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Cognitive and affective mechanisms of pain modulation and their neuronal and neurochemical correlates

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ABBREVIATIONS

ACC	Anterior cingulate cortex
BOLD	Blood oxygen level dependent
CBD	Cannabidiol
dlPFC	Dorsolateral prefrontal cortex
fMRI	Functional magnetic resonance imaging
GABA	γ-aminobutyric acid
HPA axis	Hypo-thalamic-pituitary-adrenal axis
NFR	Nociceptive flexion reflex
NMDA	N-methyl-D-aspartate
OFC	Orbitofrontal cortex
PAG	Periaqueductal grey
PET	Positron emission tomography
PFC	Prefrontal cortex
PRISM	Pictorial representation of illness and self measure
RVM	Rostral ventromedial medulla
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
SIA	Stress-induced analgesia
SIH	Stress-induced hyperalgesia
THC	Tetrahydrocannabinol

1 INTRODUCTION

Pain has a protective function which helps us to avoid further injury and to anticipate future harm and therefore has a central role in our lives. However, if pain becomes chronic, its regulation becomes essential. Healthy individuals have a number of mechanisms to inhibit unnecessary pain and thereby avoid unnecessary suffering. To understand those mechanisms in healthy individuals and to find alterations of them in patients with chronic pain, may offer paths for treating their pain. The goal of this dissertation was to further characterize stress and controllability as two factors that contribute to pain regulation. In the following chapters, a number of pain regulatory mechanisms will be introduced and previous work on behavioral, neuronal and neurochemical aspects will be described. Where possible, alterations in chronic pain will be briefly addressed. The effects of stress and control will be discussed in more detail.

1.1 Cognitive and emotional modulation of pain

The experience of pain is modulated by cognitive and emotional factors. While a positive emotional state inhibits the experience of pain, negative emotional states increase pain perception (Villemure & Bushnell, 2009). Cognitive factors that modulate the perception of pain include the attentional focus of the individual on pain (Torta, Legrain, Mouraux, & Valentini, 2017), the predictability (Arntz, van Eck, & de Jong, 1991) or controllability (Bräscher, Becker, Hoeppli, & Schweinhardt, 2016) of the context in which the individual perceives the pain, and the expectation, which the individual has towards a painful stimulus. But especially chronic pain also affects the cognitive and emotional processing of an individual. For example, it was shown that performance in an attentional task was worse in patients with chronic pain. In this study, patients with high chronic pain intensities were particularly impaired, while patients with low pain intensities were able to compensate the attentional demands of their chronic pain by switching their attention between the cognitive task and the pain (Eccleston, 1995). Hence the cognitive and emotional modulation of pain can be viewed in a feedback loop between pain, emotion and cognition (Bushnell, Čeko, & Low, 2013), see Figure 1. In the following a number of pain modulatory factors are discussed, without however claiming completeness.

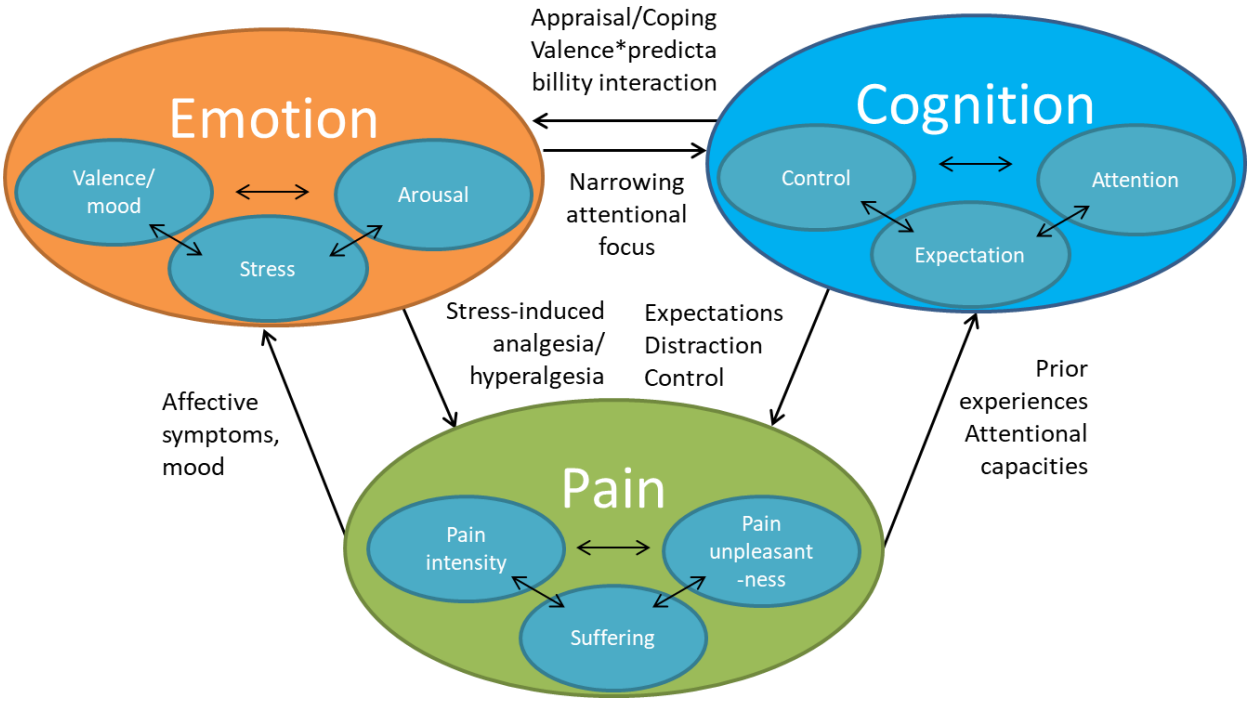


Figure 1: *A common theoretical framework illustrating the effects of cognitive and emotional factors on pain, reversed effects of pain on cognition and emotional state, as well as reciprocal within each domain. Based on (Bushnell et al., 2013).*

The attentional modulation of pain has been shown by several studies using distraction paradigms. For example, in a study on somatic and visceral pain, an auditory and a painful stimulus were presented to the subjects. Subjects were either asked to attend to the painful stimulus (attention) or to attend to the auditory stimulus (distraction). They showed reduced pain intensity ratings in the distraction condition (Dunckley et al., 2007). Other studies used auditory distraction (Boyle, El-Deredy, Martínez, Bentley, & Jones, 2008), basic visual stimuli (Miron, Duncan, & Bushnell, 1989), the Stroop task (Bantick et al., 2002), or a mental arithmetic task (Hodes, Howland, Lightfoot, & Cleeland, 1990) to distract their participants and they support the result that focusing attention away from the stimulus has analgesic effects. Additionally, these studies showed that patients with chronic pain had deficiencies in regulating their pain by distracting themselves from the pain (Snijders, Ramsey, Koerselman, & van Gijn, 2010).

A factor that modulates the pain experience before the pain even occurs is the expectancy of the individual towards the pain, i.e. whether the individual expects to experience high pain or little pain in the future. This was shown in an experiment where subjects were trained to expect lower pain intensities when the announcement phase of a stimulus was shorter. Subjects subsequently reported lower pain ratings

when they expected lower stimulus intensities (Koyama, McHaffie, Laurienti, & Coghill, 2005). The expectations towards the pain certainly depend on prior experiences of an individual (Dar, Ariely, & Frenk, 1995) and therefore may explain part of the inter-individual variation in pain sensitivity (Coghill, McHaffie, & Yen, 2003). Expectancy and prior learning experiences have further been shown to mediate placebo and nocebo responses. Placebo analgesia can be elicited by verbal instructions that create expectations of analgesia and recall previous experiences of pain relief (Price et al., 1999). Nocebo responses, on the other hand, are elicited by verbal instructions that create expectations of hyperalgesia (Colloca, Sigaudo, & Benedetti, 2008).

A factor related to expectancy is the predictability of pain. If pain is experienced under predictable conditions, the organism can prepare for the painful event. The predictability of the painful stimulus therefore has critical influence on the experience of the pain. This was shown in healthy participants, who could tolerate lower pain intensities if the intensity of a painful stimulus was not predictable to them (Arntz et al., 1991). They perceived pain as more intense and unpleasant when the stimulus onset was either not predictable in time or by a predictive cue (Carlsson et al., 2006). Hence there are two aspects of predictability that have been shown to modulate pain: knowledge about the nature of the painful stimulation (e.g. intensity), and information about the timing of the stimulus (e.g. cue or timing) (Miller, 1981). The lack of a predictive cue can furthermore enhance the modulation of pain by an emotional context. While positive and negative pictures had little influence on the nociceptive flexion reflex (NFR) when pain was signaled by a predictive cue, positive pictures decreased NFR and negative pictures increased the NFR when this signal was lacking (Rhudy, Williams, McCabe, Rambo, & Russell, 2006).

On the one hand, this interaction between the effect of predictability on pain and the effect of the individual's emotional state on pain shows that emotional modulation of pain is not independent of cognitive processing. On the other hand, it was also shown that perceived pain intensity and also NFR were modulated by emotional pictures (Rhudy, Williams, McCabe, Nguyen, & Rambo, 2005) or emotional auditory stimuli (Stancak, Ward, & Fallon, 2013) alone. The enhancement of pain intensity by negative emotional pictures is especially pronounced if the pictures depict pain-relevant cues, such as other people in pain (Godinho, Magnin, Frot, Perchet, & Garcia-Larrea, 2006). Emotional modulation of pain is particularly interesting as it has

been shown that the analgesic effects of positive emotions are impaired in patients with fibromyalgia (Kamping, Bomba, Kanske, Diesch, & Flor, 2013), which may be caused by a general deficit in appetitive activation in this group of patients (Rhudy et al., 2013).

1.1.1 Stress-induced analgesia

A phenomenon that is closely related to pain modulation by emotional context is stress. Interestingly stress can either increase pain (stress-induced analgesia, SIA) or decrease pain perception (stress-induced hyperalgesia, SIH). Whether stress reduces or increases pain perception depends on the arousal level associated with stress (Woodhams, Chapman, Finn, Hohmann, & Neugebauer, 2017). Paradigms that used negative visual or negative auditory information (Rhudy et al., 2005; Stancak et al., 2013) to induce negative emotional states elicit only low to moderate arousal levels and tend to exacerbate pain (SIH). On the other hand paradigms that induced stress by social isolation (Puglisi-Allegra & Oliverio, 1983), exposure to mental arithmetic plus noise (Flor & Grüsser, 1999) or painful electrical stimulation (Willer, Dehen, & Cambier, 1981) elicit negative emotions with high arousal levels and inhibit pain (SIA).

Besides the intensity of the stressor, the duration of the stressful event influences the way stress modulates pain (Jennings, Okine, Roche, & Finn, 2014). Studies that successfully induced SIH used longer stressors, such as separating rats' pups from their mothers for 3 hours a day for 2 weeks (van den Wijngaard et al., 2012) or restraint stress for 1 hour and up to 40 days (Gameiro et al., 2006). On the other hand studies that successfully induced SIA used shorter stressors, such as stressing rats with 3 to 30 minutes of electrical foot shock (Lewis, Cannon, & Liebeskind, 1980) or a one-time immobilization of rats for 90 minutes (Costa, Smeraldi, Tassorelli, Greco, & Nappi, 2005). This latter study directly compared the effects of long and short stress induction on pain by also repeating the immobilization over a period of 7 days. When using this repetitive long stressor, they found hyperalgesic rather than analgesic effects.

Chronic pain conditions go along with an increased prevalence of comorbid stress-related disorders such as posttraumatic stress disorder (Vaegter, Andersen, Harvold, Andersen, & Graven-Nielsen, 2017), depression or anxiety disorders (Gerhardt et al., 2011; McWilliams, Cox, & Enns, 2003). Furthermore, a large proportion of patients

with fibromyalgia perceive stress as an aggravating factor of their pain (Okifuji & Turk, 2002) and show elevated stress reactivity (Thieme, Turk, Gracely, Maixner, & Flor, 2015) and an impaired circadian rhythm of blood cortisol levels (Crofford et al., 2004). It is thus not surprising that those patients also show altered modulation of pain by stress. In a study using the "Trierer Social Stress Test", a standardized procedure to induce psychosocial stress, it was shown that patients with fibromyalgia displayed a SIH effect on mechanical pain measures, which could not be observed in the healthy control sample (Crettaz et al., 2013). However, it should be noted that both, the patients and the control sample, showed SIH when tested with thermal pain measures. Therefore the exact mechanisms of stress modulation in chronic pain remain to be elucidated. Additional support for altered pain modulation by stress in chronic pain comes from a study that induced pain and stress by a 1 hour mental task that provoked more head and neck pain in patients with fibromyalgia compared to healthy participants (Nilsen et al., 2007). Conclusions from the latter study about mechanisms are, however, limited as pain induction and stress induction are not separable in the study design.

1.1.2 Controllability

The ability to control negative events is essential for the well-being of an individual and uncontrollable environments have been associated with mental disorders such as depression (Abramson, Seligman, & Teasdale, 1978) or post-traumatic stress disorder (Foa, Zinbarg, & Rothbaum, 1992). Early studies on the effects of uncontrollable electric stimulation were done in dogs, which learned to jump over a barrier to avoid painful stimulation. Interestingly this relatively easily learned association was not acquired by dogs that were previously exposed to uncontrollable painful stimulation (Seligman & Maier, 1967). This finding was later reproduced in a similar manner in human studies and led to the formulation of the theory of learned helplessness of depression. Depressive patients believe that they cannot control their environment, experience themselves as incompetent and therefore fail to show adaptive behavior to regulate their environment (Seligman, 1972). Although more recent data suggest that this passive behavior in the presence of painful stimulation is not learned but rather the default response to such events, the theory of learned helplessness has critically influenced therapy, clinical research as well as animal models of depressive disorders (Maier & Seligman, 2016).

Already the early studies on controllability were using painful stimulation, which was either controllable or not. Besides the effects on depression, also the effects of control on the painful experience itself were systematically studied. Interestingly, those studies showed that the analgesic effect of control seems to depend on a number of factors. The mere perception of control over a painful stimulation has analgesic effects, however, if the control is actually exerted, the analgesic effect is larger (Mohr, Leyendecker, Petersen, & Helmchen, 2012). The control action has to be exerted by the individual him- or herself and may not be exerted by a third person. Therefore external control does not reduce pain sensation, whereas internal control does (Wiech et al., 2006). These findings can in part be explained by the higher degree of predictability in a context where control is exerted by the individual.

Whether the individual had control over a painful stimulus in the past and therefore expects to have control in the future, affects the emotional response to pain but not the pain sensation itself. In an experiment where one group had control over a painful stimulus and subsequently lost control, those who lost control showed higher fear of the painful stimulation compared to a group who had not experienced prior control over the stimulation. However, the groups showed no differences in pain unpleasantness ratings. Additionally, the performance in a secondary task decreased for participants that previously had control over the pain, which speaks in favor of an increased attentional focus on the painful stimulation when control is lost (Crombez, Eccleston, De Vlieger, Van Damme, & De Clercq, 2008).

For cognitive factors such as perceived control, not only the actual control, but also the perceived control is important. Hence the appraisal of the context as controllable or not is as important as really being able to control the painful experience. The general belief that pain can be controlled by one's own actions (i.e. an internal locus of control) has been associated with lower depression levels in patients with chronic pain (Wong & Anitescu, 2017) and the general belief that pain is determined by factors such as chance or other persons mediates therapy outcomes such as functional disability and suffering in patients with chronic pain (Pereira, Roios, & Pereira, 2017). Therefore, the effect of control is determined by an interaction of personal control beliefs and the actual potential to change the painful context.

1.2 Neuronal correlates of pain and pain modulation

1.2.1 Ascending nociceptive pathways

Painful stimulation is associated with activation in brain areas such as the primary (S1) and secondary (S2) somatosensory cortex, the anterior cingulate cortex (ACC), the insula, the prefrontal cortex (PFC), the amygdala, the thalamus and the periaqueductal grey (PAG) (Duerden & Albanese, 2013). Different parts of this network process different aspects of the pain experience. The sensory aspects of pain, such as intensity, location (Vierck, Whitsel, Favorov, Brown, & Tommerdahl, 2013), or duration of the pain (Khoshnejad et al., 2017) seem to be primarily encoded in S1 and S2. The ACC on the other hand encodes primarily emotional aspects of pain such as its unpleasantness (Bliss, Collingridge, Kaang, & Zhuo, 2016). The insula has been proposed to be involved in both, sensory and affective processing of nociceptive information, whereby the posterior part of the insular cortex predominantly encodes the sensory pain dimension and the anterior part predominantly encodes the affective-motivational dimension (Lu et al., 2016).

A central gateway of incoming nociceptive information is the thalamus, which acts as a gateway in two parallel ascending nociceptive pathways. The medial pathway relays nociceptive information via nuclei in the lateral thalamus to the ACC. This pathway has been proposed to process the affective component of pain. The lateral pathway on the other hand relays nociceptive information via the lateral thalamus to the somatosensory cortices. This pathway has been suggested to be involved in the processing of the sensory component of pain. In accordance with its double role in sensory and affective processing, the insula seems to play a special role in those pathways and receives input from the lateral system, but also projects to the limbic areas, such as the amygdala (Almeida, Roizenblatt, & Tufik, 2004; Treede, Kenshalo, Gracely, & Jones, 1999).

The amygdala, known for its role in emotional learning (LeDoux, 1992), receives nociceptive inputs from the dorsal horn of the spinal cord via the parabrachial area as well as from thalamic pathways. Nociceptive inputs are predominantly received by the basolateral part of the amygdala where they are processed and forwarded as affect-related information to the central nucleus of the amygdala. The central nucleus on the other hand modulates pain behavior through projections to descending pain control centers in the brainstem (Neugebauer, 2015).

It is important to note that activity in the above described pain-related areas is not a specific response to painful stimulation, as it has been shown that the network of brain areas involved in pain processing may also be activated by non-painful stimulation (Iannetti & Mouraux, 2010). However, although not specific for pain, the modulation of responses within this network offers potential for pain modulation.

1.2.2 Descending modulation of pain

One of the first findings on the inhibition of pain was that pain behavior in response to mechanical pain stimulation could be blocked by electrical stimulation in the PAG of rats (Reynolds, 1969). The PAG receives nociceptive input from ascending pain pathways, but also cortical input from the frontal and insular cortex, the amygdala, and the hypothalamus and additionally has projections to the rostral ventromedial medulla (RVM) (Basbaum & Fields, 1984). The RVM is the major source of projections to the dorsal horn of the spinal cord where the first synapses of ascending pain pathways are, and therefore transmission of nociceptive information to higher order centers can be inhibited or enhanced (Millan, 2002).

The high level of interconnectivity of the PAG with cortical areas makes the PAG-RVM system one of the most important targets of the cortical modulation of pain. The PAG and RVM therefore act as relay stations for cortical descending pain control (Stamford, 1995). However, other pathways, such as the dorsal reticular nucleus and ventrolateral medulla have also been implicated in descending pain control (Heinricher, Tavares, Leith, & Lumb, 2009).

How higher order processes in the cortex modulate these systems of descending pain control depends on the nature of the process. For example, attention seems to modulate activity in the insula and somatosensory cortex, while emotional modulation of pain primarily depends on activity in the ACC (Bushnell et al., 2013). This was, for example, shown in a study where auditory distraction led to a decreased pain-related activity in somatosensory cortex (Dunckley et al., 2007). Imaging of the PAG further confirmed activation of descending control systems during distraction (Tracey et al., 2002) and placebo-induced PAG activation during anticipation of pain (Wager et al., 2004). The role of the ACC in the emotional modulation of pain was shown by using pleasant odors that not only reduced the experience of pain unpleasantness, but also decreased pain-related activation of the ACC. Importantly, ACC activity in this study

was also associated with activity in the PAG, which confirms the activation of descending pathways by emotional modulation of pain (Villemure & Bushnell, 2009).
A high overlap between pathways that activate descending modulation and placebo analgesia is implied by the fact that ACC activation is not only modulated by emotional context, but that ACC activation in response to a painful stimulation was reduced in a placebo condition, and this ACC hypoactivation was related to increased activity in the dorsolateral prefrontal (dlPFC) and orbitofrontal cortex (OFC) in an anticipation phase before the stimulation (Wager et al., 2004).
The function of the PFC in pain processing is manifold and includes the cognitive evaluation of the nociceptive information as well as endogenous pain regulation (Seminowicz & Moayedi, 2017). Especially the dlPFC modulates pain via attentional networks and was associated with a down-regulation of the medial thalamic pathway and a modulation of pain-related activation in the anterior insula, and thereby reducing the perceived intensity and unpleasantness of painful stimulation (Lorenz, Minoshima, & Casey, 2003; Peyron et al., 1999).

1.2.3 Neurochemical correlates of the descending modulation of pain

The most commonly known neurotransmitter system involved in pain processing is the opioid system. Opioids play an important role in the descending modulation of pain and exogenously administered opioids seem to unfold their analgesic properties via descending pain-modulatory pathways. This was shown by early studies where the injection of naloxone, an opioid receptor antagonist, into the PAG of rabbits (Tsou & Jang, 1964), or the adjacent third ventricle of rats (Yeung & Rudy, 1980) counteracted the effect of systemic morphine administration. Coupling between ACC and PAG, which was associated with behavioral and neural placebo effects and RVM activation, was blocked by administration of naloxone (Eippert et al., 2009). In a μ-opioid receptor tracer study using positron emission tomography (PET), μ-opioid receptor activation in response to painful stimulation was found in the ACC, PFC, insula, thalamus, hypothalamus, and the amygdala. This opioid receptor activation was further associated with reduced pain ratings (Zubieta et al., 2001). Hence the opioid system is not only involved in pain modulation on the level of the midbrain and brainstem, but also in cortical pain modulation.
Other transmitter systems play important roles in descending inhibition as well. For example, there is evidence that different subregions of the PAG may be involved in

different forms of analgesia. Opioid analgesia appears to be mediated by the ventrolateral PAG while the dorsolateral portion of the PAG mediates non-opioid analgesia (Bandler & Shipley, 1994). This dorsolateral PAG pathway has been characterized as a cannabinoidergic pain control system in a study which showed that the microinjection of a cannabinoid receptor agonist into the dorsolateral PAG produced analgesia in rats, while the injection into the ventrolateral PAG had no significant effect (Martin, Patrick, Coffin, Tsou, & Walker, 1995). The modulatory role of the endocannabinoid system was confirmed by a study which showed that systemic administration of a cannabinoid receptor antagonist evoked significant thermal hyperalgesia (Richardson, Aanonsen, & Hargreaves, 1997) and analgesia produced by electrical stimulation in the PAG was accompanied by release of the endocannabinoid anandamide. This analgesic effect of electrical stimulation was reversed by administration of a cannabinoid receptor antagonist into the ventricles (Walker, Huang, Strangman, Tsou, & Sañudo-Peña, 1999). The endocannabinoid system also modulates cortical pain processing areas. In a human functional magnetic resonance imaging (fMRI) study, systemic administration of THC reduced functional connectivity between the thalamus and S2 and reduced effective connectivity to the hippocampus and the anterior insula (Walter et al., 2016).

