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MULTISENSORY INTERACTION:

DIFFERENT PAIN PERCEPTION THROUGH SMELL AND TASTE

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SOMMARIO

Il dolore è una percezione fondamentale per la nostra sopravvivenza, in quanto funziona da sistema di allarme che ci segnala un pericolo. Ciononostante, capita che alcuni pazienti lamentino dolore in assenza di un effettivo danno fisico. Quando il dolore neuropatico diventa di tipo cronico, la qualità di vita di tali pazienti peggiora notevolmente, con conseguenze negative in tutti gli aspetti della vita. Ad oggi, ci si rende sempre più conto della necessità di sviluppare terapie alternative per trattare il dolore, evitare gli effetti collaterali dei farmaci e ridurre così i costi del sistema sanitario.

Il dolore può essere influenzato da molti fattori, e tra le strategie più adatte ed efficaci per modulare il dolore in modo non invasivo, si riconosce la stimolazione attraverso canali sensoriali diversi. Olfatto e gusto sono fortemente connessi a componenti emotive e cognitive, e potrebbero essere utili nel modulare la percezione del dolore, fornendo quindi opzioni alternative per il trattamento e la gestione del dolore.

In questo elaborato vengono riportate le evidenze a favore del fatto che olfatto e gusto potrebbero rappresentare degli utili strumenti in interazione multisensoriale con il dolore. Prima di tutto, vengono presentati i risultati di una revisione della letteratura nella popolazione adulta, che dimostra gli effetti delle sostanze olfattive e gustative sia sul dolore sperimentalmente indotto che sul dolore clinico: diversi tipi di odori influenzano principalmente la spiacevolezza e l'intensità del dolore (chiamate qui misure qualitative del dolore), mentre i gusti influenzano principalmente la soglia e la tolleranza al dolore (così dette misure quantitative), con risultati maggiormente contraddittori. Da questa revisione, emerge la scarsità di sperimentazioni nell'ambito del dolore clinico e di ricerca effettuata tramite l'uso del neuroimaging.

In seconda misura, è stato svolto uno studio sperimentale su soggetti sani (n = 60) dove il dolore veniva indotto tramite capsaicina applicata in crema sul dorso della mano, poiché questo metodo mima il dolore tonico neuropatico. È

stato trovato che la spiacevolezza del dolore si riduce dopo la somministrazione di una sostanza olfattiva piacevole, mentre nessun effetto è stato riscontrato sull'intensità del dolore, né tantomeno tramite altre sostanze.

Infine, un esperimento molto simile è stato condotto in una popolazione clinica che soffre di un dolore cronico orale di tipo bruciante (n = 22), mostrando che la spiacevolezza del dolore aumentava dopo le somministrazioni delle sostanze olfattiva e gustativa spiacevoli. Tale effetto, per l'olfatto, sembra essere legato alla percezione soggettiva di piacevolezza della sostanza stessa da parte dei pazienti. Inoltre, un effetto più forte è stato trovato nei pazienti con una durata di malattia più lunga. Nessun effetto è stato invece rilevato sull'intensità del dolore, né con le altre sostanze usate. Sono state inoltre raccolte anche diverse variabili cliniche e psicologiche.

Per concludere, olfatto e gusto potrebbero temporaneamente alterare la spiacevolezza del dolore tonico, sia di tipo sperimentale (e quindi indotto), che in una condizione di dolore clinico cronico. Futuri esperimenti, che magari utilizzino sostanze selezionate su misura per il paziente, potrebbero smascherare un effetto più forte, e rendere così possibile l'esplorazione di effetti più a lungo termine. E' importante non dimenticare, infine, il ruolo dell'attenzione e delle emozioni nella complessa relazione tra dolore e sensi chimici, che potrebbero ricoprire un ruolo cardine nell'eventuale efficacia di tali strategie alternative.

I tre lavori riportati in questo elaborato sono stati pubblicati su riviste scientifiche internazionali.

ABSTRACT

Pain is a fundamental perception for our survival, acting as an alarm system. Nevertheless, patients may complain about the presence of pain even without actual physical harm. When neuropathic pain becomes chronic, those patients' quality of life decreases dramatically, and more or less severe consequences on several life aspects become evident. Nowadays, alternative pain-relief therapies are increasingly necessary in order to avoid drugs shortcomings, as well as to reduce the costs for healthcare systems.

Pain can be influenced by many factors, and the combined use of other senses in a multisensory interaction represents one of the most effective strategies to alter its perception. Smell and taste are strongly linked to emotion and cognition, and they might be useful in pain modulation, providing alternative approaches for pain treatment and management.

Here, we provide evidence of the useful tools that smell and taste could represent when interacting with pain. Firstly, we report the results of a literature review on adults stating the effects of olfactory and gustatory substances on both experimental and clinical pain. Smell of different types influences pain unpleasantness and intensity (the so-called qualitative measures of pain), while taste has an effect on pain threshold and tolerance (the quantitative measures) with more contradictory results. Scarce literature is reported in clinical pain and with the use of neuroimaging measures.

Secondly, we performed an experimental study on healthy adults (n = 60) where we induced pain with capsaicin cream applied on the back of their hand. Such methodology has been chosen to resemble a sort of tonic neuropathic pain. We found evidence that pain unpleasantness is reduced after the administration of the pleasant smell condition. No effect was found on pain intensity, nor with other substances.

Thirdly, we performed a very similar experimental design on a clinical population that suffers from chronic oral burning pain (n = 22), revealing that pain unpleasantness was increased by the unpleasant smell and taste substances. With reference to the smell substances, such effect was related to the

subjective perceived pleasantness of the patients. Moreover, a stronger effect was found in patients with longer disease duration. No effect was found on pain intensity, nor with other substances. Several clinical and psychological variables were also collected.

To sum up, smell and taste could temporarily alter pain unpleasantness perception in tonic pain, both experimentally induced and in a clinical condition. Future experiments that select custom-designed substances for each patient could reveal a stronger effect in chronic pain populations and explore long-term efficacy. Within this frame, it is important not to forget the key role that attention and emotions play in the relation between pain and chemical senses, when exploring the effectiveness of those alternative options.

The three works here described have been published in international peer reviewed journals.

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1: INTRODUCTION

Every day we integrate multimodal stimuli coming from both within our body and the surrounding environment: assessing them helps us take fundamental decisions for our survival, primarily to estimate a possible danger. The perception of pain usually kicks in to warn and protect our system, urging us to act in order to remove a harmful stimulus, or to signal the presence of a disease. Sometimes, something goes wrong, and pain is present even without physical harm. In these cases, when pain is a disease itself with no apparent reason to express itself, the patients' quality of life becomes meager, affecting all aspects of their lives. For their treatment, alternative pain-relief therapies are increasingly necessary in order to avoid drugs shortcomings, as well as to reduce the costs for healthcare systems.

Growing evidence account for a multisensory interaction between pain, smell and taste, which seem to share common features also on a brain level, and such an integration might be useful in pain modulation. Smell and taste are strongly linked to emotion and cognition, leading to the idea that, together, they could help finding alternative strategies for pain treatment and management. In this work, we will first review the concept of pain. Then, we will overview how smell and taste can modulate pain, and how the study of such interaction is only at its beginning. Starting from a review of the previous literature (showing the scarce research present in adults, especially in clinical populations), we will introduce a method used to induce tonic pain (capsaicin cream), that we employed in an experiment in healthy participants. Such method can be used to resemble a sort of neuropathic pain. Finally, we will describe a work where the interaction between pain, smell and taste has been studied in a group of patients suffering from oral burning pain without a recognized organic cause, in order to explore the effects of chemosensory stimuli on neuropathic pain. Our goal is to find evidence for the possible use of chemosensory stimuli as a temporary strategy for chronic pain management, a first step towards alternative options to pharmacological drugs.

Pain

> « An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage » (Raja et al., 2020)

In 2020, after months of discussions, a panel composed of the major international experts on the subject reached a new definition of pain, trying to convey all the facets that are enclosed in its perception. Pain is a sensation which is usually, but not always, linked to a physical damage coming from within our body. Its purpose is to inform us that something is wrong, warning us that we are injured, or sick, and giving us the boost to act for survival. But pain embraces not only physical features: emotional and psychological aspects play a fundamental role in its perception. It is a multimodal experience comprising sensory, emotional, and cognitive dimensions (Rainville et al., 1997; Price, 2000; Talbot et al., 2019) that can be experienced even without an evident physical injury.

Physical damage is indeed not the primary requisite for pain perception: the new definition reports in its footnotes that pain is such a personal experience that can be influenced to various degrees by biological, psychological, and social factors (Raja et al., 2020). Pain is clearly defined by the conjunction of several aspects (Mouraux and Iannetti, 2018). Within this concept, the environment plays therefore a fundamental role in pain perception.

Pain is one of the most disabling symptoms in daily life. It can vary broadly in intensity, quality, and duration. It has varied pathophysiologic mechanisms and meanings. Most of all, it is the source of complaints from patients, who report pain as one of their major symptoms, in comorbidity with diseases or as a disease itself. Therefore, it represents a challenge for clinicians, and a burden for every kind of society. Pain surely is one of the leading causes of living with disabilities for years, producing consistent health loss (Vos et al., 2012). The economy is also strongly affected by pain with increasing health care costs, to which we must add the indirect costs of lower work productivity (Gaskin and Richard, 2012). In this regard, social disparities play a significant

role (Lee et al., 2020). For all these reasons, it is fundamental to find alternative pain management options.

Pain usually has an adaptive role: it acts as an alarm bell for the damage threat to the body (Mouraux and Iannetti, 2018), and its intrinsic unpleasantness linked to its intensity. Unfortunately, it is not always the case for pain to be adaptive: sometimes, it may have adverse effects on biological functions, and social and psychological well-being (Raja et al., 2020). These adverse effects are especially profound in the occurrence of chronicity, and even in the absence of a physical damage, deeply affecting the quality of life.

