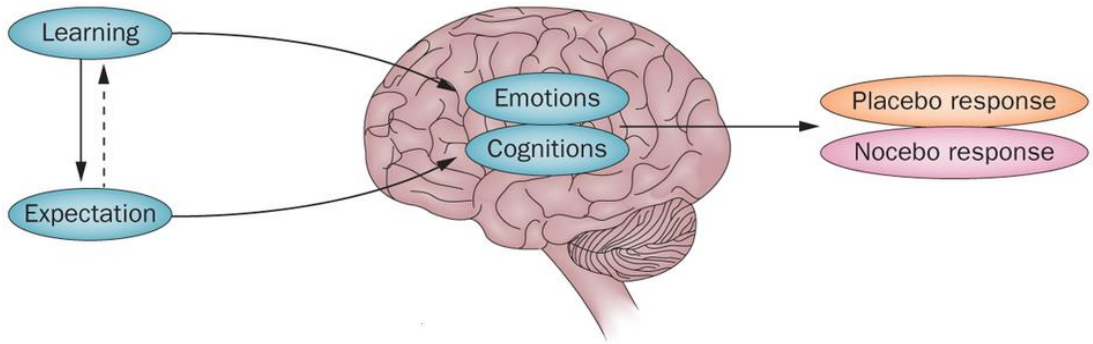
In the last decades, several studies deepened the placebo and nocebo effects highlighting the importance of the context surrounding the treatment (Benedetti, 2002; Benedetti & Amanzio, 1997; Di Blasi, Harkness, Ernst, Georgiou, & Kleijnen, 2001) and finding out the psychological (Benedetti et al., 2003; Colloca, Petrovic, Wager, Ingvar, & Benedetti, 2010; Colloca, Sigaudo, & Benedetti, 2008; Voudouris, Peck, & Coleman, 1990) and neurobiological mechanisms (Amanzio & Benedetti, 1999; Benedetti, Amanzio, Vighetti, & Asteggiano, 2006; Benedetti, Arduino, & Amanzio, 1999; Benedetti, Pollo, & Colloca, 2007; de la Fuente-Fernandez et al., 2001; Kong et al., 2008; Petrovic, Kalso, Petersson, & Ingvar, 2002).

1.1. Cognitive mechanisms

Classical theories consider expectancy and learning as the two main psychological mechanisms at the basis of the placebo and nocebo effects (Fig.1). The expectancy model places greater emphasis on the importance of verbal suggestion (Bartels et al., 2014; Colloca et al., 2010; van Laarhoven et al., 2011) and it is derived from the expectancy theory (Bootzin & Bailey, 2005; Ross & Olson, 1981) (Kirsch, 1985), by which a specific belief about what will happen in a given situation is a primary determinant of what the person will experience. On the other hand, the experience model gives priority to the importance of learning through direct experience of prior effectiveness (Bartels et al., 2014; Colloca et al., 2010; Colloca, Sigaudo et al., 2008; Wickramasekera, 1980). This model is based on Pavlov's classic discoveries and refers to an inflexible, instinctual, and automatic response (Benedetti, 2014b). Learning has

always been considered a strong factor that powerfully modulates the magnitude of the placebo and nocebo response. The length of the learning process is an important factor influencing the placebo/nocebo response; in particular, the longer the learning process, the stronger the placebo/nocebo response (Colloca et al., 2010). During the last decades, several studies tried to investigate the respective weights of expectancy and learning in inducing the placebo effect. Some evidence suggests that verbal suggestions are as strong as learning in inducing a placebo response (Colloca, Sigaudo et al., 2008; Rodriguez-Raecke et al., 2010). Nevertheless, the combination of verbally-induced expectancy and exposure to the effects of a treatment is even stronger then the effects of verbal suggestion alone (Colloca & Benedetti, 2006; Eippert, Bingel et al., 2009; Klinger, Soost, Flor, & Worm, 2007; Voudouris, Peck, & Coleman, 1985).

Figure 1. Psychological mechanisms that contribute to evoke a placebo/nocebo response



Placebo and nocebo response can be evoked through expectancies, elicited by verbal suggestion, and through a learning process, thus creating prior experience of the improvement or the worsening of a condition. *Adapted from Elsenbruch & Enck, 2015.*

Concerning the psychological mechanisms of nocebo effect, knowledge is still limited. One of the first studies on this topic showed that participants experienced localized pain when informed about potential side effects of an electrical current passing through their head (Schweiger & Parducci, 1981). This study has been later replicated by Stovner et and colleagues (2008), who showed the appearance of headache after a fake exposure to radiofrequency (Stovner, Oftedal, Straume, & Johnsson, 2008). Hence, verbal instructions are strong enough to induce discomfort (Bartels et al., 2014; Benedetti, Lanotte et al., 2007; Colloca & Miller, 2011b; van Laarhoven et al., 2011) and even to

convert painless conditions into painful perception, thus evoking negative responses comparable to those experienced through direct exposure (Colloca, Sigaudo et al., 2008; Rodriguez-Raecke et al., 2010). Additionally, verbal suggestions *per se* have stronger effects in evoking nocebo hyperalgesia than placebo analgesia (Colloca, Sigaudo et al., 2008; K. B. Jensen et al., 2012).

It is worth noting that *nocebo effect* is different from *nocebo response*. The first refers to the negative context around the patient, the treatment and the neurobiological components; the latter refers to the mechanisms that lead to the worsening of the condition (Colloca & Miller, 2011b).

Even if both verbal suggestion and conditioning are successful in evoking placebo and nocebo responses, placebo effect is primarily considered as a learning phenomenon, and the variability of its response might depend on previous experience (Colloca & Benedetti, 2006). On the other side, verbal suggestions are strong enough to induce a nocebo effect also in the absence of a learning process (Colloca, Sigaudo et al., 2008; K. B. Jensen et al., 2012).

It is worth noting that first-hand learning is not the only way to build up placebo and nocebo responses. Another mechanism that can induce a placebo and nocebo response is social learning. Namely, by observing a demonstrator's behavior, it is possible to learn how to react to a condition (Colloca & Benedetti, 2009; Colloca, Sigaudo et al., 2008; Faasse, Grey, Jordan, Garland, & Petrie, 2015; Mazzoni, Foan, Hyland, & Kirsch, 2010; Vogtle, Barke, & Kroner-Herwig, 2013; Vogtle, Kroner-Herwig, & Barke, 2016). This mechanism has been demonstrated both in animal and in human models (Heyes, 1994; Iacoboni, 2009; Olsson & Phelps, 2007). Remarkably, the effects induced by the observation of another person seem to have comparable magnitude to those induced by direct learning via conditioning procedure (Colloca, Klinger, Flor, & Bingel, 2013). These findings underline the importance of the context surrounding the participant in substantially modulating the individual pain experience.

Finally, unconscious processes affect placebo and nocebo in different ways (Egorova et al., 2015; Freeman et al., 2015; K. Jensen, Kirsch, Odmalm, Kaptchuk, & Ingvar, 2015) and this might be due to the different evolutionary nature of these responses: placebo is more related to coping and surviving behaviors, whereas nocebo has a negative component that should lead to the avoidance of dangerous situations.

1.2. Neurobiological mechanisms

The psychological mechanisms described above are related to specific neurobiological mechanisms, primarily described in the field of pain.

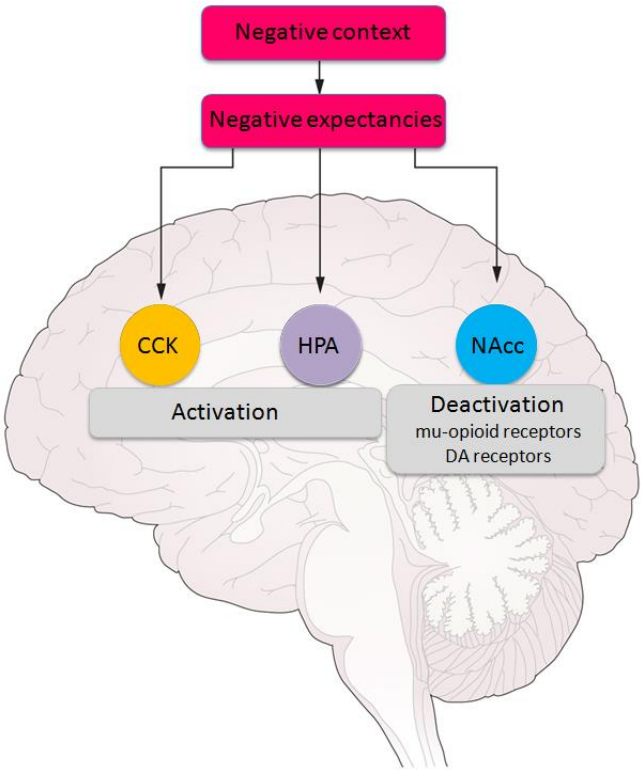
Several studies showed an activation of the dorso-lateral prefrontal cortex (dlPFC), the anterior cingulate cortex (ACC), and different subcortical areas such as the hypothalamus, amygdala, thalamus and periacqueductal grey (PAG) (Bingel, Lorenz, Schoell, Weiller, & Buchel, 2006; Eippert, Bingel et al., 2009; Lui et al., 2010; Wager et al., 2004). Specifically, the network that connects the rostral ACC (rACC) and the PAG is responsible for the responses in the somatosensory areas and the behavioral changes related to pain (Bingel et al., 2006; Eippert, Bingel et al., 2009; Lui et al., 2010; Wager et al., 2004). It is worth noting that placebo analgesia involves not only a central component, through the inhibition of pain-processing areas and somatosensory cortex (Bingel et al., 2006; Eippert, Bingel et al., 2009; Lyby, Aslaksen, & Flaten, 2011; Petrovic et al., 2002; Price, Craggs, Verne, Perlstein, & Robinson, 2007), but also a spinal inhibition (Eippert, Finsterbusch, Bingel, & Buchel, 2009). By studying the expectancy of analgesia, Wager and colleagues (2004) showed decreased activity in the rACC, insular cortex and thalamus (Wager et al., 2004). Additionally, it has been disclosed increased activity of the PFC during the expectancy of pain, along with the activation of the orbitofrontal cortex, PAG and the rACC, and decreased activity during the experience of pain (Kienle & Kiene, 1997). Craig and colleagues (2000) related the anterior cingulate and the anterior insula to the subjective experience of pain and to other emotional states (A. D. Craig, Chen, Bandy, & Reiman, 2000; Singer et al., 2004), thus underlying an involvement of the limbic and paralimbic areas.

Some authors have questioned if the neural mechanisms underlying the nocebo effect in the field of pain are the same of the placebo analgesia. In a study, the authors combined fMRI and an expectancy/conditioning manipulation model to investigate the neural substrates of nocebo hyperalgesia using heat pain on the right forearm and a sham acupuncture treatment (Kong et al., 2008). The results showed that the brain regions involved in the hyperalgesic nocebo effect included bilateral dorsal ACC, insula, left frontal and parietal operculum, orbital prefrontal cortex, and hippocampus. Basically, they found evidences that the nocebo hyperalgesic effect may be produced through the

medial system of the central pain matrix responsible for affective/emotional and cognitive aspects of pain perception (Fig. 2). Additionally, Scott and colleagues (2008) demonstrated that the nocebo response is characterized by decreased activity of the mesolimbic dopaminergic system located in the ventral basal ganglia, and of the opioid system in the rACC, OFC, insulae, thalamus, nucleus accumbens (NAcc) and PAG (Scott et al., 2008).

However, placebo and nocebo effects are not only mediated by the activation of specific brain areas, but also by the involvement of different neurotransmitters. Levine and colleagues (1978) firstly discovered the involvement of endogenous opioid system, by antagonizing the effect of the placebo analgesia with the administration of naloxone (Levine, Gordon, & Fields, 1978).

Figure 2. Nocebo-induced effects in the brain

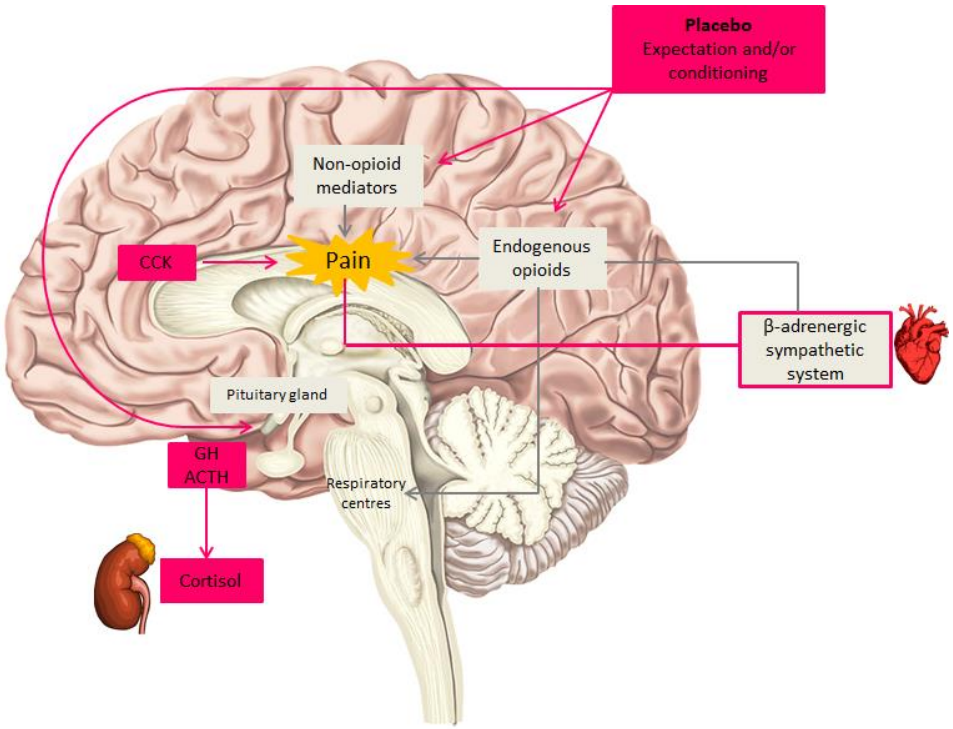


The negative context induces the development of negative expectancy. This process leads to two opposite effects in the brain: an activation of the CCK system and hypothalamic–pituitary–adrenal axis systems (related to pain transmission and anticipatory anxiety, respectively) and a deactivation of mu-opioid (μ-opioid) and dopamine (DA) receptors in the NAcc. *Adapted from Carlino, Frisaldi, & Benedetti, 2014.*

Further studies involved naloxone to study placebo analgesia and authors found that the combination of this substance with strong positive expectancy allowed to disambiguate the involvement of either opioid or non-opioid systems due to different circumstances (Amanzio & Benedetti, 1999). Specifically, the opioid system is related to the somatotopic organization, meaning that the antagonist effect of naloxone can be evoked in different body parts (Benedetti et al., 1999).

Moreover, by administering naloxone, it has been demonstrated that placebo analgesia is characterized by reduced heart rate and a decreased β-adrenergic response (Colloca & Benedetti, 2005; Pollo, Vighetti, Rainero, & Benedetti, 2003) (Fig. 3).

Figure 3. Biochemical response after placebo administration.



Pain might be reduced through expectancy and/or conditioning that activate opioid and non-opioid mechanisms. This biochemical cascade can inhibit other systems such as the respiratory and the β-adrenergic sympathetic system. Placebo analgesia is capable to act on the pituitary and the adrenal glands to simulate the effect of the analgesic drugs. On the contrary, cholecystokinin (CCK) antagonizes the endogenous opioids, thus inhibiting the placebo response. *Adapted from Colloca & Benedetti, 2005.*

Lipman and colleagues (1990) demonstrated that placebo responders showed a high level of endogenous opioid concentration in the cerebral spinal fluid (CSF)

compared to those patients considered as non-responders (Lipman et al., 1990), and it has been demonstrated that this increased concentration was found specifically in the rACC (Petrovic et al., 2002). Several studies deepened the role of the endogenous opioids thanks to the use of neuroimaging (Amanzio & Benedetti, 1999; Eippert, Bingel et al., 2009; Wager, Scott, & Zubieta, 2007; Zubieta et al., 2005), demonstrating a top down activation that involves the descending pain modulatory system.

Zubieta and colleagues (2005) demonstrated the μ-opioid activation in the dlPFC and NAcc (ipsilaterally to pain), pregenual rACC and anterior insulate cortex (controlaterally to pain) (Zubieta et al., 2005). Additionally, by using molecular imaging techniques, Scott and colleagues (2008) demonstrated the involvement of endogenous opioid, μ-opioid receptor–mediated neurotransmission when an analgesic effect is expected (Scott et al., 2008). Several additional studies investigated the neurobiological mechanisms of the nocebo effect (Colloca, Sigaudo et al., 2008; Rodriguez-Raecke et al., 2010; Sawamoto et al., 2000), showing the direct role of the CCK in the experience of a hyperalgesic nocebo effect (Benedetti et al., 2006).

1.3. Placebo and nocebo effects in motor performance

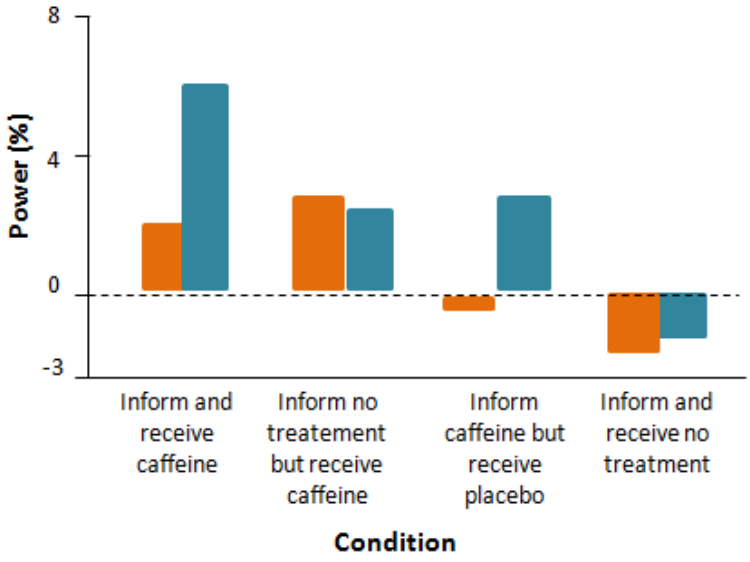
Even if placebo and nocebo effects have been widely studied in pain, these effects appear to be present even beyond the boundaries of medicine, like in motor performance.

One of the first studies investigating the placebo effect in sport performance was a within-subject study that included only men and involved military and bench press exercises (Ariel & Saville, 1972). Authors administered anabolic steroids and evaluated the maximal lift ability as outcome of the treatment. Results showed a significant improvement of motor performance during the placebo phase, that was when steroids were not administered to participants even if they thought they had received the treatment.

Since 2000s, authors tried to deepen the role of the placebo effect in sport performance. Clark and colleagues (2000) studied the cycling performance after the administration of carbohydrate supplementation. Participants received a non-caloric drink either with or without carbohydrate. Those who received the placebo showed a greater

improvement of the performance compared to those who received the carbohydrate solution (Clark, Hopkins, Hawley, & Burke, 2000). Maganaris and colleagues (2000) involved 21 athletes to study effects of deceptive administration of anabolic steroids on the maximum force production during bench press, dead lift, and squat exercises. Results showed an improvement and a maintenance of the performance in participants who believed they ingested the steroids. Since authors observed a reversal of the results when expectancy of enhancement was removed, they concluded for the central role of expectancy in evoking a placebo effect. Afterwards, Beedie and colleagues (2006) not only analyzed physiological outcome measures, such as power, heart rate, oxygen uptake and lactate during a cycling performance after the administration of different doses of caffeine, but they also considered psychological and subjective factors (Beedie, Stuart, Coleman, & Foad, 2006) (Fig.4).

Figure 4. Placebo effect in cycling performance



Averages of the power (%) expressed by both participants (orange) and placebo responders (blue) during the different experimental conditions. When participant were informed about the administration of caffeine and then received it, they showed an increased power during the cycling performance. This effect was prolonged even when participants were informed about the administration of caffeine but they received a placebo (e.g. inert substance) instead of the active component. *Adapted from Beedie, Foad, & Coleman, 2008.*

Despite authors administered an inert substance to all the participants, results showed an enhancement of cycling performance in those who believed they had ingested caffeine.

However, one of them reported to have experienced a negative effect due to the high dose of caffeine. Since the authors recorded this potential negative effect (i.e.. nocebo), they performed an additional study (2007) to investigate the role of positive versus negative beliefs on repeated 30m-sprint performances. Participants were informed they would have received an ergogenic aid. While both groups were informed about the enhancing effect of the aid on the endurance, they received information about either the positive or the negative impact of the treatment on the performance. Besides all the participants received an inert substance, the group informed about the improvement of both endurance and performance showed an enhancement of the speed. Conversely, those who were informed about the negative effect on the performance, recorded a worsening of the speed (Beedie, Coleman, & Foad, 2007). In the last decade, the placebo effect has been also studied in terms of pain endurance during a simulate sport competition (McClung & Collins, 2007), underlying the strengthening role of the preconditioning, for example with morphine (Benedetti, Pollo et al., 2007), on the placebo effect. Moreover, it has been studied the role of ergogenic placebo on muscle performance (Pollo, Carlino, & Benedetti, 2008), considering also the level of fatigue reported by the participants while lifting a weight. In all the cases, an improvement of the performance was recorded following the administration of an active substance as conditioning and/or the verbally induced expectancies. Subsequent studies tried to fill the gap on the nocebo effect in motor training, showing a detrimental performance (Corsi, Emadi Andani, Tinazzi, & Fiorio, 2016; Emadi Andani, Tinazzi, Corsi, & Fiorio, 2015; Pollo, Carlino, Vase, & Benedetti, 2012). Moreover, studies have also investigated subjective indices related to the motor performance. Authors recorded a perceived feeling of less force and a higher sense of effort at the end of the procedure compared to the beginning (Corsi et al., 2016; Emadi Andani et al., 2015), thus demonstrating that the nocebo effect has not only a behavioral component but induces also clear subjective changes.

1.5 General aims

Although many progresses have been done in the placebo and nocebo literature, there are still some open questions that need further research to be answered. In my PhD project, I tried to tackle some of these questions, by addressing the cognitive factors and neurophysiological correlates associated to placebo and nocebo effects not only in pain but also in the motor domain.

One key-question is related to the two cognitive mechanisms at the basis of placebo and nocebo effects. As previously described (par. 1.2), the main cognitive mechanisms are expectation and learning. Even if these mechanisms have been widely studied in the past years, the specific weight of each of them in modulating placebo and nocebo responses is still unknown. To cover this gap, we performed *Study 1*, in which we investigated the prevalent role of either expectation or learning in placebo and nocebo effects. The aim of this study was to investigate whether participants are more prone to be influenced by verbal suggestion or prior experience.

Moreover, we know from the literature that even if expectation and learning are considered as the main mechanisms to evoke placebo and nocebo responses, other cognitive factors should be taken into account as potential modulators. One emerging factor is how participants perceive the efficacy of the (actually inert) treatment. In addition, personality traits have been acknowledged as important modulators of the placebo/nocebo effects. Hence, in *Study 2*, by combining perception of treatment efficacy to a systematic investigation of personality traits, we tried to add new evidence on the cognitive mechanisms at the basis of these effects. We specifically focused on nocebo responses, as the literature is still scant on this side.

In a similar line of reasoning, we investigated whether expectations could be shaped by personality traits. To this aim, we used a well-validated pain paradigm in *Study 3* to better understand the advantage of measuring expectation and the shaping role of psychological factors.

A more original research line was then opened with a further study in which we combined for the first time the pain and motor fields. To this aim, we conducted *Study 4*, in which we investigated whether it is possible to induce an analgesic effect by actively involving the motor cortex (i.e. by executing a movement) and if placebo and nocebo responses further modulate this exercise-induced analgesic effect.

Finally, the neurophysiological correlates of the placebo and nocebo effects in the motor domain are still poorly understood. While there is a study on the neurophysiology of the motor-placebo effect, no study up-to-know has tried to uncover the same mechanisms in motor-nocebo. To cover this gap, we performed *Study 5*. We took advantage of a previous motor paradigm and we unraveled the activation of the motor cortex during a nocebo procedure by using the transcranial magnetic stimulation.

Overall, the studies I conducted during my PhD are linked by the need to answer some open research questions in the placebo and nocebo literature.

PART II : ON THE FACTORS INFLUENCING THE MAGNITUDE OF PLACEBO AND NOCEBO EFFECTS

Study 1 : Placebo and nocebo effects: a study on the congruency between conditioning and verbal suggestion

Despite the importance of verbal suggestion and conditioning in inducing placebo and nocebo effects, until now only few studies tried to disentangle the distinctive role of these two mechanisms (Price et al., 1999; Stewart-Williams & Podd, 2004; Voudouris et al., 1985, 1990). Linde and colleagues (2007) investigated the role of expectancy in different patients undergoing acupuncture treatment and demonstrated that expectations have a significant impact in inducing an effect in patients. Those with high expectation of benefit were more likely to obtain a better outcome, not only after the completion of the acupuncture session, but also at the follow-up (Linde et al., 2007). The amount of expectation appears to be clinically relevant for a better outcome. Bartels and colleagues (2014) tried to assess the role of verbal suggestion, conditioning or the combination of both in inducing either a placebo or a nocebo response in itch (Bartels et al., 2014). The authors confirmed that the combination of conditioning and verbal suggestion induces stronger and longer lasting placebo and nocebo effects compared to verbal suggestion or conditioning alone. In most of the studies, however, verbal suggestion and conditioning were applied in the same direction (either positive or negative) and conditioning was typically applied to reinforce the effects of verbally induced expectation. Hence, little is known on the potential contrasting effects between verbal suggestion and conditioning when they are applied in an opposite direction (e.g., one positive and one negative). Considering the current state of the art, we designed a protocol that aimed to enlarge the knowledge about these processes, by unraveling the prevalent role of either verbal suggestion or conditioning in inducing a placebo and nocebo response. To this purpose, we devised a protocol in which verbal suggestion and conditioning could be either congruent (both positive or negative) or incongruent (one positive and one negative). The question we wanted to address is whether individuals are more prone to show a placebo or nocebo effect following specific verbal instructions (independently from the type of

conditioning) or following specific conditioning (independently from the type of verbal suggestion).

Materials, methods and statistical analysis

Participants

Fifty healthy volunteers (26 women, mean age: 22.46 ± 2.40 years) were recruited from the student population of the University of Verona. Subjects were randomly divided into four groups defined by the type of verbal suggestion (either positive or negative) and conditioning (either positive or negative) (Tab.1).

Table 1. Characteristics of the four groups

Groups	Verbal Suggestion (VERB)	Conditioning (COND)	N	Age (mean±SE)
1	POSITIVE	POSITIVE	13 (7M; 6F)	22.23 ± 2.17
2	POSITIVE	NEGATIVE	14 (7M; 7F)	22.50 ± 1.99
3	NEGATIVE	NEGATIVE	11 (6M; 5F)	24 ± 3.10
4	NEGATIVE	POSITIVE	12 (5M; 7F)	21.25 ± 1.76

Participants were randomly assigned to four groups derived from the combination of verbal suggestion (positive vs. negative) and conditioning (positive vs. negative).

The resulting four groups were composed as follows (Tab.1): 13 subjects (all but one right-handed) entered the verb^{+}cond^{+} group; 14 subjects (all but one right-handed) entered the verb^{+}cond^{-} group; 11 subjects (all but one right-handed) entered the verb^{-}cond^{-} group; 12 subjects (all but one right-handed) entered the verb^{-}cond^{+}. All the participants signed an informed consent in which the procedure was described. They also self-declared to have no history of neurological, psychiatric, or other medical problems. The real purpose of the study was omitted and participants were debriefed at the end of the experiment.

Motor task

We used a slightly modified version of previous paradigms suitable to induce placebo (Fiorio et al., 2014), as well as nocebo effects in the motor domain (Corsi et al., 2016; Emadi Andani et al., 2015). Participants were asked to perform a motor task consisting of abduction movements of the right index finger against a piston connected to a force transducer. To help the participant in performing the task by using only the muscle of interest (i.e., the first dorsal interosseous, FDI), we fixed the hand with a holder. The force impressed on the piston was visualized by a cursor on a computer screen. As soon as the participant pressed the piston, the cursor moved toward a target zone, defined by four colored lines representing different level of force for each participant (from 70% to 130% of the maximal voluntary force, MVF, which was recorded for each participant at the beginning of the procedure with 10 pressures of the piston at the maximum force). The participants could start the trial by pressing a mouse key with the left index finger when a "Start" command appeared on the screen. Soon after the mouse key-press, they were required to initiate the motor task by pressing the piston with the right finger as strongly as possible in order to move the cursor into the target zone. When entering the target zone, the cursor changed color from yellow to green, thus providing a visual feedback to the participants. The motor task consisted of 50 trials, each lasting 1100ms.

Procedure

The motor task was repeated in three consecutive sessions: baseline, conditioning and final. In the first and the last sessions, the motor task was the same as described above. After the baseline session, participants underwent a placebo or nocebo procedure consisting in the application of transcutaneous electrical nerve stimulation, TENS, at 10 Hz for 5 minutes on the FDI belly region. TENS at this frequency does not have any effect on the treated body area, nonetheless two groups of participants received positive verbal suggestion of force increase due to TENS (positive verbal suggestion, $verb^{+}$), whereas two groups of participants received negative verbal suggestion of force decrease due to TENS (negative verbal suggestion, $verb^{-}$). During the conditioning phase, a surreptitious manipulation of the cursor's excursion range was introduced in steps of 0.0029 from trial 1 to trial 35. Two groups of participants received a surreptitious amplification of the cursor's excursion range (positive conditioning, $cond^{+}$), whereas two groups of

participants received a surreptitious reduction of the cursor's excursion range (negative conditioning, $cond^{-}$). Hence, by combining the two variables (verbal suggestion and conditioning), we obtained four groups (Tab.1): $verb^{+}cond^{+}$, $verb^{-}cond^{-}$, $verb^{+}cond^{-}$ and $verb^{-}cond^{+}$. Of notice, two groups ($verb^{+}cond^{+}$, $verb^{-}cond^{-}$) received a congruent conditioning with respect to the verbal suggestion, thus representing typical placebo and nocebo groups, respectively. Conversely, two groups ($verb^{+}cond^{-}$ and $verb^{-}cond^{+}$) received a conditioning opposite to the verbal suggestion.

Behavioral and subjective measures

Force during the motor task was measured in two ways: (1) by considering the mean value of the peak force amplitude in the 50 trials of each session ($Force_{peak}$) and normalizing it to the MVF (Normalized $Force_{peak}$), and (2) by calculating the percentage of strong pressures ($Strong_{press}$) in each session, considering the total number of trials in each session ($N_{tot\ trials}$) and the number of trials in which the peak force was above the mean value computed in the baseline ($N_{strong\ trials}$).

Namely, these indexes were defined as follow:

$$Normalized\ Force_{peak} = \frac{Force_{peak}}{MVF} \times 100\% \quad (1)$$

$$Strong_{press} = \frac{N_{strong\ trials}}{N_{tot\ trials}} \times 100\% \quad (2)$$

We measured subjective *Expectation* after TENS application by using a 7-points Likert scale, ranging from -3 (expectation of worse performance) to +3 (expectation of better performance). After the execution of the motor task in the conditioning and final sessions, subjects were asked to report two measures: the perceived efficacy of TENS in modulating the force (*TENS efficacy*), by means of a 10 cm visual analog scale (VAS), ranging from 0 (not effective) to 10 (very effective); the estimated the level of force (*Perception of force*) applied during the execution of the task by means of a 10 cm VAS scale, ranging from 0 (very weak) to 10 (very strong). Additionally, after the conditioning and final sessions we asked the subjects to judge how strong they felt with respect to the

baseline session by means of 7-points Likert scale, ranging from -3 (much weaker) to +3 (much stronger). In order to control for the level of fatigue, the perceived *Sense of effort* was measured in each session after the execution of the motor task, by means of the Borg scale (Borg, 1970), ranging from 6 (rest) to 20 (maximal effort).