Endocannabinoid signaling depends on retrograde signaling, where the release of endocannabinoids from the post-synaptic cell modulates activity of the pre-synaptic neuron (Wilson & Nicoll, 2001). Therefore cannabinoid action is not independent of other neurotransmitter systems. For example, it could be shown that analgesia after systemic administration of a cannabinoid receptor agonist was neutralized by injection of an agonist of the GABA (γ-aminobutyric acid) receptor type A into the RVM (Meng, Manning, Martin, & Fields, 1998). Hyperalgesia induced by systemic administration of an endocannabinoid receptor antagonist was also blocked by the administration of two antagonists of the NMDA (N-methyl-D-aspartate) receptor (Richardson, Aanonsen, & Hargreaves, 1998), which represents an interaction between the endocannabinoid and the glutamatergic transmitter system in descending pain modulation.

Overall, the facilitation of nociception is associated with activity in transmitter systems such as glutamate, histamine, cholecystokinin, melanocortin, and prostaglandins. The inhibition of nociception includes transmission involving GABA, glycine, vasopressin, oxytocin, adenosine, endogenous opioids, and endocannabinoids. A

comprehensive discussion of all the systems exceeds the scope of this introduction. For a review see Butler & Finn (2009) or Millan (2002).

1.2.4 Stress-induced analgesia

Lesion studies in rats have provided first insight into the neuronal representation of SIA and include data supporting the involvement of brain areas of the descending pain modulatory pathways. Lesions to the central nucleus of the amygdala were reported to impede SIA induced by 20 minutes of intermittent foot shock (Werka, 1994). SIA induced by a cold water swim test was further suppressed by lesions in the hypothalamus (Truesdell & Bodnar, 1987) and lesions of the frontal cortex reduced SIA after repetitive short electrical foot shocks, but could not block SIA induced by repetitive 25 second long electrical stimulation (Meagher, Grau, & King, 1989). It therefore seems likely that different types of stressors unfold their analgesic potential via different pathways.

Other studies using suppression or enhancement of neurotransmission in specific brain areas have additionally shown the involvement of the PAG. Microinjection of an opioid receptor antagonist into the ventrolateral PAG of rat pups suppressed SIA induced by exposure to an unfamiliar adult male rat (Wiedenmayer & Barr, 2000). For the involvement of opioidergic neurotransmission in SIA, substantial evidence has been accumulated. Systemic administration of an opioid receptor antagonist suppressed the effects of a stressor on human NFR (Willer et al., 1981). In studies in rodents, naloxone-induced suppression of SIA was shown to be dose dependent (Bodnar, Kelly, Spiaggia, Ehrenberg, & Glusman, 1978) and to depend on opioid receptors that are located in the PAG as shown by microinjections of naloxone into the PAG instead of systemic administration (Miczek, Thompson, & Shuster, 1985).

Whether the hypo-thalamic-pituitary-adrenal (HPA) axis, a system known to regulate neuroendocrine reactions to stress, is involved in SIA is still a topic of debate (Gaab et al., 2017). It was shown that SIA induced by cold water swim stress was diminished after hypophysectomy in rats (Bodnar, Glusman, Brutus, Spiaggia, & Kelly, 1979). On the other hand, SIA was successfully induced in hypophysectomized rats after transauricular electrical stimulation and was suppressed by naloxone administration. Therefore opioid-mediated SIA, induced by brief electrical shocks, seems to be independent of HPA axis functioning (Lewis, Cannon, Chudler, & Liebeskind, 1981).

Early studies implicate that the opioid involvement in SIA depends on the nature of the stressor. While a short and intermittent stressor produced opioid dependent SIA, SIA induced by continuous stress seems to depend on other neurotransmitter systems (Lewis et al., 1980). Non-opioid SIA may be mediated via cannabinoidergic pathways as is suggested by evidence showing that microinjection of a cannabinoid receptor antagonist into the basolateral amygdala attenuated SIA induced by continuous foot shock (Connell, Bolton, Olsen, Piomelli, & Hohmann, 2006) as well as the blockade of the cannabinoid receptor type 1 (CB_1) in the PAG, whereas an opioid receptor antagonist injection into the PAG had no effect on this type of SIA (Hohmann et al., 2005). Other systems that seem to be involved in the mediation of opioid or non-opioid SIA include GABA (Lau & Vaughan, 2014), Serotonin (Yesilyurt et al., 2015), Oxytocin (Robinson et al., 2002) and Glutamate (Onodera et al., 2001).
Human studies on the neurochemical mechanisms of descending inhibitory pathways in SIA are rare. One of those studies could show that the re-exposure to a stimulus which was previously paired with a stressor, induced a SIA response, which was attenuated by systemic administration of an opioid receptor antagonist (Flor, Birbaumer, Schulz, Grüsser, & Mucha, 2002). Salivary cortisol was not associated with the SIA effects in a study using a psychosocial stress paradigm, which suggests that the HPA axis does not seem to mediate this type of SIA (Gaab et al., 2017). There are also only few studies on the neuronal representation of SIA in higher cortical areas in humans. One of those studies showed that SIA effects were associated with activation in the ACC by using fMRI during painful stimulation before and after stress (Yilmaz et al., 2010).
Despite of the growing evidence on the neuronal mechanisms of SIA there are still some gaps in the human literature: 1) It is unknown whether animal findings on the endocannabinoid mediation of SIA can be translated to the human brain. 2) The localization of opioid and cannabinoid action in mediating SIA has not been assessed in humans.

1.2.5 Controllability

There is increasing evidence that the pain modulatory effect of control over a painful stimulation is mediated by an activation of descending pathways via projections of the PFC.

In studies using fMRI in humans, uncontrollable compared to controllable painful stimulation was associated with higher activity in the ACC, insula, S2 and PAG (Salomons, Johnstone, Backonja, & Davidson, 2004; Salomons, Johnstone, Backonja, Shackman, & Davidson, 2007). On the other hand higher anticipatory activation of the ventrolateral PFC during controllable pain was correlated with stronger control-induced analgesia (Salomons et al., 2007). The functional connectivity of the PFC-PAG axis was related to control-induced analgesia in a safety signaling paradigm (Wiech et al., 2014) and pain facilitation during uncontrollable pain was associated with functional connectivity between insula and medial PFC, whereas pain inhibition was associated with functional connectivity between insula and dorsolateral PFC (Bräscher et al., 2016). Therefore PFC activity is not only involved in the activation of the descending inhibitory pain control system in the PAG via top-down processing, but also in descending facilitation during uncontrollable pain. By inhibition of the dorsolateral PFC using transcranial magnetic stimulation its inhibitory effects could be suppressed, which additionally confirms this pathway. Interestingly this effect was only found for the affective dimension of pain, but not the sensory pain component (Borckardt et al., 2011).

PFC activation was further shown to be associated with the coping behavior of the participants in a study on exerted control. Control-related analgesia was not only related to PFC activation, but this activation was also correlated with the subjects' personal belief that pain can be controlled by their own action (internal locus of control). External locus of control on the other hand was negatively associated with this top-down modulation of control-related analgesia (Wiech et al., 2006).

The frontal modulation of control-induced analgesia was divided into modulation by perceived controllability and modulation by exerted controllability: The OFC and mediofrontal cortex selectively responded to perceived control, whereas the PFC responded to exerted control (Mohr et al., 2012).

Despite this fair amount of insight into neural mechanisms of control-induced analgesia some questions remain unanswered: (1) Studies on neurochemical correlates of control-induced analgesia are lacking. (2) The effect of control-induced analgesia on different dimensions of the painful experience remains to be elucidated because of the heterogeneity of the studies in respect to their outcome measures.

1.3 Dimensions of pain and suffering

A better understanding of the mechanisms that drive pain and pain relief is as important as a clear understanding of the outcome measures that we use to assess pain (Ballantyne & Sullivan, 2015). Fifty years ago pain has been described as a multidimensional phenomenon in terms of three dimensions of pain processing: the "sensory-discriminative" dimension, which encompasses intensity as well as spatial and temporal information of the nociceptive input, the "motivational-affective" dimension, which encompasses the aversive drive of the nociceptive input and the "cognitive-evaluative" dimension that puts the nociceptive input in relation to past experiences or control actions (Melzack & Casey, 1968).

In the last decades the "sensory-discriminative" and the "motivational-affective" dimension, mostly just labelled as pain intensity and pain unpleasantness have received most attention in research. Although pain intensity and unpleasantness are highly correlated with each other (Chapman et al., 2001), hypnotic suggestions have been used to selectively alter pain affect without changing the perceived intensity in response to noxious stimuli (Rainville, Duncan, Price, Carrier, & Bushnell, 1997) and also to alter pain intensity with little effect on pain unpleasantness (Rainville, Carrier, Hofbauer, Bushnell, & Duncan, 1999). Furthermore, pain intensity and pain unpleasantness have been related to differential facial response patterns (Kunz, Lautenbacher, LeBlanc, & Rainville, 2012).

Further evidence for separate pain dimensions stems from the case of a stroke patient with lesions in primary and secondary somatosensory cortex, who showed unilateral loss of the sensory-discriminative pain component with a preserved motivational-affective pain component (Ploner, Freund, & Schnitzler, 1999). This effect of lesions in the somatosensory cortex was confirmed in rats with lesions in the hind limb area compared to rats which underwent a sham procedure. The lesioned rats showed a loss of the sensory pain component, indicated by higher paw withdrawal thresholds, but a preserved motivational-affective pain component, as indicated by preserved avoidance of an area that was associated with noxious stimulation (Uhelski, Davis, & Fuchs, 2012).

Later theories extended the pain intensity and pain unpleasantness dimensions by subdividing them into three dimensions. Fields (1999) proposed the term algosity for a sensory stimulus quality that uniquely identifies pain in contrast to other unpleasant stimuli such as itch, the term primary unpleasantness to describe the unpleasantness

of the painful stimulus, which is directly related to its sensory properties and the term secondary unpleasantness to describe an experience that reflects a higher level process which is largely determined by memories and contextual features. An analogous concept was introduced by Price (2000): Next to the nociceptive sensations evoked by a noxious stimulus, he distinguished between the immediate pain unpleasantness, which encompasses feelings that pertain to the present or short-term future and the secondary pain affect, which includes feelings directed towards long-term implications of having pain, such as suffering.

This dimension of suffering that was newly introduced in both the theoretical concepts of Fields (1999) and Price (2000) has been underestimated in its clinical importance and often neglected in research on chronic pain (Ballantyne & Sullivan, 2015). This is especially surprising, given that suffering is the most important factor that drives patients to seek medical attention (Carnevale, 2009). Suffering is a personal experience and has been described as a pronounced state of distress, which threatens the physical or psychological integrity of a person through helplessness, loss of control, and concerns about the future (Cassel, 1982).

With experimental pain paradigms in healthy volunteers Bustan et al. (2015) and Brunner et al. (2017) have shown that suffering can be part of the painful experience that is influenced by enduring characteristics of the person, such as fear of pain and increased private self-consciousness (Brunner et al., 2017). It is unclear from the empirical perspective which other factors affect the experience of pain-related suffering and what might rather affect the aspect of pain intensity or (primary) pain unpleasantness.

1.4 Aims and hypotheses

The aim of this dissertation was to gain further insight into pain inhibitory factors such as stress and controllability.

In study 1 healthy volunteers (n=19) were tested on 3 separate days. In a randomized, double-blinded cross-over design the activation of descending inhibitory pathways by stress was tested after systemic administration of CBD, THC and placebo. Activation of descending pain inhibition was assessed with ratings of pain intensity and pain unpleasantness and the BOLD response in the fMRI. The first hypothesis addressed the role of endocannabinoid signaling in SIA in humans:

1.1. The administration of THC will enhance the reduction of pain intensity and pain unpleasantness ratings after a mental stressor.
1.2. The administration of CBD will suppress the reduction of pain intensity and pain unpleasantness ratings after a mental stressor.

The second hypothesis addresses the modulation of underlying brain circuits involved in cannabinoid-mediated SIA:

2.1. SIA after administration of THC is associated with increased BOLD responses in the amygdala, PAG and ACC.
2.2. SIA after administration of CBD is associated with decreased BOLD responses in the amygdala, PAG and ACC.

In study 2 healthy volunteers (n=26) were tested to assess pain inhibition by manipulation of controllability. Pain inhibition was measured with ratings of pain intensity, pain unpleasantness and pain-related suffering, the electromyogram of the corrugator muscle, skin conductance responses and heart rate.

3.1. Controllable painful stimulation is rated with lower pain intensity, pain unpleasantness and pain-related suffering, when compared to uncontrollable painful stimulation.
3.2. Differences between controllable and uncontrollable painful stimulation in the rating of pain-related suffering will be higher than for pain intensity or pain unpleasantness.

2 ORIGINAL CONTRIBUTIONS

2.1 The influence of tetrahydrocannabidiol and cannabidiol on pain perception and endogenous pain control[1]

[1]Löffler, M., Kamping, S., Grimm, O., Andoh, J., Rohleder, C., Leweke, M., Flor, H. (2018). The influence of tetrahydrocannabinol and cannabidiol on pain perception and endogenous pain control. Article submitted for publication.

Abstract

The role of the endocannabinoid system in pain processing is increasingly gaining attention. Evidence for its involvement in endogenous pain control mechanisms mainly derives from animal research. So far, little is known about the effects of exogenously administered cannabinoids on endogenous pain inhibition.

We employed functional magnetic resonance imaging (fMRI) during stress-induced analgesia (SIA) in 19 healthy humans and examined the influence of the administration of 10mg Tetrahydrocannabinol (THC) or 600mg Cannabidiol (CBD) versus placebo in a within-subjects design. Stress was induced by a cognitively demanding task with increasing noise levels. Electrical perception thresholds, pain thresholds, pain tolerance and perceived pain in response to repetitive electrical painful stimulation were assessed before and after stress exposure. We expected that THC, as an up-regulator of endocannabinoid signaling, would enhance SIA, while CBD, which interferes with cannabinoid signaling, should reduce SIA.

We found that THC administration was associated with a disruption of within-session habituation to the painful stimulus without an enhancement of SIA. CBD neither affected habituation nor SIA. On the neuronal level, we found a modulation of frontal processing of pain by THC in line with assumptions about cannabinoid-mediated pain suppression.

Our results suggest that systemic administration of cannabinoids might interfere with some aspects of the endogenous control of pain. Possible mechanisms of pain suppression are discussed.

Introduction

Analgesic properties of cannabinoids have been shown in a variety of chronic pain patient populations [45; 46] and they are commonly applied for pain relief [69]. On the neuronal and neurochemical level a number of studies support the role of the endocannabinoid system in the central processing of pain. CB1 receptors are expressed in a number of brain regions involved in pain control [29; 65], and thalamic CB1 [57] as well as spinal CB1 and CB2 receptors were upregulated in rat models of chronic neuropathic pain [43; 77]. Microinjection of a CB1 receptor agonist showed antinociceptive CB1 action in the amygdala, thalamus, superior colliculus and the noradrenergic A5 region [50].

In human studies THC and CBD are frequently used drugs to modulate activity of the endocannabinoid system. These exogenously administered cannabinoids target the endocannaboid system with its cannabinoid receptors type 1 (CB1) and type 2 (CB2), their ligands 2-Arachidonoylglycerol (2-AG) and Anandamide and the anandamide degrading enzyme fatty-acid amide hydrolase (FAAH) and the 2-AG-degrading enzyme monoacylglycerol lipase (MGL). While THC acts as a cannabinoid receptor agonist, and unfolds most of its pharmacological effects selectively at the CB1 and CB2 receptors, CBD acts as a partial antagonist at the CB1 receptor [21], but other modes of CBD action may include the inactivation of FAAH, modulation of endocannabinoid reuptake into the cell or interactions with the transient receptor potential channels of vanilloid type-1 and the serotonin receptor 5-HT1A, but remain unclear [47]. Brain imaging has revealed that THC and CBD have opposite effects on activation in the amygdala, anterior cingulate cortex, insular cortex, medial and lateral prefrontal cortex [11], areas also involved in pain processing [23].

However, the exact mechanisms of cannabinoid pain modulation are still unclear. On the one hand studies on the analgesic properties of exogenous administered CB1 agonists or smoked marijuana found analgesic effects [18; 28]. Other studies found no pain modulation [51] or even hyperalgesic effects [8; 51; 68]. A possible mechanism of CB-mediated analgesia is the activation of the descending pain pathway. This pathway was shown to be activated by stress and mediated by eCB signaling [16; 19], however, most of the available data were assessed in animals. These animal studies targeted different areas of the descending inhibitory pain pathway. Administration of rimonabant, a CB1-selective inverse agonist, into the dorsolateral PAG attenuated fear-conditioned analgesia, which was associated with

increased levels of anandamide in the dorsolateral PAG [52]. Inhibiting the degradation of the endogenous cannabinoids anandamide or 2-AG in the PAG, increased the effects of non-opioid stress-induced analgesia (SIA) in rats [31]. Injection of rimonabant into the basolateral nucleus of the amygdala inhibited SIA in rats [17]. Additionally, SIA in mice was prevented by systemic administration as well as local injection of a CB1 receptor agonist into the PAG [40]. Evidence that CB1 knockout mice do not show antinociception after stress exposure in a forced swim test [66] further supports the role of the endocannabinoid system in endogenous pain inhibition via the descending inhibitory pain pathway.

In the present fMRI experiment we investigated the role of the endocannabinoid system in human endogenous pain inhibition. We used blocks of repetitive painful electrical stimulation before and after a cognitive stressor to test the effects of THC and CBD on SIA. We hypothesized that THC would enhance, whereas CBD would disrupt SIA compared to a placebo condition. This modulation of the SIA effect was expected to be associated with increased BOLD response in the amygdala, PAG and ACC after administration of THC, but reduced activity in the same areas after the administration of CBD.

Methods

Subjects

Twenty healthy male volunteers participated in the study. One subject withdrew from participation because of vertigo after substance administration on visit 2. The final sample consisted of 19 male, right-handed volunteers between 19 and 35 years of age (mean age: 24.74, standard deviation (SD) = 4.617; mean body weight: 76.263 kg, SD=9.182, range 63-99 kg). The study was approved by the Ethics Committee of the Medical Faculty Mannheim, University of Heidelberg, Germany and the Federal Institute for Drugs and Medical Devices (BfArM). Written informed consent was obtained from each subject prior to the study and on each examination day (four times in total). Exclusion criteria were cardiovascular or neurological disorders, brain injury, acute or chronic pain, pain medication, lifetime and current substance abuse or dependence and any mental disorders. The subjects were examined by a psychologist using the German version of the Structured Clinical Interview for DSM IV Axis I disorders [72] to exclude subjects with a mental disorder. Additionally, all participants were examined by a registered psychiatrist and blood sampling was

performed for medical chemistry and drug screening. None of the participants reported any physical or mental disorders and their drug tests were negative. Two participants were smokers (one subject with two cigarettes a day, the other with 14 cigarettes a day), the others were non-smokers.

Experimental procedure

In a randomized, double-blind, placebo-controlled study the acute effects of orally administered THC (10 mg), CBD (600 mg) and placebo (vehicle) on SIA were compared. Each participant took part in all arms of the study. Blinding was performed at the University Pharmacy of the University of Heidelberg. Unblinding took place after all participants completed the study. The experiment was spread over four separate days. The first day was a diagnostic session where participants underwent a psychological and medical examination including drug screening. Testing sessions were conducted on days two to four, all separated by at least one week (>3 times elimination half-life of THC: [20] and CBD: [48]). The same tests were performed in each session (see Figure 1 & Table 1).

Monitoring of pharmacokinetics and mental state of the participants

To improve the interindividual comparability of absorption rates and pharmacodynamics, the participants received a standard meal on arrival. Cannabinoids have been shown to modulate mood, anxiety [49] and dissociative symptoms [54]. These symptoms were therefore assessed during the experiment. To monitor pharmacokinetics and mental state, blood samples were taken and the participants completed the state version of the German version of the Spielberger State-Trait Anxiety Inventory (STAI-S) [39], the German version of the Positive and Negative Affect Scale (PANAS) [35], self-assessment manikin (SAM) ratings of valence and arousal [13] and a short version of the Dissociation-Tension-Scale acute (DSS-4) [59; 60]. Blood samples and questionnaire measurements were taken three times: immediately before substance administration (t0), 1 hour after substance administration (t1) and at approximately three hours after substance administration (t2).