As pain holds a vital role in everyday life, researchers started to question whether a specific area of the brain could specifically spike in response to a painful stimulus. As the pain experience is such a complex facet, an author first theorized a network of areas responding to such stimulation (the Neuromatrix; Melzack, 1989, 2005), that soon after has been called by other authors *Pain matrix* (Iannetti and Mouraux, 2010). In the last two decades, a debate on whether the areas activated by a painful stimulus – namely the primary somatosensory area (S1), the secondary somatosensory area (S2), the anterior cingulate cortex (ACC), and the insula – truly respond only to a painful stimulus or not, has been arisen (to see a detailed review, see Iannetti and Mouraux, 2010). The pursuit is to find whether those areas are, in fact, pain specific. A painful stimulus is required to be unpleasant, salient, and relevant (Mouraux and Iannetti, 2018), but other non-painful stimuli can have similar characteristics (e.g., the unexpected sound of a crashing car); it is worth noting that those areas also activate for social rejection as well (Eisenberg et al., 2003).

Other brain areas have been identified to respond to pain, and they are related to the two different pathways that carry nociceptive information to the brain. In contrast to pain, nociception indicates activity that arises in the nervous system in response to a noxious stimulus (Raja et al., 2020). There are two pathways carrying nociceptive information: the anterolateral spinothalamic one (linked to the somatosensory cortex and the lateral thalamus, parietal operculum, and insula), and the medial spinoreticulothalamic one (linked to the insula,

amygdala, hippocampus, S2, parabrachial nucleus, locus caeruleus, periaqueductal gray substance, intralaminar and medial thalamic nuclei, thalamic ventral caudal parvocellular nucleus, and ventral caudal portae) (Talbot et al., 2019; Fil et al., 2013). The lateral pathway carries sensory-discriminative pain facets to S1 and S2 and refers to the spatial, temporal, and quantitative (intensity) features of pain, whereas the medial pathway carries affective-motivational pain facets to the insula and the ACC (Albe-Fessard et al., 1985; Avenanti et al., 2005), evoking unpleasantness and triggering a protective response (Talbot et al., 2019; Price, 2000). The complex and multidimensional nature of pain has its core in these two dimensions (sensory-discriminative and affective-motivational) and, clearly, when trying to measure pain as much objectively as possible, it cannot be forgotten that it will always be related to the person's interpretation, and that will be integrated in the pain evaluation.

Even though it is not our aim to unravel whether specific areas exist in the brain for pain perception, a network that consistently spikes and assesses the different features of pain is definitely present. As pain is unpleasant, salient, and relevant (Mouraux and Iannetti, 2018), dealing with it is vital in order to find alternative options in interventions, avoiding collateral effects, or in the absence of effective treatments. Alternative options for pain management are necessary, to alleviate painful conditions at least temporarily: the pharmaceutical industry drives the research on analgesic drugs, concomitantly with new and alternative treatments (Tracey & Mantyh, 2007; Oertel & Lötsch, 2013). Placebo drugs administrations and the so-called placebo effect (using the context to positively affect the patients' expectancies) aim in such direction, resulting in useful models to assess and advance personalized pain modulation (Colloca, 2019). Complementary treatments are required to make bearable painful routines of healthcare practices. The effect of placebo has been proved, and, for instance, it linked together aspects as pain and exercise (Colloca et al., 2018; Fiorio, 2018). The multidimensionality of pain allows us to consider the mind-body interaction, where not only is the social context able to influence pain, but also other sensory modalities might too. Starting from this point, we wondered whether we could explore the pain-smell-taste interaction, given their be-

havioural relevance as significant salient stimuli. A cross-modal sensory approach that takes emotions into account (being them relevant for both pain and for chemosensory stimuli) is of interest to better comprehend the brain mechanism of pain perception (Jo et al., 2021).

Pain perception is strongly influenced by natural variations in brain activation related to several factors (arousal and attention, stimulus types, psychological contexts, or treatments; Atlas et al., 2014). As placebo effect depends on many factors, the context in which pain is experienced could be one of the facets that alters it. Environmental cues alert and influence our senses, as they represent the vehicle through which we experience and acknowledge the world.

Pain, smell and taste

Together, pain, smell and taste are all crucial for our survival. They convey relevant and salient information that, for instance, tells us if we are in a dangerous environment, or if something is bad for us. On the other hand, odours and tastes together usually create flavours (Prescott et al., 2004; Shepherd, 2006). Olfactory stimuli are processed centrally by the human brain in the piriform cortex, the amygdala, and the orbitofrontal cortex, coming from the olfactory bulb and without crossing in the thalamus (Gottfried, 2006; Lundström et al., 2011). Instead, gustatory stimuli intersect in the thalamus and then are processed in the insula, operculum, and orbitofrontal cortex (Huart et al., 2009; Small, 2010). Like pain, chemical senses are strictly connected to emotion and cognition (Phillips and Heining, 2002; Krusemark et al., 2013), and growing evidence highlights common substrates between pain, smell, and taste, in the ACC, amygdala, orbitofrontal and insular cortices: these areas play a role in the brain's reward system, motivational behaviour, and emotional processing (Iannilli et al., 2009; Lötsch et al., 2016; Low and Fitzgerald, 2012; Small and Apkarian, 2006; Todd, 2010; Zald and Pardo, 1997).

The research line exploring the role of the chemical senses (smell and taste) in interaction with pain in animals and infants reports a huge amount of

evidence for the idea that specific substances could – at least temporarily – alter pain perception (see, for instance, Lehrner et al., 2005; Jahangeer et al., 1997; Nakama-Kitamura, 2014; Foo and Mason, 2005, 2009; Foo et al., 2009; Stevens et al., 2016; De Clifford-Faugere et al., 2020). In adults, such literature is still scarce, but promising, as we will see in detail in chapter 2.

Given all these reasons, the perception of painful, smell, and taste stimuli could interact on various levels and modulate each other. Evidence from the molecular level stated for an intersection between smell and pain in humans and animals: to perceive a painful stimulus we need the gene SCN9A coding for the voltage-gated sodium channel Nav1.7, and that very same channel is also a crucial requirement for the sense of smell experience (Jo et al., 2021; Heimann et al., 2013; Ahn et al., 2011; Weiss et al., 2011; Zufall et al., 2012). Another close link between olfaction and pain can also be found in patients with migraine reporting osmophobia (i.e., the aversion or hypersensitivity to odours; Gossrau et al., 2022; Delussi et al., 2021). Indeed, an odour stimulation during an acute migraine attack while in fMRI showed higher activation of the rostral pons, a structure involved in the trigemino-nociceptive pathway in migraine pathophysiology (Stankewitz and May, 2011). On a more figurative way, odours are hardly describable with words, but their hedonic attribute, meaning whether we find them pleasant or not, is always certain (Yeshurun and Sobel, 2010). The pleasantness of an odour is linked to the internal state of emotions and homeostasis, and therefore generates a given pleasantness, which can interact with pain, if present. Pleasantness therefore represents the principal perceptual axis of smell:

> "an odor object is not the odor of the banana but rather an integration of the pleasantness of the banana odor with the subjective state at which it was encountered" (Yeshurun and Sobel, 2010).

Similarly, we could state that pain perception is the integration of multiple aspects experienced at once altogether with pain. Odours, pain, and emotions as well can all be detected and discriminated, and yet it might sometimes be difficult to describe them with words and to call them by their name.

Perception of pain and perception of taste are usually combined and mediated by transient receptor potential (TRP) channels that detect visceral pain and taste (Aroke et al., 2020). The flavour (taste plus smell) is a complex sensation that activates the trigeminal and glossopharyngeal nerves, allowing chemesthesis, and in turn, it might generate pungency or irritation (Shepherd, 2006; Auvray & Spence, 2008). Therefore, TRP channels play a fundamental role and have a wide range of sensory capacities (Aroke et al., 2020; Green & Schullery, 2003).

Capsaicin as a model for experimental pain

Capsaicin (*8-methyl-N-vanillyn-6-nonenamide*) is a natural active component of chili peppers. In mammals, peripheral exposure to capsaicin causes neuronal excitation by binding to a calcium channel vanilloid receptor (TRPV1), stimulating subsets of polymodal C and Aδ nociceptive fibers (Story & Cruz-Orengo, 2007). Capsaicin triggers a burning and itching pain perception and can be mistaken as bitter taste if used to stimulate the mouth (Just et al., 2007; Lim & Green, 2007). Such a qualitative similarity points out a common function as sensory signals of potentially harmful stimuli of these two sensations. Thus, capsaicin is a well-known chemical method able to induce tonic pain through a burning sensation.

Tonic pain is a long-lasting pain perception that mimics chronic or neuropathic pain, and usually, in interaction with chemical senses, it has been induced with temperature (Eggleston et al., 2010; Kakeda et al., 2010; Lewkowski et al., 2003, 2008; Pepino & Mennella, 2005; Prescott & Wilkie, 2007; Priya et al., 2015; Villemure et al., 2003). Capsaicin-induced hyperalgesia is a proper chemical experimental approach to delve into pain processing. It is a model used to mimic neurogenic hyperalgesia symptoms, as observed in neuropathic pain (van Amerongen et al., 2016), and it has been used to assess both primary and secondary hyperalgesia (O'Neill et al., 2012; Valeriani et al., 2003, 2005). In chapter 3 it will be shown how smell and taste can modulate pain perception experienced with capsaicin cream by healthy adults.