Data Analysis

A first repeated measures analysis of variance (rmANOVA) was carried out to assess the effects of group as between-subject factor (4 levels: $verb^{+}cond^{-}$, $verb^{-}cond^{-}$, $verb^{+}cond^{+}$, $verb^{-}cond^{+}$) and session as within-subject factor (2 levels: baseline vs. final) with regards to the behavioral parameters ($Normalized\ Force_{peak}$, $Strong_{press}$) and subjective parameters (feeling of force, expectation, TENS efficacy and sense of effort).

To uncover any specific effect of verbal suggestion and conditioning on the behavioral and subjective data, we ran a second rmANOVA with verbal suggestion ($verbal^{+}$, $verbal^{-}$) and conditioning ($cond^{+}$, $cond^{-}$) as between-subject factors and again session (baseline vs. final) as within-subject factor. In order to evaluate the magnitude of the placebo and nocebo effects, we calculated the difference between the final and the baseline session (delta, Δ) for the behavioral and subjective parameters in each group.

All the analyses were followed by post-hoc comparisons including 2-tailed t-tests for paired samples and either one-way ANOVA or 2-tailed t-tests for independent samples. Bonferroni correction has been applied where necessary.

The level of significance was set at $p < 0.05$. All the data are expressed as mean ± SE. Analyses were performed using SPSS Statistics 21 software (SPSS Inc).

Results

The four groups did not statistically differ for age (one-way ANOVA, $F_{(3,49)} = 0.961$, $p = 0.419$) and education (one-way ANOVA, $F_{(3,49)} = 2.482$, $p = 0.073$), and were comparable for gender distribution (Chi-square test, $\chi^2 = 0.422$, $df = 3$, $p = 0.936$).

The MVF measured during the calibration phase did not differ between groups ($vs^{+}cond^{-}$: 18.32 ± 0.70 N, $verb^{-}cond^{-}$: 17.33 ± 0.84 N; $verb^{+}cond^{+}$: 19.23 ± 0.78 N, $verb^{-}cond^{+}$: 18.02 ± 0.58 N; one-way ANOVA, $F_{(3,49)} = 1.132$, $p = 0.346$).

First ANOVA with the 4 groups

Behavioral data. ANOVA on Normalized $Force_{peak}$ revealed no significant effect of session ($p = 0.234$) and group ($p = 0.409$). Conversely, the interaction session × group was significant ($F_{(3,46)} = 4.33$, $p = 0.009$). Post-hoc comparison revealed significant differences between sessions in the $verb^{-}cond^{-}$ group ($p = 0.037$) and a tendency also in $verb^{-}cond^{+}$ group ($p = 0.056$), due to lower levels of force recorded in the final (group $verb^{-}cond^{-}$: 90.17 ± 3.21; group $verb^{-}cond^{+}$: 89.11 ± 3.22) compared to the baseline session (group $verb^{-}cond^{-}$: 97.70 ± 3.16; group $verb^{-}cond^{+}$: 93.06 ± 3.67). Significant differences were also found in Δ values ($F_{(3,46)} = 4.37$, $p = 0.009$), in particular between group $verb^{+}cond^{-}$ and group $verb^{-}cond^{-}$ ($p = 0.026$).

ANOVA on $Strong_{press}$ disclosed a no effect of session ($p = 0.371$) and group ($p = 0.102$). The interaction session × group was slightly significant ($F_{(3,46)} = 2.81$, $p = 0.050$). However, post-hoc analysis reveals no differences between groups.

Subjective data. ANOVA on the feeling of force showed a significant effect of session ($F_{(1, 46)} = 16.58$, $p < 0.001$), due to lower values in final (5.15 ± 0.25) compared to baseline session (6.20 ± 0.20), and of group ($F_{(1,46)} = 3.80$, $p = 0.016$), due to differences between the $verb^{+}cond^{-}$ group (6.61 ± 0.35) and the $verb^{+}cond^{+}$ group (5.02 ± 0.37) ($p = 0.018$). Moreover, the interaction session × group was significant ($F_{(3, 46)} = 3.92$, $p = 0.014$). Post-hoc comparisons revealed that in the final session group $verb^{+}cond^{-}$ (6.75 ± 0.49) reported higher feeling of force values than group $verb^{-}$ $cond^{+}$ (4.32 ± 0.38, $p = 0.006$) and group $verb^{+}cond^{+}$ (4.60 ± 0.40, $p = 0.017$), suggesting that group $verb^{+}cond^{-}$ felt stronger after the conditioning procedure than the other two groups. Moreover, a difference between sessions was found in group $verb^{-}cond^{-}$ ($p = 0.022$) and in group $verb^{-}cond^{+}$ ($p = 0.001$), due to lower values of feeling of force in the final (group $verb^{-}cond^{-}$: 4.94 ± 0.68; group $verb^{-}cond^{+}$: 4.32 ± 0.40) compared to baseline session (group $verb^{-}cond^{-}$: 6.66 ± 0.39; group $verb^{-}cond^{+}$: 6.23 ± 0.56), suggesting that these two groups felt weaker after the procedure. Differences were also found in Δ value comparing baseline and final session ($F_{(3,46)} = 3.98$, $p = 0.014$), due to significant differences between group $verb^{+}cond^{-}$ and $verb^{+}cond^{-}$ ($p = 0.022$).

Analysis of expectation showed no significant effect of session ($p = 0.210$) and session × group interaction ($p = 0.231$). Conversely, the factor group was significant ($F_{(3,46)} = 36.11$,

$p < 0.001$), due to higher expectation scores in group verb^{+}cond^{-} (0.90 ± 0.18) than verb^{-}cond^{-} (-1.04 ± 0.18) and verb^{-}cond^{+} (-0.88 ± 0.19), and higher scores in group verb^{+}cond^{+} (1.05 ± 0.18) than group verb^{-}cond^{-} (for all comparisons, $p < 0.001$).

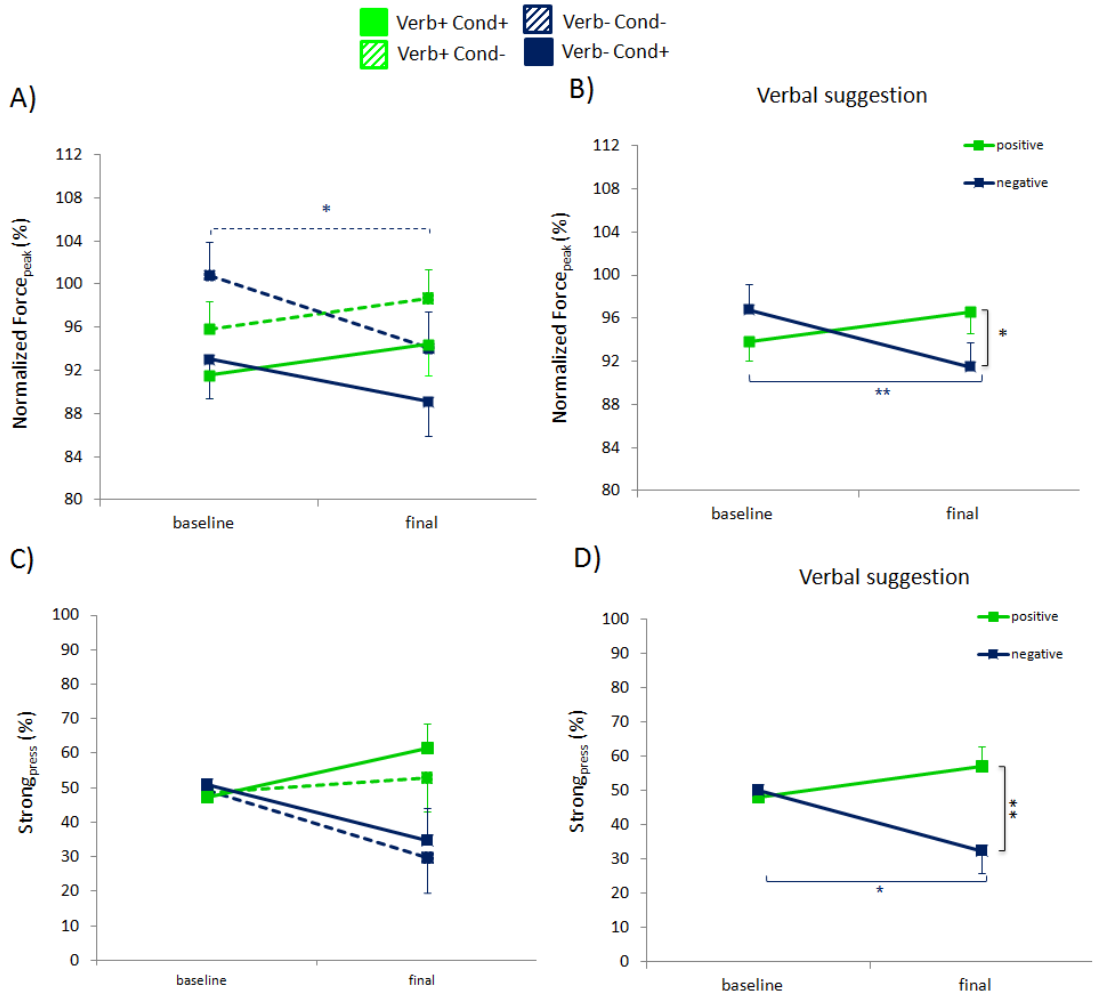
Analysis of judgment of TENS efficacy showed a significant effect of session ($F_{(1,46)} = 6.00$, $p = 0.018$), due to higher efficacy scores after the second (4.17 ± 0.36) compared to the first application (3.28 ± 0.35). No significant effect of session × group interaction ($p = 0.108$) and group ($p = 0.218$) has been found.

ANOVA on the sense of effort showed a significant effect of session ($F_{(1, 46)} = 8.15$, $p = 0.007$), due to higher effort scores reported in the final (12.46 ± 0.29) compared to baseline session (11.88 ± 0.29). No significant effect has been found for group ($p = 0.162$). However, the interaction session × group was significant ($F_{(3, 46)} = 8.39$, $p < 0.001$). Post-hoc comparisons showed differences between groups in the final session ($p = 0.026$), due to a tendency to statistical difference between group verb^{+}cond^{-} and verb^{-}cond^{+} ($p = 0.063$). Moreover, a difference between sessions was found in group verb^{+}cond^{-} ($p = 0.045$), group verb^{-}cond^{-} ($p = 0.025$) and group verb^{-}cond^{+} ($p = 0.005$). All these groups reported higher sense of effort in the final (group verb^{-}cond^{-}: 12.82 ± 0.69; group verb^{-}cond^{+}: 13.08 ± 0.48) compared to the baseline session (group verb^{-}cond^{-}: 11.72 ± 0.79; group verb^{-}cond^{+}: 11.16 ± 0.53), except for group verb^{+}cond^{-}, that reported higher values in the baseline (11.64 ± 0.55) compared to the final (10.92 ± 0.60) session. Additionally, differences were found in Δ value comparing baseline and final session ($F_{(3,46)} = 8.39$, $p < 0.001$), due to significant differences between group verb^{+}cond^{-} and verb^{-}cond^{-} ($p = 0.019$), verb^{+}cond^{-} and verb^{-}cond^{+} ($p < 0.001$), verb^{+}cond^{+} and verb^{-}cond^{+} ($p = 0.010$).

Second ANOVA with two between-subject factors

Behavioral data. ANOVA on the Normalized Force$_{peak}$ revealed a significant interaction session × verbal suggestion ($F_{(1,46)} = 12.21$, $p = 0.001$). Post-hoc analysis showed a difference between positive (verb^{+}: 96.60 ± 1.97%) and negative verbal suggestion (verb^{-}: 89.62 ± 2.23%) in the final session ($p = 0.023$). Moreover, negative verbal suggestion induced a reduction of force in the final (89.62 ± 2.23%) compared to the baseline session (95.24 ± 2.44%) ($p = 0.004$) (Fig.5 A,B).

Figure 5. Behavioral data



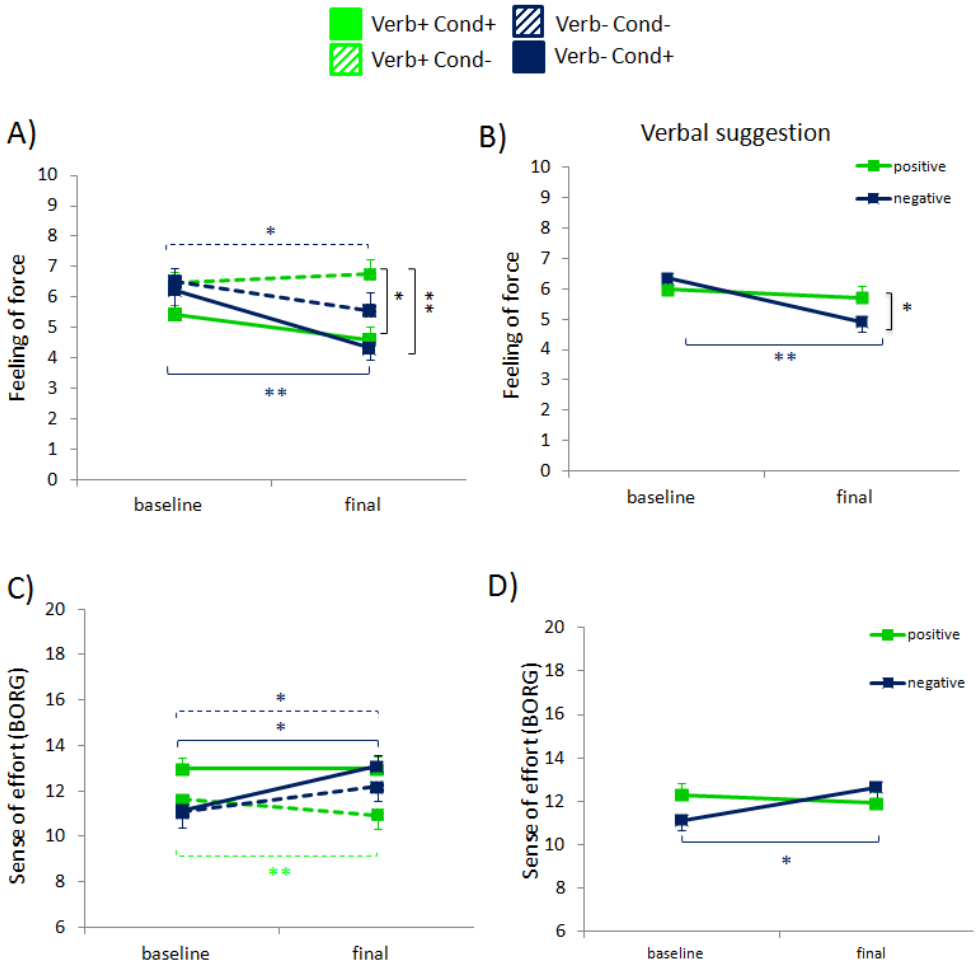
The level of force tended to increase from baseline to final session in groups that received a positive verbal suggestion (green) compared to those who received negative verbal suggestion (blue), independently from the direction of the conditioning **(A, B)**. We observed the same tendency by considering the percentage of strong pressures **(C,D)**. *$p < 0.010$; **$p < 0.050$.

ANOVA on $Strong_{press}$ disclosed a significant effect for verbal suggestion ($F_{(1,46)} = 6.14$, $p = 0.017$), due to lower $Strong_{press}$ with negative (41.19 ± 3.38) compared to positive verbal suggestion (52.56 ± 3.11). The interaction session × verbal suggestion was significant ($F_{(1, 46)} = 7.92$, $p = 0.007$). Post-hoc analysis revealed lower $Strong_{press}$ with negative (32.43 ± 6.78) than with positive verbal suggestion (56.96 ± 6.06%), specifically in the final session ($p = 0.009$). Moreover, with negative verbal suggestion, $Strong_{press}$ were lower in the final compared to baseline session (50.09 ± 0.99%) ($p = 0.018$) (Fig.5 C,D).

Subjective data. ANOVA on the feeling of force showed a significant effect of conditioning ($F_{(1,46)}$ = 7.96, p = 0.007), due to lower values with positive (5.14 ± 0.26) compared to negative conditioning (6.21 ± 0.27). The interaction session × verbal suggestion was significant ($F_{(1,\ 46)}$ = 8.86, p = 0.005). Post-hoc analysis showed differences between groups in the final session ($t_{(48)}$ = -2.02 , p = 0.049), due to lower scores of force evaluation during the main task in the negative (4.62 ± 0.38) compared to positive group (5.71 ± 0.38).

Moreover, negative verbal suggestion group perceived less force in final (4.62 ± 0.38) compared to baseline session (6.43 ± 0.34) ($t_{(48)}$ = -5.0, p < 0.001) (Fig.6 A,B).

Figure 6. Subjective data



The perceived feeling of force decreased from baseline to final session in groups that received negative verbal suggestion (blue) compared to those who received positive verbal suggestion (green), independently from the direction of the conditioning **(A, B)**. Moreover, negative verbal suggestion group (blue) rated a

higher sense of effort in final compared to baseline session, whilst positive verbal suggestion group (green) tended to be stable across sessions **(C,D)**. *p < 0.010; **p < 0.050.

Further analysis on expectation showed a significant effect of verbal suggestion ($F_{(1,46)}$ = 108.12, p < 0.001), due to higher worsening expected with negative (-0.96 ± 0.13) compared to positive verbal suggestion (0.97 ± 0.13).

ANOVA on the judgment of TENS efficacy showed a significant effect of verbal suggestion ($F_{(1,46)}$ = 4.32, p = 0.043), due to lower scores with negative (3.09 ± 0.45) compared to positive verbal suggestion (4.36 ± 0.41). Moreover, the interaction session × conditioning × verbal suggestion was significant ($F_{(1,46)}$ = 5.50, p = 0.023). Post-hoc analysis disclosed higher scores of efficacy after the second application (4.00 ± 0.60) compared to the first one (1.77 ± 0.62) for the combination of positive conditioning and negative verbal suggestion (p = 0.001).

Further ANOVA on the sense of effort showed a significant effect of interaction session × verbal suggestion ($F_{(1, 46)}$ = 20.86, p < 0.001). Post-hoc analysis showed higher effort scores in the final (12.96 ± 0.40) compared to baseline session (11.43 ± 0.46) with negative verbal suggestion (p < 0.001) (Fig.6 C,D).

Discussion

The current study aimed at investigating the prevalent role of either verbal suggestion or conditioning in modulating the placebo/nocebo response in the motor field. From a behavioral point of view, we found a worsening in force production and a reduction in the percentage of strong pressures in the final compared to the baseline session in those groups who received negative verbal suggestion (e.g. $verb^{-}cond^{-}$, $verb^{-}cond^{+}$). This result confirms previous findings from our group (Corsi et al., 2016; Emadi Andani et al., 2015). Conversely, results are not that clear if we consider groups who received positive verbal suggestion (e.g. $verb+cond^{-}$, $verb^{+}cond^{+}$). In Fiorio and colleagues, authors recorded an enhancement of force production and an increasing in the percentage of strong pressures, while in the current study we noticed a non-significant tendency to increase in these parameters (Fiorio et al., 2014). This may be due to methodological differences between the studies. First, since the current study is slightly longer than the previous one (Fiorio et al., 2014), the duration of the task and the potential muscle weariness could explain the less evident increase of force in the verb+cond+ group. Second, the previous study (Fiorio

et al., 2014) involved verbal suggestion of increased motor performance and a following conditioning phase in which we surreptitiously strengthen the given information. In the current study, the group verb+cond- underwent a conditioning phase that was the opposite compared to the verbal suggestion. Thus, the negative conditioning phase disconfirmed the positive verbal suggestion. Despite the prevalent role of the verbal suggestion in inducing a placebo response, the conditioning phase may have attenuated the effect of the information on motor performance, thus resulting in a tendency but not in a significance of force improvement. It is worth noting that, by considering verbal suggestion as factor (positive vs. negative), we found that in the final session negative verbal suggestion induced significant lower values of force compared to positive verbal suggestion. The same pattern was disclosed by analyzing the percentage of strong pressures. From a subjective point of view, we found a significant difference in the feeling of perceived force of participants who received positive compared to negative verbal suggestion. Namely, the negative verbal suggestion group perceived to be significantly weaker in the final compared to the baseline session. The positive verbal suggestion group was likely stable between sessions, thus creating a gap with the negative verbal suggestion group at the end of the experiment. Consistently, the latter group reported to perceive a higher sense of effort in the final compared to the baseline session, while positive verbal suggestion group did not disclose any variation of the perceived effort. All together, these findings suggest that verbal suggestion has a more prominent role than conditioning in influencing motor performance and perception, and to a bigger extent in the case of nocebo.

However, some limitations of the present research should be mentioned. The main weakness is the absence of a control group. By recruiting a control group, we could have ruled out other factors, like fatigue, in inducing force decrements in the nocebo verbal suggestion group. Namely, since the task consisted of many trials, it is reasonable to expect a worsening of motor performance merely due to fatigue (Crupi et al., 2013; Robbins et al., 2010).

To our knowledge, this is the first study aimed to highlight the prevalent role of either verbal suggestion or conditioning in a placebo/nocebo paradigm. Further studies are needed and future steps might include the recording of the neurophysiological correlates, to unravel any corticospinal difference linked to the prevalent role of verbal suggestion

in modulating placebo and nocebo responses. Finally, it might be useful to consider that the psychological aspects of both placebo and nocebo effects should include not only cognitive factors, like verbal suggestion and conditioning, but also the dispositional traits that characterize each single person. In the next studies, we investigated the role of personality traits in modulating the placebo and nocebo responses.

Study 2 : Changes in perception of treatment efficacy modulate the magnitude of the nocebo effect and are related to personality traits

In the previous study, we demonstrated that verbal suggestion could have a stronger influence than conditioning in modulating placebo and nocebo responses. Recent evidence, though, suggests that other cognitive factors could be taken into account, in addition to expectation and learning, when studying these effects.

With this regard, an emerging factor in the placebo/nocebo literature is related to the way in which participants perceive the treatment in terms of choice (Bartley, Faasse, Horne, & Petrie, 2016) and also in terms of its effectiveness (Pecina, Stohler, & Zubieta, 2014). Recently, it was demonstrated that the discrepancy between subject's expectation and the perception of treatment effectiveness could evoke a placebo response (Pecina et al., 2014). Beliefs about treatments and perception of treatment effectiveness have an impact in the clinical context (Horne & Weinman, 1999; Sidani et al., 2009). Hence, these factors could represent an important psychological construct to be considered in nocebo, as well as placebo, studies in which the treatment does not have any active effect *per se*, but only simulates an active therapy in a particular psychosocial context (Benedetti, 2014c).

The current study is as an explorative investigation of the role of perception of treatment effectiveness on the nocebo effect in motor performance. More precisely, we investigated changes in perception of treatment effectiveness by adopting a conditioning procedure, in which the participant was surreptitiously exposed to the inert effects of a treatment and implicitly learned to associate the application of the treatment to force decrements (Emadi Andani et al., 2015). This study aims to investigate whether and how strongly individuals perceive the treatment as effective when passing from the conditioning phase, in which the effect of the treatment is present, to the test session, in which the effect is removed and the nocebo response can be measured.

Additionally, as we know from studies in pain perception, individuals differ in the tendency to express a nocebo response, depending on dispositional traits. Nocebo hyperalgesia is negatively predicted by optimism (Geers, Helfer, Kosbab, Weiland, & Landry, 2005; Geers, Wellman, Fowler, Helfer, & France, 2010) and positively predicted by anxiety (Colloca & Benedetti, 2007; Colloca et al., 2010; Geers et al., 2010). Moreover, in the context of social observation, dispositional empathy and pain

catastrophizing are associated to nocebo effects (Swider & Babel, 2013; Vogtle et al., 2013). In the current explorative investigation, the second aim is to investigate whether changes in perception of treatment effectiveness could be related to some personality traits. With regards to the first aim of the study, based on the evidence that perception of treatment effectiveness can influence the clinical outcome (Sidani et al., 2009), we anticipate that individuals who perceive at the end of the nocebo procedure that the treatment is as strongly effective as during the conditioning will also show a more pronounced nocebo-induced reduction of force than those who less consistently perceive its effectiveness. With regards to the second aim, based on the studies demonstrating stronger nocebo hyperalgesia in individuals with low levels of optimism and high levels of anxiety (Colloca & Benedetti, 2007; Colloca et al., 2010; Geers et al., 2005; Geers et al., 2010), the hypothesis is that the same traits could be associated to the tendency to strongly perceive a negative effect of the treatment on motor performance.

Materials, methods and statistical analysis

Participants

Forty-one healthy volunteers (18 women, mean age: 22.66 ± 3.05 years; all but three right-handed) were recruited from the student population of the University of Verona. Participants read and signed the informed consent, and self-declared to have no history of medical problems, including neurological and psychiatric disease. Due to the deceptive nature of the study, only after completing the whole experimental procedure, the nocebo nature of the study was explained. The study was approved by the local ethical committee of the Department of Neurological and Movement Sciences at the University of Verona. The study was conducted in accordance with the approved guidelines.

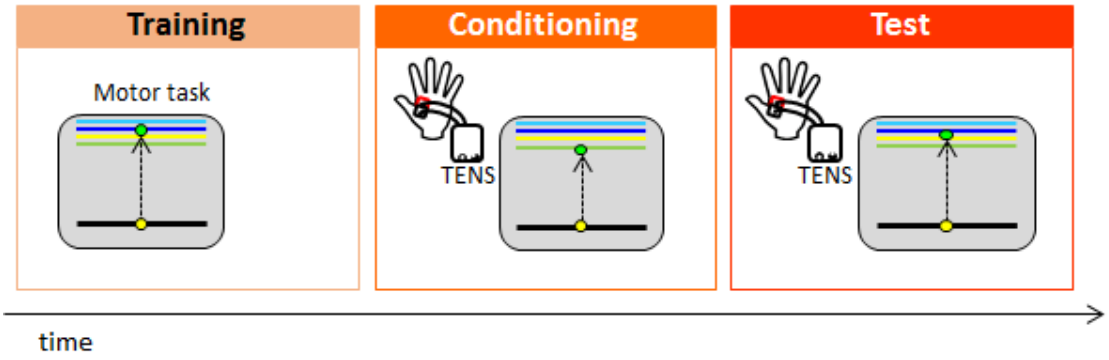
Motor task

We took advantage of the motor task previously described in Study 1. However, to better induce a nocebo effect, we modified the range delimited by the target zone. Namely, the color lines displayed on the monitor represented the 60%, 80%, 100% and 120% of the subject's MVC.

Procedure

After general instructions, 5 trials were allowed the subjects to familiarize with the task. Before starting the nocebo procedure, all the participants went through a training in which they had to perform the motor task for 50 trials. In the conditioning session, an inert treatment (TENS) was applied for 5 minutes over the region of the FDI muscle, together with the deceptive verbal instructions that it had the effect of reducing the recruitment of muscle fibers, with a consequent decrease in force production. The intensity of TENS was set to produce a slight sensation on the skin, without producing muscle contraction or discomfort. Soon after TENS, participants performed the motor task (50 trials) with a surreptitious, stepwise, pre-programmed reduction of the excursion of the cursor. Subjects were unaware of this manipulation that was meant as a procedure to condition them about the effects of TENS in reducing force. To monitor subjects' belief about TENS, we asked them to judge its efficacy on a visual analog scale (VAS) (Fig.7).

Figure 7. Schematic representation of the experimental protocol



After a first training session, a conditioning session was performed in which an inert treatment (TENS) was applied together with verbal instructions that it could induce a decrease of force and with a manipulation in which the visual feedback was surreptitiously reduced. After the motor task was completed, subjects were asked to judge TENS efficacy on a VAS ranging from 0 (not effective) to 10 (very effective). Afterwards, the procedure was repeated again in the test session, but this time the (fake) effect of TENS was removed. *Adapted from Corsi et al, 2016.*

More precisely, after the execution of the motor task, subjects were asked to report how much the TENS treatment was effective in reducing their force, on 0-10 VAS (0, not effective; 10, very effective). In the test session, TENS was applied again with the same

verbal instructions. Subjects then repeated the motor task (50 trials), but this time without any manipulation, that is without the surreptitious reduction of the cursor's excursion range. After the motor task, TENS efficacy score was measured again with the VAS. The delta of TENS efficacy scores between the test and conditioning sessions was considered as a proxy of the strength of perception of treatment efficacy.

Behavioral data

Force was evaluated by considering two indices. The first index consisted on the mean value of the peak force amplitude normalized to the MVC (Normalized $Force_{peak}$). The second index included the percentage of strong pressures ($Strong_{press}$) that considers the number of trials in which the peak force exceeded the mean value recorded in the training session. While the mean normalized force peak is a measure of the overall force level during a session, the percentage of strong pressures is a measure of consistency of behavior throughout a session.

Subjective data

In addition to TENS efficacy scores, other subjective variables were evaluated during the procedure, such as: *perception of force* (how strong they felt during the motor task) by means of a 10 cm VAS (0, very weak; 10, very strong); *sense of effort* (the perceived effort measured after the execution of the motor task, by using the Borg scale (6=rest, 20=maximal effort); *expectation* (how they expected their performance will be) on a 7-points Likert scale, ranging from -3 (much worse) to +3 (much better). Expectation scores were measured twice, after each TENS application.

Personality Questionnaires

After the whole experimental procedure, we administered some personality questionnaires in order to evaluate the possible role of personality traits. All the questionnaires were computerized (E-prime, version 2.0, Psychology Software Tools, Inc.), in order to facilitate data collection and processing. In particular, we took into account: *i) State-Trait Anxiety Inventory (STAI)*(Spielberg, 1983). This is a 40-items inventory divided in two scales: STAI-I measures the level of anxiety in a precise moment and STAI-II refers to an individual's usual anxiety tendency. In our study STAI-I was

administered twice, before and after the procedure, to control for any change in the anxiety level across sessions, while STAI-II was applied only at the end of the procedure; *ii) Life-Orientation Test-Revisited (Lot-R)*(Scheier et al., 1994). This is a 10-items scale developed to assess individual differences in generalized optimism versus pessimism. Four items are fillers and the total score is obtained by summing the answers of 6 real items; *iii) Temperament and Character Inventory (TCI)* (C.R. Cloninger et al., 1994). This 240-items questionnaire provides a personality profile in the context of a biopsychological model. It is made of four temperament and three character scales. The temperament dimensions are heritable traits and include novelty seeking (NS), harm avoidance (HA), reward dependence (RD) and persistence (P). The character dimensions may be related to different cognitive systems and include self-directedness (SD), cooperativeness (C) and self-transcendence (ST). One subject did not complete the TCI questionnaire due to recording problems; *iv) Multidimensional Iowa Suggestibility Scale (MISS)* (Kotov et al., 2004). This is a 95-items questionnaire to measure social and psychological suggestibility defined as a tendency to accept messages from others. It is made of five suggestibility sub-scales: consumer suggestibility, persuadability, physiological suggestibility, physiological reactivity and peer conformity. To the purposes of the current study, we considered the total suggestibility score; *v) Intrinsic Motivation Inventory (IMI)*(Markland & Hardy, 1997; Ryan, 1982). This is a 37-items multidimensional inventory to assess participants' subjective experience related to a target activity in laboratory experiments. It is divided in different scales assessing participant's interest/enjoyment, perceived competence and choice, effort perceived during the laboratory activity, value/usefulness of the performed activity and pressure and tension felt during the activity. To the purposes of the current study, we considered the total intrinsic motivation score.

Data analysis

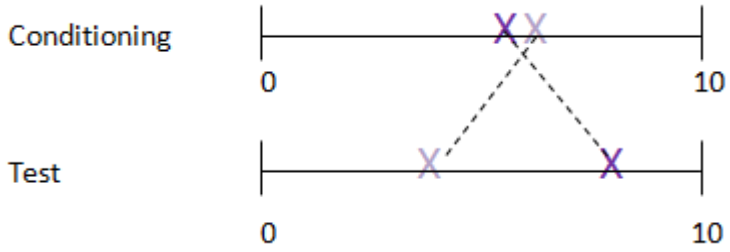
The perception of treatment effectiveness computed as difference between the test and the conditioning session (Δ TENS effectiveness) was the variable of interest in our study was. The two sessions (conditioning and test) were characterized by different visual feedbacks: during the conditioning session, there was a surreptitious reduction of the cursor's excursion range that should simulate the effect of TENS on force decrease. The

cursor's reduction was not applied in the test session. Hence, Δ TENS effectiveness represents whether and how strongly participants perceived the treatment as effective in the test compared to the conditioning session. This variable (Δ TENS effectiveness) was handled following two different statistical approaches.