Table 1: *Time line of the experimental procedure for each condition (placebo, THC and CBD) for blood samples (t0, t1, t2), drug administration (drug), threshold determinations (thresh pre, thresh post) and painful stimulation blocks (Pain stim pre, pain stim post) before and after the stress induction (stress). All values refer to time since substance administration in minutes.*[1]

Timing	**t0**	**drug**	**t1**	**Thresh pre**	**Pain stim pre**	**stress**	**Pain stim post**	**Thresh post**	**t2**
	M±SD	**M**	**M±SD**	**M±SD**	**M±SD**	**M±SD**	**M±SD**	**M±SD**	**M±SD**
Placebo	-5±3	0	62±1	74±4	99±4	113±4	134±8	140±5	199±9
THC	-5±3	0	61±3	77±9	102±9	115±9	136±9	143±10	205±14
CBD	-6±3	0	62±3	77±10	103±9	117±9	138±10	145±10	209±14

[1] M: mean, SD: standard deviation

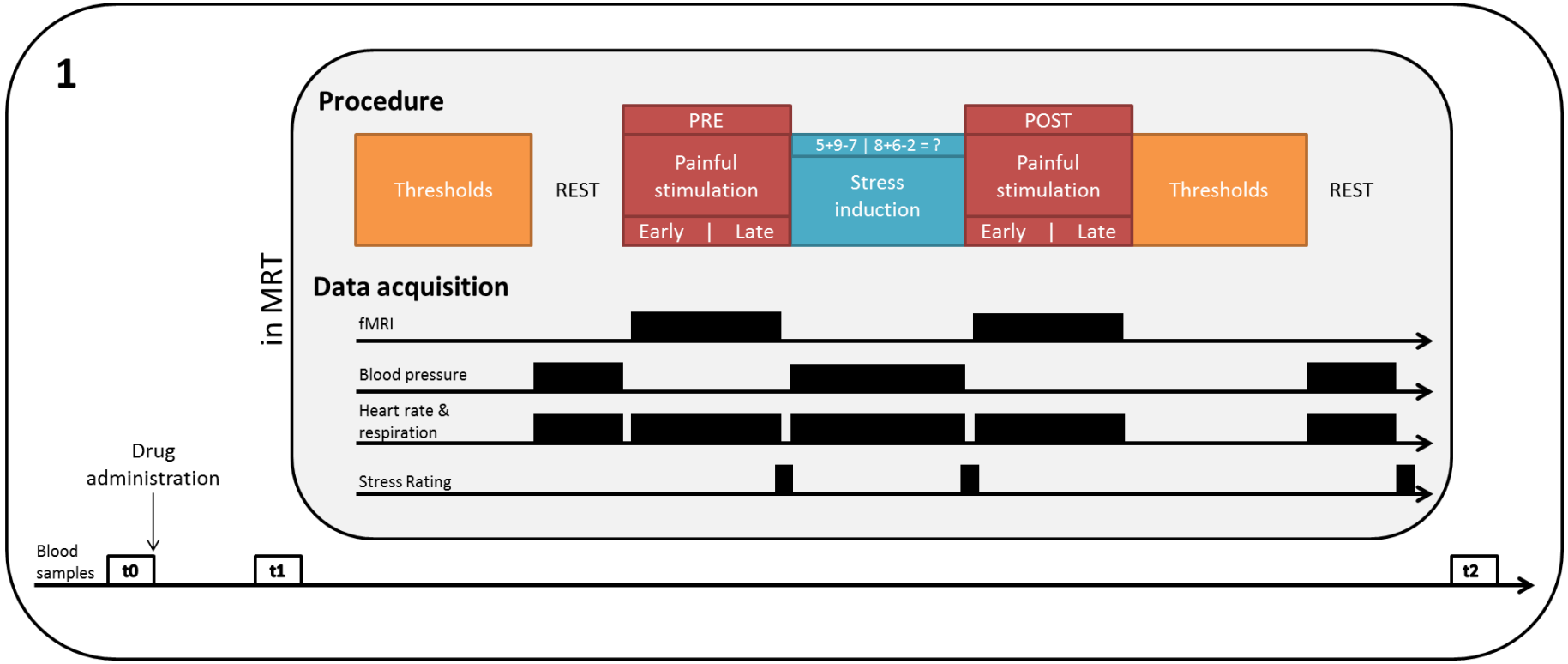


Figure 1: *Structure of the experiment from left to right. At t0 baseline blood levels of THC and CBD were determined. At t1 blood levels were determined at approximately 1 hour after drug administration (see Table 1 for timing details). At t2 blood levels were determined at the end of the experiment. Pain thresholds (orange) and painful stimulation (red: 10 stimulation blocks with duration 11.76 sec each, stimulus duration 2ms, 105 stimuli, inter stimulus interval = 112 ms, which were always followed by off blocks of 11.76 seconds duration) were implemented before (PRE) and after (POST) stress induction with a cognitive demanding task. Blood pressure and heart rate was recorded at baseline (pre-baseline), during the stressor (stress) and after the stressor (post-baseline). Functional magnetic resonance (fMRI) imaging was implemented during painful stimulation. For post-acquisition correction for physiological noise cardiac and respiration data were acquired during fMRI scanning.*

Blood samples were centrifuged at 4°C for 5 min at 4000 rpm. Supernatant was transferred into glass vials (Hycultec GmbH, Beutelsbach, Germany) and immediately frozen and stored at –80°C until liquid-liquid extraction of THC and CBD. During the extraction process, deuterated standards of THC and CBD were added to the serum aliquots, allowing for quantification of cannabinoid blood levels by isotope-dilution LC-MS/MS. The LC-MS/MS system comprised an Agilent 1200 HPLC system (Agilent Technologies® Waldbronn, Germany) coupled to an API 5000 triple quadrupole mass spectrometer (AB Sciex® Darmstadt, Germany). Samples were injected into a Synergi Hydro-RP C18 column (150 x 2 mm, 4 µm, Phenomenex® Aschaffenburg, Gemany) and eluted using a methanol/water gradient with 0.1 % formic acid at a flow rate of 0.5 mL/min. THC and CBD were quantified by tandem electrospray mass spectrometry in positive ion mode (ES+). Product ions were monitored in multiple reaction monitoring (MRM) mode.

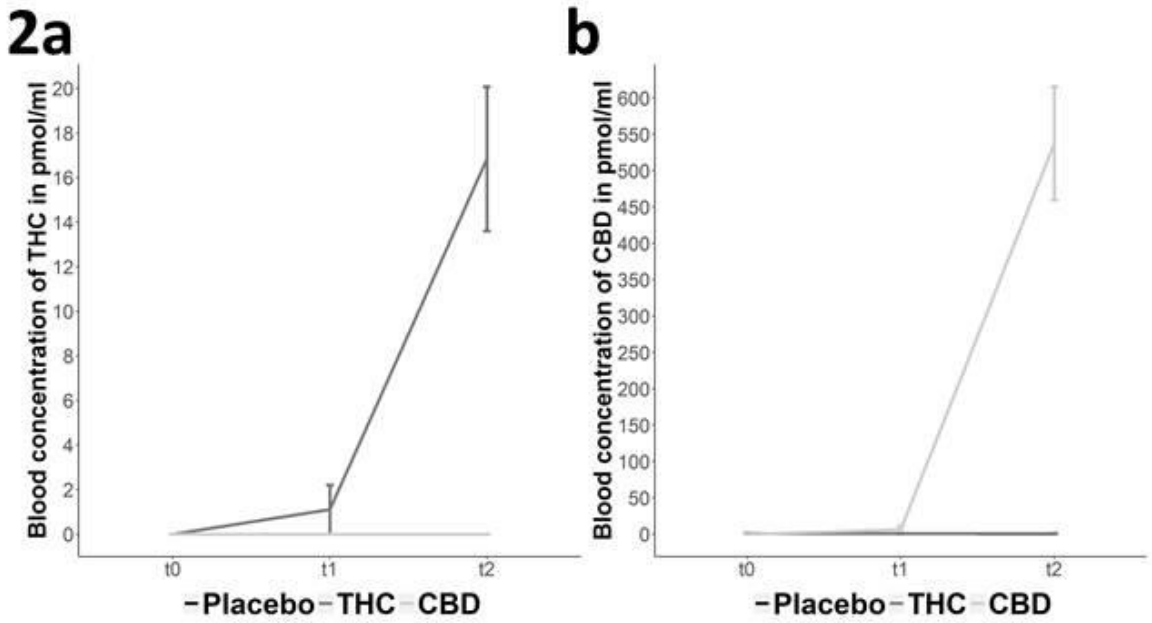


Figure 2: *Blood concentrations of THC and CBD. Black lines: Placebo condition; Grey lines: THC condition; Light grey lines: CBD condition. (a) THC blood concentration is depicted in picomoles per milliliter (pmol/ml) for each condition (Placebo, THC and CBD) and each time point (t0: baseline blood levels, t1: blood levels at approximately 1 hour after drug administration, t2: blood levels at the end of the experiment). (b) CBD blood concentration is depicted in picomoles per milliliter (pmol/ml) for each condition (Placebo, THC and CBD) and each time point (t0: baseline blood levels, t1: blood levels at approximately 1 hour after drug administration, t2: blood levels at the end of the experiment).*

Threshold determination

After substance administration, a pair of subcutaneous needle electrodes (20 mm long, 0.35-mm uninsulated tip, 2-mm² stimulation area, model: 9013R0272, 28G, Alpine Biomed ApS, Skoviunde, Denmark) was placed subcutaneously at the left lower back, 2 cm lateral to the spine, between L1 and L3 (1 mm needle separation) for electrical stimulation. Electrical stimuli were applied using a constant current stimulator (model DS7A; Digitimer, Hertfordshire, England). The experiment was performed using Presentation® software (Version 14.0, http://www.neurobs.com/).

Perception threshold, pain perception threshold and pain tolerance were assessed before and after the stressor. The participants received ascending electrical stimulation trains (Stimulation train: 8 stimuli of 2ms, inter stimulus interval = 112msec, inter train interval = 2sec) via the subcutaneous needle electrodes and were instructed to press a button when they could feel the stimulus for the first time (perception threshold), when the stimulus was painful for the first time (pain threshold) and when they could not tolerate a higher stimulus intensity (pain tolerance). Each measure was determined four times.

After the first threshold determination, the stimulation intensity was calibrated at a perceived pain intensity of 50 percent on a VAS (endpoints “no pain” and “worst pain imaginable”) that was converted to a 0 to 100% scale. The first threshold assessment was discarded and 50 percent of the difference between pain threshold and pain tolerance were added to the pain threshold, to calculate a preliminary stimulation intensity. In test trials (duration 12.544 seconds each, stimulus duration 2ms, 112 stimuli, inter stimulus interval 112msec) the perceived pain intensity was assessed using a VAS. The stimulation intensity was adapted between the test trials to reach a pain intensity rating of 50 out of 100 points, or to reach a rating closest possible to 50. The resulting stimulation intensity of each subject was used for all further procedures.

Pain stimulation before and after stress induction

To examine changes in pain perception and BOLD response to painful stimuli, the participants received trains of electrical stimulation before and after the stressor. They were instructed to look at a fixation cross and received 10 stimulation blocks (duration 11.76 sec each, stimulus duration 2ms, 105 stimuli, inter stimulus interval = 112 ms), which were always followed by off blocks of 11.76 seconds duration. The

perceived levels of pain intensity and pain unpleasantness were assessed after the first ('Early') and the last ('Late') stimulation block using visual analogue scales (VAS) ranging from "no pain" to "worst pain imaginable", and "not unpleasant" to "extremely unpleasant".

Stress induction

The stressor used in this study was mental arithmetic combined with white noise. The mental arithmetic tasks were similar to those from the Konzentrations- und Leistungstest (Concentration and Performance Test: [24; 44]) and were presented by a female voice via earphones. Such mental arithmetic tasks have previously shown to be effective in the induction of stress [26; 76]. Each task consisted of a series of two sets of three numbers (e.g. 5,9,4 and 3,8,11) that had to be added or subtracted. If the sum of the second set was smaller than the sum of the first set, the second result had to be subtracted, if the sum of the first set was smaller than that of the second set, the first result had to be added (i.e. 18+22 in this example). In our experiment an additional third arithmetic operation had to be executed after subtraction or summation (e.g. 40*2) and the subject had to verbally report the final result (i.e. 80 in this example). Each task had to be solved within 30s. In total subjects had to solve 30 tasks on each experimental day, resulting in a duration of the stressor of 15 minutes.

To account for individual cognitive performance, five parallel versions of the mental arithmetic task with varying difficulty (based on the arithmetic operations) were prepared. During the diagnostic session on day one, the individual difficulty level was determined for each participant. For that purpose, 5 tasks of the lowest level of difficulty were presented. If the participant solved at least four of those, the next level was presented. If the participant solved at least four tasks at the second level again, the next level was presented. This was continued until the participant made more than one error within a level or the highest level was reached. One level above the resulting difficulty level was then used on the second day of the experiment for stress induction. The difficulty had to be increased for 5 participants and decreased for one participant between the sessions. The level was considered as too easy if a participant solved more than 20 and too difficult if a participant solved less than 5 tasks. On each day the same set of tasks was used, but presented in a different pseudorandomized order. In order to increase the stressfulness of the task white

noise was presented continuously and increased from 65 to 80 dB from the first to the last arithmetic calculation.

Heart rate, blood pressure, and ratings of the stressor

To assess physiological effects of the stressor, blood pressure was measured with the MR compatible Criticare 506N vital signs monitor (Criticare Systems Inc., Waukesha, USA), using a sampling rate of one per minute. Heart rate was assessed with the built-in pulsoxymeter of the MR, using a sampling rate of 50Hz. Heart rate and blood pressure were measured during a 5 minute resting interval immediately before the stress phase, throughout the 15 minutes of the stress phase and during a 5 minute resting phase at the end of the experiment.

The participants were asked to verbally rate how stressed (0 = not stressed – 100 = extremely stressed) and how relaxed (0 = not relaxed – 100 = extremely relaxed) they were immediately before the stress phase, immediately after the stress phase (indicating how stressed/relaxed they were during the stress phase), and immediately after the last heart rate measurement.

Magnetic resonance imaging

Magnetic resonance images were obtained on a 3-T TRIO Siemens (Erlangen, Germany) scanner (TR = 1.96 seconds, TE = 30 ms, flip angle 76°, slice thickness 3 mm, 36 slices, field of view 220 mm, 3.4 X 3.4 X 3.0 mm voxel size) using an echo planar (EPI) T2* sensitive sequence. Parallel acceleration technique (iPAT) with generalized auto-calibrating partially parallel acquisition (GRAPPA) reconstruction was used with an acceleration factor of 2. Field map images (TR = 468 ms, TE1 = 4.92 ms, TE2 = 7.38 ms, flip angle 60°, slice thickness 3 mm, 36 slices, field of view 220 mm, 3.4 X 3.4 X 3.0 mm voxel size) were obtained for post-acquisition correction of gradient field effects. Cardiac and respiration data were acquired with built-in pulsoxymeter and respiration belt for post-acquisition correction for physiological noise. Additionally, a high-resolution magnetization prepared rapid gradient (3D MPRAGE) (slice thickness 1 mm, TR = 2.3 seconds, TE = 3.03 msec, flip angle 9°, 1 X 1 X 1 mm voxel size) was obtained for each subject on each session.

Statistical analysis

The data were analyzed using R software [63]. Linear mixed effects models were employed for the analysis of ratings and thresholds. Mixed models were implemented using lme4 in R [7] and lmerTest [37] to provide F statistics for fixed effects. The participants' intercepts were entered into the models as subject-level random effects, the estimation method was restricted maximum likelihood (REML), and the covariance matrix was unstructured. All post-hoc tests were corrected for multiple comparisons using Bonferroni corrections.

Stress ratings, blood pressure and heart rate were analyzed using the within-subject factors ‘drug’ (THC, placebo, CBD) and ‘stress’ (‘pre baseline’, ‘stress’, ‘post baseline’). Pain intensity ratings and pain unpleasantness ratings were analyzed using the within-subject factors ‘drug’ (THC, placebo, CBD), ‘time’ (early, late) and ‘stress’ (‘pre’, ‘post’). Perception threshold, pain threshold and pain tolerance were analyzed using the within-subject factors ‘drug’ (THC, placebo, CBD) and ‘stress’ (‘pre’, ‘post’). T-Tests were used to compare initial perception and pain thresholds and pain tolerance.

fMRI analysis

Functional MRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version (5.00), part of FSL (FMRIB's Software Library, http://www.fmrib.ox.ac.uk/fsl). At the first level (within-subjects), preprocessing involved several stages. The first three EPI volumes were deleted to eliminate tissue relaxation artifacts. Motion was corrected using MCFLIRT (motion correction based on FMRIB's Linear Image Registration Tool: [32]), and the resulting six motion-correction parameters were used as regressors in the design matrix. We included nuisance regressors for time points corresponding to motion outliers using the FSL motion outliers program (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLMotionOutliers), which defined outlier time points using the upper threshold for creating box plots or the 75th percentile plus 1.5 times the interquartile range. Non-brain structures were removed using BET (Brain Extraction Tool: [58]). The data were spatially smoothed using a 5-mm Gaussian kernel of full-width at half maximum. Each dataset was normalized by a single scaling factor (“grand mean scaling”), whereby each volume in a 4D dataset is normalized by the same value, to allow for cross-subject statistics to be valid. High-pass temporal filtering with a 100-s cut-off was used to remove low-frequency drifts. The resulting denoised time series data were analyzed using a general linear model

(GLM) approach. Registration to MNI152 standard space was carried out using FNIRT nonlinear registration [3; 4]. Painful stimulation trials were modeled as three separate factors of interest (Stimulation Block 1 to 5: “early stimulation”, stimulation block 6 to 10: “late stimulation” and “rating”), and the estimated motion parameters, motion outliers and physiological noise (cardiac and respiration data, preprocessed using physiological noise modeling (PNM), [14]) for each subject were included as nuisance regressors to reduce spurious activations because of cardiac cycle, thereby increasing statistical sensitivity. Areas of significant fMRI responses were determined using clusters identified by a $z > 2.3$ threshold and a corrected cluster threshold of $p = 0.05$ assuming a Gaussian random field for the Z-statistics [74].

Results

Effects of stressor and drugs on perceived stress and autonomic stress responses

The analysis of perceived stress ratings across all substances showed a significant effect of stress ($F(2,144)=176.382$; $p<.001$) with higher stress levels during the stressor compared to the pre ($t(18)=10.42$, $p<.001$) and post baseline ($t(18)=10.46$, $p<.001$). There was no significant drug effect on perceived stress ($F(2,144)=0.51$; $p=.60$) and no significant interaction of drug x stress for perceived stress ($F(4,144)=0.34$; $p=.85$). Participants experienced high levels of stress in all conditions, see Figure 3.

Blood pressure (systolic: $F(2,144)=19.07$; $p<.001$; diastolic: $F(2,144)=39.88$; $p<.001$) was significantly increased during the stress phase, with higher blood pressure during the stressor compared to the pre (systolic: $t(18)=5.90$, $p<.001$; diastolic: $t(18)=7.16$, $p<.001$) and post baseline (systolic: $t(18)=3.94$, $p<.001$; diastolic: $t(18)=3.83$, $p=.001$). At the end of the experiment blood pressure had not fully returned to baseline but remained at a higher level than during the pre baseline (systolic: $t(18)=5.85$, $p<.001$; diastolic: $t(18)=5.58$, $p<.001$). Additionally, we found a significant main drug effect on diastolic blood pressure ($F(2,144)=4.46$; $p=.013$), however, post-hoc-tests between the drugs did not yield significant differences (all $t(18)<1.91$, $p>.18$).

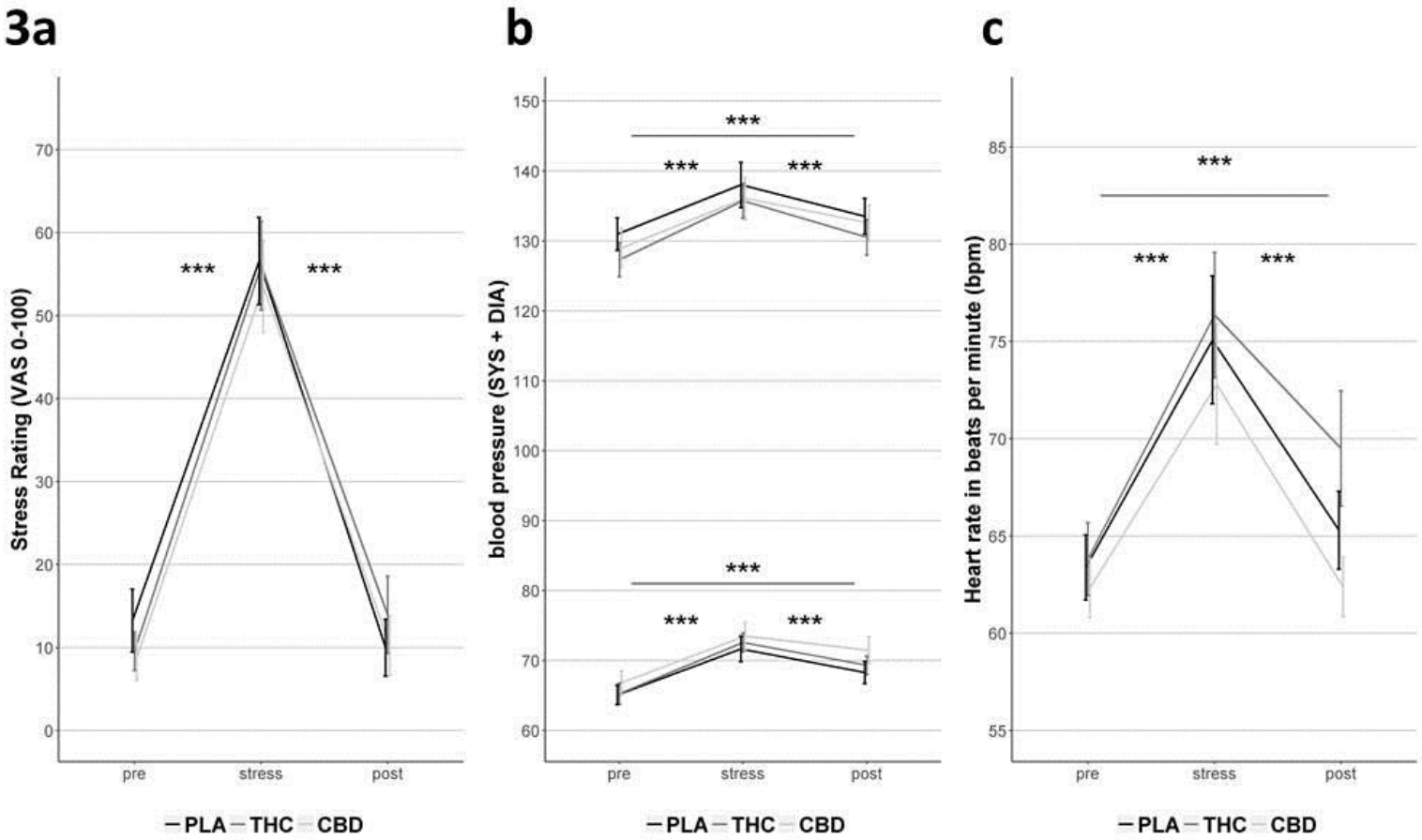


Figure 3: *Perceived stress and psychophysiological changes induced by the stressor. Black lines: Placebo condition (PLA); Grey lines: THC condition; Light grey lines: CBD condition. All graphs show data before (pre), during (stress) and after the stressor (post). Error bars display the standard error of the mean. Asterisks indicate significant post-hoc tests at ***p<.001. (a) Shows stress ratings on a visual analogue scale (VAS) ranging from "not stressed" to "extremely stressed", transformed to values ranging from 0 to 100, (b) displays systolic and diastolic blood pressure, (c) shows heart rate in beats per minute (bpm).*

The analysis of heart rate across all substances showed a significant effect of stress ($F(2,143.02)=54.21$; $p<.001$) with higher heart rate during the stressor than during the pre ($t(18)=5.35$, $p<.001$) or post baseline ($t(18)=4.87$, $p<.001$). At the end of the experiment heart rate had not fully returned to baseline, but remained at a higher level than during the pre baseline ($t(18)=3.17$, $p=.005$). Additionally, we found a significant main drug effect on heart rate ($F(2,143.02)= 6.00$; $p=.003$), with higher heart rate in the THC compared to the CBD condition ($t(18)=2.82$, $p=.03$). The heart rate in the THC condition ($t(18)=1.53$, $p=.14$) and the CBD condition ($t(18)=1.83$, $p=.12$) did not differ significantly from placebo. There was no significant interaction of drug x stress ($F(4,143.02)=1.08$; $p=.37$).