Burning Mouth Syndrome as a model for clinical pain

Oral burning pain with no causative reasons is usually defined as Burning Mouth Syndrome (BMS), a chronic disorder not universally accepted yet, though diagnostic criteria have been proposed (Kim & Kho, 2018). Pathophysiological, neuropathological, and psychological factors have been suggested as contributory factors (Borsani et al., 2014; Galli et al., 2017; Feller et al., 2017; Yoo et al., 2018; Kim and Kho, 2018). Patients with oral burning pain demonstrate relevant comorbid psychological conditions (Freilich et al., 2020; Galli et al., 2017; Klasser et al., 2016). This multifactorial disorder has been defined in different ways such as burning mouth, stomatodynia, oral dysesthesia, glossopyrosis, and glossodynia (Périer and Boucher, 2019; Klein et al., 2020). It mainly afflicts menopausal or post-menopausal women and increases in prevalence with advancing age (Kohorst et al., 2015; Teruel and Patel, 2019). Generally, pain is continuous and of moderate/severe intensity, but it can fluctuate, worsen late in the day, and remit towards the evening. The tongue is most often involved, but pain may be reported anywhere in the oral cavity. Patients may complain about dysgeusic phenomena (different perception of taste or phantom tastes in the absence of gustatory stimuli) or xerostomia although salivation is normal (Jääskeläinen, 2012). Oral pain has a negative impact on daily life and general well-being (Sardella et al., 2006). Many different treatments are offered (e.g., chlorhexidine oral rinses, benzodiazepines, antihistamine medications, anti-inflammatory drugs, antifungal agents, vitamins, topical steroids, capsaicin, psychotherapy); but despite advances in our understanding of this disorder it remains a challenge for clinicians (McMillan et al., 2016; Su et al., 2020).

The effect of administration of smell and/or taste substances has not been systematically evaluated in chronic oral burning pain. To our best knowledge, only one pilot study involving three patients with BMS reported a rapid reduction in pain intensity after the administration of sucralose, an artificial sweetener about 600 times sweeter than sucrose (Hirsch et al., 2011). In chapter 4

the results of an experiment involving patients with oral burning pain experiencing smell and taste stimuli will be presented.

Outline

As stated before, the need to explore new perspectives of pain modulations requires different approaches. Considering the lack of data regarding pain-smell-taste interaction, in this series of experiments we studied how pain, both experimentally induced and of neuropathic origin, can be modulated by olfactory and gustatory substances. First, an overview of the previous research will be presented (chapter 2): a literature search to gather evidence on pain-smell-taste interactions was run, and potential results are discussed. The review represents a first step to dig into the pain-smell-taste framework, to acknowledge current gaps and possible research questions. Then, two experiments will be reported: one run in healthy participants where we induced tonic pain (chapter 3), and another in a clinical population (chapter 4). Finally, a general discussion on the impact of this work in relation to the previous literature and the future possible steps in research will be discussed. The methodological choices were drawn from the review, that highlighted a gap in the pain-smell-taste framework with regards to induced tonic pain, and specifically through the use of capsaicin cream. We employed this method specifically to allow a possible comparison with a clinical pain, poorly studied in interaction with smell and taste.

The three works here presented have been published in international peer-reviewed journals (Cecchini et al., 2020; Sandri et al., 2021; Sandri et al., 2022).

2: A LITERATURE REVIEW ON PAIN-SMELL-TASTE INTERACTION

In the last twenty years, some studies have explored the link between pain perception and various olfactory or gustatory stimuli and the mechanisms underpinning these effects. This interplay is, however, not well documented. Here we provide a review where we comprehensively give an account of the studies that have investigated pain modulation with the application of smell and taste substances, through different paradigms, under both experimental and pathological pain conditions, and of studies on painful clinical procedures in adults.

Methods

Electronic databases PubMed and Scopus were used to implement the literature search. The terms used were: ("Pain" [MeSH] OR "Pain perception" [MeSH] OR "Analgesia" [MeSH]) AND ("Smell" [MeSH] OR "Odorants" [MeSH] OR "Taste" [MeSH] OR "Taste perception" [MeSH]) in PubMed; while in Scopus the advanced search applied was ("Pain" OR "Analgesia" OR "Pain perception") AND ("Smell" OR "Odorants" OR "Odors") OR ("Taste" OR "Taste perception"). The literature search collected published articles up to the 20th of August 2020. Titles and abstracts were screened according to the following including criteria: (1) English written published papers; (2) experimental studies in adult populations (either healthy or clinical adults; psychophysical, neurophysiological, and neuroimaging studies); and (3) pain measures investigations (pain threshold, pain tolerance, pain intensity, pain unpleasantness). After the first screening, full-text articles were checked for eligibility.

Results

2404 potentially relevant articles were retrieved during the primary literature search, of which 2373 were excluded because they did not meet the inclusion criteria based on title/abstract screening (Figure 1). The remaining studies

were read fully, and ultimately 30 studies were included in the review (Tables 1, 2). A narrative review was preferred due to differences in study methodologies that made difficult comparing the studies. For instance, studies have often investigated only one or two outcome pain measures, they varied in the methodology that induced pain in healthy population experiments, and very few studies explored the topic on clinical populations. Given all those differences, before going into the detailed results, we will overview the possible ways in which pain has been studied in interaction with smell and taste. We finally divided results by pain-smell and pain-taste interaction.

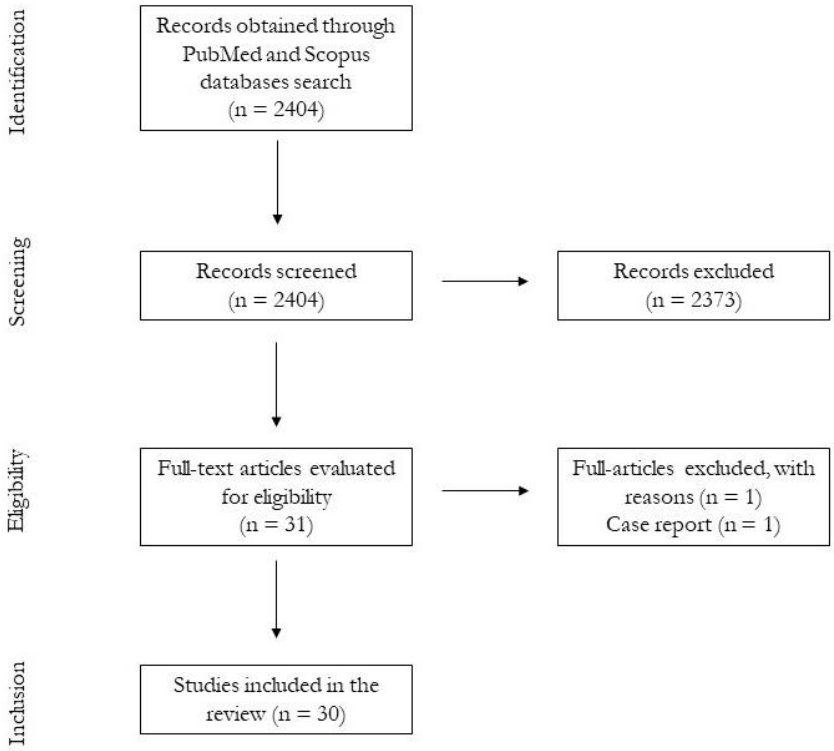

Figure 1. Flow of literature search.

Authors Year	Experimental pain method	Pathological pain condition	Painful procedure	Substances	Design	Smell test	Control condition for substances	Inclusion criteria for participants (n)	Pain threshold effect	Pain intensity effect	Pain tolerance effect	Pain unpleasantness effect
Marchand & Arsenault, 2002	Hot circulating bath test			Most pleasant, most unpleasant, most neutral individually chosen from 10 odours	Within	×	■	× 20 males and 20 females	-	■ Reduced with the pleasant odour (only in women)	-	■ Reduced with the pleasant odour (only in women)
Villemure et al, 2003	Thermode stimulation			Most preferred and most disliked individually chosen from 27 odours	Within	■	×	■ 5 males and 10 females	-	×	-	■ Reduced with pleasant and increased with unpleasant odour
Gedney et al, 2004	Thermode stimulation / pressure algometer / ischemical procedure			Lavender oil, rosemary oil, distilled water	Within	×	■	× 13 males and 13 females	×	■ Reduced with the essential oil of lavender (only in men)	×	■ Reduced with the essential oil of lavender (only in women)
Aou et al, 2005	Mechanical pain			"green" odour, control (isoamyl acetate or woody odour)		×	■	×	■ Increased with green odour	-	-	-
Martin, 2006	CPT			Lemon odour, machine oil odour, no substance	Between	×	■	■ 30 males and 30 females	-	■ Increased with the unpleasant odour	-	-
Villemure et al, 2006		Neuropathic pain		10 unpleasant, 10 pleasant odours		×	■	- 1 male	-	■ Increased with unpleasant, decreased with pleasant odours	-	■ Increased with unpleasant, decreased with pleasant odours

Prescott & Wilkie, 2007	CPT			Caramel (sweet), civet (unpleasant), after-shave lotion (pleasant), no odour	Between	×	■	■ 28 males and 66 females	-	×	■ Longer during the sweet-smelling condition	-
Villemure & Bushnell, 2009	Thermode stimulation			Most preferred and most disliked individually chosen between 6 odours	Within	■	×	■ 5 males and 9 females	-	×	-	■ Reduced with pleasant and increased with unpleasant odour
Toet et al, 2010		Patients of dental clinic		Orange, apple, no odour	Between	×	■	× 117 males and 102 females	-	×	-	-
Renner and Schreiber, 2012	Phasic nicotine stimulation			Tonic menthol stimulation, placebo (air)	Within	×	■	■ 10 males and 10 females	-	×	-	-
Bartolo et al, 2013	Electrical stimulation			Vanillin, N-valeric acid, distilled water	Within	■	■	■ 11 males and 10 females	-	■ Decreased with pleasant, increased with unpleasant odour	-	-
Masaoka et al, 2013	Electrical stimulation			Lavender odour, no-odour litmus strip	Within	■	■	■ 14 males and 10 females	-	■ Decreased with lavender oil	-	■ Decreased with lavender oil
Bagheri-Nesami et al, 2014		Patients with end-stage chronic renal failure	Needle insertion (for dialysis)	Lavender essence, placebo (lavender essence diluted in almond oil to produce smell without its properties)	Between	■	■	■ 46 experimental group (52.2% males) and 46 control group (60.9% males)	-	■ Decreased with lavender essence	-	-
Kaviani et al, 2014		Pregnant women in labor		Lavender essence, distilled water	Between	×	■	■ 160 females	-	■ Decreased with essential lavender oil	-	-

Riello et al, 2019	Electrical stimulation			Banana odour, fish odour, air	Within	■	■	■ 14 males and 14 females	×	■ Decreased with pleasant odour	×	■ Decreased with pleasant odour
Gossrau et al, 2020	Electrical stimulation	Chronic Back Pain		Rose, peach, vanilla, chocolate odours	Between	■	■	■ 28 training group (6 males and 22 females) and 14 non-training group (5 males and 9 females)	■ Increased after 4w olfactory training	-	-	-
Cecchini et al, 2020	Capsaicin cream			Banana odour, fish odour, air	Within	■	■	■ 15 males and 15 females	-	×	-	■ Decreased with pleasant odour

Table 1. Survey of published studies investigating smell-pain interaction.