In the first statistical approach, the polarity of the difference (positive or negative) was considered as means to define two groups (Fig. 8).

Conceptually, the two groups belong to two categories with opposite patterns of subjective perception: participants with a positive difference perceived the effect of the treatment more strongly in the final than in the conditioning session, whereas those with a negative difference were not so strongly consistent in their perception of treatment effectiveness.

Figure 8. TENS efficacy score calculation to define the two groups



Example of answers of participants who gave higher scores of TENS efficacy in the test compared to the conditioning session (dark purple, positive Δ TENS effectiveness score), and participants who gave lower scores in the test compared to the conditioning (light purple, negative Δ TENS effectiveness score). *Adapted from Corsi et al, 2016.*

Based on this assumption, we aimed at characterizing the two categories in terms of sample size, gender distribution, performance at the motor task, and personality traits by adopting a mixed design with between and within factors. The hypothesis in this analysis is that the group with a positive TENS difference (indicating stronger perception of the negative effects of the treatment effectiveness in the test session) should also show a more pronounced nocebo effect in the behavioral (reduction of force) and subjective outcomes (feeling weakness and effort) than the group with a negative difference (indicating less perception in the negative effects of the treatment effectiveness in the test session). Based on studies in pain perception (Geers et al, 2010; Colloca & Benedetti, 2007), we also

hypothesize that the group with a positive TENS difference should present higher levels of anxiety and lower levels of optimism than the groups with a negative difference.

To this purpose, first we checked that the two groups (positive vs. negative Δ TENS effectiveness) had similar MVC and motor performance (in terms of normalized $Force_{peak}$) in the training session, by means of t-test for independent samples. Then, we analyzed the behavioral parameters (normalized $Force_{peak}$, $Strong_{press}$) and the subjective parameters (TENS effectiveness scores, subjective perception of force, sense of effort and expectation scores) in the two crucial experimental conditions (conditioning vs. test) by means of repeated measures analyses of variance (rmANOVAs) with Group as between-subject factor (positive vs. negative Δ TENS effectiveness) and Session as within-subject factor (conditioning vs. test). In addition to all the indices of performance described above, we computed also the difference (Δ) between the test and the conditioning sessions, as computational measure to evaluate the changes of the behavioral and subjective variables between the two groups. The Δ of the two groups for each variable was analyzed by means of t-test for independent samples. The mean scores of the two groups at the personality questionnaires were analyzed with t-tests for independent samples. In all the analyses, post-hoc comparisons were executed by means of t-tests for paired or independent samples, using the Bonferroni correction for multiple comparisons where necessary. The level of significance was set at $p < 0.05$.

In a second statistical approach, we adopted a within design by considering all the participants together and by considering the difference in perception of treatment effectiveness as a continuum (from negative to positive values). The rationale for this approach is to prove whether changes in perception of treatment effectiveness were associated to changes in performance at the motor task and to personality traits. To this purpose, the difference in TENS effectiveness scores was correlated with the difference between the test and the conditioning sessions at the motor task (i.e., Δ normalized $Force_{peak}$, Δ $Strong_{press}$, Δ subjective perception of force, Δ sense of effort and Δ expectation). The hypothesis in this analysis is that more positive TENS effectiveness difference (indicating stronger perception in the negative effects of the treatment effectiveness in the test session), would be associated to a stronger nocebo effect measured as changes in the behavioral and subjective variables. Because we had a specific hypothesis about the direction of these correlations, 1-tailed test was used.

Difference of TENS effectiveness scores was correlated also with the personality variables. Although for some personality traits (like anxiety and lower levels of optimism) we could have specific hypotheses about the direction of the correlations (Geers et al, 2010; Colloca & Benedetti, 2007), for other traits (like intrinsic motivation and the temperament and character dimensions), it is not possible to make precise hypotheses and therefore 2-tailed test was used. No multiple comparisons were applied to the correlations, due to the explorative nature of the study.

All the data are expressed as mean ± s.e.m. Analyses were performed by using SPSS Statistics 21 software (SPSS Inc.).

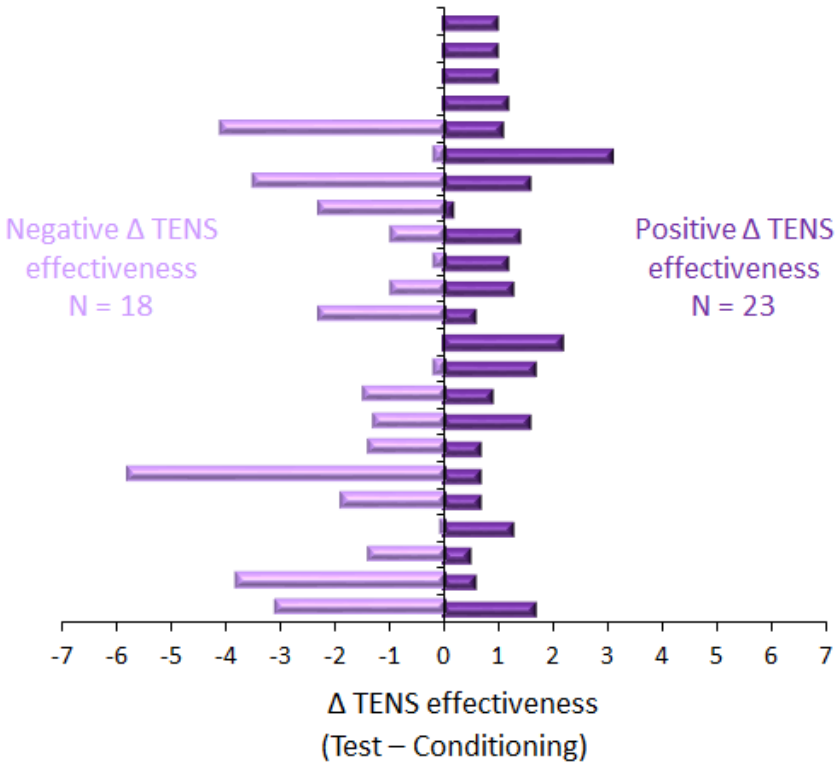
Results

Mixed design

Levine's test revealed homogeneous variances of all the data, apart from feeling of force ($p = 0.002$), which was analyzed with non-parametric tests (Mann-Whitney test for comparisons between groups and Wilcoxon signed-ranks test for comparisons across sessions). Following the first statistical approach, we found that 56.1% of the subjects (23 out of 41 subjects) had a positive difference of TENS effectiveness scores, indicating that they perceived more effect in the test than in the conditioning session.

Among these, 11 were females and 12 males, the mean age of the group was 22.57 ± 3.27 years and mean education was 15.3 ± 1.49 years. The remaining 43.9% of the subjects (18 out of 41) had a negative difference of TENS effectiveness scores, indicating that they perceived less effect of TENS in the test than in the conditioning session (Fig.9). Among these, 7 were females and 11 males, the mean age of the group was 22.78 ± 2.84 years and mean education was 15.29 ± 1.72 years. The two groups did not statistically differ for age (independent samples t-test, $t_{(39)} = -0.218$; $p = 0.828$) and for gender distribution (Chi-square test, $\chi^2 = 0.327$, df = 1, $p = 0.567$). Moreover, the two groups did not statistically differ for MVC, measured in the initial calibration phase (positive Δ TENS effectiveness: 20.53 ± 0.60 N, negative Δ TENS effectiveness: 19.41 ± 0.83 N, independent sample t-test, $t_{(39)} = 1.122$, $p = 0.269$).

Figure 9. Description of the positive and negative Δ TENS effectiveness groups.



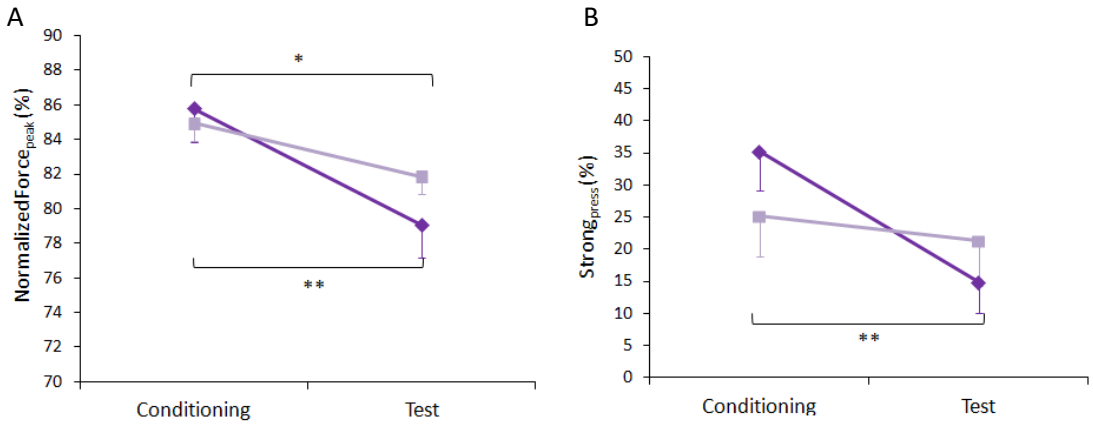
By subtracting the scores of TENS efficacy in the test and conditioning sessions, it turned out that 23 participants had a positive difference (positive Δ TENS effectiveness) and 18 had a negative difference (negative Δ TENS effectiveness). *Adapted from Corsi et al, 2016.*

Finally, the two groups had also similar normalized $Force_{peak}$ in the training session (positive Δ TENS effectiveness: 89.33% ± 1.46, negative Δ TENS effectiveness: 90.1% ± 1.54, independent sample t-test, $t_{(39)} = -0.351$, $p = 0.727$). These findings suggest that at the beginning of the experimental procedure, the two groups had comparable behavioral performance.

ANOVA on normalized $Force_{peak}$ measured during the nocebo procedure, revealed a significant effect of session ($F_{(1,39)} = 32.65$, $p < 0.001$, $\eta^2 = 0.456$), due to lower values in the test (80.45% ± 1.56) compared to the conditioning (85.38% ± 1.40) session and a non-significant effect of group ($F_{(1,39)} = 0.112$, $p = 0.740$, $\eta^2 = 0.003$). The interaction session × group was significant ($F_{(1,39)} = 4.46$, $p = 0.041$, $\eta^2 = 0.103$). Post-hoc comparisons showed that the group with positive Δ TENS effectiveness was weaker in test 79.06% ± 1.88) compared to the conditioning (85.81% ± 2) session ($p< 0.001$) and also the negative Δ TENS effectiveness group was weaker in the test (81.83 % ± 2.58) compared to the

conditioning (84.94% ± 1.87) session (p = 0.023) (Fig. 10A). Analysis of Δ $Force_{peak}$ disclosed a significant difference between groups ($t_{(39)}$ = -2.111, p= 0.041).

Figure 10. Behavioral data



A) Normalized Force. Representation of the force of the two groups in the conditioning and test sessions. A significant decrease of force could be observed only in the group with positive Δ TENS effectiveness score (Dark purple). **B) Percentage of Strong Pressures.** Representation of the $Strong_{press}$ in the two groups and in the two sessions. A significant reduction of strong pressures was found in the group of negative Δ TENS effectiveness score. **p < 0.01, *p < 0.050. *Adapted from Corsi et al, 2016.*

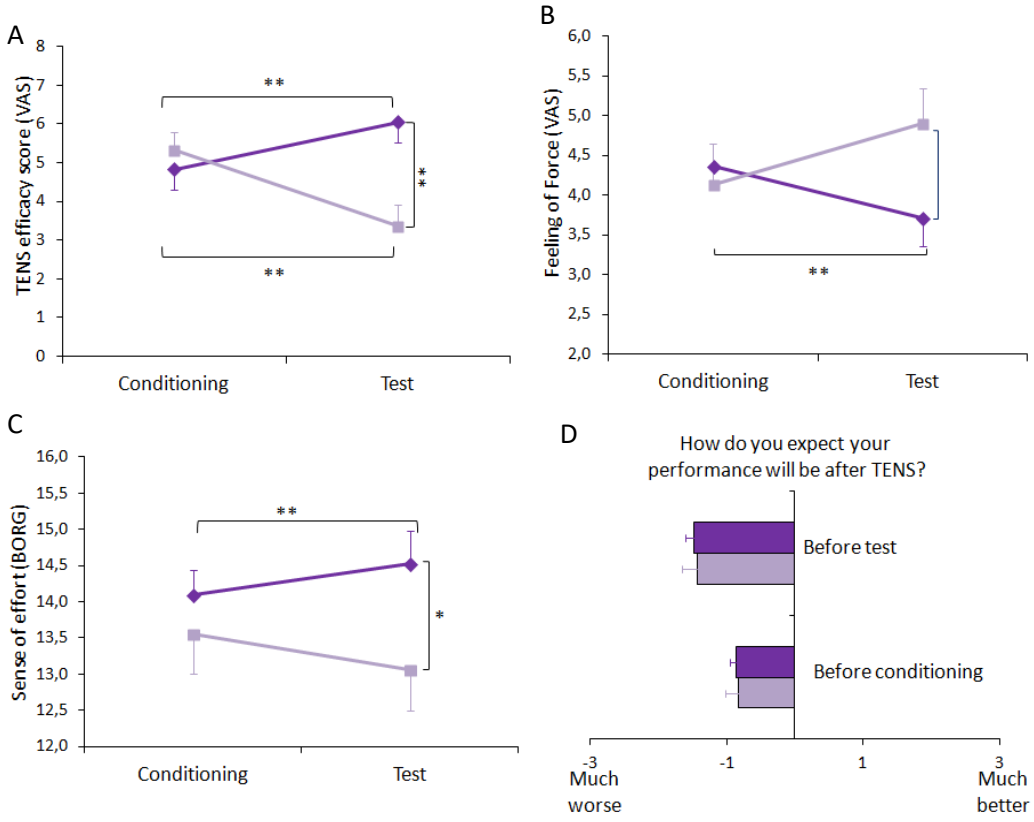
These findings suggest that positive and negative Δ TENS effectiveness groups had a different nocebo response in terms of behavioral outcome.

ANOVA on $Strong_{press}$ disclosed a similar pattern of results with a significant effect of session ($F_{(1,39)}$ = 16.33, p < 0.001, η^2 = 0.295), due to lower values in the test (18.12% ± 4.23) than in the conditioning (30.22% ± 4.55), but no effect of group ($F_{(1,39)}$ = 0.45, p= 0.834, η^2 = 0.001). The interaction session × group was significant ($F_{(1,39)}$ = 7.59, p= 0.009, η^2 = 0.163). Post-hoc comparisons showed that the positive Δ TENS effectiveness group had a significant reduction in the percentage of strong pressure in the test (14.87% ± 4.81) compared to the conditioning (35.22% ± 6.16) session (p < 0.001), while no difference was found in the negative Δ TENS efficacy score group (p = 0.199) (Fig.10B). Analysis of Δ $Strong_{press}$ disclosed a significant difference between groups ($t_{(39)}$ = -2.955, p= 0.006). These findings confirm a different nocebo effect in performance between positive and negative Δ TENS effectiveness groups.

TENS efficacy scores were analyzed to confirm that the two groups were not only qualitative, but also quantitatively different concerning their belief in the treatment efficacy. ANOVA on TENS efficacy scores showed that on average the two groups had significantly different judgments about TENS, especially in the test session (that is in the second application of TENS).

The factor session was significant ($F_{(1,39)} = 4.23$, $p = 0.047$, $\eta^2 = 0.098$), due to lower values in the test (4.69 ± 0.38) than in the conditioning (5.07 ± 0.36) session, but no effect of group ($F_{(1,39)} = 2.27$, $p = 0.140$, $\eta^2 = 0.055$) was found. The session × group interaction was significant ($F_{(1,39)} = 76.74$, $p < 0.001$, $\eta^2 = 0.663$). Post-hoc comparisons showed that positive Δ TENS effectiveness group reported higher values in the test (6.03 ± 0.51) compared to the conditioning session (4.82 ± 0.51, $p < 0.001$), while the opposite pattern was found in the negative Δ TENS effectiveness group, with lower values in the test (3.37 ± 0.55) than in the conditioning session (5.32 ± 0.49, $p < 0.001$) (Fig.11A).

Figure 11. Subjective data



a) TENS efficacy scores. In the conditioning session the two groups had similar scores of TENS efficacy, whereas in the test session the group of positive Δ TENS effectiveness score had higher scores than the

group of negative Δ TENS effectiveness score. Moreover, while the group of positive Δ TENS effectiveness score had higher scores in the test compared to the conditioning session, the groups of negative Δ TENS effectiveness score had the opposite pattern, with lower scores in the test compared to the conditioning session. This suggests that the two groups were not only qualitatively, but also quantitatively different in their belief. **b) Subjective perception of force.** The group of positive Δ TENS effectiveness score felt weaker in the test compared to the conditioning session, whereas negative Δ TENS effectiveness score had a stable feeling of force. **c) Sense of effort at the BORG scale** (Borg, 1970). The group of positive Δ TENS effectiveness score showed a significant increase of sense of effort in the test compared to the conditioning session. **d) Expectation of performance**. Expectation was measured soon after TENS application and before task execution. The scores of the two groups were not different both before the conditioning session and before the test session. All the data are expressed as mean values and standard errors (s.e.m.). **p < 0.01, *p < 0.050, ~p = 0.051. (Dark purple: Positive Δ TENS effectiveness group; Light purple: Negative Δ TENS effectiveness group). *Adapted from Corsi et al, 2016.*

Moreover, the two groups had significantly different values in the test session (p = 0.001). Analysis of the Δ TENS efficacy scores confirmed a different pattern between the two groups ($t_{(39)} = 7.943$; p < 0.001). These findings confirm a different belief in the treatment between positive and negative Δ TENS effectiveness group.

Wilcoxon signed-ranks test on force perception disclosed that the participants with positive Δ TENS effectiveness had lower scores in the test (3.71 ± 0.36) compared to the conditioning (4.36 ± 0.28) session (Z = -2.5, p= 0.012), whereas participants with negative Δ TENS effectiveness showed no difference between sessions (Z = -1.66, p= 0.098) (Fig.11B). Mann-Whitney test on the Δ force perception showed a significant difference between the two groups (U = 96.5, Z = -2.905, p= 0.004). These findings suggest that positive and negative Δ TENS effectiveness groups had a different nocebo response even in terms of subjective feeling of force.

ANOVA on the sense of effort revealed a non-significant effect of session ($F_{(1,39)} = 0.025$, p= 0.876, η^2 =0.001) and group ($F_{(1,39)} = 2.36$, p= 0.133, $\eta^2 = 0.057$). However, the session × group interaction was significant ($F_{(1,39)} = 5.05$, p= 0.030, $\eta^2 = 0.115$). Post-hoc comparisons showed that the positive Δ TENS effectiveness group perceived a higher level of effort in the test (14.52 ± 0.45) than in the conditioning (12.52 ± 0.3) session (p < 0.001), while the negative Δ TENS effectiveness group had no differences between sessions (p = 0.217). Moreover, the two groups were different in the test session (p=

0.049) (Fig.11C). Analysis of Δ sense of effort disclosed significant differences between groups ($t_{(39)}$ = 2.247, p= 0.030). Hence, from these findings we can conclude that the sense of effort after a nocebo procedure is related to the amount of belief in the negative effects of the treatment.

Analysis of expectation scores showed that the factor session was significant ($F_{(1,39)}$ = 16.62, p < 0.001, η^2 = 0.299), due to more negative values in the test (-1.45 ± 0.12) compared to the conditioning session (-0.84 ± 0.10) (Fig.11D). Conversely, the factor group ($F_{(1,39)}$ = 0.04, p= 0.838, η^2 = 0.001) and the session × group interaction ($F_{(1,39)}$ < 0.01, p= 0.994, η^2 = 0.000) were not significant. Moreover, the analysis of Δ expectation scores showed no significant differences between groups (p= 0.994).

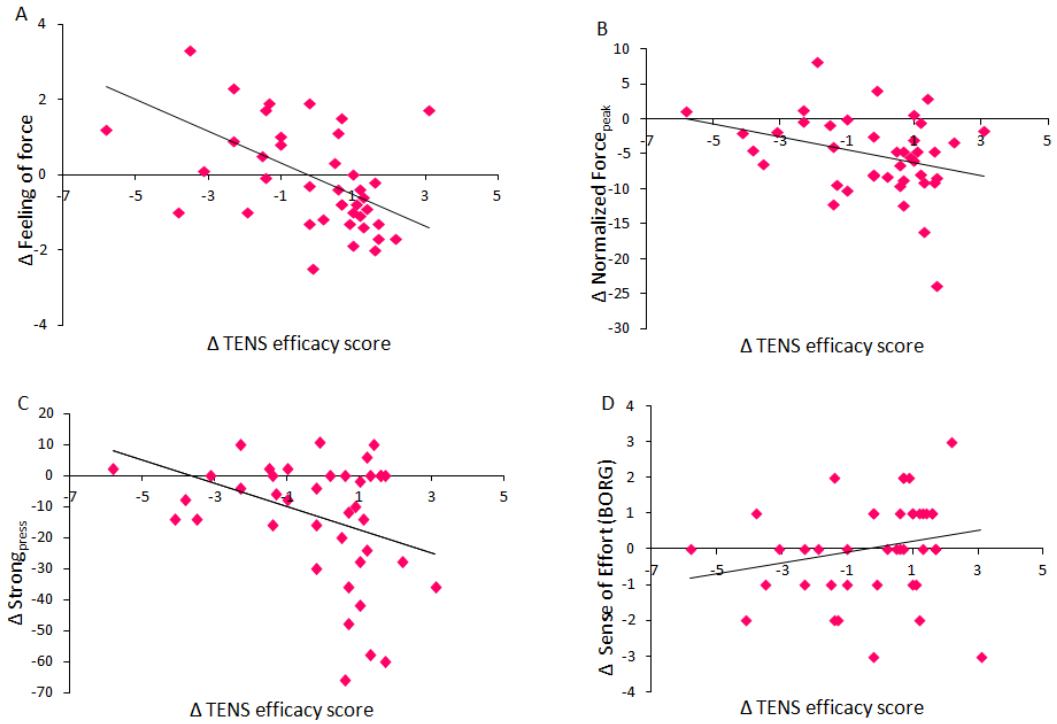
These findings suggest that expectation was not different between positive and negative Δ TENS effectiveness groups and therefore it could be unrelated to the amount of persistence of belief in the treatment.

With regards to the personality traits, test for independent samples revealed that the positive Δ TENS effectiveness group had higher STAI-II (assessing trait anxiety) scores (46.04 ± 1.73) than the negative Δ TENS effectiveness group (40.33 ± 1.93) ($t_{(39)}$ = 2.200, p = 0.034), indicating that the former was generally more anxious than the latter. Analysis of Lot-R (assessing optimism and pessimism) revealed a nearly significant difference between the two sub-groups ($t_{(39)}$ = -2.012, p = 0.051) with lower scores in participants with positive Δ TENS effectiveness (12.61 ± 0.83) compared to those with negative Δ TENS effectiveness (15.22 ± 1.02), suggesting that the former were more pessimist than the latter.

Within design

Following the second statistical approach, we found that Δ TENS effectiveness scores negatively correlated with Δ normalized $Force_{peak}$ (rho = -0.284, p = 0.036), with Δ $Strong_{press}$ (rho = -0.282, p = 0.037) and with Δ subjective perception of force (rho = -0.542, p < 0.001) and positively correlated with Δ sense of effort (rho = 0.278, p = 0.039). These findings suggest that a more positive difference in perception of TENS effectiveness was associated to a more pronounced reduction of force, reduction of feeling of force and stronger sense of effort during the nocebo procedure (Fig.12A-D).

Figure 12. Correlations between Δ TENS efficacy scores and behavioral data.

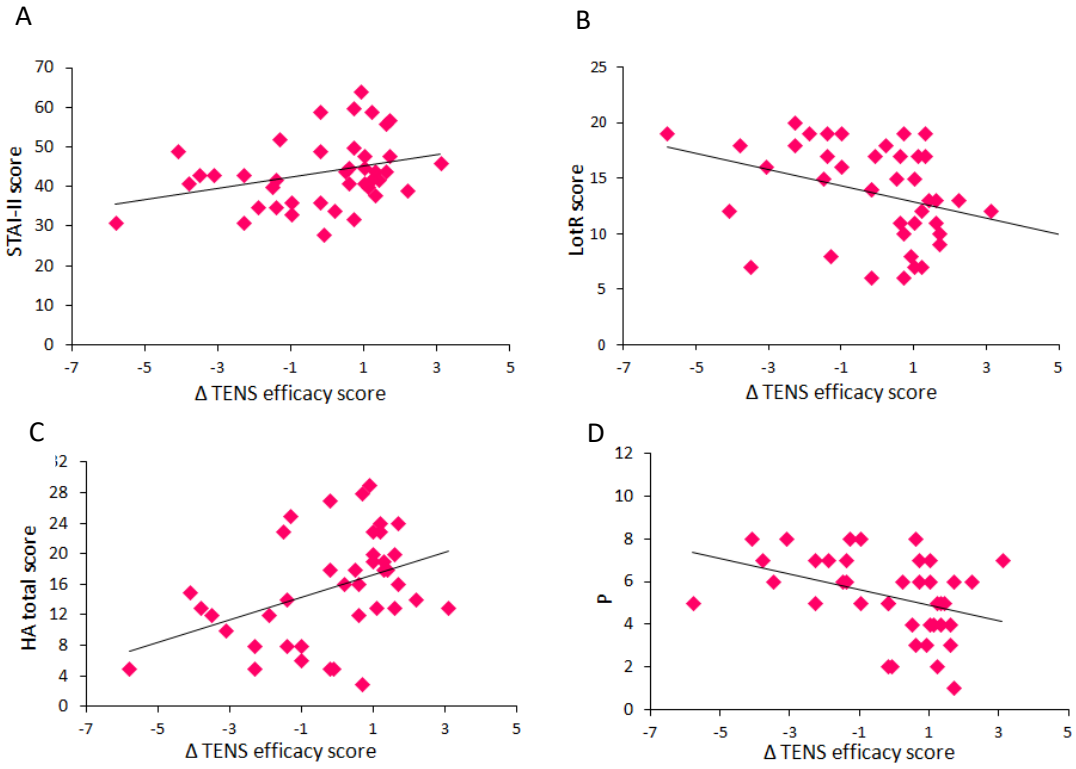


A) Δ TENS effectiveness scores negatively correlated with subjective perception of force, with Δ normalized Forcepeak **(B)**, and with Δ Strongpress **(C)**. **D)** Δ TENS effectiveness scores positively correlated with Δ sense of effort. *Adapted from Corsi et al, 2016.*

With regards to personality traits, scores at the STAI-II positively correlated with the Δ TENS effectiveness scores (rho = 0.335, p = 0.033), suggesting that the higher the anxiety trait, the stronger the perception of the negative effects of TENS in the test session (Fig.13A). A negative correlation with the Δ TENS effectiveness scores and Lot-R (rho = -0.373, p =0.016), suggesting that the lower dispositional optimism the stronger the perception of the negative effects of TENS in the test session (Fig.13B).

Analysis of the TCI revealed positive correlations between Δ TENS effectiveness scores and harm avoidance scale (assessing behavioral inhibition) (rho = 0.321, p = 0.044) (Fig.13C), indicating that the most inhibited individuals more strongly perceived the negative effects of TENS in the test session.

Figure 13. Correlations between Δ TENS efficacy scores and personality traits.



A) Δ TENS efficacy scores and STAY-II. Trait anxiety positively correlated with the Δ TENS efficacy scores, suggesting that higher levels of trait anxiety were associated to higher tendency to believe in the negative effects of the treatment. **B) Δ TENS efficacy scores and Lot-R.** Optimism scores negatively correlated with the Δ TENS efficacy scores, suggesting that lower levels of optimism were associated to higher tendency to believe in the negative effects of the treatment. **C) Δ TENS efficacy scores and TCI**. Harm avoidance total score positively correlated with Δ TENS efficacy scores, suggesting that more inhibited, pessimist and worried individuals had a higher tendency to believe in the negative effects of the treatment. **D) Δ TENS efficacy scores and TCI**. TCI-persistence negatively correlated with Δ TENS efficacy scores, suggesting that individuals who resisted less to fatigue had a higher tendency to believe in the negative effects of the treatment. *Adapted from Corsi et al, 2016.*

A negative correlation was found with persistence (assessing perseverance despite of fatigue or frustration) (rho = -0.367, p = 0.020), suggesting that those individuals who were weaker in resisting to fatigue perceived more strongly the negative effects of TENS (Fig.13D). No other correlations were found to be significant.

Discussion

The findings of this study show that changes in the perception of treatment effectiveness are associated to the magnitude of the nocebo effect in motor performance and to personality traits. By dividing the subjects in relation to changes in the perception of TENS effectiveness we found that force production was differently affected in the two sub-groups. Although reduction of normalized force was observed in both sub-groups, a more pronounced decrease of percentage of strong pressures was found in individuals with a positive difference of perception of TENS effectiveness. The two indexes of performance represent slightly different aspects: Normalized force is a measure of the mean force level in relation to the MVC, whereas the percentage of strong pressures gives a measure of the consistency of behavior. Namely, the latter parameter of motor performance represents how consistently participants pressed the piston above a certain value (as determined in the training session). This finding suggests that individuals who strongly believed in the negative effects of the treatment were also less able to exert strong pressures on the piston than those who less consistently perceive the effects of the treatment. The negative correlations between changes in perception of treatment effectiveness and force reduction indicate that the more the negative effects of the treatment are perceived at the end of the nocebo procedure the stronger is the reduction of force. Overall, the behavioral results unmask a link between changes in perception of treatment effectiveness and the magnitude of the nocebo effect. The mechanisms at the basis of these findings, however, remain to be clarified in future studies.

With regards to the subjective variables, participants with a positive difference of perception of TENS effectiveness felt more weakness and effort in the test than in the conditioning session, while those with a negative difference of perception of TENS effectiveness did not modify the feeling of force and sense of effort across sessions.

An interesting question in the placebo/nocebo literature is the definition of a placebo/nocebo-prone personality (Darragh, Booth, & Consedine, 2015; Pecina et al., 2013). The inspection of dispositional factors revealed that personality traits like anxiety and TCI-harm avoidance (which is indicative of inhibited behavior (Cloninger, 2008; Cloninger et al., 1994)) were positively correlated with the tendency to perceive the negative effects of the treatment, whereas optimism and TCI-persistence (which implies

continuing and persevering despite fatigue and lack of reward (Cloninger, Svrakic, & Przybeck, 1993)) were negatively correlated.

These findings well fit with the notion that optimism and anxiety modulate nocebo, as well as placebo, responses (Colloca et al., 2010; Geers et al., 2005; Geers et al., 2010; Morton, Brown, Watson, El-Deredy, & Jones, 2010).

In the current explorative study, we could speculate that personality traits could have bias the perception of words and performance during the nocebo procedure, thus resulting in different perception of treatment effectiveness at the end of the procedure. Based on previous evidence, we could hypothesize that more anxious and less optimistic individuals deployed more attention to the verbal information conveyed by the experimenter about the negative effects of the treatment in worsening performance. Alternatively, these individuals could have paid more attention toward the progressively shorter cursor's excursion range, thus amplifying the perception of the negative feedback during the conditioning phase. Although the current study does not allow disambiguating between the two alternatives, we hypothesize that these processes could have led to a stronger perception of treatment effectiveness at the end of the nocebo procedure.

Some methodological limitations of the current study should be mentioned. One limit is that we cannot draw conclusions on the causal relation between changes in perception of treatment efficacy, personality and force decrements. The division of the subjects was made *post-hoc*, based on the difference of the treatment efficacy scores between the test and the conditioning sessions. In other words, we first defined a variable to divide the two groups, i.e., the change of subjects' perception of treatment efficacy, and afterwards we extrapolated the dispositional traits. Moreover, the mechanisms at the basis of the force decrements, sense of weakness and sense of effort remain to be uncover. Potential interest for future studies could be to manipulate expectation and perception of treatment effectiveness in a factorial design in order to investigate which factor plays a major role in the motor nocebo effect, similarly to the study by Peciña and colleagues in placebo analgesia (Pecina et al., 2014).