Blood concentration levels of THC and CBD

Blood concentrations of THC (t0: 0±0 pmol/ml; t1: 1.11±4.82 pmol/ml; t2: 16.84±14.12 pmol/ml) and CBD (t0: 0±0 pmol/ml; t1: 6.47±20.32 pmol/ml; t2: 537.56±340.06 pmol/ml) showed a clear peak at t2 (see Figure 2).

Effects of THC and CBD on pain perception and endogenous pain inhibition

The analysis of thresholds across all substances showed a significant effect of stress with higher perception thresholds ($F(1,90)=11.49$; $p=.001$), higher pain thresholds ($F(1,89.99)=12.24$; $p<.001$) and higher pain tolerance ($F(1,84.995)=16.4$; $p<.001$) after the stressor. There was a significant drug effect on perception threshold ($F(2,90)=3.73$; $p=.03$), post-hoc-tests between the drugs, however, did not yield significant differences (all $t(18)<1.91$, $p>.21$), and no significant drug effect on pain threshold ($F(2,89.99)=1.72$; $p=.19$) or pain tolerance ($F(2,84.995)=1.02$ $p=.36$) and no significant interaction of drug x stress for perception threshold ($F(2,90)=0.40$; $p=.67$), pain threshold ($F(2,89.99)=0.04$; $p=.96$) or pain tolerance ($F(2,84.995)=0.15$; $p=.86$). This indicates reduced sensitivity to painful and non-painful stimuli after exposure to stress, which was, however, not modulated by THC or CBD (see Figure 4c).

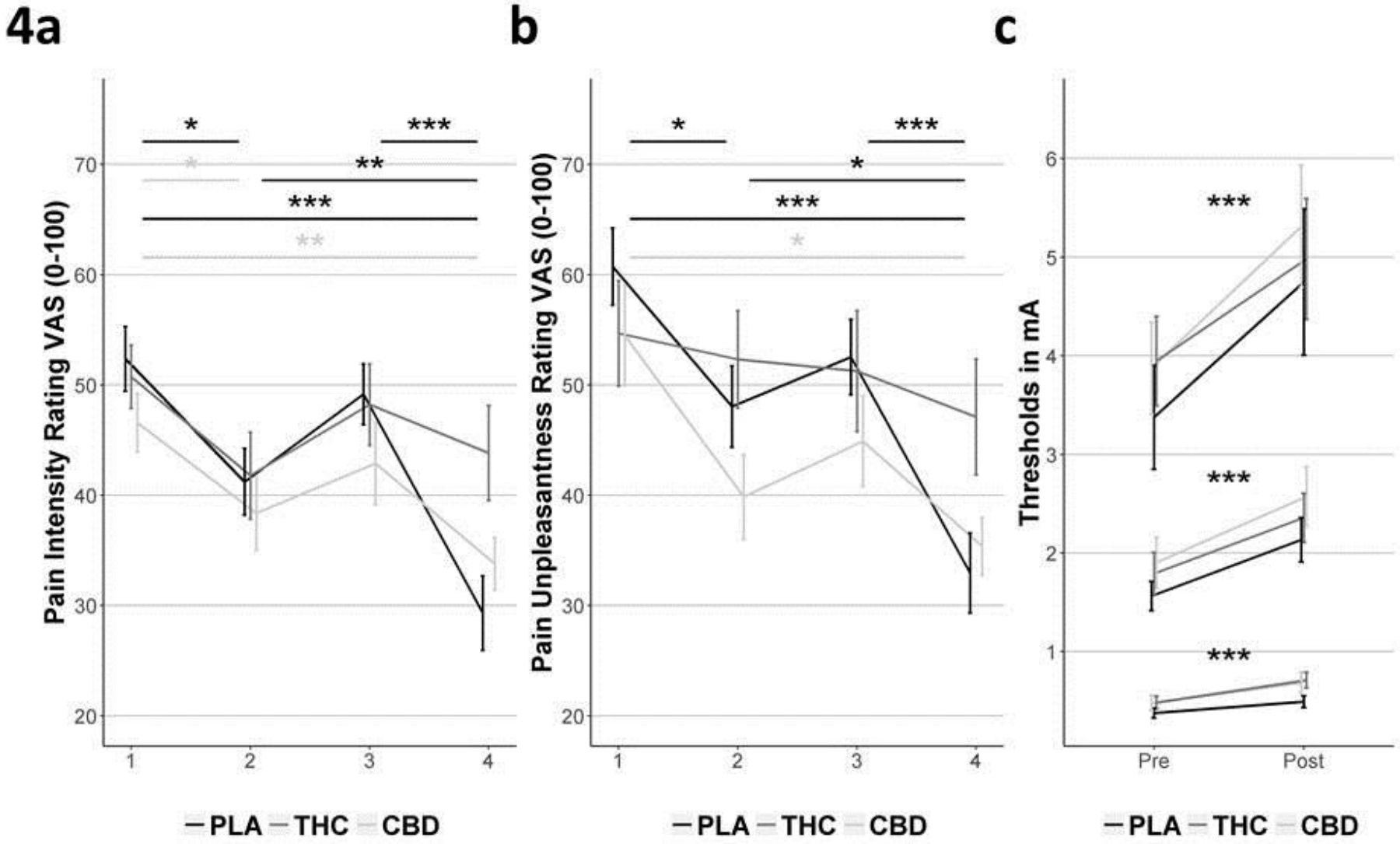

4a
b
c
Pain Intensity Rating VAS (0-100)
Pain Unpleasantness Rating VAS (0-100)
Thresholds in mA
PLA THC CBD
Pre
Post

Figure 4: *Pain ratings and thresholds for each condition and each time point. Black lines: Placebo condition (PLA); Grey lines: THC condition; Light grey lines: CBD condition. (a) The x-axis depicts the time during the experiment in relation to the stressor (1= Early|Pre, 2=Late|Pre, 3=Early|Post, 4=Late|Post). The y-axis depicts pain intensity ratings on a visual analogue scale (VAS) ranging from "no pain" to "worst pain imaginable", which was transformed to values from 0 to 100. Error bars show the standard error of the mean. Asterisks indicate color-coded significant post-hoc tests at *p<.05, **p<.01 and ***p<.001. (b) The x-axis depicts the time during the experiment in relation to the stressor (1= Early|Pre, 2=Late|Pre, 3=Early|Post, 4=Late|Post). The y-axis shows pain unpleasantness ratings on a visual analogue scale (VAS) ranging from "not unpleasant" to "extremely unpleasant", which was transformed to values ranging from 0 to 100. Error bars show the standard error of the mean. Asterisks indicate color-coded significant post-hoc tests at *p<.05, **p<.01 and ***p<.001. (c) The x-axis depicts the time before (Pre) and after (Post) the stressor. The y-axis depicts electrical currents of pain tolerance, pain threshold and perception threshold (from top to bottom) in milliampere (mA). Error bars show the standard error of the mean. Asterisks indicate significant main effect of time at ***p<.001. Black lines: Placebo condition; Grey lines: THC condition; Light grey lines: CBD condition.*

The analysis of pain intensity ratings (see Figure 4a) across all substances showed a significant effect of drug ($F(2,197.04)=4.49$; $p=.012$). Pain intensity ratings decreased from early to late stimulation blocks ($F(197.04=42.47$; $p<.001$) and from the pre to the post stressor phase ($F(1,197.04)=6.59$; $p=.011$). The same effects of drug ($F(2,197.08)=4.42$; $p=.013$), time ($F(1,197.08)=23.98$; $p<.001$) and stress ($F(1,197.08)=12.80$; $p<.001$) were found for the pain unpleasantness ratings (see Figure 4b). Post-hoc tests showed that pain unpleasantness ratings were significantly higher in the THC condition than in the CBD condition ($t(18)=2.64$; $p=.05$), but no significant differences were found when THC ($t(18)=.88$; $p=.39$) or CBD ($t(18)=1.91$; $p=.11$) were compared to placebo. For pain intensity ratings, post-hoc tests showed no significant differences between the drugs (all $t(18)<1.95$; $p>.19$).

A significant interaction effect for time x drug was found in pain unpleasantness ratings ($F(2, 197.08)=3.16$; $p=.04$), but not in pain intensity ratings ($F(2, 197.04)=2.79$; $p=.06$). There were no other significant main or interaction effects for

pain ratings (all F<1.90; p>.14). Separate analyses comparing THC with placebo and CBD with placebo showed that the drug x time interaction effect was driven by the comparison between THC and placebo. The interaction drug x time was significant for pain intensity (F(1,125.1)=4.98; p=.027) and pain unpleasantness ratings (F(1,126)=5.46; p=.02), when THC was compared to placebo. Participants reported strong decreases of pain intensity and unpleasantness during the stimulation in the placebo condition, those decreases were less pronounced under the influence of THC. When CBD was compared to placebo, this interaction effect showed a trend towards significance for pain intensity ratings (F(1,125.04)=3.74; p=.06), indicating stronger decreases of pain intensity in the placebo condition than in the CBD condition, but was insignificant for pain unpleasantness ratings (F(1,125.15)=0.79; p=.38).

Effects of drugs on anxiety, mood and dissociative symptoms

The analysis of the DSS-4 across all substances showed a significant effect of time (F(2,143.97)=4.95; p=.008) and a significant interaction of time x drug (F(4,143.97)=3.11; p=.02), but no main effect of drug (F(2,143.97)=2.27; p=.11). These effects reflect two subjects who reported dissociative symptoms or tension on the DSS-4 at the end of the THC condition (6.5 and 3 points on a 0 to 9 scale). All other subjects reported little or no dissociative symptoms (<1.5 points on the DSS-4).

The analysis of the PANAS revealed that positive affect decreased over time (F(2,144)=25.03; p<.001). Positive affect was highest at the beginning of the experiment and decreased 1 hour after drug administration (t(18)=3.63; p=.001) and decreased again until the end of the experiment (t(18)=3.83; p=.001). There was no significant drug effect (F(2,144)=0.008; p=.99) or drug x time interaction effect (F(4,144)=0.08; p=.99) on positive affect. Negative affect was not affected by drug or time effects (all F<2.39; p>.09).

The analysis of the anxiety ratings of the STAI-S revealed no significant drug or time effects (all F< 2.79; p>.06). Overall, this suggests that participants where in a less positive mood towards the end of the experiment, which was, however, not affected by drug administration.

fMRI recordings

Pre- and post-stress phase

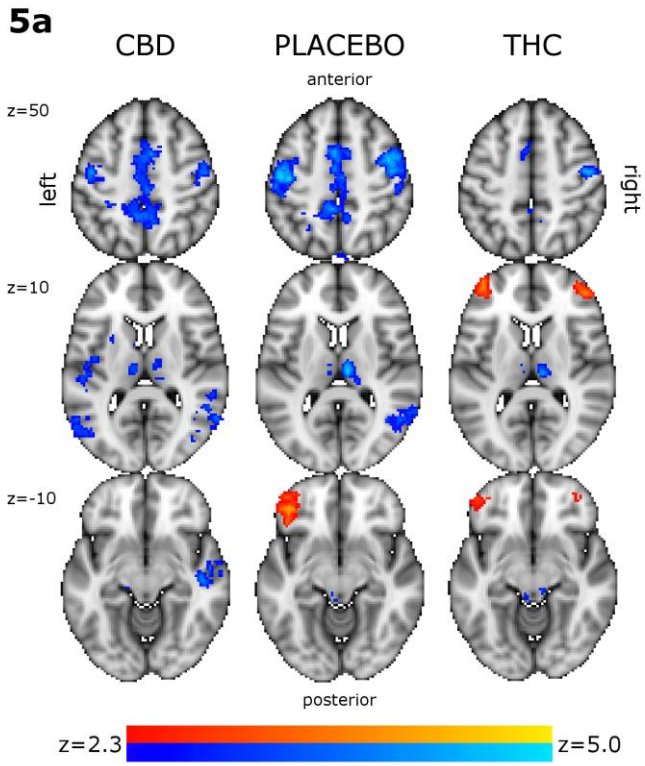
Independent of the administered drug, the BOLD response to painful stimulation increased from the pre to post stress condition in the ACC, precuneus, thalamus and PAG. In the CBD and placebo conditions, the BOLD response additionally increased in primary motor cortex and middle temporal cortex. Placebo and CBD further showed increases in the primary somatosensory cortex. In the placebo and THC condition we found decreased activity in the frontal pole after the stressor as compared to before (see Figure 5a and Table 2).

When stress-related change in the BOLD response was compared between THC and placebo (interaction effect drug x stress), we found that the BOLD response increased from pre to post stress in the left S1 in the placebo condition, while in the THC condition, we found the opposite effect (see Figure 5 and Table 2). We observed no such differences between CBD and placebo or CBD and THC.

Early and late stimulation blocks

Within pre- and post-stress phases the subjects showed a reduction of perceived pain intensity and unpleasantness (see above), which was modeled by comparing early and late stimulation blocks. In all conditions, the subjects showed significantly higher activation in the early than in the late stimulation blocks in the bilateral insula and the ACC. Significant decreases of activity in S2 were bilateral in the CBD and placebo condition but unilateral in the right S2 in the THC condition. In the placebo condition, the subjects additionally showed a significant increase in the BOLD response in the right middle frontal gyrus (MFG). We found no significant increases in the BOLD response from early to late stimulation blocks (see Figure 6a and Table 3).

The comparison of the decreases from early to late stimulation blocks in the placebo and the THC conditions (interaction effect drug x time) revealed that the decrease was significantly higher in the right MFG and middle temporal gyrus (MTG) in the placebo condition compared to the THC condition (see Figure 6 and Table 3).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THC
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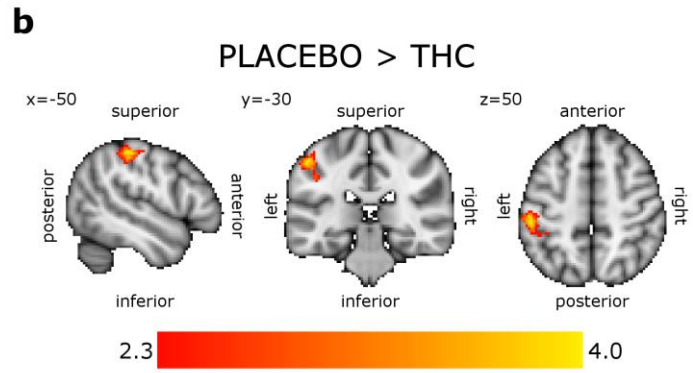
right
z=10
z=-10
posterior
z=2.3
z=5.0


b
PLACEBO > THC
x=-50
superior
posterior
anterior
inferior
y=-30
superior
left
right
inferior
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anterior
left
right
posterior
2.3
4.0

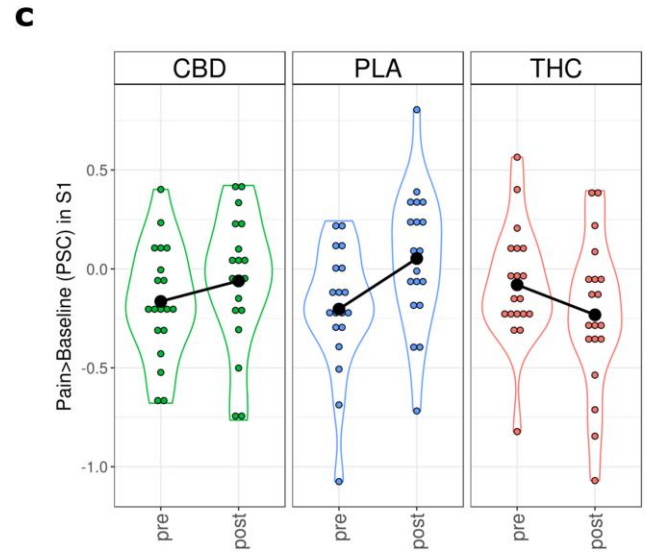
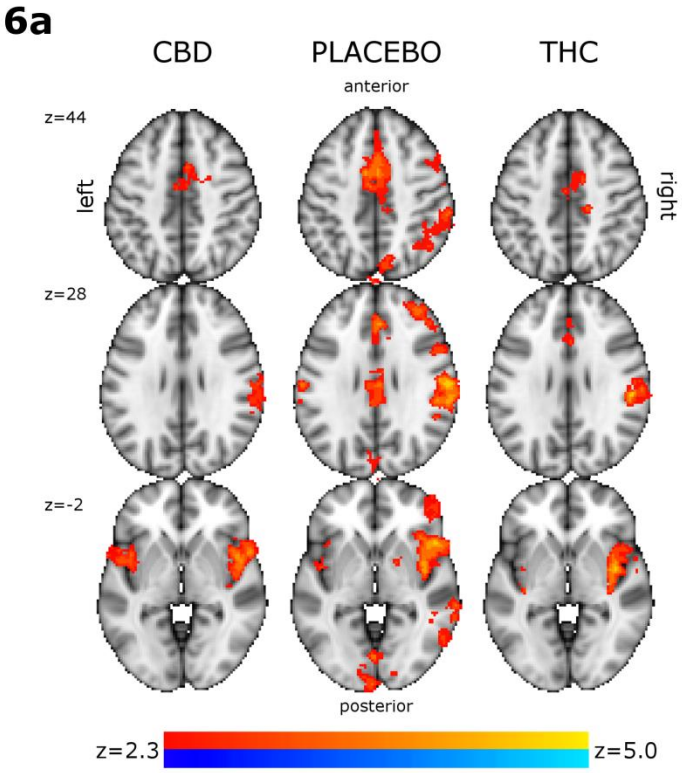

c
CBD
PLA
THC
Pain>Baseline (PSC) in S1
0.5
0.0
-0.5
-1.0
pre
post
pre
post
pre
post

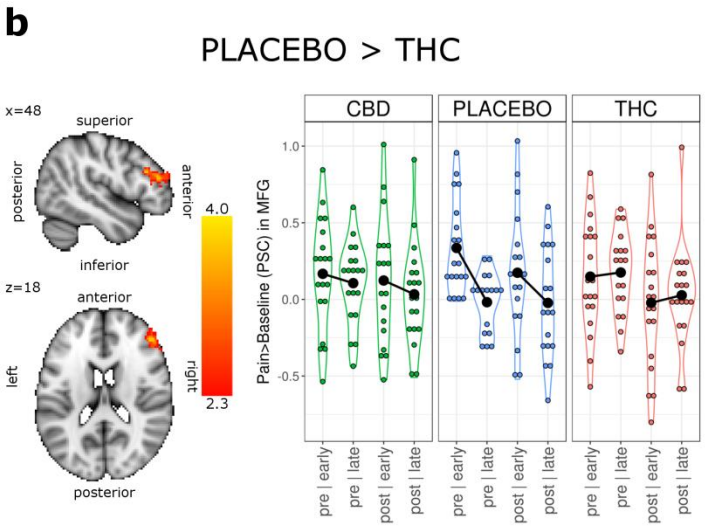
Figure 5: *Change in BOLD response from pre to post stress, separately for each condition and compared between the placebo and THC condition: (a) Red and yellow voxels depict z-values of clusters with significantly increased BOLD response before the stressor, compared to after the stressor (pre(pain>baseline)>post(pain>baseline)). Blue voxels depict clusters with significantly increased BOLD response after the stressor, compared to before the stressor (pre(pain>baseline)<post(pain>baseline)). z-coordinates are in Montreal Neurological Institute (MNI) space. (b) Pre>Post contrast compared between drugs. Yellow and red voxels depict the location of a S1 cluster where the change from pre to post stress is different between the placebo and the THC condition. x, y, and z-coordinates are given in MNI space. (c) illustrates the nature of the interaction between drug (THC vs. placebo) and stress (pre vs. post): After placebo administration BOLD response to painful stimulation was reduced in S1 but returned to baseline after the stressor. After THC administration BOLD response to painful stimulation was reduced after the stressor, but not at baseline before the stressor. Colored circles depict single subjects, black circles and black lines depict respective mean values. Single subject values were extracted from a 10mm sphere around the peak voxel of the contrast shown in Figure 5b.*

Table 2: *Activation during painful stimulation compared between the pre and post-stress phase: pre > post and pre < post, separately for each condition and the difference in stress related BOLD signal changes between the different conditions. Placebo(pre > post) ≷ THC(pre > post).*[1]

		Brain area	Coordinates (MNI 152 space) peak [x y z]	Maximum z-value
CBD	pre(pain>baseline) > post(pain>baseline)	-	-	-
	pre(pain>baseline) < post(pain>baseline)	Bilateral M1/ACC/Precuneous	[0 -34 62]	4.37
		Right middle temporal gyrus	[56 -50 14]	3.41
		Bilateral thalamus/PAG	[-10 -28 -2]	3.53
		Left S2/insula	[-38 -20 16]	3.75
		Right S1	[44 -22 54]	3.47
		Left S1	[-44 -10 52]	3.85
		Left putamen	[-26 8 -4]	3.62
		Left middle temporal gyrus	[-56 -60 8]	3.44
		Right superior temporal gyrus	[48 -20 -10]	3.57
PB	pre(pain>baseline) > post(pain>baseline)	Left frontal pole	[-42 40 -10]	4.06
	pre(pain>baseline) < post(pain>baseline)	Bilateral ACC/Precuneous	[4 -2 60]	4.58
		Right M1	[44 -6 54]	4.26
		Bilateral Precuneous	[2 -72 34]	3.36
		Bilateral Thalamus/PAG	[8 -22 8]	4.29
		Right lateral occipital cortex/middle temporal gyrus	[56 -60 12]	3.83
THC	pre(pain>baseline) > post(pain>baseline)	Left frontal pole	[-40 54 8]	3.74
		Right frontal pole	[42 46 6]	3.76
	pre(pain>baseline) < post(pain>baseline)	Bilateral ACC/precuneous	[-4 -2 44]	4.09
		Bilateral thalamus/PAG	[-4 -26 2]	4.13
		Right S1	[46 -10 52]	4.27
PB(pre(pain>baseline)<post(pain>baseline)) > THC(pre(pain>baseline)<post(pain>baseline))		Left S1	[-50 -30 50]	3.89

[1]PB: Placebo, CBD: Cannabidol, THC: Tetrahydrocannabinol, M1: Primary motor cortex, ACC: anterior cingulate cortex, S1/S2: Primary and secondary somatosensory cortex, PAG: periaqueductal grey.