Summary of articles investigating pain modulation with olfactory substances. Articles could be of three types: pain experimentally induced ("experimental pain method"), clinical pain ("pathological pain condition"), or induced with a painful procedure ("painful procedure"). Substances applied and experimental design is provided. Moreover, in the table it is possible to check whether the reported works also assessed participants' olfactory status ("smell test"), and if a control condition was provided in the experiment ("control condition for substances"), as well as a list of inclusion criteria and number of participants ("inclusion criteria for participants"). Finally, effects on four pain measures are reported. Legend: ×, not present/effect not found; ■, present/effect found; -, not applicable/not investigated. Abbreviations: CPT, Cold Pressor Test

Authors Year	Experimental pain method	Pathological pain condition	Painful procedure	Substances	Design	Taste test	Control condition for substances	Inclusion criteria for participants (n)	Pain threshold effect	Pain intensity effect	Pain tolerance effect	Pain unpleasantness effect
Mercer & Holder, 1997	Pressure algometer			Palatable food (chocolate-chip cookie), unpalatable food, neutral food, nothing	Between	×	■	■ 40 females	×	×	■ Increased with palatable food	×
Lewkowski et al, 2003	CPT			Table sugar, quinine hydrochloride, spring water	Within	×	■	■ 32 males and 40 females	×	×	■ Increase only in people with high blood pressure with sweet taste	×
Pepino & Mennella, 2005	CPT			Sucrose solution, water	Within	×	■	■ 164 females	×	-	×	-
Lewkowski et al, 2008	CPT			Sucrose solution, water, nothing	Within	×	■	■ 36 males and 22 females	-	×	■ Increase only in people with high blood pressure with sucrose	×
Kakeda et al, 2008	CPT			Sucrose solution, distilled water	Within	×	■	■ 13 males	■ Increased with sucrose	×	×	-
Eggleston et al, 2010	CPT			Sucrose solution, water, sucrose solution with cocoa	Within	×	■	■ 21 males	-	×	■ Increased with sucrose	-
Kakeda et al, 2010	CPT			Tasteless gelatin capsule, glucose tablets	Within	×	■	■ 5 males and 7 females	■ Increased with sweet condition	-	-	-

Kakeda & Ishikawa, 2011	CPT			Sucrose solution, distilled water	Within	×	■	■ 20 males and 20 females	■ Increased with sucrose only in men	×	×	-
Hirsch et al, 2011		Burning Mouth Syndrome		Granulate sucralose	Within	■	×	- 1 male and 2 females	-	■ Reduced with sucralose	-	-
Horjales-Araujo et al, 2013	Jaw muscle pain & Thermal stimulation			Sweet, bitter, neutral gelatins	Within	×	■	■ 18 males and 13 females	×	×	×	-
Priya et al, 2015	CPT			Sucrose solution, distilled water, nothing	Within	×	■	■ 40 males	■ Increased with sucrose	-	■ Increased with sucrose	-
Riello et al, 2019	Electrical stimulation			Sucrose, quinine hydrochloride, aqueous solutions	Within	■	■	■ 14 males and 14 females	×	×	×	■ Decreased with sweet taste
Mooney et al, 2020	Thermal stimulation			Sucrose solution, sucralose solution, water	Within	×	■	■ 5 males and 22 females	×	×	-	-
Cecchini et al, 2020	Capsaicin cream			Sucrose, quinine hydrochloride, aqueous solutions	Within	■	■	■ 15 males and 15 females	-	×	-	×
Duan et al, 2020	Mechanical algometer & CPT			Sweet, spicy, placebo (water) gelatin solutions	Within	×	■	■ 30 males and 30 females	■ Increased both with spicy and sweet stimulation	-	-	-

Table 2. Survey of published studies investigating taste-pain interaction.

Summary of articles investigating pain modulation with gustatory substances. Articles could be of three types: pain experimentally induced ("experimental pain method"), clinical pain ("pathological pain condition"), or induced with a painful procedure ("painful procedure"). Substances applied and experimental design is provided. Moreover, in the table it is possible to check whether the reported works also assessed participants' gustatory status ("taste test"), and if a control condition was provided in the experiment ("control condition for substances"), as well as a list of inclusion criteria and

number of participants ("inclusion criteria for participants"). Finally, effects on four pain measures are reported. Legend: ×, not present/effect not found; ■, present/effect found; -, not applicable/not investigated. Abbreviations: CPT, Cold Pressor Test

How to study pain

Pain measures

We can document and label pain measures either quantitatively (through the use of a device to measure units in a discrete system, such as temperature or time) or qualitatively (asking individuals to rate their subjective experience). This distinction between quantitative and qualitative measures serves to simplify the experimental results. A quantitative measurement can determine the pain threshold and the tolerance to pain. The pain threshold describes the intensity of the stimulation at which an individual refers a change in sensation from painless to slightly painful. Such measure characterizes the sensory-discriminative component of pain. Alternatively, pain tolerance describes the intensity of stimulation at which an individual refers an intolerable painful sensation, characterizing the affective-cognitive component of pain (Antonini et al., 2018). Qualitative measures are based instead on an individual's subjective rating on a visual (VAS) or a numerical scale (NRS) of pain intensity (the sensory-discriminative dimension) and unpleasantness (the affective dimension; Rainville, 2002).

Experimental pain

Pain can be induced in many different ways. Stimuli can be physical, chemical, mechanical, or thermal; they can be applied on subjects to elicit a painful sensation, for a brief amount of time to several minutes. Water (hot or cold) can be used, as well as hot thermal stimulation, heat, and electrical stimuli. The temperature for hot is usually set around 45-50 °C, then adjusted according to each participant's sensitivity (Marchand and Arsenault, 2002). A famous experimental paradigm implemented to stimulate painful sensations is the cold pressor test (CPT). It can be used with cold water or ice (Martin, 2006; Prescott and Wilkie, 2007; Lewkowski et al., 2003; Pepino and Mennella, 2005; Lewkowski et al., 2008; Kakeda et al., 2008; Eggleston et al., 2010; Kakeda et al., 2010; Kakeda et al., 2011; Duan et al., 2020). Another way to stimulate pain is through the submaximal effort tourniquet test (Moore et al., 1979), a physical test where ischemic pain is produced. Here, the participants raise their arm above the heart where circulation is blocked with a blood pressure cuff; then

they lower their arm and start carrying out hand-grip exercises (Gedney et al., 2004). Electrical stimuli can be applied through electrodes attached to an electrophysiological amplifier and stimulator (called digitimer or digital stimulator; Bartolo et al., 2013; Masaoka et al., 2013; Riello et al., 2019). Heat stimuli can induce pain by means of a contact thermode stimulator directly posed on the skin (Villemure et al., 2003, 2009; Gedney et al., 2004; Horjales-Araujo et al., 2013). Both with heat and electricity, stimuli can be presented once or as series of repeated pulses. A grade pressure algometer can provoke mechanical pain (pressure) progressively adding compressive force at a constant rate of grams per second (Gedney et al., 2004; Aou et al., 2005; Mercer & Holder, 1997; Duan et al., 2020). Muscle pain can be stimulated with a hypertonic saline injection into the deep masseter muscle (Horjales-Araujo et al., 2013), and capsaicin can elicit a chemesthetic burning sensation (Cecchini et al., 2020). Finally, many odorants can instigate the trigeminal nerve, triggering different sensations as burning, cooling, and stinging pain (Hummel et al., 2016).

How to study smell and taste

Perceptual status assessment

Usually, olfactory and gustatory perceptual status of participants are not investigated, making it difficult to determine whether participants have a smell or taste impairment. Few are the experiments that screened participants with validated tests (Masaoka et al., 2013; Riello et al., 2019; Cecchini et al., 2020; Gossrau et al., 2020; Hirsch et al., 2011), or that employed an experimental paradigm set up to reveal gross dysfunction in perceiving the experimental stimuli (Villemure et al., 2003, 2009; Bartolo et al., 2013). Others assessed through self-report any history of chemosensory dysfunction that would preclude taking part into the experiment (Martin, 2006; Kakeda et al., 2008; Eggleston et al., 2010), or assessed the smell ability with alcohol swabs (Bagheri-Nesami et al., 2014).

Substances' delivery

Olfactory and gustatory stimuli can be conveyed through several methods. The use of an olfactometer is usually the most commonly preferred to deliver olfactory substances, as

it allows to precisely control timing and odour inhalation, that usually happens through a nasal canula inserted in the nose (Villemure et al., 2003, 2006, 2009; Renner & Schreiber, 2012; Bartolo et al., 2013; Riello et al., 2019; Cecchini et al., 2020). Odours can also be present in the testing room with the use of a diffuser or humidifier (Gedney et al., 2004; Martin, 2006; Toet et al., 2010), where participants do not always know of the ongoing stimulation. Olfactory stimuli (essences) could also be placed on cotton balls/pads (Marchand & Arsenault, 2002; Villemure et al., 2006, 2009; Bartolo et al., 2013), or on litmus strips (Masaoka et al., 2013), or diluted in a solvent contained in airtight bottles or tied inside an air-permeable mask (Villemure et al., 2003, 2006, 2009; Bartolo et al., 2013). Pen-like odour-dispensing devices can also be used, where an odour is dissolved in a solution or there is a tampon imbibed with the liquid (Gossrau et al., 2020).