Despite this unconventional way of proceeding, our study shows for the first time an association between changes in the perception of treatment effectiveness, personality traits and force production and may inspire further investigations aiming at explaining

individual differences in the placebo and nocebo effect not only in motor performance but also in other fascinating domains, such as that of pain.

In the following study, by taking advantage of a well-validated pain paradigm, we investigated whether expectations could be shaped by personality traits.

Study 3 : Placebo and nocebo effects: The advantage of measuring expectations and psychological factors

As demonstrated in the study described above, personality factors can influence placebo and nocebo effects both in pain (Colagiuri, Schenk, Kessler, Dorsey, & Colloca, 2015; Colloca & Grillon, 2014) and in the motor domain (Corsi et al, 2016). Factors such as dispositional optimism (Geers et al., 2005; Geers, Kosbab, Helfer, Weiland, & Wellman, 2007; Geers et al., 2010; Morton, Watson, El-Deredy, & Jones, 2009; Nes & Segerstrom, 2006), hypnotic suggestibility (De Pascalis, Chiaradia, & Carotenuto, 2002), somatic focus (Geers, Helfer, Weiland, & Kosbab, 2006; Johnston, Atlas, & Wager, 2012), empathy (Colloca & Benedetti, 2009; Hunter, Siess, & Colloca, 2014; Rutgen, Seidel, Riecansky, & Lamm, 2015; Rutgen, Seidel, Silani et al., 2015), neuroticism (Pecina et al., 2013), altruism (Pecina et al., 2013), social desirability (Gelfland, Gelfland, & Rardin, 1965), fear of pain (Flaten, Aslaksen, Finset, Simonsen, & Johansen, 2006; Lyby, Aslaksen, & Flaten, 2010; Zubieta, Yau, Scott, & Stohler, 2006), locus of ego-resilience (Pecina et al., 2013), anxiety (Ober et al., 2012; Staats, Staats, & Hekmat, 2001), pessimism (Corsi et al., 2016; Geers et al., 2005), pain catastrophizing (Vogtle et al., 2013), harm avoidance and have been linked to placebo and nocebo effects.

In particular, optimism, the active behavioral and mental coping ability of individuals to face adversity, has been linked to proneness to show higher placebo analgesic effects (Geers et al., 2005; Geers et al., 2007; Geers et al., 2010). Attention toward the body, referred as somatic focus, is related to larger placebo analgesic effects and higher positive expectations (Geers et al., 2006). Empathic resonance and concern for others have been linked to placebo analgesia as well (Colloca & Benedetti, 2009; Hunter et al., 2014; Rutgen, Seidel, Riecansky et al., 2015; Rutgen, Seidel, Silani et al., 2015). Hypnotic susceptibility and responsiveness to verbal suggestions influence placebo analgesia (Huber, Lui, & Porro, 2013). Other factors such as Neuroticism-Extraversion-Openness to experience (NEO), NEO Altruism, NEO Straightforwardness, NEO Angry Hostility and Ego-Resiliency, have been coupled with a 25% variance in behavioral placebo responses to pain and 27% of the μ-opioid system activation in the nucleus accumbens (Pecina et al., 2013).

Conversely, anxiety (Staats et al., 2001), harm avoidance and persistence (Corsi et al., 2016) and pain catastrophizing (Swider & Babel, 2013; Vogtle et al., 2013) have been associated with nocebo effects. Anxiety and harm avoidance correlate positively with nocebo effects, while optimism and persistence correlate negatively with nocebo effects in the context of the motor system (Corsi et al., 2016). In the present study, our aim was to investigate how distinct positive and negative personality factors estimate the likelihood of placebo and nocebo effects. Moreover, we aimed to establish the relationship among trial-by-trial expectations of pain reduction and increase, and placebo/nocebo effects, and personality. We hypothesized that using aggregated personality factors and expectations would allow us to better estimate placebo and nocebo responses in a laboratory setting using a well-established conditioning model (Colloca et al, 2010).

Materials, methods and statistical analysis

Participants

We recruited 50 participants from Baltimore, MD, USA to enroll a total of 46 healthy participants (24 women; 27.41 ± 1.07 years). Four participants were excluded: two of them did not meet the inclusion criteria and two were unreliable with respect to the pain reports (during the experimental phases, they were not able to constantly detect the different levels necessary to create a conditioning effect). Upon arrival, participants signed a consent form to study pain modulation. Participants with cardiovascular and neurological diseases, family or personal history of psychiatric conditions, personal history of drug abuse, acute or chronic pain, color blindness, impaired hearing, pregnancy and current use of painkillers and any other medication, were excluded from participating in this study. On the day of the experiment, a toxicology drug test was also performed to exclude any recent use of marijuana, cocaine, opiates such as hydrocodone, oxycodone and hydromorphone, amphetamine, methamphetamine, ecstasy/MDMA and phencyclidine. Participants who reported use of tobacco or nicotine over the last year were also excluded. This study was carried out in accordance with the recommendations of the UMB Institutional Review Board with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the UMB Ethics Committee (Prot #

HP00065783). Due to the use of deception, a debriefing written form was given to each participant at the end of the study participation offering to withdraw the data from the study. None of them opted to do so. Participants were compensated for their participation ($90).

Pain assessment

A well-validated paradigm (Colloca et al., 2010) was used to explore placebo and nocebo responses to a contact heat thermal painful stimulation.

Individual pain sensitivity and tolerance were measured in each participant using the ATS Medoc Pathway system (Medoc Advanced Medical System, Rimat Yishai, Israel). A 3 × 3cm thermode was placed on the dominant forearm as confirmed by the Edinburgh Handedness Inventory. The baseline temperature delivered by the Medoc equipment was 32°C. Ascending series of stimulations starting from warm sensation to maximum tolerable pain were delivered, while the participant was asked to stop the machine as soon as she felt a warm sensation, low, medium and high pain. Each level was assessed four times and averaged to determine the intensities of stimulations to be used during the acquisition and testing phases of the conditioning paradigm. After exploring the individual pain sensitivity during the calibration phase in which participants were asked to report low, medium and high levels of pain three times, we defined the three painful stimulations by subtracting 3° Celsius starting from the highest reported level (e.g. 49°C, 46°C, 43°C) in order to standardize the relative differences in the delivered pain intensity. The intensities of stimulation were also rated to ensure correspondence to individual experience of low, medium and high pain.

Placebo and nocebo manipulation

Three visual cues (red, yellow, and green) were displayed on a computer placed one meter apart from a chair in a quiet lab. Participants were told that the green, yellow and red lights would anticipate the delivery of a low, medium and high level of pain, respectively. During the acquisition phase of the classical conditioning paradigm, 18 painful stimulations were delivered at the three levels of pain corresponding to an individual low, medium, and high pain (e.g. 6 red, 6 yellow, 6 green presentations). Unbeknown to the participants, during the testing phase, the remaining 9 stimulations presented with the

three color cues were set at same medium control level (e.g. 3 red, 3 yellow, 3 green presentations), in accordance with a previously described paradigm (Colloca et al., 2010). The sequence of the color cues presentation was randomized across participants using four distinct sequences. This change in the pain levels allowed us to explore how first-hand experience of low and high pain during the acquisition phase results in placebo and nocebo responses during the testing phase. Participants rated the experienced pain immediately after the painful stimulation using the VAS scale (from 0=no pain to 100=maximum tolerable pain). Pain reports were collected using Celeritas Fiber Optic Response System (Psychology Software Tools, Inc., Sharpsburg, PA, USA). Moreover, expectations were measured. The terms 'expectation' and 'expectancy' have been often used in an interchangeable way. Herein, we adopted the term 'expectation' to refer to verbalized and measurable constructs as compared to 'expectancies' defining psychophysical predictions that can be present without full awareness (i.e., implicit expectancies). Participants were asked to rate their expectations of the upcoming stimulation immediately before the delivery of the thermal stimulation using a VAS anchored from 0=no pain to 100=maximum tolerable pain.

During each trial, the visual cue was presented for 4 seconds. Immediately after the presentation of the cue, participants were asked to rate their expectation (5 seconds) about the upcoming stimulus. The thermal stimulation lasted for 10 seconds. Then participants were asked to rate their perceived pain (5 seconds) and an inter-trial interval followed with a variable timing (8-10 seconds). The procedure and the delivery of painful stimulations were controlled by scripts pre-programmed in Eprime (Psychology Software Tools, Inc., Sharpsburg, PA, USA; version 2.0). To prevent habituation, the presentation of visual cues during both phases was pseudo-randomized using four pre-programmed sequences.

Psychological questionnaires

Participants completed a comprehensive battery of psychological questionnaires, which were chosen to cover distinct psychological factors that we hypothesized to be linked to placebo and nocebo effects. In particular, for the placebo-related factors, we included optimism, reward, suggestibility, empathy and sensation-seeking and motivation. We used the following questionnaires: 1. Life-Orientation Test-Revisited, Lot-R (Scheier et

al., 1994) to assess generalized optimism versus pessimism; 2. Behavioral Inhibition and Behavioral Activation Scale, BIS/BAS (Carver & T.L., 1994) to investigate dispositional sensitivity to the behavioral inhibition system (BIS) and the behavioral activation system (BAS); 3. Multidimensional Iowa Suggestibility Scale, MISS (Kotov, Bellman, & Watson, 2004b) to investigate the main components of suggestibility; 4. Interpersonal Reactivity Index, IRI (Davis, 1980) to measure the participant's dispositional empathy in different situations; 5. Sensation Seeking (SS) (Zuckerman, 1994) to measure the necessity to find and experience new situations; 6. Tri-dimensional Personality Questionnaire, TPQ (C. R. Cloninger, Przybeck, & Svrakic, 1991) to assess novelty seeking (NS), harm avoidance (HA), and reward dependence (RD); 7. and the Intrinsic Motivation Inventory (IMI) (Markland & Hardy, 1997) to assess participants' experience during the experimental procedure that was just performed. For the nocebo-related psychological factors included measurements of various aspects of anxiety (e.g. state, severity, and sensitivity), catastrophizing, neuroticism, fear of pain, depression, feelings of worry. The following inventories were used: 1. State and Trait Anxiety Inventory, STAI (Spielberger, 1983) to investigate anxiety either in a precise moment (STAI-Y1) or as a general tendency (STAI-Y2); 2. Anxiety Sensitivity Index, ASI (Reiss, Peterson, Gurskey, & McNally, 1986) to assessed beliefs of sensations that could have harmful consequences; 3. Beck Anxiety Inventory, BAI (A.T. Beck, Epstein, Brown, & Steer, 1988) to measure experience of anxiety symptoms during the previous two weeks; 4. Beck Depression Inventory, BDI (A. T. Beck, Ward, Mendelson, Mock, & Erbaugh, 1961) to include items relating to depression, cognitions, as well as physical symptoms; 5. Mood and Anxiety Symptom Questionnaire, MASQ (Haigh, Moore, Kashdan, & Fresco, 2011) to assess depressive symptoms and anxiety symptoms; 6. Pain Catastrophizing Scale, PCS (Sullivan, Bishop, & Pivik, 1995) to assess catastrophizing impacts on pain experience; 7. Neuroticism – Extroversion – Openness Inventory (NEO) – Five Factory Inventory (FFI) (P. T. Costa, Jr., & McCrae, 1992; P. T. Costa & McCrae, 1985) to investigate Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness; 8. Fear of Pain Questionnaire, FOP (Osman, Breitenstein, Barrios, Gutierrez, & Kopper, 2002) to measure fear levels to different types of physical pain; 9. Penn State Worry Questionnaire, PSWQ (Meyer, Miller, Metzger, & Borkovec, 1990) to measure the trait of worry in different situations.

We also administered the Positive and Negative Affective Schedule, PANAS (Crawford & Henry, 2004), that investigates the relationships between positive and negative affect with personality states and emotions.

Data analysis

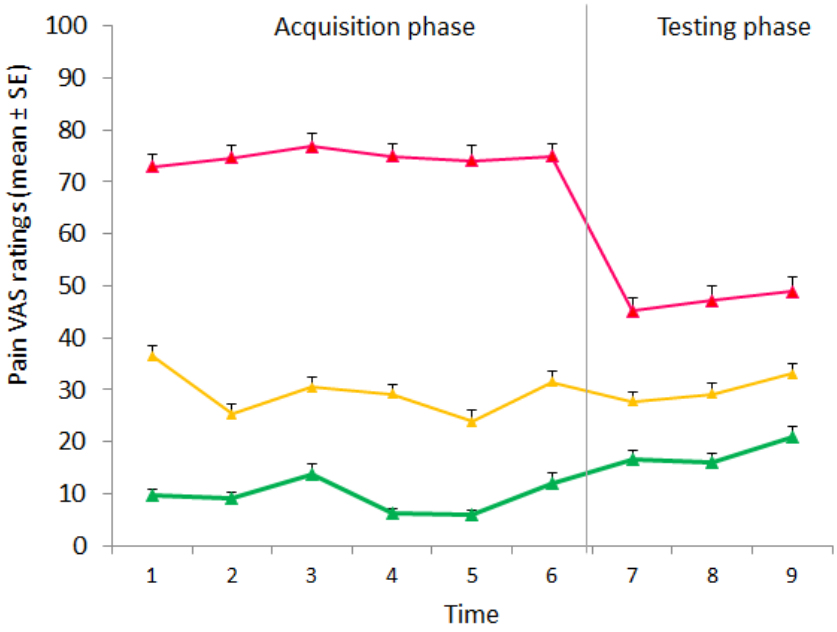
Repeated measure ANOVA was calculated using VAS pain and expectations ratings to test the main effect of the factor condition (red, yellow, and green) and time (trials) set both as within-subjects factors. F-tests were followed by the Bonferroni *post-hoc* tests for multiple comparisons. VAS pain scores from the testing phase were further averaged across trials to calculate the difference between yellow-green and red-yellow pain scores, respectively. Similarly, delta scores for expectations were averaged to be correlated with placebo and nocebo factors. The above psychological questionnaire scores were used in both simple correlation and multivariate analyses. We analyzed psychological questionnaire scores using both Spearman correlation and stepwise multiple regression model analyses in which the questionnaires were modeled to predict placebo and nocebo responses. All the analyses were carried out using the SPSS software package (SSPS Inc., Chicago, Illinois, USA, vers.21). To minimize alpha errors, the level of significance was set at $p \leq 0.005$.

Results

Acquisition phase (conditioning)

We performed separate analyses for the VAS pain reports related to the acquisition and testing phases of the conditioning paradigm. We found that during the acquisition phase, participants learned to distinguish the low, medium and high levels of painful stimuli (main effect of condition: $F_{(2,88)} = 203.970$, $p < 0.001$). The average pain score for red-associated stimuli was 74.73 ± 2.36 using an average intensity of pain equal to 47.52 °C, the average pain score for yellow was 29.55 ± 1.54 using an average pain equal to 44.55 °C and the average pain score for green was 9.37 ± 0.96 when an average pain equal to 41.51 °C out of 50 °C was delivered.

Figure 14. Time course of placebo and nocebo responses



Trial-by-trial representation of the average of pain ratings for the three conditions (control=yellow; placebo=green; nocebo=red) during the acquisition (trials 1-6) and the testing (trials 7-9) phases. Participants learned to distinguish the low, medium and high levels of painful stimuli over the acquisition phase. *Corsi & Colloca, 2017*

The factor Time was significant ($F_{(5,220)} = 7.359$, $p < 0.001$) as well as the condition × time interaction ($F_{(10,440)} = 5.324$, $p < 0.001$) (Fig.14).

Testing phase (placebo and nocebo responses)

During the testing phase, when the level of pain was set at the same control (yellow) intensity, repeated measure ANOVA revealed a significant effect of condition ($F_{(2,88)} = 96.04$, $p < 0.001$), time ($F_{(2,88)} = 7.553$, $p = 0.001$) with a non-significant condition × time interaction ($F_{(4,176)} = 0.378$, $p = 0.824$) indicating no extinction over the entire experimental session. Post-hoc Bonferroni tests indicated that the red stimuli (average VAS: 46.98 ± 2.46) were perceived as higher than the yellow control stimuli (average VAS: 29.96 ± 1.78) ($p<0.001$) and green (average VAS: 17.86 ± 1.70) were rated as lower than the yellow stimuli ($p < 0.001$) indicating both robust placebo and nocebo effects. The distribution and magnitude of placebo and nocebo responses ranged from no effects to large changes in pain modulation (Fig. 15).

Figure 15. Distribution of placebo and nocebo effects.

Each bar represents a single study participant. The green bars represent the magnitude of the placebo effect (yellow-green VAS scores). The red bars represent the magnitude of the nocebo effect (yellow-red-VAS score). It is worth noting that the individual placebo and nocebo responses range from no responses at all to medium to large effect. *Corsi & Colloca, 2017*

Placebo responses were significantly correlated with the hypoalgesic effect experienced during the acquisition phase (green– yellow ratings; Placebo: r = 0.388, p= 0.008) but nocebo hyperalgesic responses appeared to be independent of the experienced high pain (r = 0.080 p = 0.598). Moreover, being prone to experience a placebo response did not imply being also prone to experience a nocebo response, as indicated by the absence of significant correlation between individual placebo and the nocebo responses (r = -0.113 p = 0.454).

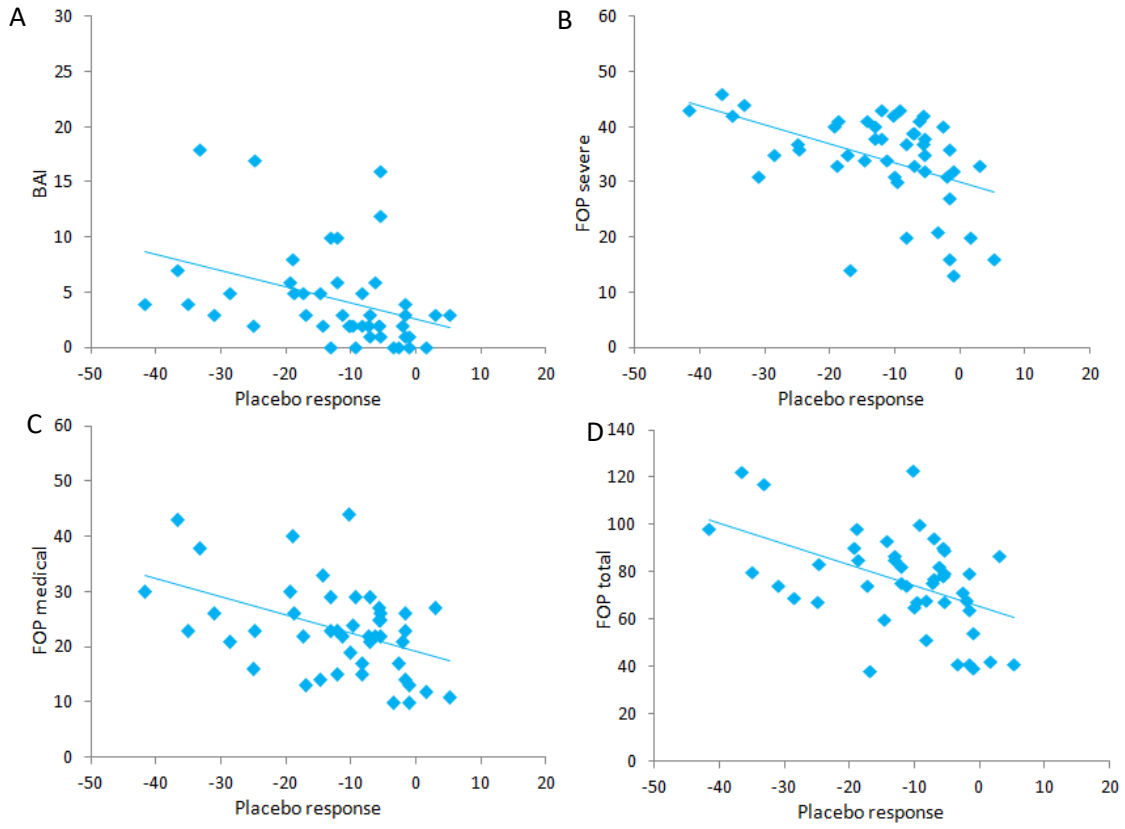
Similarly to placebo and nocebo VAS effects, expectations during the acquisition phase differed across the three conditions ($F_{(2,88)}$ = 515.152, p < 0.001), with average

expectation of high, medium and low upcoming pain equal to 75.63 ± 2.09, 34.74 ± 1.61 and 11.30 ± 0.98, respectively. Expectations were considered over time ($F_{(5,220)}$ = 3.392, p = 0.006) with a significant condition × time interaction ($F_{(10,440)}$ = 7.542, p < 0.001). During the testing phase expectations for high, medium and low pain [expectancy of high pain (70.61 ± 2.45), medium pain (33.87 ± 1.81) and low pain (9.54 ± 0.93] were staidly different across the three conditions ($F_{(2,88)}$ = 441.355, p < 0.001) with a main effect of time ($F_{(2,88)}$ = 8.092, p = 0.001), and a significant interaction condition × time ($F_{(4,176)}$ = 13.156, p < 0.001). Importantly, we found that positive expectations correlated with placebo responses (r = 0.412, p = 0.002) and similarly negative expectations correlated with nocebo effects (r = 0.351, p = 0.008).

Personality correlations and predictors

We then explored the effects of personality factors on placebo and nocebo effects.

Figure 16. Correlations between placebo response and personality traits

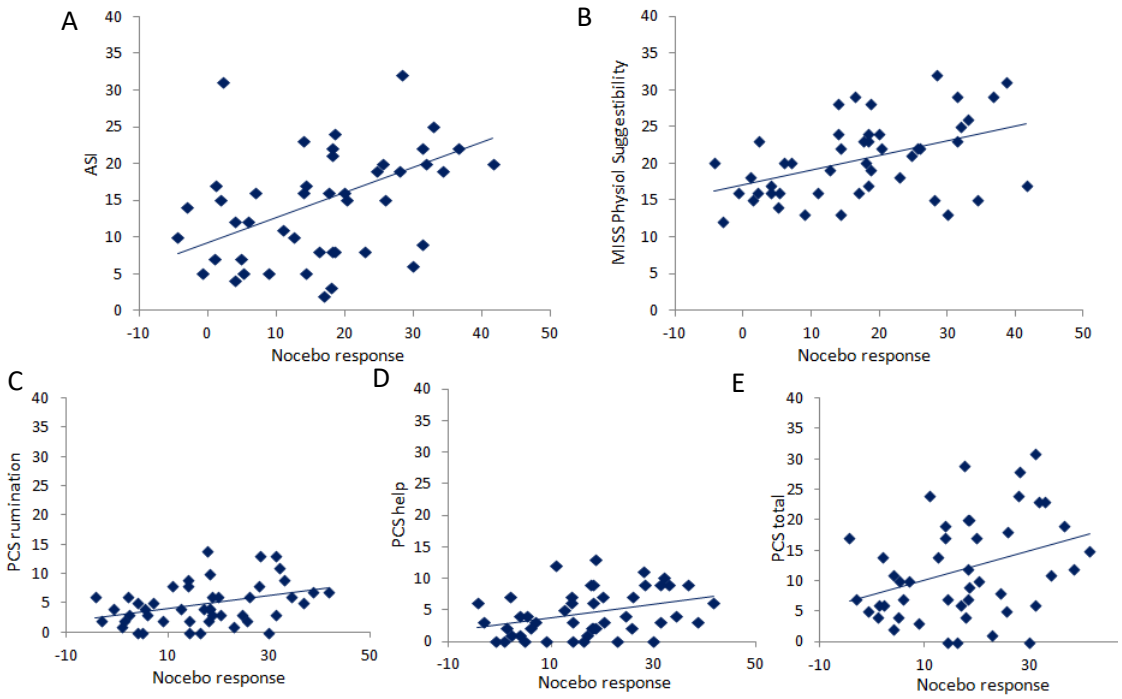


Placebo response negatively correlates with the level of anxiety and the fear of pain (FOP, severe, medical and total score). *Corsi & Colloca, 2017*

First, we ran a series of correlations analyses and found that placebo responses were negatively correlated with severity of anxiety (BAI: r = -0.485, p = 0.001), and fear of pain (FOP, severe: r = -0.490, p = 0.001, d=-3.336; medical fear, r = -0.416, p = 0.004, d=-2.584; total fear r = -0.435, p = 0.003, d=-3.924) (Fig.16A-D).

By the contrary, nocebo responses were positively correlated with anxiety sensitivity (ASI, r = 0.460, p = 0.001, d=0.137), physiological suggestibility (MISS: r = 0.438, p = 0.002, d=-0.278) with a trend for catastrophizing tendency (PCS rumination: r = 0.352, p = 0.016, d=1.118; PCS helplessness: r = 0.366, p = 0.012, d=1.146; PCS total: r = 0.343, p = 0.020, d=0.369) (Fig.17A-E).

Figure 17. Correlations between nocebo response and personality traits



Nocebo response positively correlates with the sensitivity to anxiety (ASI) and the physiological suggestibility (subscale of MISS). Additionally, nocebo response tends to correlate with different scales of the catastrophizing scale (rumination, helplessness, total score). *Corsi & Colloca, 2017*

Moreover, we considered the hypothesized psychological factors taken together in order to identify their relationship with the dependent variables (e.g. placebo and nocebo VAS) using stepwise multiple regression models.

The significant values are reported in Tables 2 and 3. Motivation (value/utility and pressure/tense subscales) and suggestibility (physiological reactivity and persuadability subscales) accounted for 51% of variance in placebo responses (Table 2).

Table 2. Stepwise multiple regression models for the prediction of placebo responses.

Dependent variable	Predictor Variables	R^2	β	t	p
Placebo hypoalgesia	*Model 1* MISS Physiol	21.6	 .464	 3.438	 .001
Placebo hypoalgesia	*Model 1* MISS Physiol *Model 2* MISS Physiol IMI Value	21.6 35.4	 .464 .577 -.389	 3.438 4.452 -2.999	 .001 <.001 .005
Placebo hypoalgesia	*Model 1* MISS Physiol *Model 2* MISS Physiol IMI Value *Model 3* MISS Physiol IMI Value MISS Persuadability	21.6 35.4 42.7	 .464 .577 -.389 .579 -.371 .270	 3.438 4.452 -2.999 4.687 -2.993 2.280	 .001 <.001 .005 <.001 .005 .028
Placebo hypoalgesia	*Model 1* MISS Physiol *Model 2* MISS Physiol IMI Value *Model 3* MISS Physiol IMI Value MISS Persuadability *Model 4* MISS Physiol IMI Value MISS Persuadability IMI Pressure	21.6 35.4 42.7 51.0	 .464 .577 -.389 .579 -.371 .270 .463 -.335 .344 .319	 3.438 4.452 -2.999 4.687 -2.993 2.280 3.745 -2.871 3.006 2.617	 .001 <.001 .005 <.001 .005 .028 .001 .007 .005 .012

Conversely, ASI, NEO-openness-extraversion and depression taken together accounted for 49.1% of variance in nocebo responses (Table 3).

Table 3. Stepwise multiple regression models for the prediction of nocebo responses.

Dependent variable	Predictor Variables	R^2	β	t	p
Nocebo hyperalgesia	*Model 1* ASI	20.3	 .451	 3.349	 .002
Nocebo hyperalgesia	*Model 1* ASI *Model 2* ASI NEO_O	20.3 33.3	 .451 .498 -.364	 3.349 3.966 -2.897	 .002 <.001 .006
Nocebo hyperalgesia	*Model 1* ASI *Model 2* ASI NEO Openness *Model 3* ASI NEO Openness NEO Extraversion	20.3 33.3 42.9	 .451 .498 -.364 .493 -.472 .329	 3.349 3.966 -2.897 4.197 -3.796 2.660	 .002 <.001 .006 <.001 <.001 .011
Nocebo hyperalgesia	*Model 1* ASI *Model 2* ASI NEO Openness *Model 3* ASI NEO Openness NEO Extraversion *Model 3* ASI NEO Openness NEO Extraversion BDI	20.3 33.3 42.9 49.1	 .451 .498 -.364 .493 -.472 .329 .448 -.413 .387 .267	 3.349 3.966 -2.897 4.197 -3.796 2.660 3.919 -3.388 3.197 2.218	 .002 <.001 .006 <.001 <.001 .011 <.001 .002 .003 .032

Discussion

In the current study, we investigated the influence of expectations and hypothesized psychological factors on placebo and nocebo effects elicited by a well-established model of conditioning and heat thermal painful stimulation. Placebo hypoalgesic responses were negatively correlated with severity of anxiety and fear of pain (e.g. medical fear, severe and total fear). On the contrary, nocebo hyperalgesic responses were positively correlated with anxiety sensitivity, suggestibility and catastrophizing (trend only). Moreover, a stepwise regression modeling showed that aggregate scores of motivation (value/utility and pressure/tense subscales) and suggestibility (physiological reactivity and persuadability subscales) accounted for the 51% of the variance in the placebo responses. By contrast, the aggregation of anxiety, openness, extraversion and depression accounted for the 49.1% of the variance in the nocebo responses. Importantly, expectations were highly correlated with placebo and nocebo effects and psychological factors did not influence level of expectations towards reduction or increase of pain.

Consistently with previous studies (Colloca & Benedetti, 2006, 2009; Colloca et al., 2010; Colloca, Sigaudo et al., 2008; Lui et al., 2010), we found that visual cues associated with prior experiences of low and high pain elicit strong placebo and nocebo effects with a distribution ranging from no responses to low modulation of pain, to medium and high reductions and increases. Studies on placebo hypoalgesia and nocebo hyperalgesia have shown a substantial inter-individual variability and distinct personality factors have been associated with placebo and nocebo effects (Colagiuri, Schenk et al., 2015; Colloca & Grillon, 2014). In our study, severity of anxiety as well as fear of pain (e.g. medical, sever and total fear) were linked to reduced placebo responsiveness to pain. Severity of anxiety including symptoms of depression, feelings of hopelessness and irritability, guiltiness or feelings of being punished, as well as physical symptoms such as fatigue, correlated negatively with placebo responses with higher severity of anxiety linked to lower reduction of pain induced by positive expectations. High levels of fear of pain referring to the dispositional tendency to have negative emotions towards pain and pain anticipation have been also associated with placebo- and nocebo-induced pain modulation (Aslaksen & Lyby, 2015; Lyby et al., 2010). We found that fear of medical pain correlates with low hypoalgesic responses and this is consistent with the parallel enhancement of nocebo induced by fear of pain and other medical procedures (Aslaksen & Lyby, 2015).

When we looked at the nocebo effect, we found a positive correlation with anxiety sensitivity, physiological suggestibility and catastrophizing.

The inclusion of an extensive battery of questionnaires related to personality factors allowed us to reveal that expectations may predict placebo and nocebo effects independently of personality factors making it an useful tool for health care providers.

Several studies have emphasized the need for exploring the impact of personality factors as at least one of the possible ways to interpret and understand the large variability in placebo hypoalgesic and nocebo hyperalgesic responses. To our knowledge, this is the first study that explores how distinct psychological factors can predict placebo hypolagesic responses and nocebo hyperalgesic responses, and the potential influence of personality factors in shaping positive and negative expectancies. Collectively, the complexity and variability in placebo- and nocebo-induced pain responses highlight a need to better understand the multidimensionality of pain and its modulation related to individual expectations and psychological factors. More specifically, since knowledge on the nocebo effect is still poor, additional studies might be performed to point out the different mechanisms (neurobiological, psychological, personological) that can account for the nocebo response. This approach would facilitate to develop strategies to identify nocebo-vulnerable pain patients in order to optimize the psychosocial and therapeutic context in which the clinical encounter occurs, with the ultimate purpose of improving clinical outcomes.

PART III : THE INTERPLAY BETWEEN PAIN PERCEPTION AND MOVEMENT EXECUTION

Study 4 : How motor-induced analgesia shapes placebo and nocebo effects in a heat model of human pain

While Part II of my PhD thesis was dedicated to the investigation of the main cognitive factors and personality traits that could modulate placebo and nocebo responses, in Part III, I aimed at investigating the potential overlap between placebo and nocebo effects in pain and in the motor domain. Within the last three decades, studies on placebo and nocebo effects have greatly expanded our knowledge about brain functioning and pain modulation.