6a
CBD
PLACEBO
THC
anterior
z=44
left
right
z=28
z=-2
posterior
z=2.3
z=5.0


b
PLACEBO > THC
x=48
superior
posterior
anterior
inferior
4.0
2.3
z=18
anterior
left
right
posterior
CBD
PLACEBO
THC
Pain>Baseline (PSC) in MFG
1.0
0.5
0.0
-0.5
pre | early
pre | late
post | early
post | late

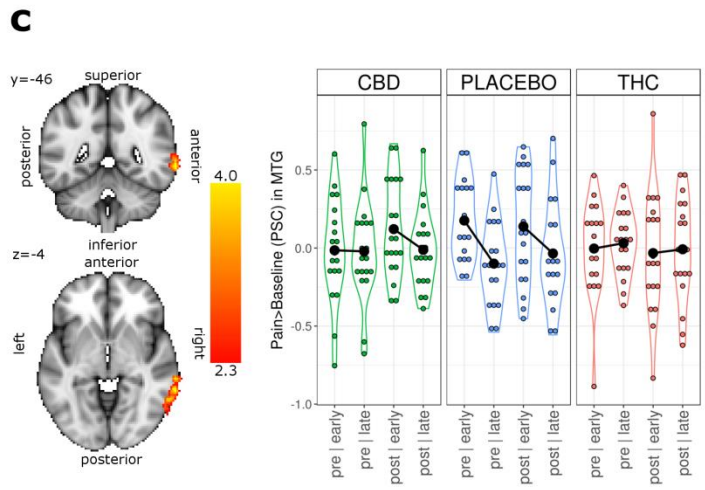

c
y=-46
superior
posterior
anterior
inferior
4.0
2.3
z=-4
anterior
left
right
posterior
CBD
PLACEBO
THC
Pain>Baseline (PSC) in MTG
0.5
0.0
-0.5
-1.0
pre | early
pre | late
post | early
post | late

Figure 6: *Change in BOLD response from early to late stimulation blocks, separately for each condition and compared between the placebo and THC condition. (a) Red and yellow voxels depict clusters with significantly increased BOLD response in early pain trials, compared to late pain trials (early(pain>baseline)>late(pain>baseline)). Blue voxels depict clusters with significantly increased BOLD response in late stimulation trials, compared to late stimulation trials (early(pain<baseline)>late(pain<baseline)). z-coordinates are Montreal Neurological Institute (MNI) coordinates. (b) Early>Late contrast compared between drugs. Left: Yellow and red voxels depict the location of a cluster in the middle frontal gyrus (MFG) where the change from early to late pain stimulation trials is different between the placebo and the THC condition. x, y, and z-coordinates are given in MNI space. Right: The graph illustrates the nature of the interaction between drug (THC vs. placebo) and stress (early vs. late): After placebo administration BOLD response to painful stimulation was increased in MFG in early, but not late pain stimulation trials. After THC administration BOLD no such early/late difference was observed. Colored circles depict single subjects, black circles and black lines depict respective mean values. Single subject values were extracted from a 10mm sphere around the peak voxel of the contrast shown in Figure 8a. (c) Left: Yellow and red voxels depict the location of a cluster in the middle temporal gyrus (MTG) where the change from early to late pain stimulation trials is different between the placebo and the THC condition. x, y, and z-coordinates are given in MNI space. Right: The graph illustrates the nature of the interaction between drug (THC vs. placebo) and stress (early vs. late): After placebo administration BOLD response to painful stimulation was increased in MTG in early, but not late pain stimulation trials. After THC administration BOLD no such early/late difference was observed. Colored circles depict single subjects, black circles and black lines depict respective mean values. Single subject values were extracted from a 10mm sphere around the peak voxel of the contrast shown in Figure 8b. PSC: percent signal change; MFG: Middle frontal gyrus; MTG; Middle temporal gyrus; CBD: Cannabidiol; THC: Tetrahydrocannabinol;*

Table 3: *Activation during painful stimulation compared between the early and late stimulation phase: early > late and early < late, separately for each condition and the difference in habituation related BOLD signal changes between the different conditions. Placebo(early > late) ≷ THC(early > late).* [1]

		Brain area	Coordinates (MNI 152 space) peak [x y z]	Maximum z-value
CBD	early(pain>baseline) > late(pain>baseline)	Right insula/S2	[58 12 0]	3.9
		Left insula/S2	[-38 2 -6]	3.55
		ACC/M1	[2 -10 60]	3.25
	early(pain>baseline) < late(pain>baseline)	-	-	-
Placebo	early(pain>baseline) > late(pain>baseline)	Right insula/S2/MFG	[60 -18 28]	4.55
		ACC/M1	[-2 -10 48]	4.05
		Right precuneous	[10 -74 40]	3.89
		Left insula/S2	[-62 -18 16]	4.11
	early(pain>baseline) < late(pain>baseline)	-	-	-
THC	early(pain>baseline) > late(pain>baseline)	Right insula/S2	[34 8 8]	4.54
		Right M1	[22 -32 68]	3.69
		ACC	[-6 34 6]	3.34
		Left insula	[-36 -16 6]	4.05
	early(pain>baseline) < late(pain>baseline)	-	-	-
PB(early(pain>baseline)>late(pain>baseline)) > THC(early(pain>baseline)>late(pain>baseline))		right MFG	[48 38 18]	3.58
		right MTG	[68 -46 -4]	4.1

[1]PB: Placebo, CBD: Cannabidol, THC: Tetrahydrocannabinol, M1: Primary motor cortex, ACC: anterior cingulate cortex, S2: Secondary somatosensory cortex, MFG: Middle frontal gyrus, MTG: middle temporal gyrus.

Discussion and conclusions

This study aimed to investigate the effects of THC and CBD on stress-induced inhibition of pain. SIA and habituation to pain were compared between a THC, CBD and a placebo condition.

The stress ratings, heart rate and blood pressure indicated that the cognitive stress plus noise induced significant stress. Perception and pain thresholds as well as tolerance levels and pain ratings were reduced after the stressor. On the neuronal level, the SIA effect was accompanied by a reduction of the BOLD response in the bilateral frontal pole and an increase in the ACC, precuneus, thalamus and PAG after, compared to before the stressor. In the placebo condition the BOLD response to painful stimulation increased from pre to post stressor in the ipsilateral S1, while no such increase was found for THC. However, neither THC nor CBD altered the sub- or suprathreshold pain perceptions post stress. Additionally, we observed that THC interfered with the decrease of self-reported pain levels to repetitive painful stimulation that could be observed in the placebo and CBD condition. In the placebo condition unpleasantness ratings of painful electric stimulation showed a stronger decrease from early to late stimulation trials than in the THC condition. In the placebo condition this habituation to the painful stimulus was accompanied by decreases of the BOLD response in the MFG and MTG from early to late stimulation trials. In the THC condition no such decrease was observed.

Stress-induced analgesia

Our data on SIA after systemic administration of different cannabinoid agents have multiple implications. First, we successfully induced stress and SIA in the placebo condition. This SIA response was accompanied by activation of ACC, precuneus, thalamus and PAG, in accordance with previous human work that showed involvement of the ACC [76]. Animal work has further shown the involvement of PAG activity in the descending inhibition of pain. Electrical stimulation of the ventrolateral PAG reduced pain sensitivity in formalin and tail-flick test in rats [22]. The injection of an opioid receptor antagonist [70] suppressed SIA, thereby confirming PAG involvement in SIA.

Our second finding relating to SIA was that, in the ipsilateral S1, the change in activity from pre to post stress differed between placebo and THC. This result was mainly driven by a decrease of activity in the placebo condition before the stressor.

Under THC we did not observe similar changes (see Figure 6b). A deactivation of S1 in response to pain has been found in studies using tonic painful stimulation, such as 3 minutes of a hot water bath in humans [5] or 7 seconds of mechanical stimulation in squirrel monkeys [64]. This deactivation has been related to the inhibition of tactile sensitivity in S1 cortex [15]. Further, it is important to note that the deactivation we found was ipsilateral to the stimulated body site. Interhemispheric inhibition in ipsilateral S1 has been shown in a study using median nerve stimulation in human subjects [55]. Together with the high density of CB1 receptors in S1 [29; 65], this suggests that THC might interfere with interhemispheric inhibitory processes.

Our third finding related to SIA was the lack of modulation of SIA by the administration of THC or CBD. This was unexpected, since studies in rodents have shown that the suppression of endocannabinoid signaling by injection of rimonabant, a CB1 receptor antagonist, into the basolateral amygdala [17], the dorsolateral PAG or RVM [61] suppressed SIA, while increased endocannabinoid signaling induced by increasing 2-AG concentrations in the ventrolateral PAG enhanced SIA [31]. We could not translate these findings to the endocannabinoid system in humans. High interspecies overlaps in CB1 receptor distribution in neocortex, primary somatosensory cortex, amygdala and PAG [30] make it unlikely that interspecies differences are responsible for the discrepancy between our human and previous work done in rodents. It is possible that the type of stressor contributed to these differences. The cognitive stressor we used has repeatedly been employed to induce stress in humans [2; 38; 67]. This type of continuous stressor may differ in the involvement of the cannabinoid system similar to differences of involvement of the opioid system in analgesia [42]. Previous animal studies used brief painful foot shocks to induce stress and measured pain thresholds, for example, by the use of the tail-flick, to quantify the SIA effect [17; 31; 61]. This procedure of stimulating at one body site and measuring pain thresholds at another, however, may involve central habituation to painful stimulation, which have been shown in humans, where repetitive painful stimulation led to pain attenuation in a non-stimulated limb [56] and pain attenuation was accompanied by activation in pain processing areas, such as thalamus, insula, SII and the putamen, while activity in the rostral ACC, an area involved in endogenous pain control, increased over time [12]. It therefore seems likely that the habituation-like non-associative learning effect in our painful

stimulation, but not the effects induced by our cognitive stressor, resemble the effects found in previous studies on SIA.

Non-associative learning/habituation effects

In our experiment, habituation to painful stimulation was impaired when THC, a CB1 receptor agonist, was administered. This effect was represented on the affective rather than the sensory dimension of pain, which is in line with previous work that has related the pain modulatory effects of THC to the affective-motivational component of pain [41]. Habituation was accompanied by decreases in the BOLD response in pain-related areas such as SII and Insula, which have previously been shown to decrease with habituation to pain [12]. As mentioned above, the habituation to pain in our study might resemble the effects found in previous studies on SIA, as they had used painful stimulation as a stressor. Therefore the analgesic effect of the stressor may have acted via central habituation to painful stimulation. The endocannabinoid system has repeatedly been linked to memory and learning mechanisms [1; 36]. Habituation, as a form of non-associative learning, has been associated with the CB1 receptor which mediates fear extinction via habituation processes, as shown in CB1 knockout mice, which were impaired in habituation of the fear response to a tone after sensitization with an inescapable footshock [33; 34].

Further, we found that, in the MFG and MTG, the change in activity from early to late stimulation differed between placebo and THC. In both cases this reflects an early activation in the placebo condition which returns to baseline during late stimulation. Initial activation and therefore also the reduction of activity over time were not present in the THC condition. A review of 34 conditioning study revealed MFG as consistently being engaged in high-threat processing [53]. Further, MFG was suggested to be suppressed in response to emotional distractors [75] and in response to an emotional scene, when the attention was targeted elsewhere [25]. The right MFG has repeatedly been associated with response inhibition, for example in Go/NoGo tasks, with a major role in monitoring and directing attention [62]. Damage to that area significantly attenuated the reaction time to a stop signal in a human lesion study [6]. The MTG and MFG have further been associated with semantic memory retrieval [27] and shown to have interconnectivity with the executive control network (ECN) [73]. The administration of THC may therefore have interfered with a monitoring and retrieval network involving inhibitory control over threatening stimuli. Since these

findings were not predicted in our study on SIA, future research should (1) determine the reproducibility of our findings on habituation and (2) clarify the neuronal mechanisms involved. Attentional, memory and emotional networks may all play important roles and have all been associated with cannabinoid signaling.

Limitations

For most participants, neither THC nor CBD blood concentrations showed a peak at t1, in contrast to blood concentrations at t2. The time between blood samples at t1 and the beginning of the SIA experiment was approximately 16 minutes. Hence, more frequent blood sampling would have been desirable. Previous studies have shown clear drug effects after oral administration of the same doses between one and two hours after substance administration [10; 11; 71], and the blood levels in our experiment showed a peak at the end of the experiment. Thus, the time window for our experiment should still have been appropriate.

Future studies could clarify the nature of these effects by including additional study arms. Cannabinoids have been shown to be involved in placebo analgesia [9], a natural history condition without drug/placebo administration could therefore control for placebo effects. Including different types of stressors and a condition without stress could furthermore clarify the analgesic mechanisms that THC interfered with in our study.

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Conflict of Interest

There is no conflict of interest.

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2.2 The impact of controllability on pain and suffering[2]

[2] Löffler, M., Kamping, S., Brunner, M., Bustan, S., Kleinböhl, D., Anton, F., Flor, H. (2018). The impact of controllability on pain and suffering. Pain reports, in revision with minor revisions.

Abstract

Introduction: Chronic pain and pain-related suffering are major health problems. The lack of controllability of experienced pain seems to greatly contribute to the extent of suffering. This study examined how controllability affects the perception of pain and pain-related suffering, and the modulation of this effect by beliefs and emotions such as locus of control of reinforcement, pain catastrophizing, and fear of pain.

Methods: Twenty-six healthy subjects received painful electric stimulation in both controllable and uncontrollable conditions. Visual analogue scales and the "Pictorial Representation of Illness and Self Measure" (PRISM) were used to assess pain intensity, unpleasantness, pain-related suffering and the level of perceived control. We also investigated nonverbal indicators of pain and suffering such as heart rate, skin conductance and corrugator electromyogram.

Results: Controllability selectively reduced the experience of pain-related suffering, but did not affect pain intensity or pain unpleasantness. This effect was modulated chance locus of control but was unrelated to fear of pain or catastrophizing. Physiological responses were not affected by controllability. In a second sample of twenty-five participants we varied the instruction to reflect less personal involvement in controllability of the pain. The effect of controllability on pain-related suffering was only present when a high degree of personal emotional involvement was implied.

Discussion: Our data suggest that the additional measure of pain-related suffering may be important in the assessment of pain and may be more susceptible to the effects of perceived control than pain intensity and unpleasantness. We also show that this effect depends on personal involvement.

Introduction

Pain perception is modulated by cognitive and emotional variables such as predictability [12,38], controllability [7,57], attentional focus [1,46], or fear of pain [30,43,44]. Studies in healthy volunteers showed that controllable situations reduce pain intensity [7,39,57] and unpleasantness [7], however, controllability did not always change pain perception [20,27,47]. Although actual control was more effective than perceived control alone in reducing pain [39], this dissociation cannot fully explain these ambiguous findings, as exerted control did not reduce pain intensity [56] and perceived control was found to decrease pain intensity [7] in other studies. Anxiety [56], helplessness [57] or pain unpleasantness [7] were not assessed consistently across studies, although these variables may modulate the effects of control on pain perception. Carnevale [13] suggested that suffering is the most important factor that drives patients to seek medical attention. This is also true for patients with chronic pain [3]. We have demonstrated that pain-related suffering is an additional component of pain that can be assessed independently of pain intensity and unpleasantness [10]. The lack of controllability might be especially important for the experience of pain-related suffering in both experimental pain studies and in patients [6,16]. Ongoing but unsuccessful efforts to influence the pain make patients especially vulnerable to suffering [53]. In addition to observing the effects of control on verbal reports of pain and suffering, a secondary goal of the study was to see if there are also effects on physiological indicators of pain and suffering such as skin conductance responses (SCR), corrugator electromyogram (EMG) and heart rate (HR) [8]. This would indicate multi-level effects and would permit bias-free assessments of pain and suffering also in groups that may not easily give verbal reports such as children or incapacitated persons.

The effect of the experimental manipulation of controllability is modulated by individual differences in the perception of control. Wiech et al. [56] reported that exerted control over painful stimulation led to reduced pain perception in half of the subjects while the other half showed increased pain intensity ratings. The authors hypothesized that the individuals' locus of control might explain these inter-individual variations. We therefore examined locus of control of reinforcement, which is the degree to which people believe that they have control over the outcome of positive or negative events in their lives as opposed to external forces beyond their control

[34,35,45]. We hypothesized that an internal locus of control would modulate higher effects of uncontrollability on pain than an external or chance locus of control.

The current study examined the influence of controllability on pain intensity, unpleasantness and pain-related suffering in two experiments that differed in the level of personal involvement. We hypothesized that control over pain would positively affect all three dimensions, with the strongest reduction related to suffering. We expected reductions in SCR and EMG, but not HR. A high chance locus of control, the belief that powerful others control one's life, high catastrophizing and high fear of pain were assumed to reduce the positive effects of perceived control on suffering.

Methods

Participants

Twenty-six right-handed subjects (13 male) between 18 and 43 years of age (mean: 25.5, standard deviation (SD) = 5.81) participated in the study (sample 1). Twenty-five right-handed subjects (8 male) between 20 and 49 years of age (mean: 25.32, SD = 5.79) participated in study 2 (sample 2). Sample sizes were based on power calculations to detect large effects (effect size d>0.5) in a within-subjects comparison, which have previously been reported in a similar study design [57]. The Ethics Committee of the Medical Faculty Mannheim, University of Heidelberg, Germany, approved the study and written informed consent was obtained from each participant. Exclusion criteria were cardiovascular or neurological disorders, brain injury, acute or chronic pain, current use of pain medication, pregnancy, lifetime and current substance abuse or dependence and any other mental disorders. The subjects were screened by a psychologist using the German version of the Structured Clinical Interviews for the Diagnostic and Statistical Manual IV (SCID) [58] Axis I to exclude subjects who fulfilled the criteria for a mental disorder.

Apparatus and application of painful stimuli

Pain processing was investigated in response to a series of painful electrical stimuli applied under conditions of controllability vs. uncontrollability. A pair of subcutaneous needle electrodes (20 mm long, 0.35-mm uninsulated tip, 2-mm² stimulation area, model: 9013R0272, 28G, Alpine Biomed ApS, Skoviunde, Denmark) were placed at the left upper back, at the mid-trapezius muscle, (1mm needle separation). The

stimulation site was chosen to mimic a clinical condition, like chronic back pain as closely as possible under experimental conditions and to allow similar experiments in chronic back pain patients. Needle electrodes mainly activating Aδ fibers [26] were chosen to elicit a rapid and sharp painful sensation. The invasive character of these needles was also expected to result in sufficiently high suffering ratings to avoid floor effects in the rating data. Electric stimuli (2ms stimulus duration, 400V, inter stimulus interval 500ms) were applied using a constant current stimulator (model DS7A; Digitimer, Hertfordshire, England). The experiment was performed using Presentation® software (Version 14.0, http://www.neurobs.com).

Psychophysical thresholds and stimulus calibration

The electrical stimulation parameters were determined individually, first by assessing pain-related thresholds by the method of limits. For this purpose, perception threshold, pain threshold and pain tolerance were assessed during four ascending series of electric stimuli. The participants were instructed to press a button when they felt the stimulus for the first time (perception threshold), when the stimulus was painful for the first time (pain threshold) and when they could no longer tolerate the stimulus intensity (pain tolerance). Each threshold was acquired once per ascending series. The first ascending series was discarded as a practice trial, to exclude early fatigue and/or sensitization. Thus, the average of 3 ratings per threshold served as the final parameter. A painful stimulus intensity was preset at 70% of the interval between pain threshold and pain tolerance.

Next, the painful stimulus intensity was adjusted to a perceived pain intensity of 70% on a visual analogue scale (VAS) with the endpoints “no pain” (0) to “extreme pain” (100). Magnitude estimates of pain intensity were assessed during three test trials (duration 10 seconds each). Stimulus intensity was adjusted to reach a VAS rating of about 70%. The resulting individual stimulus intensity of each subject was used for all further procedures (sample 1: mean: 8.75, SD = 11.2; sample 2: mean: 10.35, SD = 18.38, values in mA).

Experimental procedure

We employed a within-subjects design, where each subject received painful electric stimulation in a controllable and an uncontrollable condition. The experiment consisted of four blocks in which participants received eight series of painful

stimulations (see Figure 1a). Each of the four blocks comprised four controllable and four uncontrollable trials, which were presented in an intermixed order (randomized within each block).

In the controllable condition, the participants had the possibility to stop the painful electric stimulation via a button press. They were instructed to press the button when the stimulation became intolerable. In the uncontrollable condition, the participants were informed that the duration of the stimulation was randomly determined by the computer. In reality, we used a yoked control design to match the length of the stimulations in the uncontrollable condition with those in the controllable condition [57]. The duration of the four uncontrollable trials was predetermined by the duration of the four controllable trials of the preceding block. The first block started with a controllable trial. Durations of the uncontrollable trials of the first block were predetermined randomly by one of the preceding controllable trials of the same block. This led to comparable durations of the controllable and uncontrollable trials (sample 1: controllable: 22.8 ± 19.03 sec, uncontrollable: 22.33 ± 18.33 sec; sample 2: controllable: 16.9 ± 15.05 sec, uncontrollable: 18.2 ± 15.28 sec). The blocks were separated by 1-minute breaks.

Due to our experimental manipulation, the exerted control had to be accompanied by increased perceptions of control in the controllable trials. We therefore assessed perceived controllability of the pain during the controllability and uncontrollability trials after each block of 8 trials. The same VAS was used for the controllable and uncontrollable trials. The VAS was 800 pixels (23.5 cm) long and ranged from “not at all controllable” to “extremely controllable”, with a visual angle of 16.7°. The pixels were linearly transformed to values of 0 to 100. Perceived controllability differed significantly between the controllable and uncontrollable conditions (sample 1: $t(25)=12.91$, $p<.001$, $d=2.53$; sample 2: $t(24)=6.96$, $p<.001$, $d=1.39$). The participants rated perceived controllability higher when they were able to stop the stimulation compared to the uncontrollable condition.