Gustatory substances are usually found in solid form. These can be either ingested or retained in the mouth (Mercer & Holder, 1997; Kakeda et al., 2010; Horjales-Araujo et al., 2013; Duan et al., 2020); when they are in liquid form, they need to be kept in the mouth for the necessary time (Lewkowski et al., 2003, 2008; Pepino & Mennella, 2005; Kakeda et al., 2008, 2011; Eggleston et al., 2010; Priya et al., 2015; Mooney et al., 2020); or sprayed directly on participants' oral cavity (Riello et al., 2019; Cecchini et al., 2020).

Smell and taste evaluation

Generally, participants have been asked to evaluate the stimuli, and especially the odours, indicating how much they like each substance on a scale (VAS, NRS), or their mood perception after olfaction stimulation. Indeed, pleasantness represents the principal perceptual axis of smell (Yeshurun and Sobel, 2010). Authors can employ such methodology either in a pilot phase, to select the best olfactory substances for the experiment (for example, Marchand & Arsenault, 2002; Villemure et al., 2003), or to choose the best solution concentration (Martin, 2006; Prescott & Wilkie, 2007; Toet et al., 2010). It can also be used as control, to determine whether the substance was perceived as planned (Martin, 2006; Riello et al., 2019; Cecchini et al., 2020).

Pain-smell interaction

A variety of methodologies and odours have been used in literature, making it hard to compare results across studies. Most of them focused on the qualitative pain measures (intensity and unpleasantness), while only few investigated pain thresholds and/or pain tolerance (Table 1).

Quantitative pain measures were little explored with odours: one study found higher pain threshold mechanically induced with a "green" odour, but information on the number of subjects tested and the experimental design was not available (Aou et al., 2005). Other two studies reported no modification at pain threshold for electrical, thermode, pressure algometer and ischemic stimulation (Gedney et al., 2004; Riello et al., 2019). Higher pain tolerance was found with CPT in the sweet-smelling condition (Prescott & Wilkie, 2007), but not with the use of other painful stimuli (electrical, thermode, pressure algometer stimulation, ischemic procedure; Gedney et al., 2004; Riello et al., 2019).

Pain intensity reports have also been inconsistent. One study referred reduced pain intensity induced with three different methods (thermode, pressure algometer, ischemic) and associated with essential oil of lavender, but only in men (Gedney et al., 2004). Other authors reported lower pain intensity after thermal stimulation only in women, when the most pleasant odour selected for each participant was delivered (Marchand & Arsenault, 2002). Pain intensity was increased with the unpleasant odour of machine oil at CPT (Martin, 2006), while other studies reported that pain intensity decreased and increased after pleasant (vanillin) and unpleasant (N-valeric acid) odours (Bartolo et al., 2013), and decreased with lavender oil (Masaoka et al., 2013), through the use of electrical stimuli. Similarly, the pleasant odour of banana reduced pain intensity after electrical stimulation, while no effect was found for the unpleasant odour (fish; Riello et al., 2019). Pain intensity was not modified by odours also in studies where pain was induced by hot or cold thermal stimulations (Villemure et al., 2003, 2009; Prescott & Wilkie, 2007), nor with capsaicin cream applied to the back of the right hand (Cecchini et al., 2020), nor with phasic nicotine stimuli to induce burning and stinging pain (Renner & Schreiber, 2012).
Regarding pain unpleasantness, odours convergently modulates it. Pain unpleasantness was reduced by pleasant odours and/or increased by unpleasant ones with different pain induced methods (Marchand & Arsenault, 2002; Gedney et al., 2004; Masaoka et al., 2013;

Riello et al., 2019; Cecchini et al., 2020). In particular, Villemure and collaborators linked such effect to the subjects' mood (Villemure et al., 2003, 2009): a pleasant odour, inducing positive mood, decreased pain-related activity within the ACC, medial thalamus, S1, and S2 (Villemure et al., 2009).

Regarding the effect of odours on clinical pain, some daily-life smells have been found to exacerbate the neuropathic pain in one patient (with neuropathic pain secondary to cervical myelopathy of unclear origin, most often impacting the right hand, wrist, and elbow but also the hip and spine; Villemure et al., 2006). This patient rated pain intensity and unpleasantness after smelling unpleasant and pleasant odours: the pain decreased and increased according to the odours' valences. While smelling the unpleasant odour, authors also found higher activation in pain processing areas (e.g., thalamus, amygdala, insular cortex, ACC). Recently, a 4-week olfactory training program was implemented in patients with chronic low back pain (Gossrau et al., 2020). Results demonstrated increased pain threshold at electrical cutaneous stimulation, indicating that olfactory training might desensitize pain perception circuits.

Finally, pain intensity in patients treated in dental clinics was not influenced by orange and apple odours (Toet et al., 2010). The same measure was although found reduced when using essential lavender oil after needle insertion in patients with end-stage chronic renal failure (Bagheri-Nesami et al., 2014), and in pregnant women in labour (Kaviani et al., 2014).

Pain-taste interaction

To date, most studies that fulfilled our inclusion criteria have investigated quantitative pain measures induced with CPT and have administered sweet substances (Table 2). Pain threshold was increased at the CPT in adult males when they held sugar diluted in water or sugar tablets in their mouth (Kakeda et al., 2008, 2010, 2011; Priya et al., 2015), and also after spicy and sweet stimulation at the CPT and the pressure algometer (Duan et al., 2020). However, few other studies did not find a relevant effect of sugar preparations on pain threshold measured with CPT (Lewkowski et al., 2003; Pepino & Mennella, 2005), nor with pressure algometer in conjunction with sweet soft drinks and palatable food (Mercer & Holder, 1997). Pain threshold at thermal stimulation did not change with bitter or sweet

tastes (Horjales-Araujo et al., 2013) or with electrical stimulation (Riello et al., 2019) or after heat thermal stimulation (Mooney et al., 2020).

Pain tolerance was instead increased with sugar at the CPT (Lewkowski et al., 2003; Eggleston et al., 2010; Priya et al., 2015), and with palatable food during the algometer pressure test – but only in women (Mercer & Holder, 1997). Nevertheless, no effect on pain tolerance was found at CPT, nor with thermal or electrical stimulation (Riello et al., 2019; Pepino & Mennella, 2005; Kakeda et al., 2008, 2011; Horjales-Araujo et al., 2013). Regarding the qualitative pain measures, none of the studies here reported found a relevant effect of taste on pain intensity (Riello et al., 2019; Cecchini et al., 2020; Mercer & Holder, 1997; Lewkowski et al., 2003, 2008; Kakeda et al., 2008, 2011; Eggleston et al., 2010; Horjales-Araujo et al., 2013; Mooney et al., 2020). Besides, pain unpleasantness was not modified by taste when pain was induced with the pressure algometer (Mercer & Holder, 1997), CPT (Lewkowski et al., 2003, 2008; Pepino & Mennella, 2005), or capsaicin cream (Cecchini et al., 2020). It was reduced with sucrose administration only with brief electrical skin stimuli (phasic pain; Riello et al., 2019).

Regarding neuroimaging studies, only one explored the taste-pain interaction in healthy adults. Authors found activation of pain-related neural networks (e.g., ACC, insula, posterior parietal cortex, thalamus) with the CPT, and those areas were less active during the sweet taste condition (Kakeda et al., 2010).

To date, with regard th the effect of gustatory substances on clinical pain, only one study has explored the interaction in three patients with Burning Mouth Syndrome (BMS), that were resistant to any other treatment (Hirsch et al., 2011). The authors found immediate decrease of pain intensity scores after sucralose administration.

Discussion

What follows are the results emerged from the literature search of the review:
- odours have an effect on the qualitative measures (especially pain unpleasantness) of experimental pain (Marchand & Arsenault, 2002; Villemure et al., 2003, 2009; Gedney et al., 2004; Martin, 2006; Bartolo et al., 2013; Masaoka et al., 2013; Riello et al., 2019; Cecchini et

al., 2020), while pain threshold and pain tolerance are not significantly affected by them (Gedney et al., 2004; Aou et al., 2005; Prescott & Wilkie, 2007; Riello et al., 2019);

- experimentally induced pain studies with gustatory substances show more contradictory, and therefore less robust, results. Pain threshold and tolerance appear to be modulated by taste substances, but pain intensity does not (Riello et al., 2019; Cecchini et al., 2020; Mercer & Holder, 1997; Lewkowski et al., 2003, 2008; Kakeda et al., 2008, 2010, 2011; Eggleston et al., 2010; Horjales-Araujo et al., 2013; Priya et al., 2015; Mooney et al., 2020; Duan et al., 2020). No firm conclusion can be drawn on pain unpleasantness due to the scarcity of studies indagating this measure;

- although only two studies applied neuroimaging methods to study the interaction between pain, smell and taste separately, there might be a general overlap between areas' activations involving the thalamus and ACC (Villemure & Bushnell, 2009; Kakeda et al., 2010);

- there is still very scarce information regarding interaction of pain and smell, and pain and taste in pathological conditions.

Olfaction fulfils a neurobiological role, strongly connected to mood and the affective domain: it has even been linked to depression (Croy et al., 2014) and odour sensitivity has been related to personality traits (Croy et al., 2011). Other evidence revealed how smell substances exert beneficial effects on physiological and psychological processes both in animals and humans (Lehrner et al., 2005; Jahangeer et al., 1997; Nakama-Kitamura, 2014), where also attention needs to be carefully considered, and that could explain the effect found on pain unpleasantness, a measure of the affective pain component.