Interestingly, researches have suggested that the motor system can produce an analgesic effect due to the connections of the motor system with the descending modulatory pain pathway areas. This relationship was clearly demonstrated by means of motor cortex (M1) stimulation, but the principle of its effect is still poorly understood.

Peyron and colleagues (2007) applied a 4-contact electrodes array on M1 in patients suffering by medically refractory neuropathic pain, in order to stimulate M1 and to study the focal cerebral blood flow changes in regions with high density of opioid receptors (Peyron, Faillenot, Mertens, Laurent, & Garcia-Larrea, 2007). Results showed that pain relief after the stimulation of M1 lasted several hours, and that there were local hemodynamic changes in cortical and subcortical areas that were functionally interrelated, such as anterior mid-cingulate cortex (aMCC), pregenual anterior cingulate cortex (pgACC), thalamus, periacqueductal gray (PAG), and striatum. To deepen this effect, other studies have been conducted on neuropathic pain patients. These studies converged in indicating the inhibitory effect of M1 stimulation at the thalamic and spinal level (Garcia-Larrea et al., 1999; Tsubokawa, Katayama, Yamamoto, Hirayama, & Koyama, 1991) and the increase of endogenous opioids secretion in the aMCC, prefrontal cortex (PFC) and cerebellum after the stimulation (Maarrawi et al., 2007). These areas contain a high density of opioids receptors and are involved in the cortical-subcortical network activated during opioid analgesia. An additional study conducted by Maarrawi

and colleagues (Maarrawi et al., 2007) investigated whether the distribution of endogenous opioids receptors predicts the magnitude of pain relief elicited by the stimulation of the M1. Positron emission tomography (PET) results showed that the higher the density of opioids receptors available in the thalamus, insula and PAG, the stronger the probability to benefit from the stimulation of the M1. However, the potential influence of the stimulation of the M1for pain relief is still underappreciated and knowledge is still missing on whether executing a movement could reduce painful perception. Moreover, we do not know yet whether the placebo effect in this context could have an additional analgesic value.

Therefore, we designed an experiment to investigate the relationship between pain modulation, expectancy and motor activity. We think that this approach can build up new knowledge on the relation between pain perception and defensive-like actions, as well as on the role of positive and negative expectancy in modulating motor-induced analgesic effects. To understand whether pain processing is affected by the execution of an active movement we delivered painful stimulations with or without concurrent movement execution. Moreover, since expectancy plays a critical role in modulating pain perception, we also studied the role of positive and negative expectations on motor-induced analgesia. We predict a hypoalgesic effect when the heat painful stimulation is delivered during the movement execution and an enhancement of hypoalgesia during the placebo procedure. Additionally, we predict that negative expectation of increased pain will reduce or reverse this analgesic movement-related effect.

Materials, methods and statistical analysis

Participants

We studied the effect of placebo hypoalgesia and nocebo hyperalgesia by adopting a well-validated model (Colloca & Benedetti, 2006, 2009; Colloca et al., 2010; Colloca, Pine, Ernst, Miller, & Grillon, 2016; Colloca, Sigaudo et al., 2008).

We recruited a total of 50 participants from the University of Maryland Baltimore area. Four participants were excluded either because they did not meet the inclusion criteria or because their unreliability with respect to the pain reports. Thus, a sample of 46 healthy participants (24 women) aged from 18 to 53 years (mean age 27.41 ± 1.07) was included

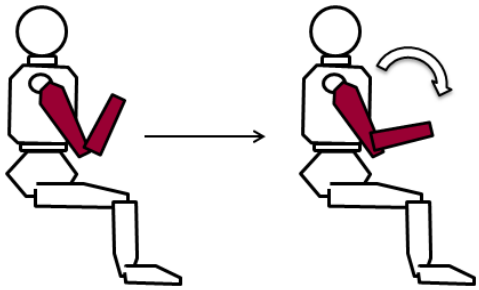
in this research. Potential participants were screened over the phone to determine the eligibility before scheduling the appointment. Those who met inclusionary but do not met exclusionary criteria were invited to participate and underwent an additional self-report checklist prior to the experiment implementation. We used a urine toxicology analysis to exclude any drug abuse. The drug screen included primary drugs or metabolites for marijuana, cocaine, opiates, amphetamine, methamphetamine, ecstasy, phencyclidine, hydrocodone, oxycodone and hydromorphone. Exclusion criteria included: cardiovascular, neurological diseases, pulmonary abnormalities, kidney and liver disease, history of cancer within past 3 years, history of chronic pain disorder, any psychiatric condition, lifetime alcohol/drug dependence, impaired hearing, pregnancy or breast-feeding, abnormal blood pressure values, nicotine smokers during the last six months, color-blindness and a history of surgery performed on the arm, shoulder, wrist or hand. Participants signed the informed consent and the Health Insurance Portability and Accountability Act (HIPAA) form as approved by the University of Maryland Baltimore Institutional Review Board (IRB, Prot # HP00065783). In the consent form participants were informed that we were interested in exploring how feeling pain can change body movements, and the whole procedure was disclosed. Additionally, it explained that the participation in the study was entirely voluntary and even if they initially consented to participate, they could withdraw at any time. The potential risks related to the painful stimulation, movement execution, breach of privacy and confidentiality were outlined. Since we used elements of deception, participants were debriefed at the end of their study participation and they completed a written exit form which provided them detailed information and the opportunity to withdraw their data from the study. None of the participants chose to withdraw their data from the study. Participants were monetarily compensated for their time (90$).

Force assessment and movement

The maximum voluntary force (MVF) for each participant was assessed by performing four isometric movements with the dominant arm, using the Biodex 4 Pro machine (Biodex Medical System, Shirley, New York, USA). This is a sophisticated and reliable machine that allowed us to assess and control participants' force during the experiment. Participants were comfortably seated on a chair with the upper part of the body stabilized

with belts across the shoulders. Both dynamometer and seat orientation were set at 90°. For each participant, we set the height of the dynamometer in order to have the center of rotation matching the participant's elbow position. The arm was fixed with an additional strap in order to help participants to perform correctly the movement. The MVF was measured using an isometric protocol characterized by 1 repetition for 4 positions (e.g. 15°, 30°, 45°, 60°). Each repetition required 5 seconds of contraction time followed by 10 seconds rest. At the end of this assessment we computed the mean of the MVF recorded for each position and we calculated the 30% of the MVF. This value allowed us to calibrate the subsequent extension-flexion motor task in order to prevent fatigue (Fig.18).

Figure 18. Motor task



Participants were asked to performed extension-flexion movements of the dominant arm with the aid of the Biodex machine.

During the experimental procedure, we created isotonic protocols linked together. We controlled for the level of force (by using the 30% of MVF) and speed (by setting the velocity equal to 60°/sec for all the participants), thus standardizing the task for all the participants. The range of motion that defined the amplitude of the movement was set at 80° for both the assessment and the experimental phases.

Pain assessment and stimulation

Before starting the experiment, pain threshold and pain tolerance were measured using the procedure described in Study 3.

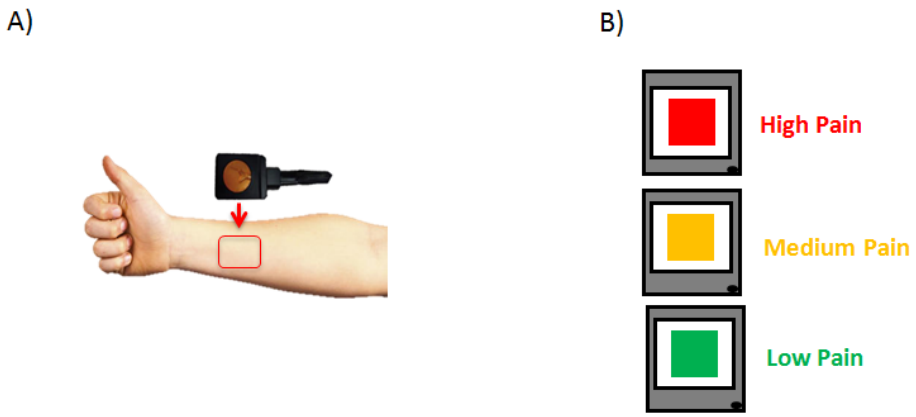
Procedure

Before the force and pain assessment, we measured weight, height, body mass index (BMI) and blood pressure. The experimental procedure consisted of three phases: baseline, acquisition and testing.

The baseline consisted of 10 trials: 5 in which participants completed a motor movement and 5 in which they were at rest. During all the baseline trials, the participants received a medium level of painful stimulation to the volar forearm and it served as a control phase. Before the acquisition phase began, participants were told that a red cue would have informed them about the delivery of a moderately high level of painful stimulation, a yellow cue about a medium level of pain and a green cue about a low painful stimulation (Fig.19 A,B). In this phase, participants received the three levels of pain while performing the arm extension and flexion movement during half of the trials and at rest in the other half (control trials).

Before each stimulation, participants rated their expectancy of the upcoming pain stimulus on a visual analogue scale (VAS), ranging from 0 (no pain) to 100 (maximum tolerable pain). After each painful stimulus was delivered, participants rated their experienced pain using a VAS scale, from 0 (no pain) to 100 (maximum tolerable).

Figure 19. Pain stimulation and visual color cues

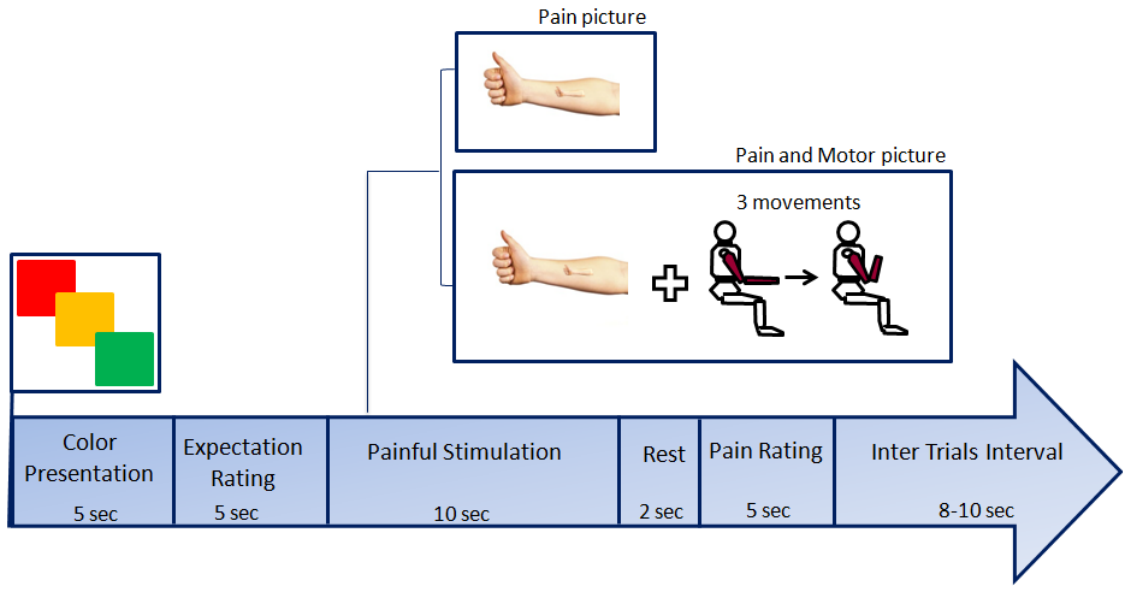


A) Thermal stimulation. The probe was applied on the participant's dominant volar forearm and remained stable during all the experiment. **B) Visual color cues.** Participant saw three different colors (red, yellow, green) on a computer screen and they were told that these colors would have informed them about the delivery of three distinct levels of pain (high, medium, and low, respectively).

Each visual cue (red, yellow and green) was presented 12 times, for a total of 36 trials divided in two blocks of 18 trials each. One half of the painful stimulations were given with repetitions of elbow extension and flexion movements set at 30% of participants' MVC. The remaining painful stimulations were given at rest.

Afterwards, there was the testing phase, in which participants were presented each visual cue (red, yellow and green) 6 times, for a total amount of 18 painful stimulations. Nine painful trials were intermixed with three elbow extension-flexion movements set at 30% of the MVF. However, pain intensity was surreptitiously changed to the medium level in all the cues (as in the baseline). Namely, all thermal stimulations were delivered at a medium intensity regardless of the color cue presented. This procedure allowed us to evaluate any change in pain perception due to subjects' expectation according to the type of cue (and independent of the physical stimulation that was the same in all the trials). Participants continued to give ratings of their expected and experienced pain on the VAS scales. Two different pictures were displayed during the experiment and inform the participant whether to perform the movement or to rest (Fig.20).

Figure 20. Timeline of the experimental trial



Soon after the color presentation, participants were asked to rate their level of expectation about the upcoming stimulus. Then the painful stimulation followed for 10 seconds (at rest or during the extension-flexion movement). After 2 seconds of rest, participants rated their level of perceived pain. Finally, an inter-trials-interval followed with variable timing to avoid abituation.

Moreover, at the end of each block, participants rated their level of fatigue on a Borg scale (Borg, 1970). In order to maintain constant attention throughout the experimental procedure, participants were asked to count the number of visual stimuli presented during the acquisition and testing phase trials.

Data Analysis

Repeated measure analysis of variance was performed on both VAS expectation score and VAS pain score considering colors (red, yellow, green) and time as within-subject factors for each phase (either acquisition or testing) and each condition (either Pain or PainMovement). Under Pain, we mean a condition in which painful stimulation was delivered at rest; under PainMovement we mean a condition in which painful stimulation was delivered during movement. Additional analyses were performed by adding the conditions (Pain, PainMovement) in the general linear model, thus obtaining three within-subject factors (i.e. colors, time, conditions). Moreover, to test for the main effect of sex on placebo and nocebo effects, this variable was successively included in the model as between subject factor.

F-tests were followed by the Bonferroni *post-hoc* tests for multiple comparisons. T-tests for paired samples were carried out to compare the mean values of the conditions during all the phases (baseline, acquisition, testing). All the analyses were performed using the SPSS software package (SSPS Inc., Chicago, Illinois, USA, vers.21). The level of significance was set at $p < 0.050$.

Results

We recorded different participants' characteristics. On average, the weight was equal to 168.23 ± 5.14 Libras and the height was equal to 5'25.21 ± 7.64 inches, thus providing a Body Mass Index of 26.00 ± 0.71. The general blood pressure was within the guidelines provided by the American Heart Association (Systolic: 120.19 ± 2.00; Diastolic: 75.15 ± 1.27; BPM: 66.36 ± 1.45).

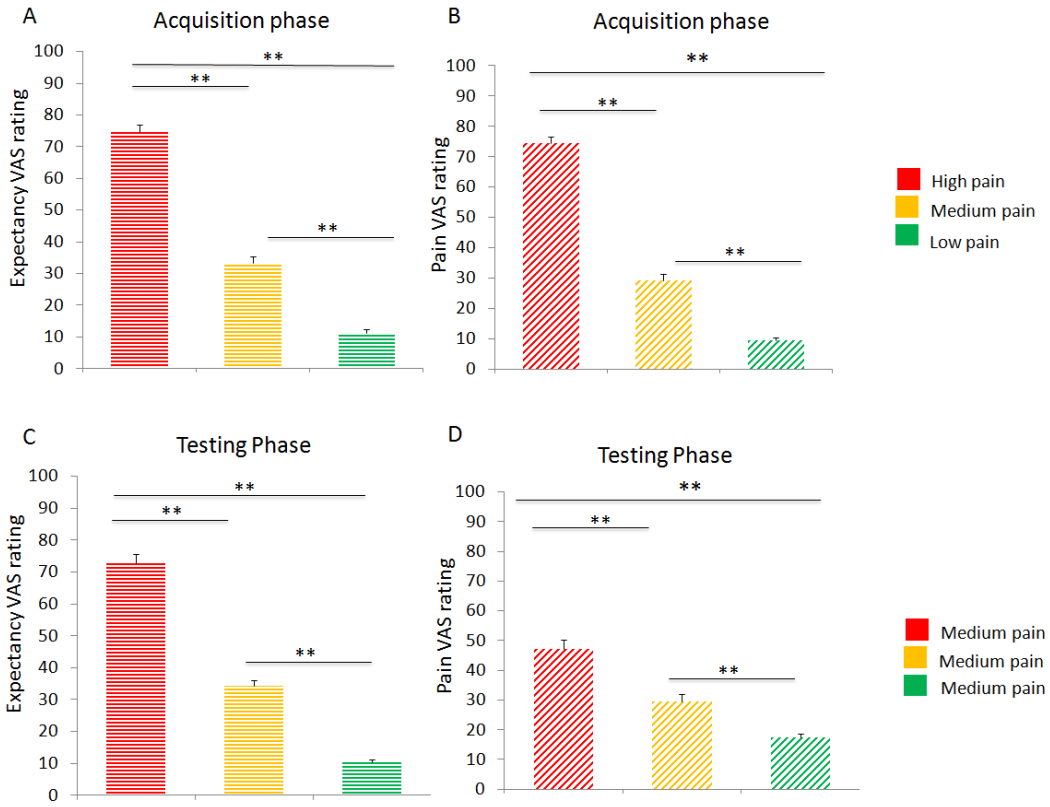
During the acquisition phase, the average of intensity of pain stimulation was equal to 47.52 °C for red-associated stimuli, equal to 44.55 °C for yellow, and equal to 41.51 °C for green. As anticipated above, during the testing phase, the intensity of painful

stimulation was set to the medium level of pain (average of 44.55 °C) for all the three visual colored cues. All the temperatures are considered as out of 50 °C.

Pain condition

The analysis showed that during the acquisition phase, participants expected to receive three different levels of pain, accordingly to the presented color cues (main effect of color: $F_{(2,90)} = 517.992$, $p < 0.001$). The factor time was significant ($F_{(5,225)} = 3.493$, $p = 0.005$), as well as the color × time interaction ($F_{(10,450)} = 7.610$, $p < 0.001$) (Fig.21A).

Figure 21. Averages of expectation and pain scores rated by participants



Before starting the acquisition phase, participants expected to receive three different levels of pain, accordingly to the previous verbal information **(A)**. Moreover, they clearly perceived three different levels of pain during the stimulations **(B)**, meaning that our conditioning procedure was successful. We found the same pattern also during the testing phase that is when we delivered the same medium level of pain for all the three conditions. Participants not only continued to expect the three levels of pain **(C)**, but also perceived them **(D)**. ** $p < 0.001$

Moreover, during this phase participants clearly learned to distinguish the low, medium and high levels of painful stimuli (main effect of color: $F_{(2,90)} = 496.205$, $p < 0.001$). The factor time was significant ($F_{(5,225)} = 7.511$, $p < 0.001$) as well as the color × time interaction ($F_{(10,450)} = 5.484$, $p < 0.001$). Post-hoc Bonferroni tests indicated that the red stimuli (average VAS: 46.99 ± 2.43) were perceived as higher than the yellow control stimuli (average VAS: 29.91 ± 1.76) ($p<0.001$) and green (average VAS: 17.88 ± 1.67) were rated as lower than the yellow stimuli ($p< 0.001$) indicating both robust placebo and nocebo effects (Fig.21B).

During the testing phase, when the level of pain was set at the same medium (yellow) intensity, repeated measure ANOVA on Expectation revealed a significant effect of color ($F_{(2,90)} = 449.294$, $p < 0.001$), time ($F_{(2,90)} = 8.102$, $p = 0.001$) and color × time interaction ($F_{(4,180)} = 13.336$, $p < 0.001$)(Fig.21C). Repeated measure ANOVA on Pain ratings revealed a significant effect of color ($F_{(2,90)} = 97.467$, $p < 0.001$) and time ($F_{(2,90)} = 7.774$, $p = 0.001$). However the color × time interaction was not significant ($F_{(4,180)} = 0.391$, $p = 0.815$) (Fig.21D).

PainMovement condition

As described for the previous condition, in the acquisition phase participants expected to receive three different levels of pain (main effect of color: $F_{(2,90)} = 601.958$, $p < 0.001$). The factor time was significant ($F_{(5,225)} = 2.413$, $p = 0.037$) as well as the color × time interaction ($F_{(10,450)} = 10.604$, $p < 0.001$). Moreover, also during the testing phase participants distinguished the low, medium and high levels of painful stimuli (main effect of color: $F_{(2,90)} = 335.788$, $p < 0.001$). The factor time was not significant ($F_{(5,225)} = 0.896$, $p = 0.484$). The color × time interaction was significant ($F_{(10,450)} = 6.494$, $p < 0.001$). Post-hoc Bonferroni tests indicated that the red stimuli (average VAS: 39.79 ± 2.66) were perceived as higher than the yellow control stimuli (average VAS: 23.97 ± 1.85) ($p<0.001$) and green (average VAS: 13.02 ± 1.35) were rated as lower than the yellow stimuli ($p < 0.001$) indicating both robust placebo and nocebo effects.

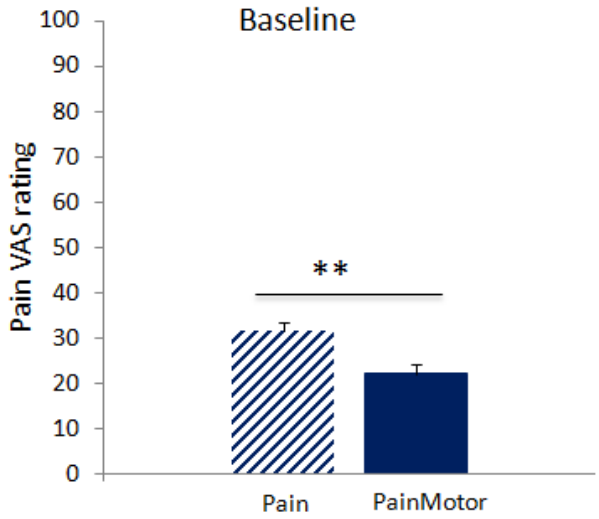
During the testing phase, when we delivered the same medium level of pain, repeated measure ANOVA on Expectation revealed a significant effect of color ($F_{(2,90)} = 325.191$, $p < 0.001$). Time factor was not significant ($F_{(2,90)} = 2.066$, $p = 0133$). The color × time interaction was significant ($F_{(4,180)} = 14.039$, $p < 0.001$). Repeated measure ANOVA on

Pain ratings revealed a significant effect of color ($F_{(2,90)}$ = 88.653, p < 0.001). Time was not significant ($F_{(2,90)}$ = 14.91, p = 0.231). However the color × time interaction was significant ($F_{(4,180)}$ = 4.136, p = 0.003). Post-hoc Bonferroni tests indicated that the red stimuli (average VAS: 46.98 ± 2.46) were perceived as higher than the yellow control stimuli (average VAS: 29.96 ± 1.78) (p<0.001) and green (average VAS: 17.86 ± 1.70) were rated as lower than the yellow stimuli (< 0.001) indicating both robust placebo and nocebo effects.

Pain vs. PainMovement condition

Results showed significant differences at baseline between Pain and PainMovement conditions ($t_{(45)}$ = 7.328, p < 0.001), due to higher pain levels rated in the Pain condition (31.54 ± 2.05) compared to PainMovement condition (22.05 ± 2.11) (Fig.22).

Figure 22. Averages of pain scores rated by participants during the Pain and PainMovement conditions.



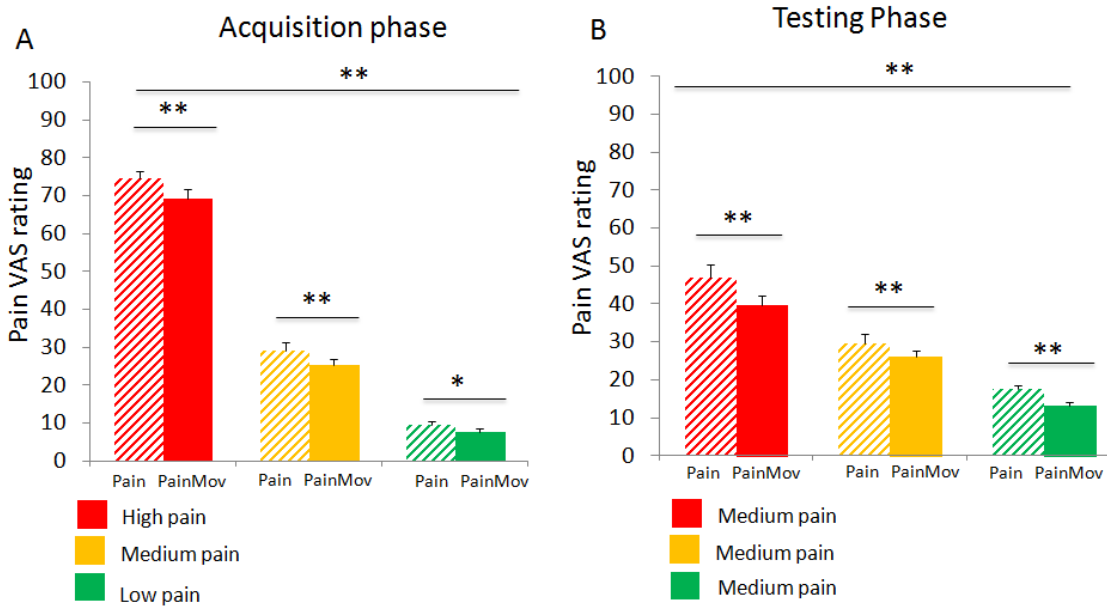
Participants perceived as less painful the heat thermal stimulation delivered while performing the movement (PainMov) compared to the condition at rest (Pain) ** p < 0.001

By including Pain and PainMovement conditions as within subject factor along with color ad time, our analysis on Expectation showed a significant effect of the following factors: color ($F_{(2,90)}$ = 600.953, p < 0.001), condition ($F_{(1,45)}$ = 6.961, p = 0.011), and time ($F_{(5,225)}$ = 2.318, p = 0.044). We found significant differences for color × condition ($F_{(2,90)}$ =

21.842, $p < 0.001$), color × time ($F_{(10,450)} = 11.051$, $p < 0.001$), and condition × time ($F_{(5,225)} = 4.186$, $p = 0.001$) interactions.

Repeated measure ANOVA on Pain ratings revealed a significant effect of color ($F_{(2,90)} = 446.750$, $p < 0.001$), condition ($F_{(1,45)} = 24.768$, $p < 0.001$), and time ($F_{(5,225)} = 3.154$, $p = 0.009$). Additional significant differences were found for color × condition ($F_{(2,90)} = 3.411$, $p = 0.037$), color × time ($F_{(10,450)} = 6.342$, $p < 0.001$), and condition × time ($F_{(5,225)} = 7.902$, $p < 0.001$) interactions. Post-hoc Bonferroni tests indicated that the stimulations delivered during the movement execution (PainMovement condition) were perceived as less painful compared to the stimulations received at rest (Pain condition) (red: $p< 0.001$; yellow: $p< 0.001$; green: $p = 0.016$) (Fig.23A).

Figure 23. Averages of pain scores rated by participants at rest (Pain) and during the movement (PainMovement).



Pain scores rated by participants while receiving the painful stimulation either at rest (Pain condition) or during the extension-flexion movements (PainMovement condition) in the acquisition **(A)** and testing **(B)** phase. During the movement execution, participants perceived less pain compare to the perceived stimulation at rest. We obtained this result not only during the acquisition phase (when three different levels of pain were delivered) but also during the testing phase that is when we delivered the same medium level of pain for all the three visual color cues.

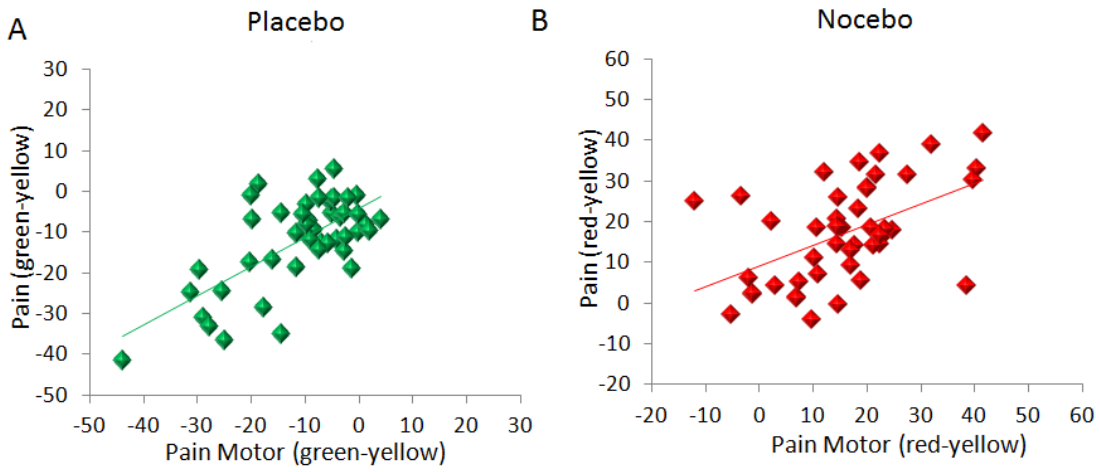
** $p < 0.001$; * $p = 0.016$

During the testing phase, when the level of pain was set at the same medium (yellow) intensity, repeated measure ANOVA on Expectation revealed a significant effect of color ($F_{(2,90)}$ = 436.830, p < 0.001), time ($F_{(2,90)}$ = 7.991, p = 0.001), color × time interaction ($F_{(4,180)}$ = 18.130, p < 0.001) and condition × time interaction ($F_{(2,90)}$ = 4.057, p = 0.021). Repeated measure ANOVA on Pain ratings showed a significant effect of color ($F_{(2,90)}$ = 109.842, p < 0.001), condition ($F_{(1,45)}$ = 33.703, p < 0.001), and time ($F_{(2,90)}$ = 7.383, p = 0.001). Additionally, a tendency to significance for color × time interaction ($F_{(4,180)}$ = 2.322, p = 0.058) was outlined. Post-hoc Bonferroni tests indicated that the stimulations delivered during the movement execution (PainMovement condition) were perceived as less painful compared to the stimulations received at rest (Pain condition) (all p < 0.001) (Fig.23B).

Correlations

Placebo responses during Pain condition were significantly correlated with the hypoalgesic effect experienced during the movement execution (PainMovement condition; r = 0.454, p = 0.002), as well as nocebo responses evoked during Pain condition were significantly correlated with the hyperalgesic effect experienced during PainMovement condition (r = 0.494, p < 0.001) (Fig.24 A,B).

Figure 24. Correlations between Pain condition and PainMovement condition.

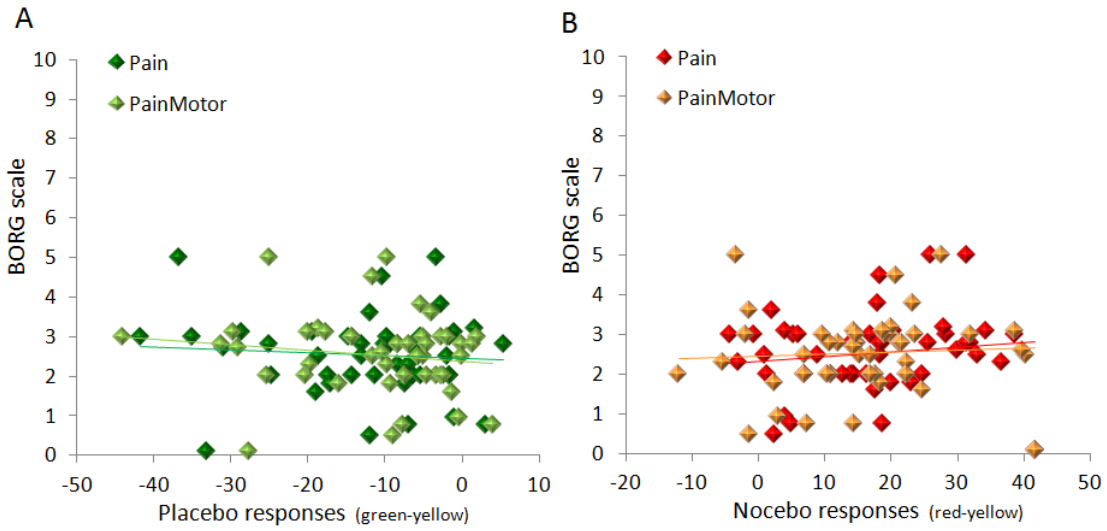


The magnitude of placebo responses obtained during Pain condition was positively correlated with the effect experienced during the PainMovement condition **(A)**. We obtained the same patters for the nocebo response **(B)**, meaning that a participant that positively respond to a placebo/nocebo paradigm at rest, is

more likely prone to experience a stronger hypoalgesic/hyperalgesic effect (respectively) during a movement execution.