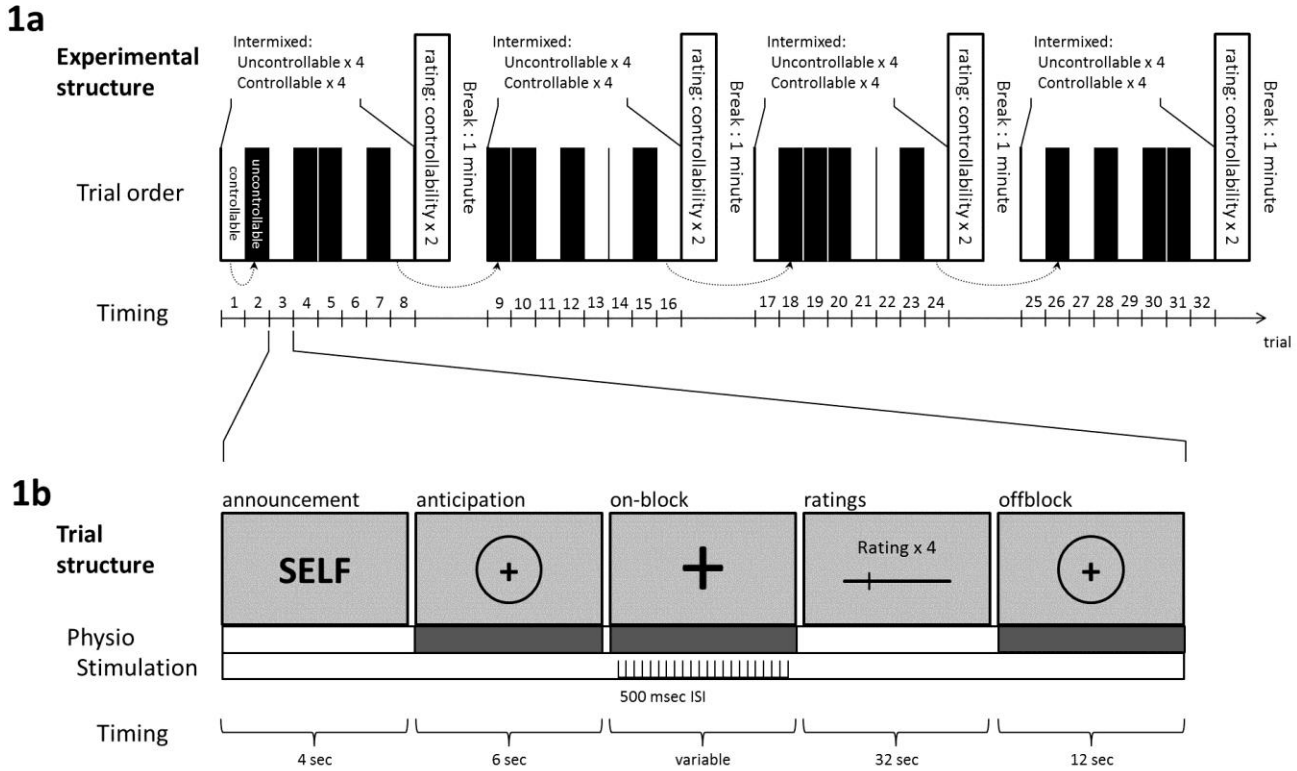

Figure 1: *Structure of the experiment: (a) The experiment consisted of four blocks with eight stimulation trials each. In half of the cases, the stimulation could be stopped by the participant (controllable condition). The remaining trials were stopped by the computer (uncontrollable condition). The duration of the self-controlled trials equaled the duration of the computer-controlled trials in the subsequent block (dotted arrows). The order of the trials was randomized within each block. (b) Each trial was announced by a slide that indicated the type of the trial. Note that in 25 participants the word 'self' was replaced by the word 'button press' to announce the controllable trials. The anticipation phase was followed by a varying interval of painful stimulation, ratings of pain intensity, pain unpleasantness and pain-related suffering. Each trial ended with an off-block lasting 12 seconds (sec). ISI: inter stimulus interval, sec seconds, msec: milliseconds.*

Instructions and trial structure

In each trial the condition was announced on the computer screen during a four seconds prestimulus time span. The stimulus was expected during the following six seconds of an anticipation phase. Controllable trials were announced during the prestimulus interval with the words: “SELF; please press the button to terminate the stimulation”. Uncontrollable trials were announced, for four seconds, with a slide stating: “COMPUTER; stimulation will be terminated by the computer”. The assessment of perceived stimulus intensity, unpleasantness and related suffering on a VAS, as well as the PRISM task, providing an alternate estimate of suffering, were presented in random order after each trial. The participants had a 32-second time frame to rate all four scales, followed by a resting time span (off block) of 12 seconds.

Ratings

The assessment of pain intensity, unpleasantness and suffering was performed on horizontal visual analogue scales (800 pixels = 235 mm) with appropriate endpoints (pain intensity: “no pain” and “extreme pain”; pain unpleasantness: “not unpleasant” and “extremely unpleasant”; suffering: “no suffering” and “extreme suffering”). The scales were presented on a computer screen at a distance of 800 mm and comprised an angular view of 16.7°. The VAS ratings were transformed to values ranging from 0 to 100.

In the absence of a gold standard on how to measure suffering [9], we complemented the “suffering” VAS with the suffering scale implemented in the “Pictorial Representation of Illness and Self Measure” (PRISM), because it performed best on quality criteria compared to other instruments for measuring suffering [33].

The PRISM task was presented as a computerized version of the original task. The participants viewed a grey screen with a fixed yellow circle in the bottom right corner, representing their self. A moveable red circle was located in the center of the screen, representing the current painful stimulation (corresponding to “illness” in the original PRISM task). The participants were instructed to estimate the importance of the painful stimulation in their life by placing the red circle in an appropriate distance to the yellow circle. According to the PRISM rationale, the amount of suffering is coded by the inverse of the distance of the centers of the red and yellow circles. Thus, the closer the red circle (painful stimulation) is found in relation to the yellow circle (self), the higher is the suffering felt under the aversive stimulation while the further the red

circle is away from the yellow circle, the lower is the suffering indicated. The raw PRISM scale values were linearly transformed to values ranging from 0 to 100, with 0 representing no and 100 representing maximal suffering.

Before the experiment, all participants were asked if they were able to discriminate between the ratings. Since suffering is a personal and individual experience conveying multiple meanings which we did not want to delimit [14], no definition was given to the participants. If the participants struggled with differentiating between suffering and any of the pain scales, they were asked: “Do you think one can suffer without being in pain – can you give an example?” and “Do you think one can be in pain without suffering – can you give an example?”. All participants confirmed those questions and were able to give examples. If participants struggled with the discrimination of pain intensity and pain unpleasantness, it was explained to them by using the radio metaphor by Price et al. (1983) [41]. In this metaphor experiencing pain is compared to listening to the sound of a radio, where pain intensity refers to the loudness of the music and pain unpleasantness refers to the quality of the music played. The participants had a practice run for each rating before the experiment commenced.

Adjustment of the experimental procedure

This experiment was initially performed in 26 participants. During the debriefing session after the experiment, four participants reported being confused by the overlap between the PRISM rating and the instructions. The controllable trials were announced with the word ‘self’, while during the PRISM task the participants had to place a token of the painful stimulation in relation to their self (yellow circle), with closer placements representing higher suffering.

The participants stated that, in the PRISM task, they were confused between two possible meanings of the word “self” on the display and did not know whether they were expected to rate the amount of their own suffering (with ratings closer to the self, indicating higher suffering) or how much they felt being the agent in control of the previous stimulation (with ratings closer to the self, indicating higher agency).

This instruction-related issue was not anticipated and therefore not systematically assessed in all participants. It can hence not be ruled out that other participants were having the same problem without reporting it. This renders a subsample analysis impossible and PRISM ratings in this sample should therefore be interpreted with

caution. To rule out this ambiguity, the experiment was repeated in another 25 participants. For those 25 participants the general instructions before the experiment were changed and referred to controllable trials as ‘stoppable by a button press’. Controllable trials were announced with a slide stating: “BUTTON PRESS; please press the button to terminate the stimulation”) and uncontrollable trials with the following slide: “COMPUTER; stimulation will be terminated by the computer”). The data of the second experiment (sample 2) were analyzed in the same way as the initial experiment (sample 1).

Questionnaires

Prior to the experiment, the participants completed the Locus of Control Scale [32], which assesses beliefs about control of reinforcement with the subscales chance (IPC-C), control by powerful others (IPC-P), and perceived mastery over one's personal life (IPC-I). The scale has good test-retest-reliability (IPC-I: r=.55, IPC-P: r=.66 and IPC-C: r=.70), internal consistency (α=.91, for IPC-I, α=.95 for IPC-P and α=.9 for IPC-C) and validity [31]. A general locus of control scale was chosen because we examined healthy individuals and the painful stimulation was not related to any health problem [54].

The participants also completed the Pain-Related Self Statements Scale (PRSS, [22]), which assesses catastrophizing and active coping. The scale is validated in German participants and has excellent reliability (α=.92 for catastrophizing and α=.88 for active coping) and validity, as shown by significantly higher values for pain catastrophizing and significantly lower values for active coping in pain patients compared to healthy controls, and low to moderate correlations with other pain-related variables such as amount of daily activity, affective distress or pain severity.

Furthermore, the participants completed the Fear of Pain Questionnaire (FPQ-III). The FPQ-III is a self-report measure designed to evaluate fears about severe, minor, and medical pain with higher scores indicating more fear. The FPQ-III has shown to be a valid and reliable instrument with good test-retest reliability, predictive validity, and internal consistency (for severe pain: α=.88, r=.69, for minor pain: α=.87, r=.73 and for medical pain α=.87, r=.76) [37,49] and has been validated in a German population. The PRSS and FPQ-III were chosen to allow for comparison with previous experimental work on pain-related suffering [8,10] and because they exist in validated German versions.

Physiological assessments

Electromyography (EMG), skin conductance responses (SCR) and electrocardiogram (ECG) were recorded and amplified using a BrainAmp ExG amplifier (Brain Products GmbH, München, Germany) and registered with a sampling frequency of 500 Hz. The data in one participant had to be discarded due to technical problems.

EMG activity was recorded from the musculus corrugator supercilii using small surface electrodes (1.5mm Ag/AgCl) that were placed in a bipolar fashion above the left eye, using the placement recommended by Fridlund and Cacioppo [24]. The SCRs were recorded from two electrodes (5mm Ag/AgCl), which were placed on the medial phalanges of digits III and IV of the left hand [4]. SCR analysis was performed using the Ledalab V3.4.6c software package for Matlab and followed the guidelines of Fowles et al. [23]. ECG was recorded using two 7-mm Ag/AgCl electrodes (Asmuth GmbH Medizintechnik, Minden, Germany), placed on the subjects' left lateral sternum at the upper and lower edges of the musculus pectoralis major. The ground electrode was placed on the right hip bone. Calculation of interbeat latencies and artefact correction were performed by the KubiosHRV software [50]. Details on preprocessing of the physiological data can be found in the supplementary material.

Statistical analysis

As explained in the methods section, two samples were assessed in two separate experimental runs, with differing instructions for the controllability condition. In the original sample (sample 1) the instructions referred to controllable trials as being stoppable by the participant. In the sample of the second experiment (sample 2) the instructions were changed and referred to controllable trials as 'stoppable by a button press'. All results are reported for both experimental runs. Statistical analyses were performed using RStudio 1.0.143 (RStudio, Inc.) with R 3.4.0 (The R Foundation for Statistical Computing).

Controllability and its influence on pain and suffering

To test the specificity of the effect of controllability on suffering compared to pain intensity and pain unpleasantness, we used an analysis of variance to examine the within-subject effects of controllability (controllable versus uncontrollable) and rating dimension (intensity versus unpleasantness versus suffering VAS versus PRISM). Effect sizes for the ANOVAs are reported as Cohen's d. We used pairwise post-hoc t-

tests (false discovery rate, FDR [5] corrected) to compare the VAS ratings for perceived controllability, pain intensity, unpleasantness and suffering as well as PRISM ratings in the controllable and uncontrollable conditions. Effect sizes for the t-tests are reported as Cohen's d.

Individual differences in the effects of controllability on pain and suffering

To explore the impact of locus of control, fear of pain and pain catastrophizing on the effect of controllability, the IPC, FPQ-III and PRSS subscales and the difference in ratings (controllable minus uncontrollable) were correlated for each rating (intensity, unpleasantness, suffering VAS, PRISM).

Sample differences in the effects of controllability on pain and suffering

To explore sample differences in the effect of controllability on the ratings, we implemented separate analyses of variance for each rating dimension (pain intensity, unpleasantness, suffering VAS and PRISM) to test the interaction of sample (sample 1 versus sample 2) and controllability (controllable versus uncontrollable). To examine the association of enduring beliefs and pain controllability, we compared the correlations of the IPC, FPQ-III and PRSS subscales with differences in controllability (controllable minus uncontrollable) between samples using Fishers'z.

Physiological assessments

For details on statistical analysis of physiological data see supplementary material.

Results

Controllability and its influence on pain and suffering

The analyses of variance using controllability (controllable versus uncontrollable) and the rating dimension (intensity versus unpleasantness versus suffering VAS versus PRISM) as within subject effects were significant for rating dimension (sample 1: $F(3,75)= 15.41$, $p<.001$, $d=1.1$; sample 2: $F(3,72)= 24.47$, $p<.001$, $d=1.43$) with lower suffering than intensity and unpleasantness ratings. The main effect of controllability was not significant in this analysis (sample 1: $F(1,25)=0.40$, $p=.53$, $d=.03$; sample 2: $F(1,24)=0.93$, $p=.34$, $d=.07$), however, we found a significant interaction for controllability X rating dimension (sample 1: $F(3,75)=6.59$, $p<.001$, $d=.12$; sample 2: $F(3,72)=5.69$, $p=.001$, $d=.11$). This illustrates a reduction of suffering VAS

(t(25)=3.42, p=.008, d=.67) in the controllable condition of sample 1, which was not present in the intensity (t(25)=-1.37, p=.26, d=.27), unpleasantness (t(25)=-1.33, p=.26, d=.26) and PRISM (t(25)=0.31, p=0.75, d=.06) ratings (see Figure 2a). For sample 2, it illustrates an increase of unpleasantness ratings in the controllable condition (t(24)=-2.11, p=.04, d=.42), which was not present in the intensity (t(24)=-1.76, p=.09, d=.35), suffering VAS (t(24)=0.78, p=.44, d=.15) and PRISM ratings (t(24)=0.18, p=.85, d=0.03), (see Figure 2b).

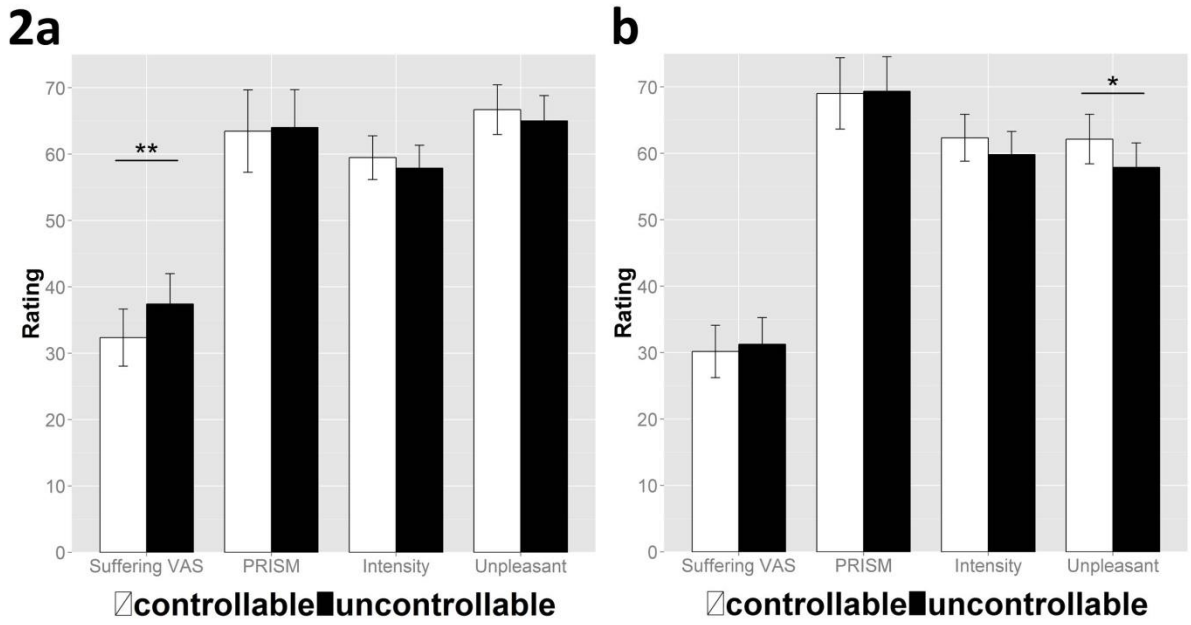

Figure 2: *Effect of controllability on ratings in the original sample (a) and the second experiment (b): Bars show mean pain intensity, pain unpleasantness, suffering VAS, and PRISM rating for the controllable (white) and the uncontrollable (black) condition, error bars depict the standard error of the mean. Asterisks show significant repeated-measures t-tests (controllable vs. uncontrollable) with *p<.05 and **p<.01. VAS: visual analogue scale; PRISM: Pictorial Representation of Illness and Self Measure.*

Table 1: *Pain and suffering scales within experimental conditions: Intensity, unpleasantness, suffering and PRISM ratings and ratings of perceived controllability are shown over all conditions.*[1]

Sample	Condition	Sample size (n)	Perceived controllability	Intensity	Unpleasantness	Suffering	PRISM
			M±SD	M±SD	M±SD	M±SD	M±SD
Sample 1 (personally relevant)	**controllable**	26	90.89±11.05	59.46±16.78	66.71±19.08	32.35±21.91	63.47±31.66
	uncontrollable	26	26.09±20.57	57.88±17.51	65.02±19.32	37.43±23.34	64.01±29.13
	significance		***	n.s.	n.s.	**	n.s.
Sample 2 (impersonal)	**controllable**	25	84.83±13.11	62.34±17.62	62.14±18.63	30.16±19.7	69.02±26.85
	uncontrollable	25	41.56±28.17	59.8±17.41	57.89±18.36	31.27±19.93	69.34±25.96
	significance		***	n.s.	*	n.s.	n.s.

[1] M: mean, SD: standard deviation, PRISM: Pictorial Representation of Illness and Self Measure, ***p<.001, **p<.01, *p<.05, n.s.: p>.05.

Individual differences in the effects of controllability on pain and suffering

Subjects with a high chance-related locus of control showed significantly more reduction in suffering VAS ratings in sample 1 (rp(24)=-.42, p=.03), but less reduction in suffering VAS (rp(23)=0.49, p=.03) and suffering as assessed with the PRISM (rp(23)=0.53, p=.02) in sample 2 in the controllable versus uncontrollable trials (see Figure 3). There was no significant influence of chance-related locus of control on the effect of experimental control for pain intensity (sample 1: rp(24)=-.38, p=.08; sample 2: rp(23)=0.35, p=.08), unpleasantness (sample 1: rp(24)=-.28, p=.17; sample 2: rp(23)=0.42, p=.05) and also not for PRISM ratings in sample 1 (rp(24)=-.02, p=.92). There was no significant effect of internal locus of control, locus of control directed to powerful others, catastrophizing, active coping or fear of minor, severe or medical pain on the difference between controllable and uncontrollable condition (all r<.35, p>.18).

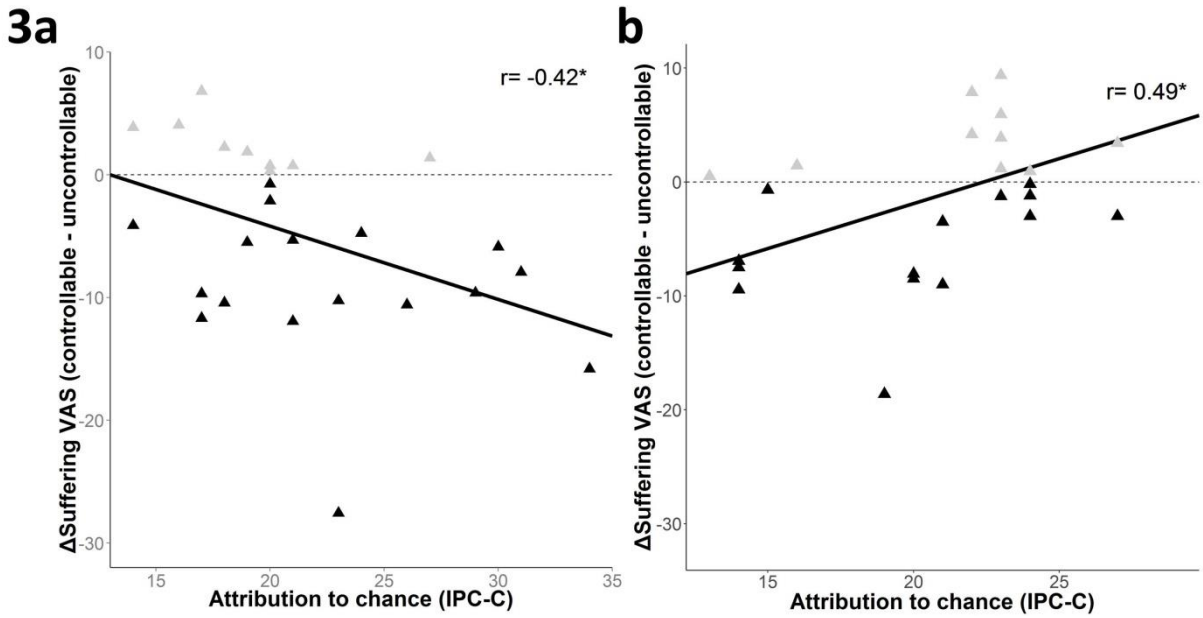

Figure 3: *Impact of attributional style on the effects of control in the original sample (a) and the second experiment (b): the x-axis shows the chance subscale of the IPC. The y axis shows the difference (Δ) in the suffering VAS ratings. Ratings in the uncontrollable condition were subtracted from ratings in the controllable condition. The black triangles depict the participants who indicated more suffering when pain could not be controlled. The grey triangles depict participants who indicated more suffering when pain could be controlled. IPC-C: Chance subscale of the internal, powerful others and chance scale, *p<.05.*

Table 2: *Correlations of attributional style, coping strategies and fear of pain with differences in ratings (controllable minus uncontrollable) for each rating (intensity, unpleasantness, suffering VAS, PRISM*[1]

	Sample 1 (personally relevant)				Sample 2 (impersonal)			
	Intensity	**Unpleasantness**	**Suffering VAS**	**PRISM**	**Intensity**	**Unpleasantness**	**Suffering VAS**	**PRISM**
	r(24); p	**r(24); p**	**r(24); p**	**r(24); p**	**r(23); p**	**r(23); p**	**r(23); p**	**r(23); p**
IPC: Internal	r=-.18 p=.38	r=-.03 p=.86	r=.2 p=.33	r=-.27 p=.19	r=.06 p=.76	r=-.15 p=.76	r=-.21 p=.76	r=-.11 p=.76
IPC: Powerful Others	r=-.23 p=.25	r=-.02 p=.91	r=-.16 p=.43	r=-.04 p=.86	r=.06 p=.95	r=.35 p=.33	r=.01 p=.95	r=.15 p=.92
IPC: Chance	r=-.38† p=.08	r=-.28† p=.17	**r=-.42*† p=.03**	r=-.02† p=.92	r=.35 p=.08	r=.42 p=.05	**r=.49* p=.03**	**r=.53* p=.02**
PRSS: Catastrophizing	r=-.13 p=.95	r=.21 p=.95	r=-.01 p=.96	r=.08 p=.95	r=-.08 p=.7	r=-.16 p=.61	r=-.16 p=.61	r=-.24 p=.61
PRSS: Coping	r=.09 p=.87	r=.22 p=.54	r=.01 p=.96	r=-.25 p=.54	r=.24 p=.38	r=.26 p=.38	r=.22 p=.38	r=-.02 p=.93
FPQ: minor	r=-.23 p=.35	r=-.24 p=.35	r=-.27 p=.35	r=-.12 p=.55	r=-.04 p=.83	r=.15 p=.63	r=.23 p=.63	r=.15 p=.63
FPQ: severe	r=-.31 p=.47	r=-.1 p=.63	r=.15 p=.62	r=-.2 p=.62	r=.04 p=.86	r=.12 p=.77	r=.17 p=.77	r=.15 p=.77
FPQ: medical	r=-.13 p=.88	r=-.03 p=.88	r=-.11 p=.88	r=-.08 p=.88	r=-.22 p=.59	r=-.01 p=.95	r=.25 p=.59	r=-.02 p=.95

[1] Correlation coefficients (r) and significance level (*p<.05, **p<.01, ***p<.001, †significantly lower than the respective correlation in sample 2) are reported. Degrees of freedom are depicted at the top. IPC-C: Internal, Powerful others and Chance scale. PRSS: Pain-Related Self Statements Scale. FPQ: Fear of Pain Questionnaire.