Regarding taste, the majority of studies retrieved from the search reported an effect on pain threshold or pain tolerance, but results are inconsistent. Some studies reported a gender effect, and future research will need to clarify such potential differences. Moreover, future studies should try out different pain induced methodologies (not only CPT) and other gustatory substances, given that nearly all studies implemented sweet substances. A few used bitter tastes but no effect on pain was detected (Riello et al., 2019; Cecchini et al., 2020; Lewkowski et al., 2003; Horjales-Araujo et al., 2013).

Taking into account the two studies that explored brain activation, ACC is known to be involved also in emotional processing and pain perception (Small & Apkarian, 2006;

Bartolo et al., 2013), while the thalamus might be involved in mental integration and regulation, and not just simply employed as a point of relay (Wolff & Vann, 2019). Future studies should also explore the interaction of chemical senses altogether in relation to pain. Finally, the initial results on clinical pain are promising and showing potential effect, but they still need to be extensively replicated.

Given the scarcity of research in clinical pain conditions, and the emerging need for alternative pain-relief options, we decided to explore if smell and taste could influence a chronic pain condition. Before carrying out the experiment in a clinical population, we wanted to assess the effect of different substances on a pain model, to evaluate the potential effectiveness of the experimental paradigm. Therefore, we employed capsaicin cream to induce pain in a group of healthy participants.

3: PAIN-SMELL-TASTE INTERACTION IN HEALTHY PARTICIPANTS

Capsaicin cream represents a unique opportunity to explore pain processing through a chemical method, as a model that mimics neurogenic hyperalgesia's symptoms (van Amerongen et al., 2016). With this methodology, the link between smell, taste and tonic pain could favour new insights with potential translational impact in many clinical chronic pain conditions, such as burning neuropathic pain.

Methods

128 volunteers (59 females, 69 males; age range; 19-33 years) were stimulated by means of capsaicin cream on the back of their right hand. After 20 minutes, subjects were asked if they perceived any pain. 60 of them (47%) perceived it and took part in the experimental procedure: 30 of them participated in the smell-taste experiment (15 females, age range: 20-28 years; mean age: 23.41 ± 2.47 years) while the other 30 took part in the taste-pain experiment (15 females, age range: 19-33 years; mean age: 24.55 ± 2.83 years). The two groups were matched for gender and age ($t_{(58)} = 1.664$, $p = 0.101$). Public announcements at the University of Verona were made for recruitment. All subjects were students at the university. Inclusion criteria were: age 18-35, normogeusia and normosmia, no pain-related pathology. Exclusion criteria were: history of otolaryngology disorders, head trauma, Bell's palsy, stroke, confirmed/presumed pregnancy, long-lasting drug therapy, allergy, neuropathies (e.g., determined by diabetes), or any other condition that may interfere with the experimental procedure, no pain after capsaicin cream application. Participants were asked not to use perfumed products and to refrain from eating or drinking coffee, food, candies/chewing gum on the experimental day as well as not to brush their teeth 15 minutes before the experiment. Participants gave their written informed consent before taking part in the study. The project has been approved by the "Review Board for Research involving Human Participants" of the University of Verona (submission n.1/2017) and it was conducted in accordance with the revised version of the Declaration of Helsinki.

Pilot study

Given that capsaicin induces high inter-individual variability in pain perception (Lötsch et al., 2015), we ran a pilot phase to test three different kinds of cream galenic preparation (3%, 4%, and 5% of capsaicin, by Peretti Pharmacy, Dossobuono, Verona) on 30 healthy volunteers (pilot group) in order to choose the cream associated with the highest rates of pain perception. We selected the appropriate medium to incorporate the capsicum extract (*fitalite cream and 96% ethyl alcohol*) and three groups of participants were stimulated with one type of cream each. Participants were then asked to rate pain intensity on a Numerical Rating Scale (NRS) ranging from 0 ("*not at all painful*") to 10 ("*extremely painful*") every 10 minutes, starting from 20 minutes to 60 minutes after cream application. A significantly increment in pain perception by capsaicin cream application has been proved after 20 minutes after the application, with a pain peak around 30-40 minutes (Farina et al., 2001; Fierro et al., 2010; Valeriani et al., 2003), and a significant decrease after 60 minutes (Farina et al., 2001; Valeriani et al., 2003).

10 participants were tested with the 3% capsaicin cream (8 women, 2 men; mean age: 30.77 ± 6.4 years), 10 with the 4% capsaicin cream (8 women, 2 men; mean age: 32.4 ± 7.3 years), and 10 with the 5% capsaicin cream (7 women, 3 men; mean age: 32.2 ± 7.6 years). The chosen cream was the one at 4% because it resulted with higher NRS intensity ratings (at 30 minutes 3% cream induced a NRS = 3.5 ± 3.4; 4% cream induced NRS = 4.3 ± 2.4; 5% cream induced NRS = 3.1 ± 2.8; see Figure 2).

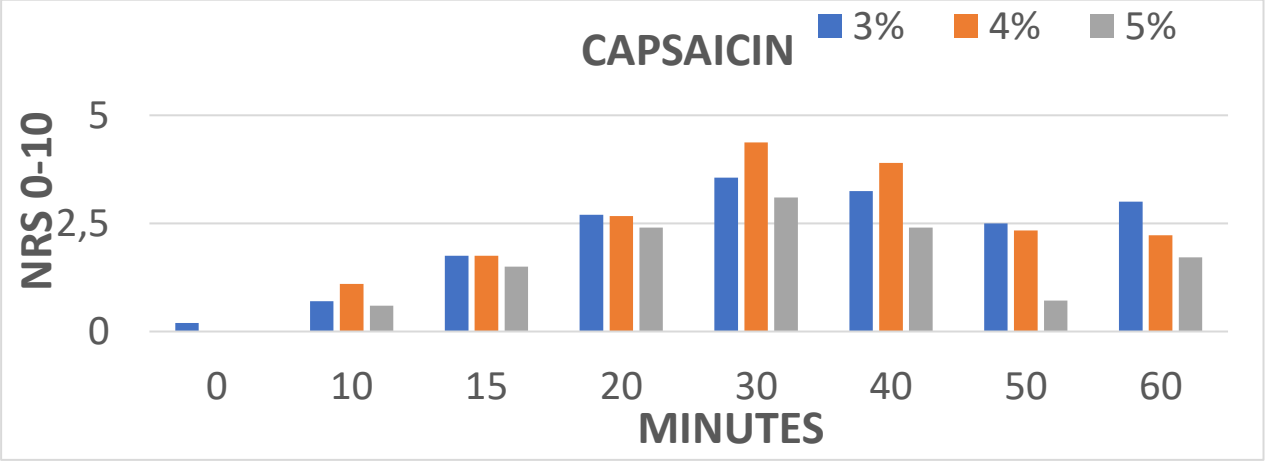

Figure 2. Perception of pain intensity in three groups with different capsaicin concentration (3%, 4%, 5%). Pain intensity was collected every 10 minutes.

Perceptual status evaluation

Before the experiment, olfactory and gustatory perceptual status was evaluated for each participant with standardized procedures. For participants who took part in the smell experiment the Sniffin' Sticks Extended Test (Burghart, Germany) was adopted to assess olfactory function. For participants who were involved in the taste experiment, the Taste Strips Test (TST, Burghart, Germany) was used to evaluate gustatory function.

Smell

The Sniffin' Sticks Extended Test (Burghart, Germany) comprises three subtests, assessing odour threshold (T), discrimination (D), and identification (I) with a forced choice experimental paradigm (Hummel et al., 2007). It uses tools that deliver odours through the tip of a pen-like device. During the first subtest (T), participants are required to indicate in which pen among three pens they perceived an odour (n-butanol). Two pens contain an odourless solution and one the odour, and they are presented in random order. The concentration of the odour is increased every time participants make a mistake, until the odour is reliably detected. That is the threshold for the participant. Then, during the discrimination subtest participants are asked to indicate which pen of three smells different. Sixteen triplets are randomly presented. During threshold and discrimination subtests participants are blindfolded to prevent visual identification of the pens dispensing odours. Finally, the identification subtest comprises sixteen odours that participants are required to name. For each odour, participants can choose from a list of four odours. The total score combines the sum of each subtest, and distinguishes normosmia (TDI $\geq$ 30.3), hyposmia (30.3 > TDI $\geq$ 16) and functional anosmia (TDI < 16).

Taste

The Taste Strips Test (TST, Burghart, Germany; Landis et al., 2009; Mueller et al., 2003) consists in filter paper strips impregnated with four different concentrations for each taste (sweet, sour, salty, bitter). Participants clean their mouth with water between each trial and place the paper strip on the tongue. They identify the taste, taking all the time they need. The test does not include umami, because Europeans are not familiar with it yet

(Cecchini et al., 2019; Landis et al., 2009). A TST score ≥ 9 indicates normogeusia and a score < 9 indicates hypogeusia.

Procedure

The experiment took place in a quiet room where participants seated comfortably with their hand placed on a table. Hyperalgesia was induced by applying 0.2 ml of 4% capsaicin cream on the back of their right hand (Figure 3b), specifically on a 2 cm^2 circle area, previously marked on the skin at the level of the first dorsal interosseous muscle. Twenty minutes after cream application, participants were asked to rate pain intensity ("How intense was the painful stimulation?") on a Numerical Rating Scale (NRS) ranging from 0 ("not at all painful") to 10 ("extremely painful"). If pain intensity was rated ≥ 1, participants were considered for the following step and asked to rate the unpleasantness of the painful stimulus ("How unpleasant was the painful stimulation?") on a NRS from 0 ("not at all unpleasant") to 10 ("extremely unpleasant") (Figure 3a). Participants rated pain intensity and unpleasantness every 10 minutes, starting from 20 minutes. Pain intensity was considered a proxy of the sensory component of pain, while pain unpleasantness was considered as indicator of the pain emotional component (Gibson & Farrell, 2004; Price, 2000; Rainville et al., 1992).