However, experiencing a placebo response does not suggest to experience also a nocebo response, as showed by the non-significant correlation between placebo and the nocebo responses for both conditions (Pain: r = -0.113 p = 0.454; PainMovement: r = -0.113 p = 0.454). Since we wanted to control for the level of fatigue, we correlated the placebo and nocebo responses with the average of the Borg scale. By considering both conditions (Pain and PainMovement), we did not find any correlation between the level of perceived fatigue (Borg scale) and the placebo (Pain: r = 0.038, p = 0.803; PainMovement: r = 0.175, p = 0.246) and nocebo (Pain: r = 0.128, p = 0.395; PainMovement: r = 0.156, p = 0.299) responses (Fig. 25 A,B).

Figure 25. Correlations between the level of perceived fatigue and placebo/nocebo response



The level of perceived fatigue did not correlate with the placebo **(A)** and nocebo **(B)** responses obtained by subtracting the yellow pain-related score from the green and the red pain score (respectively) during the testing phase.

Discussion

In the current study, we replicated the classical placebo and nocebo effects by demonstrating a reduction and an increase of pain perception, respectively, after a conditioning phase. Our procedure was successful in obtaining these responses and in

confirming previous studies (Colloca & Benedetti, 2006, 2009; Colloca et al., 2010; Colloca et al., 2016; Colloca, Sigaudo et al., 2008). Our results showed a modulation of pain perception during the testing phase consistent with the acquisition phase. Namely, during the testing phase participants perceived a high level of pain after the red cue, a medium level of pain after the yellow cue, and a low level of pain after the green cue. It is worth noting that during the testing phase we always delivered a medium level of pain, independently of the color cue. Hence, this result suggests that the expectation induced by the cue influenced pain perception.

Additionally, we designed this experiment to unravel the relation between pain perception and active motor defensive-like actions, and we first expected a hypoalgesic effect when the heat painful stimulation was delivered during the movement execution. Our results confirmed this hypothesis. In the baseline session, that is when the painful stimulations were delivered at a medium level of intensity without any visual color cue, participants perceived less pain while performing the movement (PainMovement condition) than at rest (Pain condition). Moreover, the modulation of pain perception during movement execution with respect to rest was present also in the other sessions (acquisition, testing) and for each color cue (red, yellow, green), suggesting that is independent of the stimulation (high, medium, low). More interestingly, during the testing phase this modulation resulted in an attenuation of the nocebo response and in an enhancement of the placebo response.

There would be several factors that can account for these results. An interesting model suggests that sensory perception and experience change in relation to movement execution (Timm, SanMiguel, Keil, Schroger, & Schonwiesner, 2014). Namely, whenever we perform an action, copies of our motor commands are dispatched as corollary discharges to sensory structures that predict the sensory consequences of the actions thanks to a forward model (Tsakiris & Haggard, 2005). Then, a comparator model compares the predicted and received sensory feedback and in case of a match, the real sensory perception will be attenuated (i.e. sensory attenuation). This model explains why self-generated stimulations are usually perceived as less intense and elicit reduced neural responses compared to externally generated sensory effects (Blakemore, Goodbody, & Wolpert, 1998; Blakemore, Wolpert, & Frith, 1998). This phenomenon is thought to be related to motor-based sensory prediction (Waszak, Cardoso-Leite, & Hughes, 2012). We

may speculate that sensory attenuation is the cause of the hypoalgesic effect found in our study. However, it could be argued that movement execution triggered additional cognitive processes, like attention on the moving arm or on the quality of the movement that could have interfered with pain perception, thus reducing it. Hence, whether our results are best explained by the sensory attenuation model or by other factors should be proved with further studies. For instance, it could be useful to perform a study in which the painful stimulations are delivered not on the moving but on the controlateral arm. In this way, participant would receive the painful stimulation on the dominant arm, while performing the movement with the non-dominant arm, or vice versa. If we still find a decrease of pain sensation, it means that this reduction could be induced by cognitive factors, like attention. If we will not find any reduction of pain, this would support our hypothesis of sensory attenuation.

Although we acknowledged that this is an exploratory study that requires further developments, we also think that being based on a within-subject design, the results are reliable. Namely, every single participant underwent all the conditions, including the control condition (i.e., pain perception without motor execution). The fact that participants serve as their own control provides a way of reducing the amount of error arising from the natural variance between individuals.

As mention above, further studies need to unravel the nature of this motor-induced hypoalgesia. Moreover, it would be interesting to investigate also the neurophysiological underpinnings of the motor-induced analgesia involving specific techniques, such as the transcranial magnetic stimulation. As a first step toward this aim, it would be interesting to unravel the neurophysiological mechanisms of the nocebo effect, to study if the nocebo can be consider as the negative counterpart of the placebo effect also from a neurophysiological point of view.

PART IV: THE NEUROPHYSIOLOGY OF THE NOCEBO EFFECT IN MOTOR PERFORMANCE

Study 5: Modulation of inhibitory corticospinal circuits induced by a nocebo procedure in motor performance

In the previous part, we have demonstrated that the motor system can interact with pain perception in modulating placebo and nocebo effects. However, when considering the motor domain alone, knowledge is still incomplete on how placebo and nocebo effects work. In this regard, the investigation of the neurophysiological correlates of the placebo effect in motor performance is very recent. Fiorio and colleagues (2014) demonstrated not only an increasing of force, but also an enhancement of corticospinal excitability characterized by an increase of the motor evoked potential (MEP) amplitude and a decrease of the cortical silent period duration (CSP), highlighting not only a cortical but also a corticospinal activation during this procedure (Fiorio, Emadi Andani, Marotta, Classen, & Tinazzi, 2014). However, neurophysiological underpinnings of the nocebo effect are not yet clear.

The aim of the present study is to uncover the behavioral and neurophysiological correlates of the nocebo effect in motor performance. To this end, we took advantage from a previously developed paradigm (Fiorio et al., 2014), adequately adapted to induce nocebo effects on force production, and applied transcranial magnetic stimulation to measure MEP and CSP.

From a behavioral point of view, it is reasonable to expect a nocebo-induced reduction of force. However, the prediction is less obvious for the neurophysiological correlates. If nocebo effects in the motor system are associated to the behavioral outcome, we should expect opposite results to those previously described for placebo (Fiorio et al., 2014), i.e., a decrease of corticospinal excitability. However, differently from MEP amplitude, CSP duration is not necessarily related to changes in the amount of force (Cantello, Gianelli, Civardi, & Mutani, 1992; Inghilleri, Berardelli, Cruccu, & Manfredi, 1993; Saisanen et al., 2008) and therefore it could be modulated even independently from the behavioral outcome.

Materials, methods and statistical analysis

Participants

Thirty-two healthy volunteers were included in the study and were randomly divided into two groups: 17 subjects (all but two right-handed) entered the experimental group (7 women and 10 men; mean age, 23.3 ± 2.9 years old) and 15 subjects (all but two right-handed) entered the control group (9 women and 6 men; mean age, 22.1 ± 2.2 years old). The two groups did not statistically differ for age (independent samples t-test, $t_{(30)}$ = 1.347; p = 0.188), and were comparable for gender distribution (Chi-square test, χ^2 = 1.129, df = 1, p = 0.288). Before starting the study, all the subjects received an information sheet in which the experimental procedure and the TMS technique were explained in detail. Since we were interested in condition them, the real purposes of the study were explained only at the end of the whole experimental procedure. After having read the information sheet, subjects signed a written informed consent form in which they also declared to have no history of neurological, psychiatric, or other medical problems. The study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the ethical committee of the Department of Neurological and Movement Sciences, University of Verona, Italy (approval number 47921).

Motor task

The motor task has been previously described in Study 1. However, to better induce a nocebo effect, we modified the range delimited by the target zone. Namely, the color lines displayed on the monitor represented the 60%, 80%, 100% and 120% of the subject's MVC.

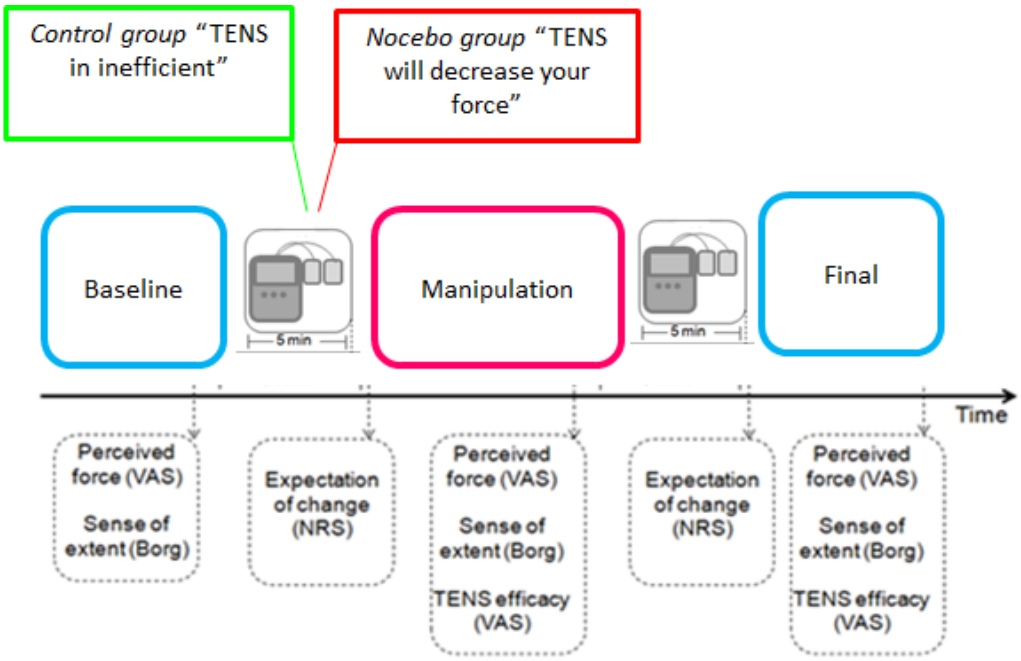
Procedure

The protocol included three sessions: baseline, manipulation and final (Fig.26). The baseline and final sessions were identical in both experimental and control groups, and consisted in the execution of the motor task previously described (Study 1).

These two sessions allowed comparing subjects' performance before and after the nocebo manipulation. Nocebo effects were obtained by applying an inert treatment (10 Hz transcutaneous electrical nerve stimulation, TENS) for 5 minutes over the region of the FDI belly. The intensity of TENS was adjusted until the subject reported a slight sensation

without muscle contraction. Subjects were also asked to report whether TENS was painful or uncomfortable. None of the participants reported these sensations. Participants of the experimental group were told that TENS would have reduced the recruitment of muscle fibers, thus resulting in a decrease of force production.

Figure 26. Timeline of the experimental protocol.



The study consisted of three sessions (baseline, manipulation and final). The two groups of participants (control and experimental) received different verbal information about the effects of TENS. Moreover, in the manipulation session the two groups underwent different procedures: the experimental group performed the motor task with a surreptitious reduction of the cursor's excursion range (conditioning procedure), while the control group performed the motor task without any reduction. *Adapted from Emadi-Andani et al, 2015.*

Because of the cutaneous sensation perceived by the subjects over the region of the hand muscle involved in the task, TENS can be expected to manipulate the subject's belief of worse motor performance. To reinforce the subjects' belief about the effects of TENS, the experimental group underwent a conditioning phase. During the manipulation session, a surreptitious reduction of the cursor's excursion range was introduced stepwise. More precisely, after TENS, the motor task was executed again, but during this session an attenuation coefficient was surreptitiously introduced, and the cursor's excursion range was gradually decreased in steps of 0.0029 from trial 1 to trial 35 and remained stable

until the end of the session (from trial 36 to trial 50). Consequently, by applying the same amount of force as in the baseline, the participants of the experimental group saw the cursor achieving lower lines of the target zone than before, and therefore believed to be weaker because of TENS. Before starting the final session, TENS was applied again together with verbal suggestion of worse motor performance. Subjects then repeated the motor task (50 trials), but this time without any manipulation, that is the same as in the baseline session. The same motor task was performed by the subjects of the control group, who also underwent the TENS application as described above, but with different verbal information. In particular, these subjects were clearly told that they have been assigned to a control group in which TENS was completely inert in modulating force. They executed the motor task three times, like the experimental group, but without reduction of the cursor's displacements in the manipulation session.

For each subject, the whole experiment took about 1.5 hours to be completed. Participants were tested at different times during the day, starting from 9.00am to 5.00pm. In the experimental group, 9 subjects were tested in the morning (9.00am-12.00am) and 8 subjects were tested in the afternoon (1.00pm-5.00pm). In the control group, 9 subjects were tested in the morning (9.00am-12.00am) and 6 subjects were tested in the afternoon (1.00pm-5.00pm). By analyzing the distribution of the subjects tested in the morning and in the afternoon, we found no differences between the two groups (Chi-square test, χ^2 = 0.161, df = 1, p = 0.688).

Behavioral and subjective data

We took advantage of the indices previously described in Study 1. Namely, to evaluate the behavioral response we recorded the Normalized $Force_{peak}$ and the $Strong_{press}$. Subjective evaluations involved Expectation, Judgment of TENS efficacy, Perception of force, and Sense of effort.

TMS task

The neurophysiological investigation was carried out in an additional task, called TMS-task, specifically devised to exclude all the possible bottom-up confounding factors that could influence corticospinal excitability, such as the actual force level, joint velocity and background electromyographic activity. This task was executed by all the subjects soon

after the main motor task and consisted in a red line visible on the PC monitor that represented the 30% of the subject's MVF. The subjects were asked to keep the cursor stable on the red line until its color changed from yellow to green (30 ± 1% MVF). When the cursor was stable for at least 500 ms, the software automatically triggered the TMS pulse, which was delivered on the FDI optimal scalp position at 100% of the resting motor threshold (rMT). For each subject, the intensity of the stimulation remained stable across the sessions, thus ruling out any effect of TMS intensity on the changes of MEP amplitude and CSP duration. The TMS-task consisted of 16 trials, each one lasting 5000 ms. If the subject was not able to maintain the cursor stable on the red line, the TMS pulse was not delivered and the trial was repeated again. Hence, the task was repeated until 16 TMS pulses were obtained. In order to avoid adaptation, the TMS pulse could be triggered randomly between 500 ms and 1300 ms after the cursor had become green. In each trial, electromyographic (EMG) activity was recorded 100 ms before and 1000 ms after the TMS pulse. A 10-seconds interval was inserted between the trials. The TMS task was performed about 7-8 minutes after the application of TENS.

TMS stimulation and EMG recording

Surface EMG was recorded from the motor point of the FDI and abductor digiti minimi (ADM) muscles of the right hand with bipolar self-adhesive Ag-AgCl electrodes (1.5×2.5 cm) in a belly-tendon montage. The ground electrode was attached to the wrist. EMG signals were band-pass filtered (20 Hz-2.5 kHz; plus 50-Hz notch) (D360, Digitimer, Welwyn Garden City, UK), amplified at a gain of 1000 (Digitimer, Hertfordshire, England), digitized at 5 kHz with laboratory interface (Cambridge Electronic Design 1401, Cambridge, England) controlled by Spike 2 (version 6, Cambridge Electronic Design) and then analyzed off-line.

A figure-of-eight coil (outer diameter of each wing 110 mm) was used to apply a biphasic single TMS pulse (STM 9000 magnetic stimulator, Ates-EBNeuro, Italy). The coil was mounted on an articulated arm and positioned tangentially to the skull at an angle of 45° to the sagittal plane (Brasil-Neto et al., 1992; Mills, Boniface, & Schubert, 1992). The FDI optimal scalp position was identified by moving the coil in small steps laterally to vertex in the left hemisphere and by delivering TMS pulses with constant intensity until stable and maximal MEPs could be evoked in the relaxed FDI muscle. The rMT was

defined as the lowest stimulus intensity able to evoke MEPs with an amplitude of at least 50 μV in at least five out of ten trials in the FDI muscle.

Peak-to-peak MEP amplitude was recorded from the two muscles (FDI_{amp} and ADM_{amp}) and the duration of the cortical silent period (CSP) was recorded from the active muscle (FDI_{csp}). The CSP duration was calculated from the onset of the TMS trigger pulse and the moment in which the rectified EMG activity, averaged over a 10 ms period, had returned to 50% of pre-stimulus values (Butler, Petersen, Herbert, Gandevia, & Taylor, 2012).

Data analysis

Before calculating the mean value of $Force_{peak}$ in each session for each subject, we removed the trials in which volunteers did not press the piston after having pressed the mouse key (2.13% for the experimental group and 1.35% for the control group).

Analyses were performed using SPSS Statistics 21 software (SPSS Inc). Repeated measures analyses of variance (rmANOVAs) were carried out to assess the effects of group (between-subject factor, 2 levels: experimental vs. control) and sessions (within-subject factor, 3 levels: baseline, manipulation, final) with regards to the behavioral parameters (normalized $Force_{peak}$, $Strong_{press}$), subjective parameters (perception of force and sense of effort) and neurophysiological parameters (MEP, CSP and background EMG). Independent samples t-test was applied to compare across groups the level of expectation (NRS scores), the judgments about the effects of TENS (VAS scores). In all the analyses, post-hoc comparisons were executed by means of 2-tailed t-tests for paired or independent samples, using the Bonferroni correction for multiple comparisons where necessary. The level of significance was set at $p < 0.05$. All data are expressed as mean ± SE.

Results

MVF as measured in the initial calibration phase did not differ between the two groups (experimental: 21.74 ± 0.88 N, control: 20.78 ± 0.81 N, independent sample t-test, $t_{(30)} = 0.80$, $p = 0.431$).

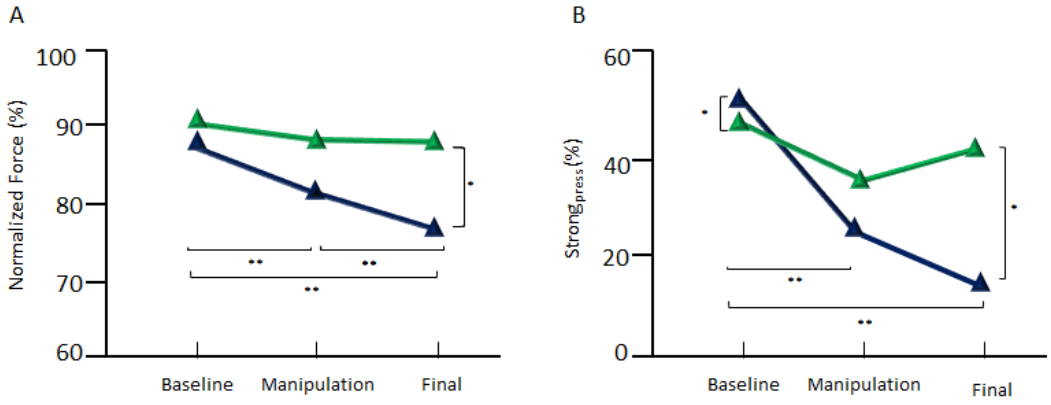
Behavioral data

ANOVA on normalized $Force_{peak}$ revealed a significant effect of session ($F_{(2,60)} = 15.16$, $p < 0.001$), due to lower values in the final (82.77 ± 2.19) compared to the baseline (89.76 ± 1.74; $p < 0.001$) session. The factor group revealed a non-significant trend ($F_{(1,30)} = 3.84$, $p = 0.059$), due to lower values in the experimental group (82.45 ± 2.44) compared to the control group (89.42 ± 2.59). More interestingly, the interaction session × group was significant ($F_{(2,60)} = 5.71$, $p = 0.005$).

Post-hoc comparisons showed that the experimental group was significantly weaker in the final (77.04 ± 2.82%) than in the manipulation (81.94 ± 2.02%) and baseline (88.36 ± 1.41%) session ($p = 0.008$ and $p < 0.001$, respectively). Moreover, the experimental group was also weaker in the manipulation than in the baseline session ($p = 0.002$).

On the other hand, the two groups had different force values in the final ($p = 0.014$), but not in the baseline ($p = 0.430$) and manipulation ($p = 0.077$) sessions (Fig.27A).

Figure 27. Behavioral data



A) Normalized force. The peak decreases in the experimental group (blue line) from baseline to final session, whereas it remains stable in the control group (green line). Moreover, the two groups have nearly different values in the final session. **B) Percentage of strong pressures.** This value decreases in the experimental group (blue line) from baseline to final session, whereas it remains stable in the control group (green line). **< 0.010; *p < 0.050. *Adapted from Emadi Andani et al., 2015.*

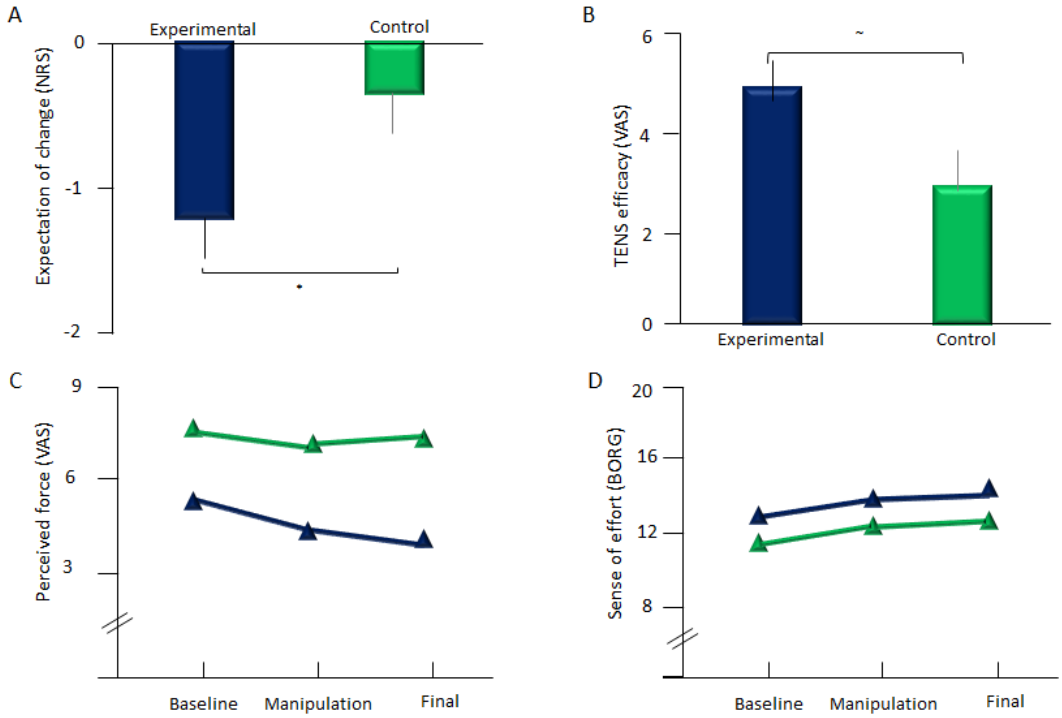
ANOVA on $Strong_{press}$ disclosed a similar pattern of results with a significant effect of session ($F_{(2,60)} = 10.09$, $p < 0.001$), due to lower values in the final (27.77 ± 5.78) than in the baseline session (48.73 ± 1.08; p =0.002), but no effect of group ($F_{(1,30)} = 2.25$, $p = 0.144$). The interaction session × group was significant ($F_{(2,60)} = 4.58$, $p = 0.014$). Post-

hoc comparisons showed that the experimental group pressed the piston more frequently weaker in the final (14.98 ± 7.16%) and in manipulation (25.29 ± 6.53) compared to the baseline (51.52 ± 1.47%) session ($p < 0.001$ and $p = 0.004$, respectively) (Fig.27B).
No difference was found in the control group between the final (40.54 ± 9.25%), the manipulation (34.27 ± 7.05%) and the baseline (45.94 ± 1.6%) sessions (for all comparisons, $p > 0.391$). Moreover, in the final session the experimental group had significantly lower values than the control group ($p = 0.035$). Conversely, in the baseline session the experimental group had significantly higher values than the control group ($p = 0.015$), while there was no difference between groups in the manipulation session ($p = 0.358$). Altogether, these findings suggest that the proposed procedure was successful in inducing a decrease of force production in the experimental group.

Subjective data

Analysis of the expectation level showed a significant difference between the two groups ($t_{(30)} = -2.31$, $p = 0.028$), due to lower values in the experimental (-1.24 ± 0.25) compared to the control group (-0.37 ± 0.28) (Fig.28A). Scores on TENS efficacy were slightly higher in the experimental (5.14 ± 0.66) than in the control group (3.02 ± 0.85) ($t_{(30)} = 1.99$, $p = 0.056$) (Fig.28B).
Analysis of force perception disclosed a significant effect of session ($F_{(2,60)} = 8.80$, $p < 0.001$), due to overall lower values in the final (5.73 ± 0.33) compared to the baseline session (6.52 ± 0.26; $p = 0.002$) and a significant effect of group ($F_{(1,30)} = 22.44$, $p < 0.001$), due to lower values in the experimental (4.67 ± 0.40) compared to the control group (7.40 ± 0.42). The session × group interaction was not significant ($F_{(2,60)} = 2.29$, $p = 0.110$) (Fig.28C).
Analysis of the sense of effort revealed a significant effect of session ($F_{(2,60)} = 11.12$, $p < 0.001$), due to higher values in the final (13.47 ± 0.43) compared to the baseline (12.07 ± 0.38; $p = 0.001$) session. Group ($F_{(1,29)} = 3.71$, $p = 0.064$) and the interaction session × group ($F_{(2,60)} = 0.54$, $p = 0.586$) were not significant (Fig.28D).

Figure 28. Subjective data.



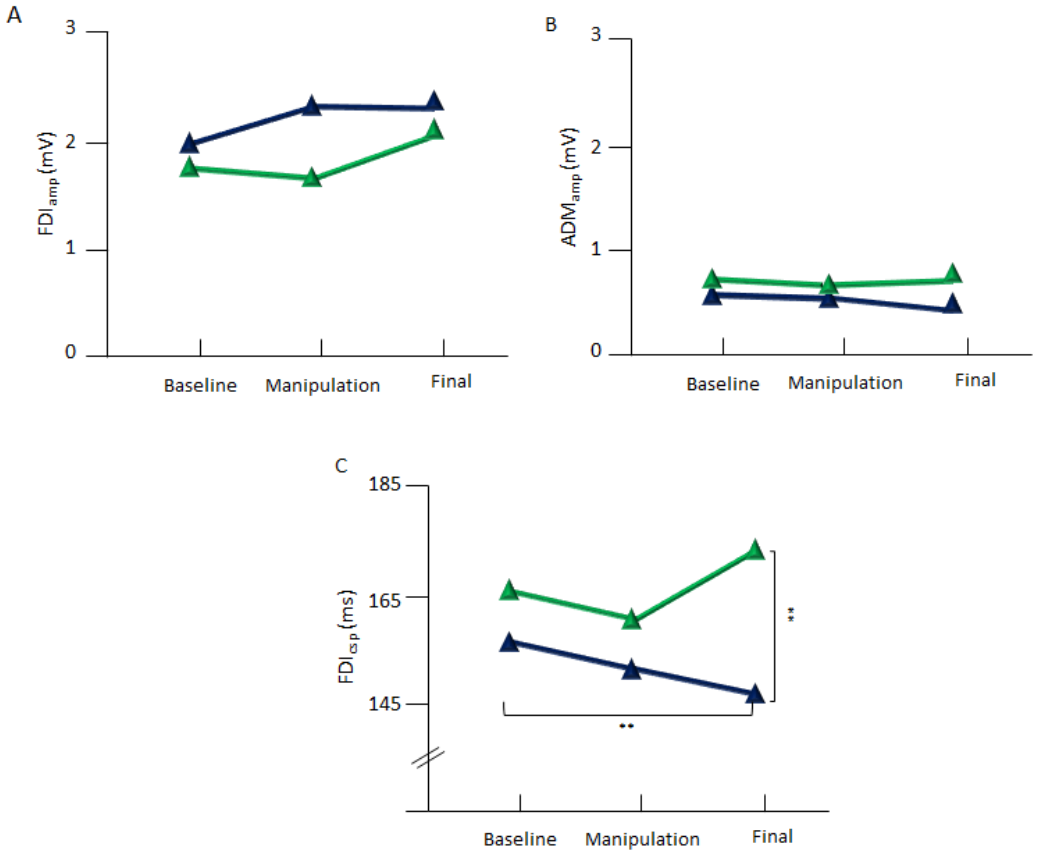
A) Scores of expectation of change in performance. The experimental group (blue bar) expected a more negative change of performance than the control group (green bar). **B) Judgments of treatment efficacy.** The experimental group (blue bar) has higher score than the control group (green bar). **C) Subjective perception of force**. In general, the experimental group (blue line) felt weaker than the control group (green line). **D) Sense of effort.** The perceived effort was overall higher in the final than in the baseline session. All the values are expressed as mean ± SE. **p < 0.010, *p < 0.050, ˜p = 0.056. *Adapted from Emadi Andani et al., 2015.*

Neurophysiological data

Independent sample t-test on the rMT of the two groups (experimental group: 59.65 ± 10.79, control group: 64.13 ± 8.98) revealed no significant differences ($t_{(30)}$ = -1.27, p = 0.214).

ANOVA on the FDI_{amp} revealed no effect of session ($F_{(2,60)}$ = 1.70, p = 0.191), no effect of group ($F_{(1,30)}$ = 1.19, p = 0.284), and no significant session × group interaction ($F_{(2,60)}$ = 1.31, p = 0.278) (Fig.29A).

Figure 29. Neurophysiological data.



A) FDI amplitude. MEP amplitude recorded from the FDI muscle was comparable in the experimental (blue line) and the control (green line) and across sessions. **B) ADM amplitude.** MEP amplitude recorded from the ADM muscle was comparable in the experimental (blue line) and the control (green line) and across sessions. **C) CSP duration.** The CSP was shorter in the final than in the baseline session in the experimental group (blue line), whereas there was a slight increase, although not significant, in the control group (green line). Moreover, the two groups had different CSP duration in the final session. All the values are expressed as mean ± SE. **p < 0.010. *Adapted from Emadi Andani et al., 2015.*

ANOVA on the ADM_{amp} revealed no effect of session ($F_{(2,60)} = 0.38$, $p = 0.683$), no effect of group ($F_{(1,30)} = 0.66$, $p = 0.422$), and no session × group interaction ($F_{(2,60)} = 2.24$, $p = 0.116$) (Fig.29B).

Analysis of the FDI_{csp} revealed no effect of session ($F_{(2,60)} = 1.09$, $p = 0.342$), but a significant effect of group ($F_{(1,30)} = 6.67$, $p = 0.015$), due to lower values in the experimental (151.78 ± 4.0) than in the control group (166.76 ± 4.22). The session × group interaction was also significant ($F_{(2,60)} = 5.39$, $p = 0.007$). Post-hoc comparisons

showed that the experimental group presented shorter FDI_{csp} duration in the final (146.17 ± 3.94 ms) than in the baseline (156.76 ± 4.39 ms; $p = 0.005$) session, but no difference was found between the manipulation (152.41 ± 3.29) and the other two sessions (for both comparisons, $p > 0.100$) (Fig.29C).

Moreover, no difference was found in the control group between the final (174.27 ± 7.94), the manipulation (160.33 ± 5.02) and the baseline (165.67 ± 4.7) sessions (for all comparisons, $p > 0.203$). In the final session, the FDI_{csp} duration of the experimental group was different from the control group ($p = 0.003$). To rule out whether the MEP amplitude was a mere reflection of the amount of applied force, we analyzed (rmANOVA) the level of the force of each group in the three sessions (baseline, manipulation, final) of the TMS-task, that is in the moment in which the TMS pulse was applied. This analysis showed no effect of session ($F_{(2,60)} = 0.65$, $p = 0.526$), no effect of group ($F_{(1,30)} = 0.01$, $p = 0.913$) and no session × group interaction ($F_{(2,60)} = 0.97$, $p = 0.384$), suggesting that during the TMS-task, the force level was the same in both groups and all the sessions. This suggests that the MEP amplitude was not influenced by the level of force exerted at the time of the TMS pulse.