Sample differences in the effects of controllability on pain and suffering

There was no significant interaction of sample and controllability for pain intensity ($F(1,49)=0.28$, $p=.60$, $d=.03$), unpleasantness ($F(1,49)=1.18$, $p=.28$, $d=.07$), suffering VAS ($F(1,49)=3.74$, $p=.06$, $d=.09$) or PRISM ratings ($F(1,49)=0.01$, $p=.93$, $d=.004$). The correlation of chance–related locus of control with the difference in controllability ratings was significantly higher in sample 2 than in sample 1 for pain intensity ($z=2.57$, $p=.01$), pain unpleasantness ($z=2.47$, $p=.01$), suffering VAS ($z=3.30$, $p=.001$) and PRISM ratings ($z=2.05$, $p=.04$). The samples did not significantly differ in any of the correlations between the rating differences in pain intensity, pain unpleasantness, suffering VAS or PRISM with the subscales of the FPQ-III (all $z<1.72$, $p>.08$), PRSS (all $z<1.27$, $p>.20$) or IPC-I and IPC-P subscales (all $z<1.40$, $p>.15$).

Physiological correlates of experimental controllability

We found a significant increase of SCR during painful stimulation ($F(2,50)=27.5$, $p<.001$, $d=1.93$), a HR deceleration during painful stimulation ($F(2,50)=14.01$, $p<.001$, $d=.29$) and an anticipatory deceleration of corrugator EMG before the onset of painful stimulation ($F(2,50)=9.89$, $p<.001$, $d=.14$). None of the physiological measures differed significantly between controllable and uncontrollable trials. For further details on physiological data see supplementary material.

Discussion and conclusions

This study shows that control over pain primarily reduces the degree of perceived suffering. This effect was modulated by the subjects' locus of control: The more participants attributed their behavior to chance, the greater was the reduction of suffering when they had control over their pain. Pain intensity and unpleasantness ratings, in contrast, were unaffected by control over pain. This effect was only present when the subjects showed a high personal involvement in the experiment initiated by an instruction that focused the person being able to stop the pain. In a second experiment, where the instructions referred to controllable trials as 'stoppable by a button press', suffering was not influenced by controllability. Here controllability increased unpleasantness ratings, while pain intensity and suffering remained unaffected. Interestingly the modulation by attribution to chance was inverse in this second sample: the more participants attributed their behavior to chance, the smaller was the reduction of suffering when they had control over their pain.

Controllability and its influence on suffering

The finding that controllability reduces suffering extends the view of the impact of controllability on the pain experience by ascribing a key role to uncontrollability in the manifestation of suffering. According to Thompson [51], the effects of control depend on the meaning the individual ascribes to control, which matches the view that the transition from pain to suffering results when patients feel out of control, and that this transition is influenced by the meaning the individual ascribes to the pain (e.g. when chest pain is mistaken as a life threatening symptom by patients with a panic disorder) [15,29]. Thus, perceived controllability may act as an assurance that one will not face an event that is beyond the limits of endurance and suffering can be relieved by changing the meaning of the pain [3,14,42] to an experience that one can cope with.

Our results could therefore shed a light on the inconsistencies of prior studies, which examined the relationship of controllability and pain perception by relying exclusively on the pain intensity and unpleasantness dimensions. Because prior studies did not assess pain-related suffering, it is not clear to what extent these measures implicitly related to suffering. Depending on the relevance of suffering for the given experimental setup, pain relief [7,39,57], no changes [20,27,47], or in some cases even increases in perceived pain [48,56] may be obtained. The choice of outcome measure is thus important for the detection of the effects of controllability. Suffering has recently been proposed as an outcome measure in patients with chronic pain in addition to pain intensity and unpleasantness measures because it encompasses aspects of helplessness, hopelessness and the feeling of being overwhelmed [3].

Controllability and its influence on pain unpleasantness

In the second study, the pain unpleasantness ratings in the controllable condition were increased and controllability was not found to alleviate suffering. Testing for sample interaction effects showed that the effects of controllability on pain and suffering did not differ between the samples (see Figure 2). As reported above, increased pain ratings in response to controllability have been found before [48,56]. Our experimental design was similar to the one used in a study on brain mechanisms of pain controllability [57], but did not implement a button press at the end of the uncontrollable trials. This was included in the original study to account for motor responses in the brain. This missing button press at the end of the uncontrollable

trials may have induced a different attentional state as compared to the controllable trials. An attentive, but non-reactive awareness was previously shown to reduce pain unpleasantness [40].

Locus of control and suffering

An external locus of control directed towards luck, fate or chance has been associated with maladaptive pain coping strategies [19] and higher levels of pain despite patient-controlled analgesia [28]. Patients with chronic pain who attributed pain to chance experienced pain more frequently and showed high pain intensity ratings [11]. Overall, chance locus of control might be associated with less physical activity, more medication abuse and higher interference of pain in daily life [11]. Internal locus of control, by contrast, has been associated with positive outcomes like lower pain scores, higher satisfaction and lower disability levels [18,28].

In sum, although an internal locus of control seems to be a resilience factor against chronic pain [2,25], it does not seem to be associated with pain perception in experimental pain [57]. Rather, attribution to chance seems to lead to worse outcome expectations. Our results suggest that attribution to chance modulates the effects of control over pain on pain-related suffering. Fear of pain or pain catastrophizing did not modulate the effects of control, which implies a specific effect of the attribution to chance.

In study 1, higher attribution to chance led to greater reduction of suffering when pain could be controlled. However, in contrast to these findings, we found the inverse relationship in our second experiment. The two studies differed in the instructions given to the participants. It may be that subjects with a high attribution to chance usually do not perceive experimental pain as controllable, but may have overcome their general feeling of uncontrollability after the explicit announcement of control as a personal capacity in study 1. This, however, cannot explain the contrasting findings of our second study. Future studies should therefore more stringently target the modulatory effects of instructions on the effects of pain controllability on suffering. Additionally cultural factors should be considered, as it has been shown that the influence of external locus of control on affective symptoms is weaker for collectivistic societies [17]. Moreover, the meaning of suffering differs between cultures and depends not only on the cultural background of the patient but also on the cultural background of the caregiver [21,36,55].

Physiological correlates of experimental controllability

In this study, neither corrugator EMG nor SCR or HR were affected by control over pain. This is in line with a study showing that changes in SCR were not associated with the failure to control pain [27]. This study, however, also showed that changes in HR were associated with the failure to control pain. We previously found that SCR and corrugator EMG, but not HR were associated with suffering [8]. Given the explorative nature of our hypothesis on physiological correlates of controllability effects and the sensitivity of those effects to the modulation by individual and contextual factors, there is a need for further research on this issue. This should address the effects of perceived and exerted control on physiological correlates or the modulatory effects of personality.

Limitations

The instructions in our experiment where not optimal for the use of the PRISM task. It therefore cannot be ruled out that this ambiguity affected other aspects of the experiment. However, as all reports of ambiguity referred to and were limited to, the PRISM rating, it is unlikely that our results were biased by misunderstandings of the instructions. A change of the instructions in a second sample did not yield any changes in the effects of control on the PRISM task. The PRISM task was developed and validated for the application in health issues like posttraumatic stress disorder [59], lung disease, psioriasis, breast cancer [60] or chronic urticaria [52]. The sensitivity of the PRISM task in an experimental setting with healthy individuals has not been tested and classical VAS measures seem to perform better in this task.

Conclusion

The present study demonstrates that controllability primarily affects suffering rather than pain severity or unpleasantness. In addition, this result helps to understand the previous inconclusive findings on the effect of controllability on pain. We propose a complex interaction between individual control beliefs and instructional context that influence the experience of suffering. Future studies should take these factors into account when studying the effects of controllability and assessing its significance in a clinical context.

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Author contribution

ML, SK, MB & HF designed the study; ML & MB collected the data; ML & DK analyzed the data, ML, SK, MB & HF wrote the paper; SB, DK & FA commented on the paper, FA & HF acquired the funding. All authors contributed to the interpretation of the data, revised the manuscript, and approved the final version of the manuscript.

Conflict of interest

There is no conflict of interest.

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SUPPLEMENTARY MATERIAL

Physiological data

We recorded physiological responses during an anticipation phase, on-blocks and off-blocks (see Figure 1a). Anticipation of pain triggers similar physiological responses as pain itself [5], and the anticipation of pain may influence the experience of pain itself [4]. To study the effect of pain (on-block) or the anticipation of pain (anticipation phase) we also recorded a phase without any stimulation or the anticipation of the same (off-block) at the end of each trial to have a baseline of physiological responses. Physiological responses have previously been divided into different components, which have also been related to different pain components. For example, HR responses to pain were related to physical pain intensity until 3 seconds of pain onset, while after 6 seconds they were related to the perceived pain experience of the subjects [3]. We therefore checked HR and EMG responses for time effects before the main analysis.

Recordings

Raw EMG signals were filtered with a 20 Hz low cut-off, fullwave rectified and integrated (time constant: 10 ms) using a digital filter. When the EMG was split up into time bins of 1 second, we found a main effect of time for uncontrollable trials in the off-blocks ($F(11,264)=5.62$, $p<.001$, $d=.16$) and the on-blocks ($F(11,264)=1.82$, $p=.04$, $d=.13$), and for the controllable trials in the off-blocks ($F(11,264)=4.46$, $p=.005$, $d=.20$). There was no significant time effect for the anticipation phase (all $F(5,120)<.84$, $p>.52$, $d<.03$). As can be seen in Figure S1, these time effects can be split up into two phases (0-6 seconds and 6-12 seconds). These phases were therefore considered separately, when analyzing the EMG. Extreme values were excluded from the analyses (cut-off 2 SDs; 3.6% of the trials). All trials of the anticipation phases, on-blocks and off blocks were averaged per participant.

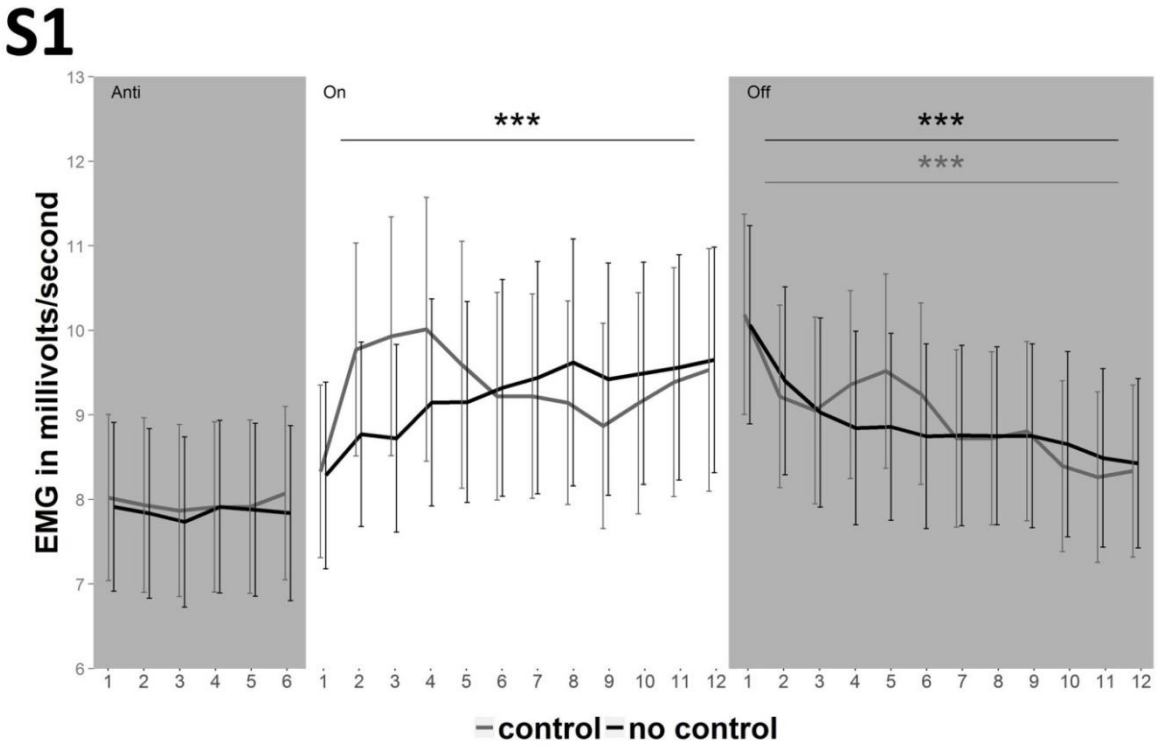

Figure S1: *Time effect in the electromyogram of the corrugator presented in time-bins of one second within each phase of a trial: lines show mean values, error bars depict the standard error of the mean of the corrugator EMG during anticipation phase (ANTI), On-blocks (ON) and Off-Blocks (OFF). Grey lines depict controllable trials, black lines depict uncontrollable trials. Asterisks show significant main effects within each block (anticipation, on-block, off-block) and condition (controllable, uncontrollable) with ***p<.001. EMG: electromyogram of the corrugator; ANTI: anticipation phase; ON: on-blocks; OFF: off-blocks;*

SCR amplitudes were quantified as the maximum response in the time window of 2–6 s in the anticipation phase, the on-block and the off-block, and were converted to microSiemens (µS). This time window was chosen because it showed the largest responses during raw data inspection. SCR amplitudes below 0.05 µS were classified as zero responses. The data were transformed using a logarithmic transformation (log10(1+SCR)) and extreme values were excluded from the analyses (cut-off 2 SDs; 4.1% of the trials). To account for habituation effects during the experiment, nonlinear detrending was performed by fitting the data to an 1/ex function using the nonlinear least squares method in R [1]. For each subject an 1/ex function was fitted to the log-transformed original data. All further analyses were

performed with the residuals of the original data from this fitted function. All trials of the anticipation phases, on-blocks and off-blocks were averaged per participant.

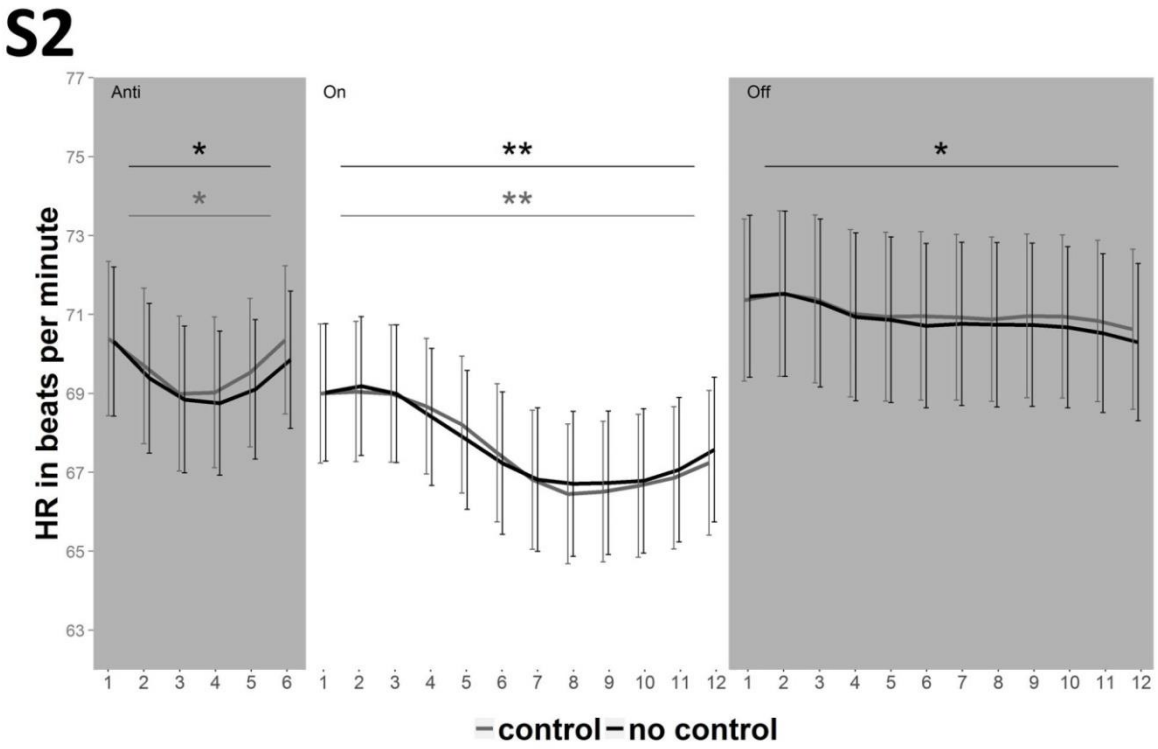

Figure S2: *Time effect in the heart rate presented in time-bins of one second within each phase of a trial: lines show mean values, error bars depict the standard error of the mean heart rate during anticipation phase (ANTI), On-blocks (ON) and Off-Blocks (OFF). Grey lines depict controllable trials, black lines depict uncontrollable trials. Asterisks show significant main effects within each block (anticipation, on-block, off-block) and condition (controllable, uncontrollable) with *p<.05 and **p<.01. HR: heart rate; ANTI: anticipation phase; ON: on-blocks; OFF: off-blocks;*

Raw ECG signals were filtered offline with a 20 Hz low cut-off digital filter. Beat detection was visually inspected and interpolated (added, removed or relocated: <1% of R-peaks), if necessary. When the HR was split up into time bins of 1 second, we found a significant main effect of time in the on-blocks (controllable: $F(11,264)=9.40$, $p=.001$, $d=.23$; uncontrollable: $F(11,264)=7.78$, $p=.004$, $d=.21$), in the anticipation phase (controllable: $F(5,120)=4.23$, $p=.03$, $d=.11$; uncontrollable: $F(5,120)=4.01$, $p=.04$, $d=.11$), in the off-blocks only for uncontrollable trials (controllable: $F(11,264)=2.78$, $p=.08$, $d=.05$; uncontrollable: $F(11,264)=4.48$, $p=.01$, $d=.06$), as can be seen in Figure S2, this time effect can be split up into three phases (0-3 seconds, 3-6 seconds, 6-9 seconds), as was previously done by Möltner et al. [3]. These

phases were therefore considered separately, when analyzing the heart rate. Heart rate (HR) was calculated as mean HR before the stimulation (anticipation phase, 6 seconds), during the stimulation (on-block, variable duration) and after each stimulation (off-block, 12 seconds). Extreme values were excluded from the analyses (cut-off 2 SDs; 4 % of the trials). All trials of the anticipation phases, on-blocks and off-blocks were averaged per participant.

Statistical analysis

For SCR, EMG and HR we examined the within-subject effects of block (on-block vs. anticipation phase vs. off-block), and controllability (controllable versus uncontrollable). For HR we additionally included the factor time (0 to 3 sec vs 3 to 6 sec vs. 6 to 9 sec). Effect sizes for the ANOVAs are reported as Cohen's d. We used pairwise post-hoc t-tests (false discovery rate, FDR [2] corrected) to compare the on-blocks, off-blocks and anticipation phases. Effect sizes for the t-tests are reported as Cohen's d.

Results

SCR showed a significant effect for block ($F(2,50)=27.5$, $p<.001$, $d=1.93$). Post hoc comparisons revealed that the SCRs were significantly higher during on- compared to off-blocks ($t(24)=5.29$, $p<.001$, $d=1.03$) and the anticipation phase ($t(24)=6.80$, $p<.001$, $d=1.33$), see Figure S3. The SCRs during the anticipation phase did not significantly differ from the SCRs during the off-blocks ($t(24)=-0.03$, $p=.97$, $d=.006$). There were no other significant main effects or interactions (all $F<2.9$, all $p>.06$, all $d<.09$).

The EMG showed a significant main effect for block ($F(2,50)=9.89$, $p<.001$, $d=.14$) with post-hoc comparisons showing that EMG during anticipation phase was lower than during off- ($t(24)=-4.57$, $p<.001$, $d=.93$) and on-blocks ($t(24)=-3.46$, $p=.003$, $d=.70$). EMG during on-blocks did not differ significantly differ from the EMG during off-blocks ($t(24)=-.55$, $p=.58$, $d=.11$), see Figure S3. There were no other significant main or interaction effects for the EMG response (all $F<2.38$, $p>.12$, $d<.04$).