The two separated experiments took place between February and November 2019. Participants were randomly assigned to the smell or taste group and matched for age and gender. Pain measures were collected at baseline, during the corresponding experiment (Figure 3a), and at the end of the experiment (second baseline, at around 30 minutes after the cream application). Olfactory and gustatory stimuli were delivered according to a factorial design with one sense (smell or taste) for each experiment and different valences following the same design of the previous work of our research group (Riello et al., 2019). Chemosensory stimuli were categorized by three valences: pleasant (sweet taste/banana odour), unpleasant (bitter taste/fish odour), and neutral (air/water). The order of types of valences was random, according to a counterbalanced design. We selected the stimuli in accordance with the idea of using stimuli with opposite and phylogenetically archaic valence: sweet taste is associated to energy intake and survival, the bitter one is related to defence

and alerts against poisonous substances (Beauchamp, 2016). Odours usually helps us process threatening or social stimuli present in the environment (Stevenson, 2010). Neutral condition served as control.

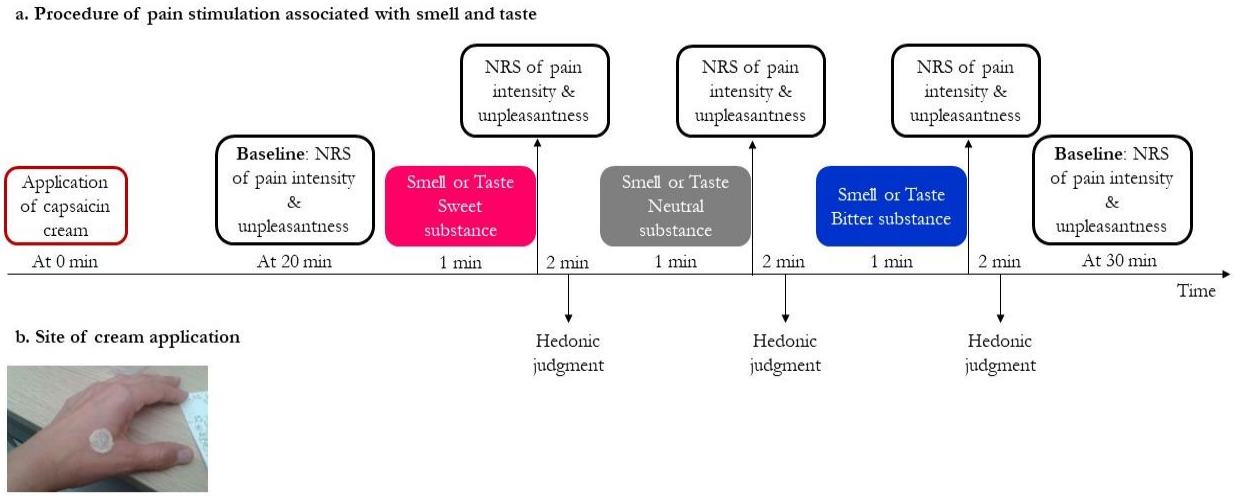

Figure 3. (a) Experimental procedure; (b) Site of cream application. NRS: Numerical Rating Scale.

We delivered the odour solutions orthonasally through a single-use birhinal cannula (Intersurgical S.p.A., Italy) attached to a home-made manual olfactometer (built at the Anatomy Section Department of Verona University according to the work of Lowen & Lukas, 2006). The chosen odours were banana extract solution as pleasant one, and fish extract solution as unpleasant one. Air was dispensed as the neutral stimulus. After 1-min stimulation, participants were asked to rate pain variables on the NRS.

The gustatory substances were manually sprayed directly on the open mouth of participants. Two aqueous saline spray solutions – corresponding to the sweet and bitter basic tastes (i.e., sucrose 10% for sweet and quinine hydrochloride 0.05% for bitter) – were applied by the experimenter several times to maintain the taste in the oral cavity. The chosen solutions are supra-threshold solutions part of the "Whole Mouth Test" generally used as rapid screening test for the four basic taste qualities (Cecchini et al., 2015). Neutral low mineral water was dispensed as neutral stimulus. Given physiological differences in sensation between the two tastes duration, the sweet solution was delivered every 10 seconds, the bitter one every 30 seconds, and the neutral solution every 20 seconds.

Each substance lasted 1 minute, after that the participants rated pain intensity and unpleasantness. We collected pain measures at the first baseline (20 minutes after cream application), in association with three substances (according to the random counter-balanced order of valance stimulation), and finally again at the second baseline (30 minutes from capsaicin cream application). There was a break of 2 minutes between each substance stimulation during which neutral air and natural low mineral water were given to clean the oral and nasal cavity. Due to individual subjectivity in hedonic judgment of smell and taste, participants rated the hedonic valence of the substances ("How do you rate the substance?") on a Likert scale from -10 ("extremely unpleasant") to 10 ("extremely pleasant"), where 0 equals to "neutral", at the end of each stimulation (e.g., after positive smell).

Statistical analyses

Pain measures collected after each stimulation were normalized to the mean of the baseline values following the equation [1].

$$\text{Normalized}_{\text{value}} = \frac{X - Bx}{Bx} \qquad [1]$$

X represents the value given by the participant after a specific stimulation and *Bx* the mean of the two baselines (collected at 20 and 30 minutes: before and after the experiment). Such normalization has been implemented to control for the inter-individual variability of pain perception.

Shapiro-Wilk test was applied to assess normality of distribution of the data; since it was violated for all the variables ($p < 0.05$), we performed non-parametric analyses. Factor valence was analysed separately for smell and taste experiments regarding intensity and unpleasantness with the two-way non-parametric Friedman's test (Friedman, 1940). Post-hoc comparisons using Wilcoxon's signed rank test was applied when significant factors were identified. These tests were also used to analyse the hedonic ratings of the substances, in order to control for our a priori choice of the substances. One-sample Wilcoxon signed rank test was used to determine whether the hedonic scores of the substances were significantly different from 0.

p-value < 0.05 was considered statistically significant and Bonferroni correction was applied for multiple comparisons to correct *p*-values ($p < 0.016$ was considered statistically

significant with three comparisons). All analyses were performed using RStudio software (version 1.3.1093 © 2009-2020 RStudio, PBC).

Results

60 participants out of 128 (47%) were enrolled. 68 participants were excluded for perceiving no pain after 20 minutes from cream application (NRS = 0). This finding is in line with the high inter-individual variability reported in pain perception with capsaicin (Lötsch et al., 2015). All participants were normosmic (mean TDI: 33.85 ± 3.22) and normogeusic (mean TST: 13.6 ± 1.4).

Mean row values (not normalized data) of pain intensity and unpleasantness NRS ratings during the two experiments can be seen in tables 3 and 4.

	Baseline	*Smell*		
		Pleasant	Unpleasant	Neutral
Intensity	3.23 ±2.10	3.23±2.53	3.83 ±2.07	3.07 ±2.49
Unpleasantness	4.33 ±2.56	3.50 ±2.62	4.40 ±2.22	4.17 ±2.82

Table 3. Mean (± standard deviation) raw values (not normalized; NRS 0-10) of Pain Intensity and Pain Unpleasantness subjective ratings in the different smell conditions.

	Baseline	*Taste*		
		Pleasant	Unpleasant	Neutral
Intensity	3.3 ±2.21	3.5 ±2.6	3.37 ±2.59	3.43 ±2.74
Unpleasantness	3.71 ±2.49	3.7 ±2.88	3.37 ±2.5	4.13 ±2.65

Table 4. Mean (± standard deviation) raw values (not normalized; NRS 0-10) of Pain Intensity and Pain Unpleasantness subjective ratings in the different taste conditions.

Friedman's test for the normalized NRS unpleasantness scores given after the olfactory substances revealed a significant factor valence (pleasant, unpleasant, and neutral, χ^2 = 6.771, *df* = 2, *p* = 0.034). Post-hoc comparisons showed an effect of pleasant versus unpleasant odours (Wilcoxon test, Z = -2.745, *p* = 0.006) and a tendency in the comparison between pleasant and neutral odours (Wilcoxon test, Z = -1.924, *p* = 0.054). See figure 5 that reports median values. This result points out that pain unpleasantness perception was significantly decreased during pleasant odour, as compared to the unpleasant odour condition.

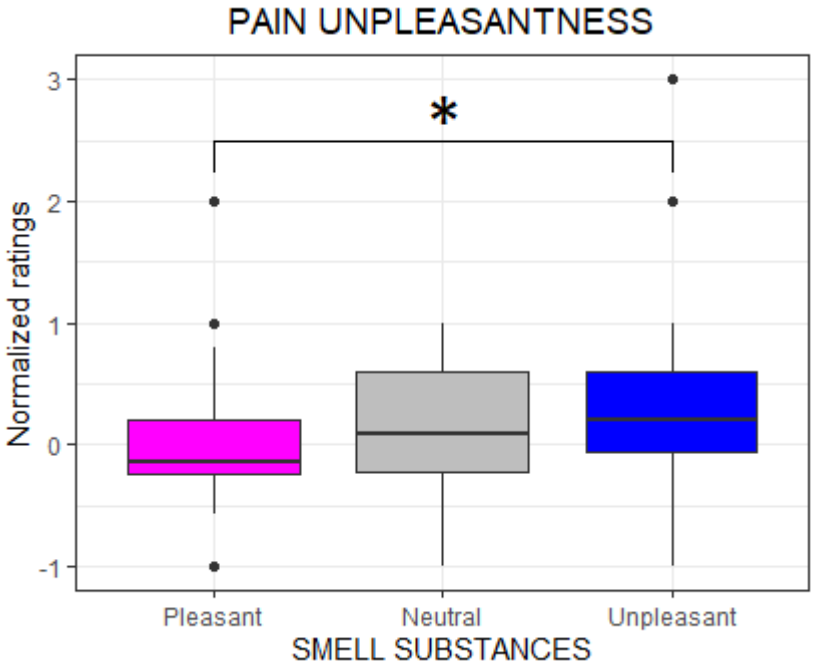

Figure 5. Box plots of normalized pain unpleasantness ratings relative to baseline (mean of two baseline values) for related to pleasant (magenta), neutral (grey), unpleasant (blue) smell stimuli. The horizontal black lines represent median values. Data are presented as normalized scores to baseline. * = p < 0.05 after application of Bonferroni correction.