Discussion

This study investigated the behavioral, subjective, and neurophysiological correlates of the nocebo effect in motor performance.

The behavioral results showed a clear reduction of force in the experimental group compared to the control group. Both our behavioral indices (e.g. the normalized force peak and the percentage of strong pressures) decreased across sessions only in the experimental group. Since motor performance in the control group did not decline, we argue that the worsening in the experimental group cannot be due to the massed repetition of the task (Crupi et al., 2013) or by a physiological reduction of force (Gandevia, 2001; Robbins, Goodale, Docherty, Behm, & Tran, 2010), but it is likely induced by the nocebo procedure. Subjects of the experimental group were told that TENS would have decreased their force and the conditioning procedure was surreptitiously applied in order to make them believe that TENS was really effective. By reducing the cursor's excursion range, subjects could see the cursor reaching lower lines of the target zone than before and were therefore conditioned about the effects of TENS in reducing force. Interestingly, in the

final session, subjects of the experimental group showed a continuous decrease of force production, even if the conditioning was removed. The negative scores at the expectation scale given by the experimental group compared to the positive scores of the control group, together with higher scores of TENS efficacy, confirm that the nocebo procedure induced a subjective state characterized by the expectation of a worse outcome. By comparing these findings to our previous study on the placebo effect, we can observe an opposite pattern of behavior: increase of force in the placebo study (Fiorio et al., 2014) and reduction in the current study. This confirms that opposite verbal instructions and conditioning procedures result in divergent behavioral outcomes.

With regard to the neurophysiological data, we found that after the nocebo procedure only the CSP duration was modulated (i.e., reduced), whereas MEP amplitude remained stable. In a previous paper on the placebo effect (Fiorio et al., 2014), the most evident neurophysiological findings were obtained in the group of participants who underwent a conditioning procedure in addition to verbal suggestion. Since MEP amplitude was not reduced and CSP duration was not increased by the nocebo procedure, the prediction to find an opposite pattern of corticospinal activation between this nocebo study and the previous placebo study (Fiorio et al., 2014) was not confirmed. We suggest that MEP amplitude in our study did not decrease – despite the reduced motor performance in the experimental group – probably because the procedure prevented a reduction of attention (Conte et al., 2007), thus maintaining MEP size stable in both groups and throughout the sessions. This raises the question why the placebo procedure resulted in a change of MEP amplitude (Fiorio et al., 2014), whereas here the nocebo procedure leaved MEPs unchanged. One possible and speculative answer is that being the placebo-induced expectation directed to a potential benefit, it is also associated to reward mechanisms (de la Fuente-Fernandez, Schulzer, & Stoessl, 2004; Lutz, Pedroni, Nadig, Luechinger, & Jancke, 2012; Schweinhardt, Seminowicz, Jaeger, Duncan, & Bushnell, 2009; Scott et al., 2007; Yu et al., 2014) that can increase MEP amplitude (Klein, Olivier, & Duque, 2012). Conversely, the nocebo procedure does not induce anticipation of reward and this fact, together with a constant level of attention throughout the sessions, could have maintained MEP amplitude stable. Despite stable MEPs, the CSP duration changed, suggesting that only the CSP is modulated by the nocebo procedure. In particular, the CSP duration was clearly shorter in the experimental group, whereas in the control group

there was an opposite tendency toward a CSP prolongation, although not significant. Since TENS was applied exactly in the same way in the two groups, the different changes in CSP cannot be explained by the effects of TENS *per se*. Instead, the main factor distinguishing the two groups was the nocebo procedure, consisting of verbal suggestion and conditioning. The core of this procedure is an induced expectation of change in performance, and therefore we propose that this cognitive function may be responsible for the CSP reduction. Even though this interpretation remains to be proven, our study clearly adds further evidence for a cognitive modulation of the CSP, by showing a reduction following a nocebo procedure. This result is similar to the one we previously observed for the placebo effect (Fiorio et al., 2014), suggesting that nocebo and placebo effects in motor performance share some common mechanisms. This means that, independently of the direction of the behavioral outcome (increase or decrease of force) or of the belief (positive or negative), the nocebo/placebo-induced expectation modulates the CSP in the same way.

It could be speculated that probably the CSP is more easily modulated than the MEP. Of note, while MEP is related to excitatory circuits and can be influenced by the activity of both cortical and spinal neurons the CSP is related to the activity of inhibitory motor circuits and, at least in the last part, is mainly generated by cortical mechanisms (Inghilleri et al., 1993). Hence, the CSP reduction in the experimental group hints at a change in the cortical inhibitory circuits. The functional significance of the involvement of inhibitory circuits in the nocebo/placebo effects in motor performance, as well as the underlying mechanisms, remain to be explained. Potentially, a brain network involved in expectation and anticipation (Kong et al., 2008; Krummenacher, Candia, Folkers, Schedlowski, & Schonbachler, 2010; Wager et al., 2004) may exert a top-down modulation on the inhibitory circuits of the primary motor area. Therefore, the reduced inhibitory activity could represent a state of alertness of the motor system that serves to deal with the expected changes of performance.

Moving forward, neurophysiological differences between placebo and nocebo effects in motor performance could be uncovered with more specific methods that allow stimulating selective cortical circuits within the motor cortex.

OVERALL CONCLUSIONS

The research on placebo and nocebo effects has been going on for the last decades and has widely expanded the knowledge on these effects. The topic of this thesis is still a central theme in neuroscience. Thus, we investigated specific aspects of this topic, from the factors that influence the magnitude of the responses to the neurophysiological underpinnings, and the relation between pain and motor system. With the aim to produce innovative researches, we investigated a still under-debate topic regarding the factors that modulate the magnitude of the placebo and nocebo effects, not only in the motor but also in pain field. To try to address this ongoing question, we performed the experiments described in Part II.

First, we developed a protocol that allowed us to shed light on the potential different role of verbal suggestion and conditioning during a placebo/nocebo procedure in motor performance. To this aim, we randomly divided the participants in four different groups: two with congruent conditioning and verbal suggestion (either positive or negative) and two with incongruent ones (one positive and one negative).

By creating an incongruent situation between the given information (verbal suggestion) and the experience (conditioning), we aimed to investigate which one was prevailing in influencing performance. Results disclosed a strong decrease of force in the groups with negative verbal suggestion, independently of the conditioning. These groups also showed a lower perception of force and a higher sense of effort at the end of the procedure compared to the beginning. These findings hint at a more prominent role of verbal suggestion compared to conditioning, especially during a nocebo procedure. As mentioned in the discussion of Study 1, we should interpret these data with caution, due to the absence of a control group. However, the results of our study are important for at least two reasons: first, the procedure we applied was successful in evoking a placebo and nocebo response, thus confirming previous studies; second, this is the first study suggesting that verbal suggestion is likely to have a preponderant role in determining an increasing (placebo) and a worsening (nocebo) of motor performance.

Moving forward with our line of research, we included in our study another important factor that could actively influence the response to nocebo effect: personality traits. Namely, we investigated whether the persistence of belief about a treatment and personality traits could account for individual differences in the magnitude of the nocebo response. We identified two different patterns as regards to the subjective evaluations about the expectation of treatment efficacy: some participants continued to believe in the treatment efficacy during all the experiment, according to the verbal suggestion, whereas other participants did the opposite (i.e. we recorded a decreasing in their level expectation about the efficacy of the treatment from one phase to the other). Results showed that people who continued to believe in the treatment efficacy had a stronger nocebo effect, as evidenced by lower levels of force, higher feeling of weakness and higher sense of effort, compared to those who did not believe that much in the treatment efficacy. Personality questionnaires revealed that traits such as optimism, self-directedness, anxiety and harm-avoidance might be crucial in determining a stronger nocebo response. The results of this study are important because, differently from previous researches, we adopted a new approach, by taking into account the changes in perception of treatment effectiveness between the experimental sessions. Moreover, this kind of investigation might contribute to address an ever-lasting topic: characterize the individual differences in the nocebo effect in motor performance.

After these original results on individual differences, we extended the interest of personality traits to the field of pain, to unravel whether these factors have a determinant role in modulating the placebo and nocebo responses. After a deeply search of the literature, we created a large battery of personality questionnaires. By considering that personality is a continuum of factors, it highlights the importance of considering distinct factors together. Thus, we took into account two sets of psychological factors related to placebo and nocebo responsiveness and used stepwise multiple regressions models to calculate hierarchies and residual components. Such an approach indicated that an aggregate score for motivation and suggestibility accounted for the 51% of the variance in the placebo hypolagesic responses whilst anxiety severity, NEO-openness-extraversion and depression considered together accounted for the 49.1% of the variance of nocebo responses, suggesting that it helps evaluate the psychological factors comprehensively. Another important finding from this study was that positive expectations were

significantly correlated with placebo responses and negative expectations were significantly correlated with nocebo responses. Despite one may argue that asking on a trial-by-trial about expectancy of the upcoming pain may have generated a sort of self-prophecy (e.g. You get what you expect, you get what you ask for), it remains an interesting finding that could be important to keep in mind every time we measure pain in real-world settings. To our knowledge, this is the first study that explores how distinct psychological factors can predict placebo hypolagesic responses and nocebo hyperalgesic responses, and the absence of influence on expectancies by personality factors.

Moving forward in enhancing the knowledge on placebo and nocebo effect, we were interested in pointing out whether is there a link connecting pain and motor field. To successfully combine these two fields, we took advantage from a well-established pain paradigm and we integrated our previous expertise in motor performance. Thus we developed a protocol made of different painful stimulations (high, medium, low) received at rest or while performing a specific and well-calibrated movement (extension-flexion of the dominant arm). Our results showed that participants perceived as less painful the heat stimulations received during the motor movement. More interestingly, this motor-induced hypoalgesia was present in all the conditions (red, yellow, green) and phases (acquisition and testing), thus resulting in an enhancement of the hypoalgesic placebo effect and an attenuation of the hyperalgesic nocebo effect. Since this was an extremely interesting but exploratory study, it is reasonable to consider that to strengthen the efficacy of the motor-induced analgesia, further studies need to be performed. Even so, this study represents a fruitful starting point to deeply investigate the active role of the motor cortex in inducing a hypoalgesic effect. Moreover, understanding these mechanisms might have important clinical implications in terms of optimizing therapeutic and rehabilitation programs, thus improving treatment outcomes in real-world settings.

By considering the paucity of studies on nocebo effect, we decided to investigate whether the nocebo effect is entirely the negative counterpart of the placebo effect. More precisely, we aimed to unravel the neurophysiological correlates of the nocebo effect. To pursue this aim we used a slightly different protocol compared to Fiorio and colleagues (2014). Our results showed that from a behavioral and subjective point of view, the placebo and nocebo effects are exactly the opposite. Placebo induces an increase of force, a higher sense of perceived force and a lower sense of effort. Conversely, nocebo effect

leads to a worsening in force production, a decrease in the perception of force and an increase of sense of effort in performing the movement required by the task. However, besides our hypothesis to find opposite neurophysiological pattern, we obtained unexpected results. The placebo effect is characterized by an enhancement of corticospinal excitability characterized by an increase of the MEP amplitude and a decrease of CSP duration, highlighting not only a cortical but also a corticospinal activation during this procedure. The nocebo effect, instead, was associated to stable MEP amplitude and a shortening of the CSP duration. The cortical silent period reflects changes at the cortical level, in particular reflecting the activation of inhibitory circuits. Since a change in the CSP was found only in the experimental groups, we speculated that it could be due to the verbal suggestion, given during the experiment, and learning experience, gained through the conditioning process. This study helped to enlarge current knowledge on the nocebo effect, showing his complex nature, and its influence at different levels (behavioral, subjective and neurophysiological).

To sum up, from these studies we have learned that: 1) people are more likely to rely on verbal suggestion than on experience when contrasting situations are presented; 2) not only verbal suggestion, conditioning and observation but also individual characteristics (e.g. personality traits) influence the magnitude of the placebo and nocebo response in pain and motor systems; 3) active movements seem to modulate pain experience in healthy volunteers; 4) nocebo effect is not exactly the opposite of the well-known placebo effect: they share common neurophysiological mechanisms.

From a general prospective, the studies included in this thesis unravel new mechanisms and characteristics of the extremely elaborate frame of the placebo and nocebo responses. Neurophysiological mechanisms of nocebo effect have been revealed, and the role of personality traits has been highlighted for both placebo and nocebo not only in pain but also in motor field. So far, most of the studies involved placebo and nocebo in a singular and distinct domain. However, we demonstrated that it is possible to combine different domains, such as pain and motor, to evoke a stronger hypoalgesic effect or to attenuate the hyperalgesic nocebo effect, thus describing new fascinating aspects of these effects. However, placebo and nocebo effects (especially in motor domain) are still an open field of research. Further studies are needed to confirm the prevalent role of verbal suggestion on conditioning procedure, to prove the causal role of sensory

attenuation in modulating pain perception through active movements, and to better delineate the role of individual differences (e.g. personality traits) in influencing the magnitude of placebo and nocebo effects.

From a practical and concrete point of view, the strength of these studies regards patients' treatment and communication. All the words and communication methods may influence the comprehension of a diagnosis and the real effect of a pharmacological treatment. Moreover, how we communicate is crucial to influence both the intensity and the severity of the experienced effects. Along these elements, we should consider all the individual characteristics strictly related to each person, such as the personality traits: the same words may affect in a different way different patients.

Recent findings are growing awareness on the patients' uniqueness in their responsiveness to treatments, with expectation of benefit playing a central role.

A reasonable and feasible solution may be to develop a personalized and individualized communicative method, based on the patient's characteristics, thus establishing a good doctor-patient relationship that leads to the best diagnostic and therapeutic outcome. Over the next few years, it will be extremely fruitful to direct researchers' efforts toward these aspects to better contextualize placebo and nocebo effects in real-world settings and better understand the real implications of placebo and nocebo effects, thus avoiding their misuse and optimizing their application.

LIST OF PUBLICATIONS

1. Emadi Andani M, Tinazzi M, **Corsi N**, Fiorio F (2015). *Modulation of Inhibitory Corticospinal Circuits Induced by a Nocebo Procedure in Motor Performance*. PlosONE 10(4): e0125223.

2. Gambina G, Valbusa V, **Corsi N**, Ferrari F, Sala F, Broggio E, Condoleo MT, Survo V, Errera P, Cagnin AC, Moretto G and Moro V (2015). *The Italian Validation of the Anosognosia Questionnaire for Dementia in Alzheimer's Disease*. American Journal of Alzheimer's Disease & Other Dementias. 30(6): 635-644.

3. **Corsi N**, Emadi Andani M, Tinazzi M, Fiorio F (2016). *Changes in perception of treatment efficacy modulate the magnitude of the nocebo effect and are related to personality traits*. Sci. Rep. 6, 30671.

4. **Corsi N** and Colloca L (2017). *Placebo and nocebo effects: The advantage of measuring expectations and psychological factors*. Front. Psychol. 8:308.

5. Blasini M, **Corsi N**, Klinger R, Colloca L (2017). *Nocebo and pain: An overview of the psychoneurobiological mechanisms*. Pain Reports, e585.

6. Moro V, Valbusa V, **Corsi N**, Bonazzi A, Condoleo MT, Broggio E, Moretto G, Gambina G. *Comprehension of written text for the clinical competence and decision making assessment in Alzheimer's Disease*. Under revision.

7. **Corsi N,** Tinazzi M, Emadi Andani M Fiorio M. *Placebo and nocebo effects: a TMS study on the congruency between conditioning and verbal suggestion.* In preparation.

8. **Corsi N,** Ludman T, Renn C, Wittenberg G, Fiorio M, Colloca L. *How motor-induced analgesia shapes placebo and nocebo effects in a heat model of human pain*. In preparation.

REFERENCES

Albu, S., & Meagher, M. W. (2016). Expectation of nocebo hyperalgesia affects EEG alpha-activity. *Int J Psychophysiol*.

Amanzio, M., & Benedetti, F. (1999). Neuropharmacological dissection of placebo analgesia: expectation-activated opioid systems versus conditioning-activated specific subsystems. *J Neurosci*, 19(1), 484-494.

Andre, J., Zeau, B., Pohl, M., Cesselin, F., Benoliel, J. J., & Becker, C. (2005). Involvement of cholecystokininergic systems in anxiety-induced hyperalgesia in male rats: behavioral and biochemical studies. *J Neurosci*, 25(35), 7896-7904.

Ariel, G., & Saville, W. (1972). Anabolic steroids: the physiological effects of placebos. *Med Sci Sports Exerc*, 4: 124-6.

Aslaksen, P. M., & Lyby, P. S. (2015). Fear of pain potentiates nocebo hyperalgesia. *J Pain Res*, 8, 703-710.

Au Yeung, S. T., Colagiuri, B., Lovibond, P. F., & Colloca, L. (2014). Partial reinforcement, extinction, and placebo analgesia. *Pain*, 155(6), 1110-1117.

Balint, M. (1955). The doctor, his patient, and the illness. *Lancet*, 268(6866), 683-688.

Bandura, A. (Ed.). (1986). *Social Foundations of Thought and Action: A Social Cognitive Theory*: Prentice Hall, Englewood Cliffs, NJ

Barsky, A. J., Saintfort, R., Rogers, M. P., & Borus, J. F. (2002). Nonspecific medication side effects and the nocebo phenomenon. *JAMA*, 287(5), 622-627.

Bartels, D. J., van Laarhoven, A. I., Haverkamp, E. A., Wilder-Smith, O. H., Donders, A. R., van Middendorp, H., et al. (2014). Role of conditioning and verbal suggestion in placebo and nocebo effects on itch. *PLoS One*, 9(3), e91727.

Bartley, H., Faasse, K., Horne, R., & Petrie, K. J. (2016). You Can't Always Get What You Want: The Influence of Choice on Nocebo and Placebo Responding. *Ann Behav Med*, 50(3), 445-451.

Bass, M. J., Buck, C., Turner, L., Dickie, G., Pratt, G., & Robinson, H. C. (1986). The physician's actions and the outcome of illness in family practice. *J Fam Pract*, 23(1), 43-47.

Beck, A. T., Epstein, N., Brown, G., & Steer, R. A. (1988). An inventory for measuring clinical anxiety: Psychometric properties. . *Journal of Consulting and Clinical Psychology*, 56(6), 893-897.

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

Beecher, H. K. (1955). The powerful placebo. *J Am Med Assoc*, 159(17), 1602-1606.

Beedie, C. J., Coleman, D. A., & Foad, A. J. (2007). Positive and negative placebo effects resulting from the deceptive administration of an ergogenic aid. *Int J Sport Nutr Exerc Metab*, 17(3), 259-269.

Beedie, C. J., Foad, A. J., & Coleman, D. A. (2008). Identification of placebo responsive participants in 40km laboratory cycling performance. *J Sports Sci Med*, 7(1), 166-175.

Beedie, C. J., Stuart, E. M., Coleman, D. A., & Foad, A. J. (2006). Placebo effects of caffeine on cycling performance. *Med Sci Sports Exerc*, 38(12), 2159-2164.

Benedetti, F. (2002). How the doctor's words affect the patient's brain. *Eval Health Prof,* 25(4), 369-386.

Benedetti, F. (2013). Responding to nocebos through observation: social contagion of negative emotions. *Pain,* 154(8), 1165.

Benedetti, F. (2014a). Drugs and placebos: what's the difference?: Understanding the molecular basis of the placebo effect could help clinicians to better use it in clinical practice. *EMBO Rep,* 15(4), 329-332.

Benedetti, F. (2014b). Placebo effects: from the neurobiological paradigm to translational implications. *Neuron,* 84(3), 623-637.

Benedetti, F., & Amanzio, M. (1997). The neurobiology of placebo analgesia: from endogenous opioids to cholecystokinin. *Prog Neurobiol,* 52(2), 109-125.

Benedetti, F., Amanzio, M., Casadio, C., Oliaro, A., & Maggi, G. (1997). Blockade of nocebo hyperalgesia by the cholecystokinin antagonist proglumide. *Pain,* 71(2), 135-140.

Benedetti, F., Amanzio, M., Vighetti, S., & Asteggiano, G. (2006). The biochemical and neuroendocrine bases of the hyperalgesic nocebo effect. *J Neurosci,* 26(46), 12014-12022.

Benedetti, F., Arduino, C., & Amanzio, M. (1999). Somatotopic activation of opioid systems by target-directed expectations of analgesia. *J Neurosci,* 19(9), 3639-3648.

Benedetti, F., Lanotte, M., Lopiano, L., & Colloca, L. (2007). When words are painful: unraveling the mechanisms of the nocebo effect. *Neuroscience,* 147(2), 260-271.

Benedetti, F., Pollo, A., & Colloca, L. (2007). Opioid-mediated placebo responses boost pain endurance and physical performance: is it doping in sport competitions? *J Neurosci,* 27(44), 11934-11939.

Benedetti, F., Pollo, A., Lopiano, L., Lanotte, M., Vighetti, S., & Rainero, I. (2003). Conscious expectation and unconscious conditioning in analgesic, motor, and hormonal placebo/nocebo responses. *J Neurosci*, 23(10), 4315-4323.

Benson, H. (1997). The nocebo effect: history and physiology. *Prev Med*, 26(5 Pt 1), 612-615.

Bingel, U., Lorenz, J., Schoell, E., Weiller, C., & Buchel, C. (2006). Mechanisms of placebo analgesia: rACC recruitment of a subcortical antinociceptive network. *Pain*, 120(1-2), 8-15.

Bingel, U., & Placebo Competence Team. (2014). Avoiding nocebo effects to optimize treatment outcome. *JAMA*, 312(7), 693-694.

Bingel, U., Wanigasekera, V., Wiech, K., Ni Mhuircheartaigh, R., Lee, M. C., Ploner, M., et al. (2011). The effect of treatment expectation on drug efficacy: imaging the analgesic benefit of the opioid remifentanil. *Sci Transl Med*, 3(70), 70ra14.

Blakemore, S. J., Goodbody, S. J., & Wolpert, D. M. (1998). Predicting the consequences of our own actions: the role of sensorimotor context estimation. *J Neurosci*, 18(18), 7511-7518.

Blakemore, S. J., Wolpert, D. M., & Frith, C. D. (1998). Central cancellation of self-produced tickle sensation. *Nat Neurosci*, 1(7), 635-640.

Bootzin, R. R., & Bailey, E. T. (2005). Understanding placebo, nocebo, and iatrogenic treatment effects. *J Clin Psychol*, 61(7), 871-880.

Borg, G. (1970). Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med*, 2(2), 92-98.

Brasil-Neto, J. P., Cohen, L. G., Panizza, M., Nilsson, J., Roth, B. J., & Hallett, M. (1992). Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity. *J Clin Neurophysiol*, 9(1), 132-136.

Butler, J. E., Petersen, N. C., Herbert, R. D., Gandevia, S. C., & Taylor, J. L. (2012). Origin of the low-level EMG during the silent period following transcranial magnetic stimulation. *Clin Neurophysiol*, 123(7), 1409-1414.

Cantello, R., Gianelli, M., Civardi, C., & Mutani, R. (1992). Magnetic brain stimulation: the silent period after the motor evoked potential. *Neurology*, 42(10), 1951-1959.

Carlino, E., Frisaldi, E., & Benedetti, F. (2014). Pain and the context. *Nat Rev Rheumatol*, 10(6), 348-355.

Carlino, E., Piedimonte, A., & Frisaldi, E. (2014). The effects of placebos and nocebos on physical performance. *Handb Exp Pharmacol*, 225, 149-157.

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

Christie, A., & Kamen, G. (2014). Cortical inhibition is reduced following short-term training in young and older adults. *Age (Dordr)*, 36(2), 749-758.

Clark, V. R., Hopkins, W. G., Hawley, J. A., & Burke, L. M. (2000). Placebo effect of carbohydrate feedings during a 40-km cycling time trial. *Med Sci Sports Exerc*, 32(9), 1642-1647.

Cloninger, C. R. (2008). The psychobiological theory of temperament and character: comment on Farmer and Goldberg (2008). *Psychol Assess*, 20(3), 292-299; discussion 300-294.

Cloninger, C. R., Przybeck, T. R., & Svrakic, D. M. (1991). The Tridimensional Personality Questionnaire: U.S. normative data. *Psychol Rep*, 69(3 Pt 1), 1047-1057.

Cloninger, C. R., Przybeck, T. R., Svrakic, D. M., & Wetzel, R. D. (1994). *The Temperament and Character Inventory (TCI): A Guide to Its Development and Use*. St. Louis, MO.

Cloninger, C. R., Svrakic, D. M., & Przybeck, T. R. (1993). A psychobiological model of temperament and character. *Arch Gen Psychiatry*, 50(12), 975-990.

Colagiuri, B., McGuinness, K., Boakes, R. A., & Butow, P. N. (2012). Warning about side effects can increase their occurrence: an experimental model using placebo treatment for sleep difficulty. *J Psychopharmacol*, 26(12), 1540-1547.

Colagiuri, B., Quinn, V. F., & Colloca, L. (2015). Nocebo Hyperalgesia, Partial Reinforcement, and Extinction. *J Pain*, 16(10), 995-1004.

Colagiuri, B., Schenk, L. A., Kessler, M. D., Dorsey, S. G., & Colloca, L. (2015). The placebo effect: From concepts to genes. *Neuroscience*, 307, 171-190.

Colloca, L. (2014). Placebo, nocebo, and learning mechanisms. *Handb Exp Pharmacol*, 225, 17-35.

Colloca, L., & Benedetti, F. (2005). Placebos and painkillers: is mind as real as matter? *Nat Rev Neurosci*, 6(7), 545-552.

Colloca, L., & Benedetti, F. (2006). How prior experience shapes placebo analgesia. *Pain*, 124(1-2), 126-133.

Colloca, L., & Benedetti, F. (2007). Nocebo hyperalgesia: how anxiety is turned into pain. *Curr Opin Anaesthesiol*, 20(5), 435-439.

Colloca, L., & Benedetti, F. (2009). Placebo analgesia induced by social observational learning. *Pain*, 144(1-2), 28-34.

Colloca, L., & Finniss, D. (2012). Nocebo effects, patient-clinician communication, and therapeutic outcomes. *JAMA*, 307(6), 567-568.

Colloca, L., & Grillon, C. (2014). Understanding placebo and nocebo responses for pain management. *Curr Pain Headache Rep*, 18(6), 419.

Colloca, L., Klinger, R., Flor, H., & Bingel, U. (2013). Placebo analgesia: psychological and neurobiological mechanisms. *Pain*, 154(4), 511-514.

Colloca, L., Lopiano, L., Lanotte, M., & Benedetti, F. (2004). Overt versus covert treatment for pain, anxiety, and Parkinson's disease. *Lancet Neurol*, 3(11), 679-684.

Colloca, L., & Miller, F. G. (2011a). Harnessing the placebo effect: the need for translational research. *Philos Trans R Soc Lond B Biol Sci*, 366(1572), 1922-1930.

Colloca, L., & Miller, F. G. (2011b). The Nocebo Effect and Its Relevance for Clinical Practice. *Psychosom Med*.

Colloca, L., Petrovic, P., Wager, T. D., Ingvar, M., & Benedetti, F. (2010). How the number of learning trials affects placebo and nocebo responses. *Pain*, 151(2), 430-439.

Colloca, L., Pine, D. S., Ernst, M., Miller, F. G., & Grillon, C. (2016). Vasopressin Boosts Placebo Analgesic Effects in Women: A Randomized Trial. *Biol Psychiatry*, 79(10), 794-802.

Colloca, L., Sigaudo, M., & Benedetti, F. (2008). The role of learning in nocebo and placebo effects. *Pain*, 136(1-2), 211-218.

Colloca, L., Tinazzi, M., Recchia, S., Le Pera, D., Fiaschi, A., Benedetti, F., et al. (2008). Learning potentiates neurophysiological and behavioral placebo analgesic responses. *Pain,* 139(2), 306-314.

Conte, A., Gilio, F., Iezzi, E., Frasca, V., Inghilleri, M., & Berardelli, A. (2007). Attention influences the excitability of cortical motor areas in healthy humans. *Exp Brain Res,* 182(1), 109-117.

Corsi, N., Emadi Andani, M., Tinazzi, M., & Fiorio, M. (2016). Changes in perception of treatment efficacy are associated to the magnitude of the nocebo effect and to personality traits. *Sci Rep,* 6, 30671.

Costa, P. T., Jr., , & McCrae, R. R. (1992). *NEO PI-R professional manual*.

Costa, P. T., & McCrae, R. R. (1985). *The NEO personality inventory manual*.

Craig, A. D., Chen, K., Bandy, D., & Reiman, E. M. (2000). Thermosensory activation of insular cortex. *Nat Neurosci,* 3(2), 184-190.

Craig, K. D. (1987). Pain and affectivity in infancy: their interdependence and independence. *Cephalalgia,* 7 Suppl 6, 115-118.

Crawford, J. R., & Henry, J. D. (2004). The positive and negative affect schedule (PANAS): construct validity, measurement properties and normative data in a large non-clinical sample. *Br J Clin Psychol,* 43(Pt 3), 245-265.

Crupi, D., Cruciata, G., Moisello, C., Green, P. A., Naro, A., Ricciardi, L., et al. (2013). Protracted exercise without overt neuromuscular fatigue influences cortical excitability. *J Mot Behav,* 45(2), 127-138.

Darragh, M., Booth, R. J., & Consedine, N. S. (2015). Who responds to placebos? Considering the "placebo personality" via a transactional model. *Psychol Health Med*, 20(3), 287-295.

Davis, M. H. (1980). A multidimensional approach to individual differences in empathy. *JSAS Catalog of Selected Documents in Psychology*, 10(85).

de la Fuente-Fernandez, R., Ruth, T. J., Sossi, V., Schulzer, M., Calne, D. B., & Stoessl, A. J. (2001). Expectation and dopamine release: mechanism of the placebo effect in Parkinson's disease. *Science*, 293(5532), 1164-1166.

de la Fuente-Fernandez, R., Schulzer, M., & Stoessl, A. J. (2004). Placebo mechanisms and reward circuitry: clues from Parkinson's disease. *Biol Psychiatry*, 56(2), 67-71.

De Pascalis, V., Chiaradia, C., & Carotenuto, E. (2002). The contribution of suggestibility and expectation to placebo analgesia phenomenon in an experimental setting. *Pain*, 96(3), 393-402.

Di Blasi, Z., Harkness, E., Ernst, E., Georgiou, A., & Kleijnen, J. (2001). Influence of context effects on health outcomes: a systematic review. *Lancet*, 357(9258), 757-762.

Doganci, B., Breimhorst, M., Hondrich, M., Rodriguez-Raecke, R., May, A., & Birklein, F. Expectations modulate long-term heat pain habituation. *Eur J Pain*.

Dworkin, S. F., Chen, A. C., LeResche, L., & Clark, D. W. (1983). Cognitive reversal of expected nitrous oxide analgesia for acute pain. *Anesth Analg*, 62(12), 1073-1077.

Egorova, N., Yu, R., Kaur, N., Vangel, M., Gollub, R. L., Dougherty, D. D., et al. (2015). Neuromodulation of conditioned placebo/nocebo in heat pain: anodal vs cathodal transcranial direct current stimulation to the right dorsolateral prefrontal cortex. *Pain*, 156(7), 1342-1347.

Eippert, F., Bingel, U., Schoell, E. D., Yacubian, J., Klinger, R., Lorenz, J., et al. (2009). Activation of the opioidergic descending pain control system underlies placebo analgesia. *Neuron,* 63(4), 533-543.

Eippert, F., Finsterbusch, J., Bingel, U., & Buchel, C. (2009). Direct evidence for spinal cord involvement in placebo analgesia. *Science,* 326(5951), 404.

Elsenbruch, S., & Enck, P. (2015). Placebo effects and their determinants in gastrointestinal disorders. *Nat Rev Gastroenterol Hepatol*, 12(8), 472-485.