HR showed a significant main effect for block ($F(2,50)=14.01$, $p<.001$, $d=.29$). Post hoc comparisons revealed that HR was significantly lower during on- compared to off-blocks ($t(24)=-3.95$, $p=.002$, $d=.80$) and the anticipation phase ($t(24)=-3.55$, $p=.002$, $d=.72$). HR during anticipation was significantly lower than during the off-

blocks (t(24)=-3.21, p=.004, d=.65), see Figure S3. Furthermore, the HR displayed a significant main effect for time (F(2,50)=7.45, p=.008, d=.06). Post-hoc comparisons showed that HR was significantly higher in the first 3 seconds after onset, compared to seconds 3 to 6 (t(23)=-5.52, p<.001, d=1.12) and decreasing again in seconds 6 to 9 (t(23)=-2.74, p=.01, d=.56). We furthermore found a significant interaction of time X block (F(4,100)=5.87, p=.01, d=.14), which represents the stronger heart rate deceleration during on- compared to off-blocks, especially 6 to 9 seconds after onset, see Figure S2. There were no other significant main or interaction effects for the HR (all F<2.04, p>.16, d<.02).

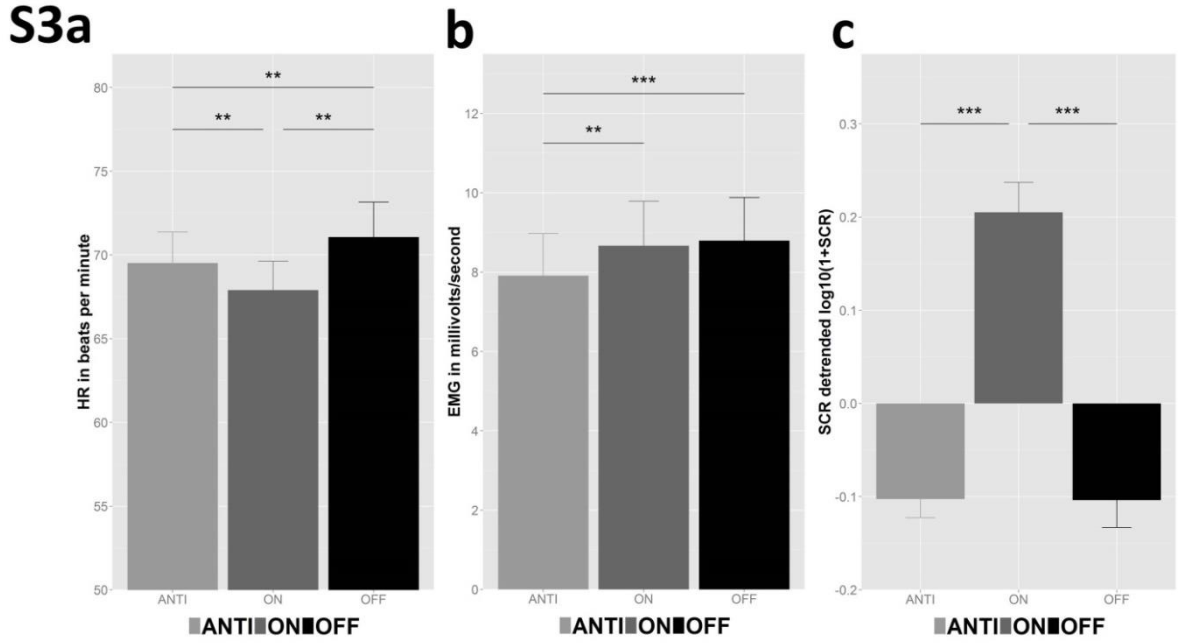

Figure S3: *Psychophysiology: Bars show mean values, error bars depict the standard error of the mean. Asterisks show significant post-hoc tests (corrected for multiple comparisons with the false discovery rate FDR) with **p<.01 and ***p<.001. (a) heart rate was lowest during on-blocks. During anticipation HR was already decreased. (b) Corrugator electromyogram was decreased during anticipation only. (c) Skin conductance responses increased during painful stimulation (on-blocks). SCR: skin conductance responses; EMG: electromyogram of the corrugator; HR: heart rate; ANTI: anticipation phase; ON: on-blocks; OFF: off-blocks;*

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3 GENERAL DISCUSSION

3.1 Study 1

Our healthy volunteers showed SIA when placebo was administered, as well as after THC or CBD administration. This is indicated by reduced pain ratings and increased pain thresholds after the stressor. However, neither THC nor CBD altered the SIA effect, and therefore the results do not support hypotheses 1.1 and 1.2. BOLD responses in the fMRI showed that the SIA effect was associated with increased brain activity in the ACC, thalamus and PAG after the stressor compared to before, independent of the administered drug. Those areas have previously been implicated in the descending inhibition of pain. Activity in the frontal pole, which was previously associated with preparatory responses to pain (Ploghaus et al., 1999), attention to pain and affective pain processing (Coghill et al., 2003), increased after the stressor in the THC and placebo condition, but not after CBD administration. Further, THC was associated with lower reductions of activity in S1, but not other areas of the descending pain pathway. CBD did not alter SIA-induced activity in any brain region that is involved in the descending inhibition of pain. Therefore the results do not support hypotheses 2.1 and 2.2. Additional analysis showed that THC, compared to the placebo condition, attenuated the habituation to a painful stimulus. Along with this habituation to painful stimulation, the activity in the MFG and MTG showed a greater reduction when a placebo was administered compared to THC.

These results suggest that the endocannabinoid system is involved in modulatory mechanisms of pain, however, in a different way than expected. In the following the absence of SIA modulation by cannabinoid action is discussed, as well as the presence of modulation of habituation by cannabinoids.

The absence of SIA modulation by different cannabinoid receptor agents contradicts rat studies, which demonstrated cannabinoid involvement in SIA (Connell et al., 2006; Hohmann et al., 2005). These contradictory findings may have two possible explanations with different implications for pain therapy and basic research on the neurochemical mechanisms of pain: (1) Conclusions from rat studies on endocannabinoid-mediated SIA cannot be generalized to human pain pathways. (2) The SIA effect that we see in our study underlies a different mechanism than those seen in prior studies.

The first explanation may relate to the lack of SIA modulation by cannabinoids in study 1. It can however hardly explain the effects that we found on habituation to pain. These results indicate that there is indeed a cannabinoid-mediated pain modulatory mechanism that is preserved in humans. Further, the endogenous cannabinoid system is conserved throughout evolution from coelenterates to humans (Salzet, Breton, Bisogno, & Di Marzo, 2000). Therefore it also seems likely that cannabinoid function and neuronal pathways are preserved in humans.

The second explanation is in line with the fact that all prior studies on endocannabinoid mediation of SIA used painful electric stimulation to induce stress. The original study, which proposed a non-opioidergic pathway for SIA has used continuous inescapable foot shock (3 minutes at 60Hz with 3 mA) to induce non-opiodergic SIA (Lewis et al., 1980). Later studies adopted this procedure, changing only the stimulation intensity to 0.9mA, and confirmed an endocannabinoid mediation of this type of SIA (Connell et al., 2006; Hohmann et al., 2005). In our study we have used a continuous stressor as well, however not a painful stimulus but rather a cognitively demanding task. Moreover, we used electrical stimulation blocks to measure pain sensitivity before and after the stressor. During this painful stimulation we found a reduction of pain sensitivity. The administration of THC, a cannabinoid receptor agonist, attenuated this pain suppression. The electrical stimuli that we used to measure pain sensitivity might therefore have induced the same cannabinoid-mediated pain suppression, which was found in animal studies using electrical stimuli to induce SIA. The second explanation, however, is not in line with the finding that opioid-mediated SIA is induced by intermittent stress, while non-opioid mediated SIA is induced by continuous stress (Lewis et al., 1980). We have used a continuous stressor, which by this rationale should induce cannabinoid-mediated SIA, while our intermittent painful stimulation should induce opioid-mediated SIA. However, previous data have shown that a continuous stressor, similar to the one we used, also induced opioid-mediated SIA (Flor et al., 2002) and supports our data which show that this type of SIA may be independent of cannabinoids. Furthermore, the stressor we used was 15 minutes long and therefore significantly longer than the 3 minutes that were used to induce non-opioid SIA in the study by Lewis et al. (1980). In the same study, 30 minutes of stressful stimulation induced opioid-mediated SIA (Lewis et al., 1980). The painful stimulation in our study, with a total of ~2 minutes (10 times 11.76

seconds) of stimulation, resembles the duration of this non-opioid stress induction much better than our mental stressor.

Habituation to repetitive painful stimulation is a form of non-associative learning and was found to be impaired in patients with chronic pain disorders (Valeriani et al., 2003), whereas intact habituation seems to be a resilience factor for chronic pain. Central processing of habituation was confirmed in a study, which showed that pain attenuation was found at the arm contralateral to the stimulation site. This effect was not modulated by the systemic administration of an opioid receptor antagonist (Rennefeld, Wiech, Schoell, Lorenz, & Bingel, 2010). On the neuronal level, habituation to pain was accompanied by a reduction of activity in the medial prefrontal cortex, ACC, thalamus, S1, S2 and insular cortex (Becerra et al., 1999). A similar network of brain regions showed decreased activation during painful stimulation in study 1. So far no study has directly investigated the modulation of habituation to painful stimulation by cannabinoids. However, the cannabinoid system was shown to be involved in a number of learning processes: Cannabinoid agents were shown to modulate fear extinction in the hippocampus (Abush & Akirav, 2010). A well-established finding is also that cannabinoids modulate working and short-term memory (Ranganathan & D'Souza, 2006). In general, cannabinoid receptor agonists seem to impair memory formation, whereas cannabinoid receptor antagonists seem to reverse these deficits or act as memory enhancers (Riedel & Davies, 2005). In our study we showed that THC, a cannabinoid receptor agonist, impaired non-associative learning, here habituation to repetitive painful stimulation. This significantly adds to our understanding of learning mechanisms in pain processing. It is, however, an incidental finding and therefore needs to be replicated in another sample. Such studies should take special care with respect to timing aspects of the experimental procedures. For example, the low-frequent blood sampling in our study caused methodological limitations, which may be overcome by implementing continuous blood monitoring.

3.2 Study 2

The healthy volunteers in study 2 indicated that they suffered less during a controllable painful stimulation compared to an uncontrollable painful stimulation, while perceived pain intensity and unpleasantness were not different between the controllable and uncontrollable painful stimulation. Therefore the results only partly

confirmed hypothesis 3.1. The significant interaction between the effect of control on pain ratings and the type of rating indicate that the effect of control on the suffering rating was more pronounced than that on pain intensity and pain unpleasantness ratings. Therefore the results support hypothesis 3.2. Additional analyses showed that the ameliorating effect of controllability on suffering was stronger in subjects with a high chance-related locus of control. In a second experiment the instructions were changed from announcing controllability as a personal capacity (first experiment) to a technical procedure (second experiment). Analysis of the second experiment showed that suffering was no longer modulated by control and that the relationship between chance-related locus of control and the effects of control was inverse. Subjects with higher chance-related locus of control showed lower reductions of suffering when pain could be controlled than when pain was not controllable. Further, pain unpleasantness ratings were higher in the controllable condition as compared to the uncontrollable condition in the second experiment.

These results have several implications. First, our data experimentally confirm theoretical work on suffering, such as the work of Cassell (1982), who postulated that controllability has a strong modulatory influence on suffering. This assumption has also been tested empirically in interview studies, where chronically ill patients (cardiovascular disease, diabetes, cancer, multiple sclerosis, lupus erythematosus and others) indicated that they suffered if loss of function was associated with loss of control over their lives (Charmaz, 1983). Along with this, an interview study with 40 participants above 70 years of age identified lack of control as a general theme of the suffering experience in later life (Black & Rubinstein, 2004). Our study was the first to systematically vary controllability in an experimental setup to assess its effects on suffering.

Second, the results of our data show that controllability was not associated with pain intensity and unpleasantness. It was previously shown that suffering is an independent component of the pain experience that can be assessed in experimental setups and is distinguishable from the pain intensity and unpleasantness dimensions (Brunner et al., 2017; Bustan et al., 2015). The present study now adds that suffering is also independently related to controllability as a specific aspect of the pain stimulation.

Third, individual locus of control moderated the effect of control on suffering. A number of enduring beliefs have been associated with suffering (see Figure 2). In a

study with patients with myofascial pain dysfunction, for example, neuroticism was related to suffering in the presence of experimental as well as clinical pain, while no such relationship was found for the sensory aspects of the pain (Harkins, Price, & Braith, 1989). Additionally, fear of pain and private self-consciousness were associated with suffering in response to experimental pain stimulation in healthy volunteers (Brunner et al., 2017). Suffering is a highly individual experience and individuals will suffer under different conditions (Cassell, 1999; Edwards, 2003). Distressing events, such as a major illness may elicit suffering or not, depending on the meaning the individual gives to such events (Thompson, 1981). If the illness is associated with loss of important functions in the patients' daily life, it will elicit higher degrees of suffering (Cassel, 1982; Cassell, 1999). A professional sprinter, for example, will most probably suffer from an injury which precludes his or her further career in sports. The same injury will induce comparable pain intensity and unpleasantness in an average person, however, this person may be suffering to a lesser degree if the injury does not impede daily-life functioning. Therefore individual beliefs and lifestyles may modulate suffering. The data of the current study did not only show that pain controllability alleviated suffering, but also that this effect was modulated by the individual beliefs of the person. In the case of our study the degree to which the individual tended to attribute events in his or her life to luck or fate. It did not matter for participants with a low chance-related locus of control if the external event (painful stimulation) was controllable or not, as they did not perceive this event as being controlled by external factors in the first place. On the other hand, participants who have a high chance-related locus of control, perceive external events (e.g. painful stimulation) as being controlled by external factors, which are out of their control. A clear indication of internal control changed the experience of the painful stimulation which then induced less suffering.

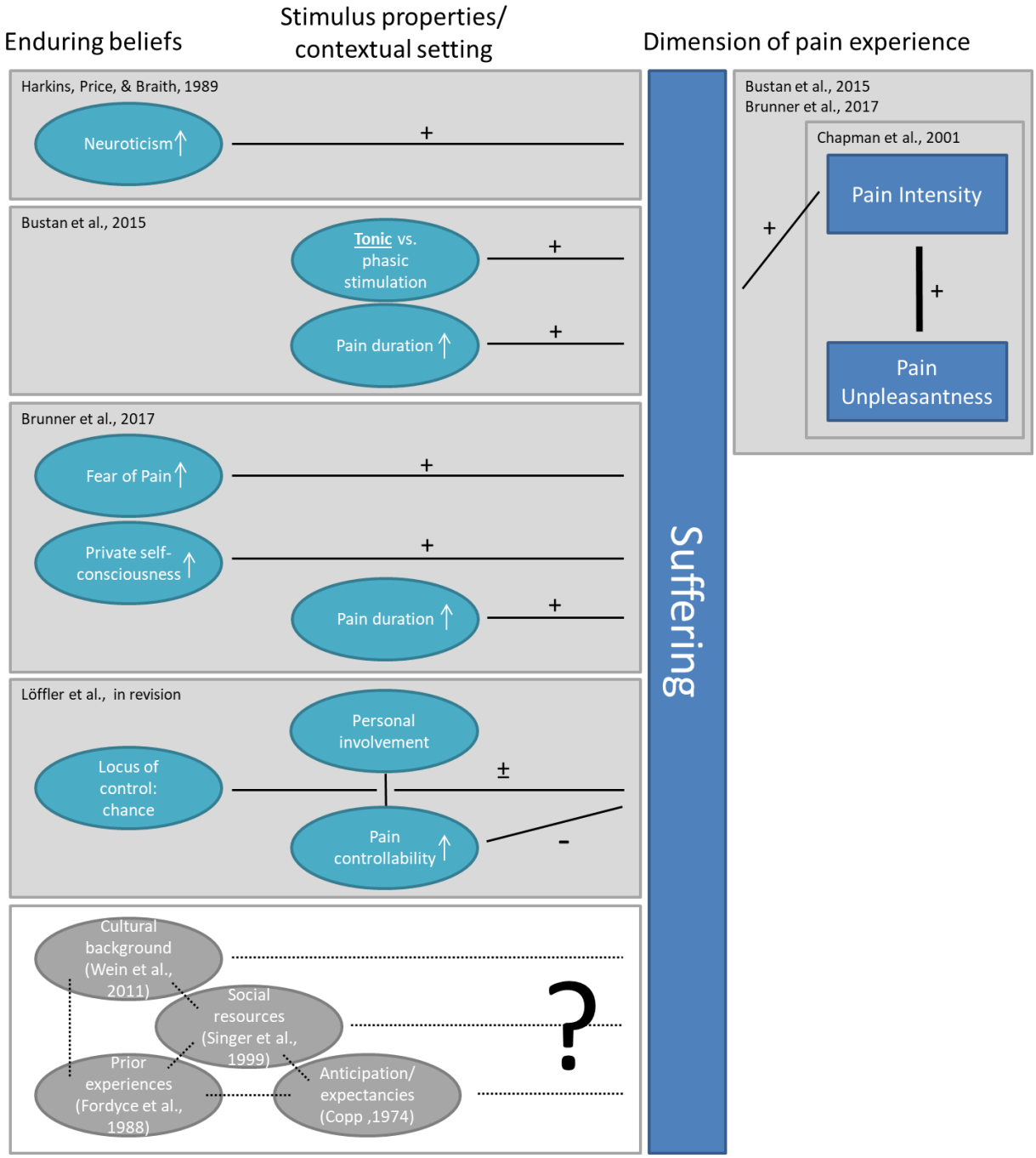

Figure 2: *A theoretical framework, based on empirical findings, illustrating how suffering is related to stimulus properties of painful events, contextual setting, traits and other pain dimensions. Lines indicate positive (+) or negative (-) relationships, or modulatory (±) effects. Dashed lines in the bottom box indicate associations which need further empirical evidence.*

3.3 Limitations

Study 1 is limited by the systemic route of drug administration, which has the disadvantage of various areas of central, but also peripheral receptor binding. In animals the role of specific sites can be investigated by using microinjections into these areas (Connell et al., 2006; Hohmann et al., 2005), which allows for a dissociation of central and peripheral effects of drug administration. In humans, less invasive methods are preferred. We have used fMRI to identify the brain areas involved in cannabinoid modulation of pain. This, however, does not allow for conclusions about receptor binding in the brain areas identified, as indirect modulation by other brain areas might be responsible for the changes in BOLD response. In future human studies this drawback could be overcome by implementing methods with, for example, positron emission tomography (PET) to identify binding sites of the systemically administered drugs. Further we have used CBD in our study to down-regulate central cannabinoid signaling by acting as an inverse agonist at the CB1 receptor. The mode of action of CBD at the CB1 receptor is, however, still relatively unclear and may involve 5-HT and vanilloid receptor types (Maione et al., 2011). The study might have benefitted from cannabinoid agents with a clearer receptor profile, such as SR141716A. However, SR141716A was shown to have severe side effects, such as severe mood disorders (Sam, Salem, & Ghatei, 2011). Another drawback related to pharmacokinetics is the relatively long experimental period without the assessment of THC and CBD concentration in the blood. We decided to use low frequent blood sampling to avoid unintended stressful events and because blood withdrawals were shown to confound BOLD signal changes (Kalisch, Elbel, Gössl, Czisch, & Auer, 2001).

Study 2 could have benefitted from the prior development of a method to measure suffering in experimental setups. As there was no gold standard we used a VAS together with the “Pictorial representation of illness and self measure” (PRISM), where the latter seemed to be less suitable for experimental use. Further, study 2 may not cover all aspects of the multidimensional phenomenon of suffering. For example, existential aspects of the suffering experience can hardly be addressed in a laboratory setup. Therefore the second study should be extended to clinical pain populations where controllability of the pain is central.

3.4 Conclusions and outlook

Both studies contributed relevant findings related to endogenous mechanisms to regulate pain and suffering in humans, but also raised further questions.

Study 1 found that the regulation of pain intensity and pain unpleasantness by habituation to repeated painful stimulation, but not SIA induced by a cognitive stressor, was modulated by THC. Cannabinoid based drugs become increasingly common in the treatment of chronic pain and the understanding of cannabinoid action becomes increasingly important. From the results of our study, it seems that THC does not increase the effects of endogenous pain inhibition by SIA, but rather impairs the habituation to pain. Future studies are needed to show if THC impairs the habituation to pain also in a clinical context.

Study 2 found that controllability reduced suffering but not pain intensity or pain unpleasantness. It therefore sheds light on previous inconsistencies in studies on the role of controllability in pain perception. Study 2 failed to confirm these results in a separate sample, however it highlights the contextual setting and enduring beliefs as interesting modulators of controllability effects on suffering.

Endogenous mechanisms to regulate pain remain a highly interesting field of research. The two studies show, that these mechanisms need to be addressed on multiple levels, reaching from the perceptual dimensions of the pain experience to neuronal and neurochemical mechanisms. Knowledge about the bodies' own mechanisms to regulate pain, and an understanding of the modulation of those mechanisms, offers the possibility to strengthen them in patients who suffer from chronic pain and thereby offer pain relief for patients.

4 SUMMARY

This dissertation presents two studies investigating stress-induced analgesia (SIA, study 1) and control-induced analgesia (study 2) as two endogenous mechanisms of pain control. In study 1 SIA was induced in 19 healthy volunteers after administration of Tetrahydrocannabinol (THC), Cannabidiol (CBD) and placebo. The SIA effect was evaluated by pain ratings and blood oxygen level dependent (BOLD) responses to suprathreshold painful stimulation, pain thresholds, BOLD responses and psychophysiological measures. The aim was to determine whether cannabinoids are involved in human descending pain control. The main result was that SIA, although successfully induced in all conditions, was not modulated by an exogenously administered cannabinoid receptor agonist or inverse agonist. However, after THC administration the habituation to painful stimulation was attenuated. This was accompanied by altered brain activation in the middle frontal gyrus and middle temporal gyrus. The results suggest that in humans, cannabinoids are involved in habituation to pain, but not in SIA.

In study 2, 26 healthy volunteers were given painful stimulation in a controllable and an uncontrollable condition. The control-induced pain relief was assessed with ratings of pain intensity, pain unpleasantness and pain-related suffering. The aim of the study was to determine which pain dimension is affected by controllability of pain stimulation. The main result was that the exertion of control over pain reduced the experience of pain-related suffering while pain intensity and pain unpleasantness were not affected. Moreover, the effect on pain-related suffering was more pronounced in individuals with a higher general belief that their environment is determined by chance. The results suggest that control over a painful stimulus does not affect the classical pain dimensions of intensity and unpleasantness, but rather the suffering that is associated with them.

The results of study 1 indicate that SIA induced by mental arithmetic tasks is not mediated via endocannabinoid pathways, whereas these pathways seem to be involved in other inhibitory pain systems. Study 2 demonstrates that control over pain alleviates the suffering rather than the pain itself. It therefore offers a therapeutic target in cases of terminally ill, were suffering but not pain can be avoided. A better understanding of behavioral, physiological and neuronal mechanisms underlying healthy human pain inhibition offers new targets for pain inhibition in chronic pain.

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