Emadi Andani, M., Tinazzi, M., Corsi, N., & Fiorio, M. (2015). Modulation of inhibitory corticospinal circuits induced by a nocebo procedure in motor performance. *PLoS One,* 10(4), e0125223.

Evans, W., & Hoyle, C. (1933). The Comparative Value of Drugs Used in the Continuous Treatment of Angina Pectoris. *Quarterly Journal of Medicine,* Jul; 2(7).

Faasse, K., Grey, A., Jordan, R., Garland, S., & Petrie, K. J. (2015). Seeing is believing: Impact of social modeling on placebo and nocebo responding. *Health Psychol,* 34(8), 880-885.

Fiorio, M., Emadi Andani, M., Marotta, A., Classen, J., & Tinazzi, M. (2014). Placebo-induced changes in excitatory and inhibitory corticospinal circuits during motor performance. *J Neurosci,* 34(11), 3993-4005.

Flaten, M. A., Aslaksen, P. M., Finset, A., Simonsen, T., & Johansen, O. (2006). Cognitive and emotional factors in placebo analgesia. *J Psychosom Res,* 61(1), 81-89.

Flaten, M. A., Simonsen, T., & Olsen, H. (1999). Drug-related information generates placebo and nocebo responses that modify the drug response. *Psychosom Med,* 61(2), 250-255.

Flor, H., & Turk, D. C. (1988). Chronic back pain and rheumatoid arthritis: predicting pain and disability from cognitive variables. *J Behav Med*, 11(3), 251-265.

Foad, A. J., Beedie, C. J., & Coleman, D. A. (2008). Pharmacological and psychological effects of caffeine ingestion in 40-km cycling performance. *Med Sci Sports Exerc*, 40(1), 158-165.

Fox, E., Russo, R., & Dutton, K. (2002). Attentional Bias for Threat: Evidence for Delayed Disengagement from Emotional Faces. *Cogn Emot*, 16(3), 355-379.

Freeman, S., Yu, R., Egorova, N., Chen, X., Kirsch, I., Claggett, B., et al. (2015). Distinct neural representations of placebo and nocebo effects. *Neuroimage*, 112, 197-207.

Gallace, A., Torta, D. M., Moseley, G. L., & Iannetti, G. D. (2011). The analgesic effect of crossing the arms. *Pain*, 152(6), 1418-1423.

Gandevia, S. C. (2001). Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev*, 81(4), 1725-1789.

Garcia-Larrea, L., Peyron, R., Mertens, P., Gregoire, M. C., Lavenne, F., Le Bars, D., et al. (1999). Electrical stimulation of motor cortex for pain control: a combined PET-scan and electrophysiological study. *Pain*, 83(2), 259-273.

Geers, A. L., Helfer, S. G., Kosbab, K., Weiland, P. E., & Landry, S. J. (2005). Reconsidering the role of personality in placebo effects: dispositional optimism, situational expectations, and the placebo response. *J Psychosom Res*, 58(2), 121-127.

Geers, A. L., Helfer, S. G., Weiland, P. E., & Kosbab, K. (2006). Expectations and placebo response: a laboratory investigation into the role of somatic focus. *J Behav Med*, 29(2), 171-178.

Geers, A. L., Kosbab, K., Helfer, S. G., Weiland, P. E., & Wellman, J. A. (2007). Further evidence for individual differences in placebo responding: an interactionist perspective. *J Psychosom Res*, 62(5), 563-570.

Geers, A. L., Wellman, J. A., Fowler, S. L., Helfer, S. G., & France, C. R. (2010). Dispositional optimism predicts placebo analgesia. *J Pain*, 11(11), 1165-1171.

Gelfland, D. M., Gelfland, S., & Rardin, M. (1965). Some personality factors associated with placebo responsivity. *Psychol Rep*, 17, 555-562.

Gensini, G. F., Conti, A. A., & Conti, A. (2005). Past and present of "what will please the lord": an updated history of the concept of placebo. *Minerva Med*, 96(2), 121-124.

Geuter, S., & Buchel, C. (2013). Facilitation of pain in the human spinal cord by nocebo treatment. *J Neurosci*, 33(34), 13784-13790.

Gold, H., Kwit, N.T., & Otto, H. (1937). The xanthines (Theobromine and Aminophylline) in the treatment of cardiac pain. *JAMA*, Jun 26; 108(26):2173-79.

Goubert, L., Crombez, G., & Van Damme, S. (2004). The role of neuroticism, pain catastrophizing and pain-related fear in vigilance to pain: a structural equations approach. *Pain*, 107(3), 234-241.

Goubert, L., Vlaeyen, J., Crombez, G., & Craig, K. (2011). Learning about pain from others: an observational learning account. *The Journal of Pain*, 12(2), 167-174

Gracely, R. H., Dubner, R., Deeter, W. R., & Wolskee, P. J. (1985). Clinicians' expectations influence placebo analgesia. *Lancet*, 1(8419), 43.

Graves, T.C. (1920). Commentary on a Case of Hystero-Epilepsy with Delayed Puberty: Treated with Testicular Extract. *The Lancet*, Dec 4; 196(5075)

Greenfield, S., Kaplan, S., & Ware, J. E., Jr. (1985). Expanding patient involvement in care. Effects on patient outcomes. *Ann Intern Med,* 102(4), 520-528.

Haggard, P., & Whitford, B. (2004). Supplementary motor area provides an efferent signal for sensory suppression. *Brain Res Cogn Brain Res,* 19(1), 52-58.

Hahn, R. A. (1997). The nocebo phenomenon: concept, evidence, and implications for public health. *Prev Med,* 26(5 Pt 1), 607-611.

Haigh, E. A., Moore, M. T., Kashdan, T. B., & Fresco, D. M. (2011). Examination of the factor structure and concurrent validity of the Langer Mindfulness/Mindlessness Scale. *Assessment,* 18(1), 11-26.

Hess, A., Kunesch, E., Classen, J., Hoeppner, J., Stefan, K., & Benecke, R. (1999). Task-dependent modulation of inhibitory actions within the primary motor cortex. *Exp Brain Res,* 124(3), 321-330.

Heyes, C. M. (1994). Social learning in animals: categories and mechanisms. *Biol Rev Camb Philos Soc,* 69(2), 207-231.

Hilty, L., Lutz, K., Maurer, K., Rodenkirch, T., Spengler, C. M., Boutellier, U., et al. (2011). Spinal opioid receptor-sensitive muscle afferents contribute to the fatigue-induced increase in intracortical inhibition in healthy humans. *Exp Physiol,* 96(5), 505-517.

Hodges, P. W. (2011). Pain and motor control: From the laboratory to rehabilitation. *J Electromyogr Kinesiol,* 21(2), 220-228.

Hodges, P. W., & Tucker, K. (2011). Moving differently in pain: a new theory to explain the adaptation to pain. *Pain,* 152(3 Suppl), S90-98.

Holmes, A., Richards, A., & Green, S. (2006). Anxiety and sensitivity to eye gaze in emotional faces. *Brain Cogn*, 60(3), 282-294.

Horne, R., & Weinman, J. (1999). Patients' beliefs about prescribed medicines and their role in adherence to treatment in chronic physical illness. *J Psychosom Res*, 47(6), 555-567.

Huber, A., Lui, F., & Porro, C. A. (2013). Hypnotic susceptibility modulates brain activity related to experimental placebo analgesia. *Pain*, 154(9), 1509-1518.

Hunter, T., Siess, F., & Colloca, L. (2014). Socially induced placebo analgesia: a comparison of a pre-recorded versus live face-to-face observation. *Eur J Pain*, 18(7), 914-922.

Iacoboni, M. (2009). Imitation, empathy, and mirror neurons. *Annu Rev Psychol*, 60, 653-670.

Inghilleri, M., Berardelli, A., Cruccu, G., & Manfredi, M. (1993). Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. *J Physiol*, 466, 521-534.

Jacobs, B. (2000). Biblical origins of placebo. *J R Soc Med*, 93(4), 213-214.

Jellinek, E. M. (1946). Clinical tests on comparative effectiveness of analgesic drugs. *Biometrics*, 2(5), 87-91.

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

Jensen, K. B., Kaptchuk, T. J., Chen, X., Kirsch, I., Ingvar, M., Gollub, R. L., et al. (2015). A Neural Mechanism for Nonconscious Activation of Conditioned Placebo and Nocebo Responses. *Cereb Cortex,* 25(10), 3903-3910.

Jensen, K. B., Kaptchuk, T. J., Kirsch, I., Raicek, J., Lindstrom, K. M., Berna, C., et al. (2012). Nonconscious activation of placebo and nocebo pain responses. *Proc Natl Acad Sci U S A,* 109(39), 15959-15964.

Johnston, N. E., Atlas, L. Y., & Wager, T. D. (2012). Opposing effects of expectancy and somatic focus on pain. *PLoS One,* 7(6), e38854.

Kaplan, S. H., Greenfield, S., & Ware, J. E., Jr. (1989). Assessing the effects of physician-patient interactions on the outcomes of chronic disease. *Med Care,* 27(3 Suppl), S110-127.

Keltner, J. R., Furst, A., Fan, C., Redfern, R., Inglis, B., & Fields, H. L. (2006). Isolating the modulatory effect of expectation on pain transmission: a functional magnetic resonance imaging study. *J Neurosci,* 26(16), 4437-4443.

Kennedy, W. P. (1961). The nocebo reaction. *Med World,* 95, 203-205.

Kidgell, D. J., & Pearce, A. J. (2010). Corticospinal properties following short-term strength training of an intrinsic hand muscle. *Hum Mov Sci,* 29(5), 631-641.

Kienle, G. S., & Kiene, H. (1997). The powerful placebo effect: fact or fiction? *J Clin Epidemiol,* 50(12), 1311-1318.

Kim, J., Ryu, S. B., Lee, S. E., Shin, J., Jung, H. H., Kim, S. J., et al. (2016). Motor cortex stimulation and neuropathic pain: how does motor cortex stimulation affect pain-signaling pathways? *J Neurosurg,* 124(3), 866-876.

Kirsch, I. (1985). Response Expectancy as a Determinant of Experience and Behavior. *American Psychologist*, 40(11):1189-1202.

Kissel, P., Barrucand, D. (1964). *Placebos et effet placebo en medecine.* Paris: Masson.

Klein, P. A., Olivier, E., & Duque, J. (2012). Influence of reward on corticospinal excitability during movement preparation. *J Neurosci*, 32(50), 18124-18136.

Klinger, R., Blasini, M., Schmitz, J., & Colloca, L. (2017). Nocebo effects in clinical studies: hints for pain therapy. *Pain Report*.

Klinger, R., Soost, S., Flor, H., & Worm, M. (2007). Classical conditioning and expectancy in placebo hypoalgesia: a randomized controlled study in patients with atopic dermatitis and persons with healthy skin. *Pain*, 128(1-2), 31-39.

Klosterhalfen, S., Kellermann, S., Braun, S., Kowalski, A., Schrauth, M., Zipfel, S., et al. (2009). Gender and the nocebo response following conditioning and expectancy. *J Psychosom Res*, 66(4), 323-328.

Kong, J., Gollub, R. L., Polich, G., Kirsch, I., Laviolette, P., Vangel, M., et al. (2008). A functional magnetic resonance imaging study on the neural mechanisms of hyperalgesic nocebo effect. *J Neurosci*, 28(49), 13354-13362.

Kotov, R. I., Bellman, S. B., & Watson, D. B. (2004a). Mulltidimensional Iowa Suggestibility Scale (MISS). In S. B. University (Ed.).

Koyama, T., McHaffie, J. G., Laurienti, P. J., & Coghill, R. C. (2005). The subjective experience of pain: where expectations become reality. *Proc Natl Acad Sci U S A*, 102(36), 12950-12955.

Krummenacher, P., Candia, V., Folkers, G., Schedlowski, M., & Schonbachler, G. (2010). Prefrontal cortex modulates placebo analgesia. *Pain*, 148(3), 368-374.

Kudel, I., Edwards, R. R., & Moric, M. (2005). The role of neuroticism, pain catastrophizing and pain-related fear in vigilance to pain: a structural equations approach. A comment on Goubert et al. (2004). *Pain,* 115(1-2), 214-216; author reply 216-217.

Levine, J. D., Gordon, N. C., & Fields, H. L. (1978). The mechanism of placebo analgesia. *Lancet,* 2(8091), 654-657.

Linde, K., Witt, C. M., Streng, A., Weidenhammer, W., Wagenpfeil, S., Brinkhaus, B., et al. (2007). The impact of patient expectations on outcomes in four randomized controlled trials of acupuncture in patients with chronic pain. *Pain,* 128(3), 264-271.

Lipman, J. J., Miller, B. E., Mays, K. S., Miller, M. N., North, W. C., & Byrne, W. L. (1990). Peak B endorphin concentration in cerebrospinal fluid: reduced in chronic pain patients and increased during the placebo response. *Psychopharmacology (Berl),* 102(1), 112-116.

Liuzza, M. T., Candidi, M., Sforza, A. L., & Aglioti, S. M. (2015). Harm avoiders suppress motor resonance to observed immoral actions. *Soc Cogn Affect Neurosci,* 10(1), 72-77.

Loehr, J. D. (2013). Sensory attenuation for jointly produced action effects. *Front Psychol,* 4, 172.

Lorber, W., Mazzoni, G., & Kirsch, I. (2007). Illness by suggestion: expectancy, modeling, and gender in the production of psychosomatic symptoms. *Ann Behav Med,* 33(1), 112-116.

Lui, F., Colloca, L., Duzzi, D., Anchisi, D., Benedetti, F., & Porro, C. A. (2010). Neural bases of conditioned placebo analgesia. *Pain,* 151(3), 816-824.

Lund, K., Petersen, G. L., Erlandsen, M., De Pascalis, V., Vase, L., Jensen, T. S., et al. (2015). The magnitude of placebo analgesia effects depends on how they are conceptualized. *J Psychosom Res*, 79(6), 663-668.

Lutz, K., Pedroni, A., Nadig, K., Luechinger, R., & Jancke, L. (2012). The rewarding value of good motor performance in the context of monetary incentives. *Neuropsychologia*, 50(8), 1739-1747.

Lyby, P. S., Aslaksen, P. M., & Flaten, M. A. (2010). Is fear of pain related to placebo analgesia? *J Psychosom Res*, 68(4), 369-377.

Lyby, P. S., Aslaksen, P. M., & Flaten, M. A. (2011). Variability in placebo analgesia and the role of fear of pain--an ERP study. *Pain*, 152(10), 2405-2412.

Maarrawi, J., Peyron, R., Mertens, P., Costes, N., Magnin, M., Sindou, M., et al. (2007). Motor cortex stimulation for pain control induces changes in the endogenous opioid system. *Neurology*, 69(9), 827-834.

Maganaris, C.N., Collins, D., & Sharp, M. (2000). Expectancy effects and strength training: do steroids make a difference? *Sport Psychologist*, 14 (Pt 3): 272-8

Markland, D., & Hardy, L. (1997). On the factorial and construct validity of the Intrinsic Motivation Inventory: conceptual and operational concerns. *Res Q Exerc Sport*, 68(1), 20-32.

Marotta, A., Ferre, E. R., & Haggard, P. (2015). Transforming the thermal grill effect by crossing the fingers. *Curr Biol*, 25(8), 1069-1073.

Mathews, A., Mackintosh, B., & Fulcher, E. P. (1997). Cognitive biases in anxiety and attention to threat. *Trends Cogn Sci*, 1(9), 340-345.

Mathis, J., de Quervain, D., & Hess, C. W. (1998). Dependence of the transcranially induced silent period on the 'instruction set' and the individual reaction time. *Electroencephalogr Clin Neurophysiol,* 109(5), 426-435.

Mazzoni, G., Foan, L., Hyland, M. E., & Kirsch, I. (2010). The effects of observation and gender on psychogenic symptoms. *Health Psychol,* 29(2), 181-185.

McClung, M., & Collins, D. (2007). "Because I know it will!": placebo effects of an ergogenic aid on athletic performance. *J Sport Exerc Psychol,* 29(3), 382-394.

Mehta, S., Rice, D., Janzen, S., Pope, J. E., Harth, M., Shapiro, A. P., et al. (2016). Mood, Disability, and Quality of Life among a Subgroup of Rheumatoid Arthritis Individuals with Experiential Avoidance and Anxiety Sensitivity. *Pain Res Manag,* 2016, 7241856.

Meyer, T. J., Miller, M. L., Metzger, R. L., & Borkovec, T. D. (1990). Development and validation of the Penn State Worry Questionnaire. *Behav Res Ther,* 28(6), 487-495.

Mills, K. R., Boniface, S. J., & Schubert, M. (1992). Magnetic brain stimulation with a double coil: the importance of coil orientation. *Electroencephalogr Clin Neurophysiol,* 85(1), 17-21.

Morton, D. L., Brown, C. A., Watson, A., El-Deredy, W., & Jones, A. K. (2010). Cognitive changes as a result of a single exposure to placebo. *Neuropsychologia,* 48(7), 1958-1964.

Morton, D. L., Watson, A., El-Deredy, W., & Jones, A. K. (2009). Reproducibility of placebo analgesia: Effect of dispositional optimism. *Pain,* 146(1-2), 194-198.

Nes, L. S., & Segerstrom, S. C. (2006). Dispositional optimism and coping: a meta-analytic review. *Pers Soc Psychol Rev,* 10(3), 235-251.

Neukirch, N., & Colagiuri, B. (2015). The placebo effect, sleep difficulty, and side effects: a balanced placebo model. *J Behav Med,* 38(2), 273-283.

Ober, K., Benson, S., Vogelsang, M., Bylica, A., Gunther, D., Witzke, O., et al. (2012). Plasma noradrenaline and state anxiety levels predict placebo response in learned immunosuppression. *Clin Pharmacol Ther,* 91(2), 220-226.

Olsson, A., & Phelps, E. A. (2007). Social learning of fear. *Nat Neurosci,* 10(9), 1095-1102.

Osman, A., Breitenstein, J. L., Barrios, F. X., Gutierrez, P. M., & Kopper, B. A. (2002). The Fear of Pain Questionnaire-III: further reliability and validity with nonclinical samples. *Journal of Behavioral Medicine,* 25 (2), 155-173.

Pagano, R. L., Fonoff, E. T., Dale, C. S., Ballester, G., Teixeira, M. J., & Britto, L. R. (2012). Motor cortex stimulation inhibits thalamic sensory neurons and enhances activity of PAG neurons: possible pathways for antinociception. *Pain,* 153(12), 2359-2369.

Pazzaglia, C., Testani, E., Giordano, R., Padua, L., & Valeriani, M. (2016). Expectation to feel more pain disrupts the habituation of laser-pain rating and laser-evoked potential amplitudes. *Neuroscience,* 333, 244-251.

Peciña, M., Azhar, H., Love, T. M., Lu, T., Fredrickson, B. L., Stohler, C. S., et al. (2013). Personality trait predictors of placebo analgesia and neurobiological correlates. *Neuropsychopharmacology,* 38(4), 639-646.

Peciña, M., Stohler, C. S., & Zubieta, J. K. (2014). Neurobiology of placebo effects: expectations or learning? *Soc Cogn Affect Neurosci,* 9(7), 1013-1021.

Petrovic, P., Kalso, E., Petersson, K. M., & Ingvar, M. (2002). Placebo and opioid analgesia-- imaging a shared neuronal network. *Science,* 295(5560), 1737-1740.

Peyron, R., Faillenot, I., Mertens, P., Laurent, B., & Garcia-Larrea, L. (2007). Motor cortex stimulation in neuropathic pain. Correlations between analgesic effect and hemodynamic changes in the brain. A PET study. *Neuroimage,* 34(1), 310-321.

Ploghaus, A., Narain, C., Beckmann, C. F., Clare, S., Bantick, S., Wise, R., et al. (2001). Exacerbation of pain by anxiety is associated with activity in a hippocampal network. *J Neurosci,* 21(24), 9896-9903.

Pollo, A., Carlino, E., & Benedetti, F. (2008). The top-down influence of ergogenic placebos on muscle work and fatigue. *Eur J Neurosci,* 28(2), 379-388.

Pollo, A., Carlino, E., Vase, L., & Benedetti, F. (2012). Preventing motor training through nocebo suggestions. *Eur J Appl Physiol,* 112(11), 3893-3903.

Pollo, A., Vighetti, S., Rainero, I., & Benedetti, F. (2003). Placebo analgesia and the heart. *Pain,* 102(1-2), 125-133.

Price, D. D., Craggs, J., Verne, G. N., Perlstein, W. M., & Robinson, M. E. (2007). Placebo analgesia is accompanied by large reductions in pain-related brain activity in irritable bowel syndrome patients. *Pain,* 127(1-2), 63-72.

Price, D. D., Milling, L. S., Kirsch, I., Duff, A., Montgomery, G. H., & Nicholls, S. S. (1999). An analysis of factors that contribute to the magnitude of placebo analgesia in an experimental paradigm. *Pain,* 83(2), 147-156.

Reiss, S., Peterson, R. A., Gurskey, D. M., & McNally, R. J. (1986). Anxiety sensitivity, anxiety frequency, and the prediction of fearfulness. *Behaviour Research and Therapy*, 24, 1-8.

Robbins, D. W., Goodale, T. L., Docherty, D., Behm, D. G., & Tran, Q. T. (2010). The effects of load and training pattern on acute neuromuscular responses in the upper body. *J Strength Cond Res,* 24(11), 2996-3007.

Rodriguez-Raecke, R., Doganci, B., Breimhorst, M., Stankewitz, A., Buchel, C., Birklein, F., et al. Insular cortex activity is associated with effects of negative expectation on nociceptive long-term habituation. *J Neurosci,* 30(34), 11363-11368.

Ross, M., & Olson, J. M. (1981). An expectancy-attribution model of the effects of placebos. *Psychol Rev,* 88(5), 408-437.

Rutgen, M., Seidel, E. M., Riecansky, I., & Lamm, C. (2015). Reduction of empathy for pain by placebo analgesia suggests functional equivalence of empathy and first-hand emotion experience. *J Neurosci,* 35(23), 8938-8947.

Rutgen, M., Seidel, E. M., Silani, G., Riecansky, I., Hummer, A., Windischberger, C., et al. (2015). Placebo analgesia and its opioidergic regulation suggest that empathy for pain is grounded in self pain. *Proc Natl Acad Sci U S A,* 112(41), E5638-5646.

Ryan, R. M. (1982). Control and information in the intrapersonal shepre: an extension of cognitive evaluation theory. *J of Personality and Social Psychology,* 43, 450-461.

Saisanen, L., Pirinen, E., Teitti, S., Kononen, M., Julkunen, P., Maatta, S., et al. (2008). Factors influencing cortical silent period: optimized stimulus location, intensity and muscle contraction. *J Neurosci Methods,* 169(1), 231-238.

Sambo, C. F., Torta, D. M., Gallace, A., Liang, M., Moseley, G. L., & Iannetti, G. D. (2013). The temporal order judgement of tactile and nociceptive stimuli is impaired by crossing the hands over the body midline. *Pain,* 154(2), 242-247.

Sawamoto, N., Honda, M., Okada, T., Hanakawa, T., Kanda, M., Fukuyama, H., et al. (2000). Expectation of pain enhances responses to nonpainful somatosensory stimulation in the anterior cingulate cortex and parietal operculum/posterior insula: an event-related functional magnetic resonance imaging study. *J Neurosci,* 20(19), 7438-7445.

Scheier, M. F., Carver, C. S., & Bridges, M. W. (1994). Distinguishing optimism from neuroticism (and trait anxiety, self-mastery, and self-esteem): a reevaluation of the Life Orientation Test. *J Pers Soc Psychol*, 67(6), 1063-1078.

Schutz-Bosbach, S., Avenanti, A., Aglioti, S. M., & Haggard, P. (2009). Don't do it! Cortical inhibition and self-attribution during action observation. *J Cogn Neurosci*, 21(6), 1215-1227.

Schwarz, K. A., Pfister, R., & Buchel, C. (2016). Rethinking Explicit Expectations: Connecting Placebos, Social Cognition, and Contextual Perception. *Trends Cogn Sci*, 20(6), 469-480.

Schweiger, A., & Parducci, A. (1981). Nocebo: the psychologic induction of pain. *Pavlov J Biol Sci*, 16(3), 140-143.

Schweinhardt, P., Seminowicz, D. A., Jaeger, E., Duncan, G. H., & Bushnell, M. C. (2009). The anatomy of the mesolimbic reward system: a link between personality and the placebo analgesic response. *J Neurosci*, 29(15), 4882-4887.

Scott, D. J., Stohler, C. S., Egnatuk, C. M., Wang, H., Koeppe, R. A., & Zubieta, J. K. (2007). Individual differences in reward responding explain placebo-induced expectations and effects. *Neuron*, 55(2), 325-336.

Scott, D. J., Stohler, C. S., Egnatuk, C. M., Wang, H., Koeppe, R. A., & Zubieta, J. K. (2008). Placebo and nocebo effects are defined by opposite opioid and dopaminergic responses. *Arch Gen Psychiatry*, 65(2), 220-231.

Segerstrom, S. C. (2000). Personality and the immune system: models, methods, and mechanisms. *Ann Behav Med*, 22(3), 180-190.

Severeijns, R., Vlaeyen, J. W., van den Hout, M. A., & Weber, W. E. (2001). Pain catastrophizing predicts pain intensity, disability, and psychological distress independent of the level of physical impairment. *Clin J Pain*, 17(2), 165-172.

Shapiro, A. K. (1968). Semantics of the placebo. *Psychiatr Q*, 42(4), 653-695.

Sidani, S., Miranda, J., Epstein, D. R., Bootzin, R. R., Cousins, J., & Moritz, P. (2009). Relationships between personal beliefs and treatment acceptability, and preferences for behavioral treatments. *Behav Res Ther*, 47(10), 823-829.

Silva, G. D., Lopes, P. S., Fonoff, E. T., & Pagano, R. L. (2015). The spinal anti-inflammatory mechanism of motor cortex stimulation: cause of success and refractoriness in neuropathic pain? *J Neuroinflammation*, 12, 10.

Singer, T., Seymour, B., O'Doherty, J., Kaube, H., Dolan, R. J., & Frith, C. D. (2004). Empathy for pain involves the affective but not sensory components of pain. *Science*, 303(5661), 1157-1162.

Sinke, C., Schmidt, K., Forkmann, K., & Bingel, U. (2016). Expectation influences the interruptive function of pain: Behavioural and neural findings. *Eur J Pain*.

Spielberger, C. D. (1983). *Manual for the State-Trait Anxiety Inventory STAI (form Y)*. Palo Alto, CA: Consulting Psychologist Press Inc.

Staats, P. S., Staats, A., & Hekmat, H. (2001). The additive impact of anxiety and a placebo on pain. *Pain Med*, 2(4), 267-279.

Starfield, B., Wray, C., Hess, K., Gross, R., Birk, P. S., & D'Lugoff, B. C. (1981). The influence of patient-practitioner agreement on outcome of care. *Am J Public Health*, 71(2), 127-131.

Stefan, K., Wycislo, M., & Classen, J. (2004). Modulation of associative human motor cortical plasticity by attention. *J Neurophysiol*, 92(1), 66-72.

Stewart-Williams, S., & Podd, J. (2004). The placebo effect: dissolving the expectancy versus conditioning debate. *Psychol Bull*, 130(2), 324-340.

Stewart, M. A. (1995). Effective physician-patient communication and health outcomes: a review. *CMAJ*, 152(9), 1423-1433.

Stovner, L. J., Oftedal, G., Straume, A., & Johnsson, A. (2008). Nocebo as headache trigger: evidence from a sham-controlled provocation study with RF fields. *Acta Neurol Scand Suppl*, 188, 67-71.

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

Swider, K., & Babel, P. (2013). The effect of the sex of a model on nocebo hyperalgesia induced by social observational learning. *Pain*, 154(8), 1312-1317.

Timm, J., SanMiguel, I., Keil, J., Schroger, E., & Schonwiesner, M. (2014). Motor intention determines sensory attenuation of brain responses to self-initiated sounds. *J Cogn Neurosci*, 26(7), 1481-1489.

Tinazzi, M., Farina, S., Tamburin, S., Facchini, S., Fiaschi, A., Restivo, D., et al. (2003). Task-dependent modulation of excitatory and inhibitory functions within the human primary motor cortex. *Exp Brain Res*, 150(2), 222-229.

Trost, Z., Strachan, E., Sullivan, M., Vervoort, T., Avery, A. R., & Afari, N. (2015). Heritability of pain catastrophizing and associations with experimental pain outcomes: a twin study. *Pain*, 156(3), 514-520.

Tsakiris, M., & Haggard, P. (2005). Experimenting with the acting self. *Cogn Neuropsychol*, 22(3), 387-407.

Tsubokawa, T., Katayama, Y., Yamamoto, T., Hirayama, T., & Koyama, S. (1991). Treatment of thalamic pain by chronic motor cortex stimulation. *Pacing Clin Electrophysiol*, 14(1), 131-134.

van Laarhoven, A. I., Vogelaar, M. L., Wilder-Smith, O. H., van Riel, P. L., van de Kerkhof, P. C., Kraaimaat, F. W., et al. (2011a). Induction of nocebo and placebo effects on itch and pain by verbal suggestions. *Pain*.

Vogtle, E., Barke, A., & Kroner-Herwig, B. (2013). Nocebo hyperalgesia induced by social observational learning. *Pain*, 154(8), 1427-1433.

Vogtle, E., Kroner-Herwig, B., & Barke, A. (2016). Nocebo hyperalgesia: contributions of social observation and body-related cognitive styles. *J Pain Res*, 9, 241-249.

Voss, M., Ingram, J. N., Haggard, P., & Wolpert, D. M. (2006). Sensorimotor attenuation by central motor command signals in the absence of movement. *Nat Neurosci*, 9(1), 26-27.

Voudouris, N. J., Peck, C. L., & Coleman, G. (1985). Conditioned placebo responses. *J Pers Soc Psychol*, 48(1), 47-53.

Voudouris, N. J., Peck, C. L., & Coleman, G. (1990). The role of conditioning and verbal expectancy in the placebo response. *Pain*, 43(1), 121-128.

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

Wager, T. D., Scott, D. J., & Zubieta, J. K. (2007). Placebo effects on human mu-opioid activity during pain. *Proc Natl Acad Sci U S A*, 104(26), 11056-11061.

Waszak, F., Cardoso-Leite, P., & Hughes, G. (2012). Action effect anticipation: neurophysiological basis and functional consequences. *Neurosci Biobehav Rev*, 36(2), 943-959.

Webster, R. K., Weinman, J., & Rubin, G. J. (2016). A systematic review of factors that contribute to nocebo effects. *Health Psychol*, 35(12), 1334-1355.

Werhahn, K. J., Behrang-Nia, M., Bott, M. C., & Klimpe, S. (2007). Does the recruitment of excitation and inhibition in the motor cortex differ? *J Clin Neurophysiol*, 24(5), 419-423.

Wickramasekera, I. (1980). A conditioned response model of the placebo effect predictions from the model. *Biofeedback Self Regul*, 5(1), 5-18.

Wolpert, D. M. (1997). Computational approaches to motor control. *Trends Cogn Sci*, 1(6), 209-216.

Yoshida, W., Seymour, B., Koltzenburg, M., & Dolan, R. J. (2013). Uncertainty increases pain: evidence for a novel mechanism of pain modulation involving the periaqueductal gray. *J Neurosci*, 33(13), 5638-5646.

Yu, R., Gollub, R. L., Vangel, M., Kaptchuk, T., Smoller, J. W., & Kong, J. (2014). Placebo analgesia and reward processing: integrating genetics, personality, and intrinsic brain activity. *Hum Brain Mapp*, 35(9), 4583-4593.

Zubieta, J. K., Bueller, J. A., Jackson, L. R., Scott, D. J., Xu, Y., Koeppe, R. A., et al. (2005). Placebo effects mediated by endogenous opioid activity on mu-opioid receptors. *J Neurosci*, 25(34), 7754-7762.

Zubieta, J. K., Yau, W. Y., Scott, D. J., & Stohler, C. S. (2006). Belief or Need? Accounting for individual variations in the neurochemistry of the placebo effect. *Brain Behav Immun*, 20(1), 15-26.

Zuckerman, M. (1994). *Behavioral expressions on biosocial bases of sensation-seeking*. New York Cambridge University Press.