Regarding the normalized NRS intensity scores in the smell experiment no significant effect was found (Friedman's test, *p* > 0.05). Regarding the taste experiment, no significant

effect was found between conditions either for normalized NRS unpleasantness nor normalized NRS intensity scores (Friedman's test, all $p > 0.05$).

Regarding the Likert scales for the hedonic ratings, all substances were perceived as intended: the pleasant odour (mean 2.62 ± 4.47) and the pleasant taste (mean 6.23 ± 2.25) as positively pleasant, and both different from zero (Wilcoxon test for odor: Z = -2.890; $p = 0.004$; for taste: Z = -4.795; $p < 0.001$); the unpleasant odour (mean -4.57 ± 3.56) and the unpleasant taste (mean -5.67 ± 3.03) as unpleasant, and both different from zero (Wilcoxon test for odour: Z = -4.263; p < 0.001; for taste: Z = -4.641; $p < 0.001$). Finally, both air (mean 0.00 ± 1.98) and water (mean -0.23 ± 0.56) were perceived as neutral and indeed they did not differ from 0 (Wilcoxon test, Z = -0.282; $p > 0.05$ for air; Z = -2.111; $p = 0.035$ for taste).

Friedman's test showed a significant difference in the ratings given for the substances between pleasant, unpleasant, and neutral for smell ($\chi^2 = 39.128$, $df = 2$, $p < 0.001$), and between pleasant, unpleasant, and neutral for taste ($\chi^2 = 58.06$, $df = 2$, $p < 0.001$). Post-hoc comparisons (Wilcoxon test) confirmed significant differences between all conditions (all $p < 0.001$). See figures 6 and 7 reporting median values.

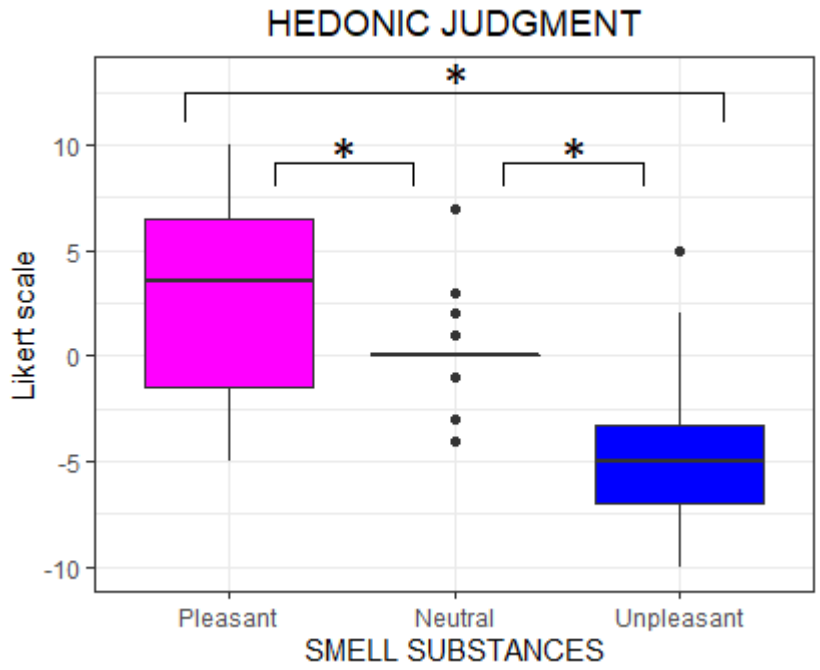

Figure 6. Hedonic values for smell substances related to pleasant (magenta), neutral (grey), unpleasant (blue) smell stimuli. The horizontal black lines represent median values. Likert

scale of hedonic ratings is represented on y axis, from -10 (very unpleasant) to +10 (very pleasant), with 0 = neutral. * = p < 0.05 after application of Bonferroni correction.

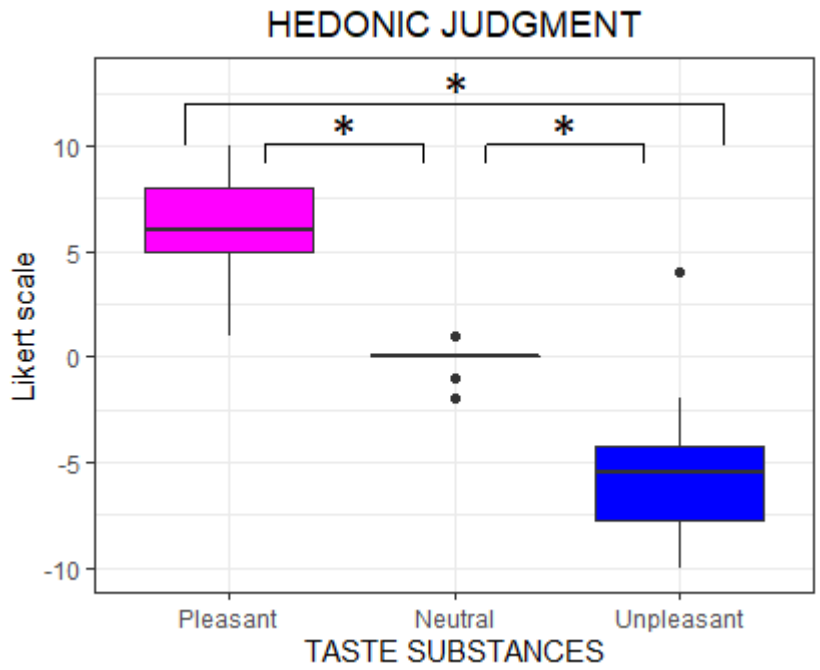

Figure 7. Hedonic values for taste substances related to pleasant (magenta), neutral (grey), unpleasant (blue) taste stimuli. The horizontal black lines represent median values. Likert scale of hedonic ratings is represented on y axis, from -10 (very unpleasant) to +10 (very pleasant), with 0 = neutral. * = p < 0.05 after application of Bonferroni correction.

Finally, correlation analyses (Spearman correlation) were run to control whether changes in perception of pain intensity and unpleasantness were associated with the substances' hedonic ratings. No significant correlations were found between the hedonic judgments and relative pain measures in the smell and taste experiments (all $p > 0.05$).

Discussion

The primary result from this work is that pleasant and unpleasant odours are able to modulate only the pain unpleasantness perception (affective pain dimension) in a context where pain was induced by a cutaneous application of capsaicin cream.

Capsaicin cream provokes a feeling that mimics neuropathic pain, and it is usually effective in half of the healthy population (Lötsch et al., 2015). As a matter of fact, 47% of our participants reported a moderate painful sensation after 20 minutes of application, representing a good starting point to explore how chemical senses work in interaction with pain. Capsaicin, producing secondary hyperalgesia, provokes a central sensitization of the spinal cord dorsal horn neurons and supra-spinal pain pathways. Such sensitization plays a vital role into chronic pain induction and maintenance (You et al., 2016). This phenomenon could be responsible for a complex pain perception, influencing the emotional component more than the sensory ones. Our results indicate that pleasant and unpleasant odours influence the processing of pain unpleasantness, while tastes do not, and pain intensity is not modulated. Given that a clear difference with the neutral stimulation has not reached statistical significance but only a tendency between the neutral and the pleasant stimulations, future research is needed to further investigate this aspect.

Nevertheless, smell clearly proved an effect on the affective component of tonic pain, confirming previous findings that sensory and emotional pain components are dissociable in tonic pain (Villemure et al., 2003). Therefore, different neural modulatory circuits might be involved. Although brain activity was not investigated in this study, it is possible to assume that some cerebral areas involved in emotional pain regulation might play a crucial part in this multisensory interaction, as previously reported (chapter 2, Villemure and Bushnell, 2009).

When a simultaneous stimulation is given, a major response is usually observed in subjects. Smell and taste are usually perceived together ("flavour perception") and could generate a multimodal central response. Instead, we decided to stimulate the two senses separately, because we were interested in disentangling the potential role of odours (usually linked also to emotions and cognition) and taste (usually linked to more direct survival instincts).

We found no evidence of pain being modulated by tastes. Results in literature are contradictory (chapter 2), as it has been found that pain was reduced with pleasant taste (for instance, Pepino and Mennella, 1995; Kakeda et al., 2010), but also not (Mooney et al., 2020). These variances could be related to the different methodologies adopted to induce pain.

Our data might help understanding the interaction between pain, smell, and taste. Understanding tonic and persistent pain that mimics neuropathic pain might lead to improvements in the therapeutic approach to pain control. In such sense, we therefore propose a similar design to test pain-smell-taste interaction in a clinical population suffering from oral burning pain of no causative reason.

4: PAIN-SMELL-TASTE INTERACTION IN A CLINICAL POPULATION

Given the promising results on pain-smell-taste interaction that we obtained in a pain model tested on healthy subjects (chapter 3), we decided to develop a similar design in a group of patients with a burning neuropathic type of pain, to address the gap found in the literature (chapter 2) and explore possible future alternatives to pain-relief drugs. We chose patients with Burning Mouth Syndrome given that a pilot with favourably results on three patients on pain-taste interaction had already been conducted (Hirsch et al., 2011).

Methods

30 patients treated at the Maxillofacial Surgery Unit outpatient services of Verona Hospital were enrolled. Inclusion criteria were: age > 18 years, currently suffering from chronic oral burning pain for at least 3 months, no oral or systemic diseases that could justify the oral pain, no concomitant factors influencing olfactory and gustatory functions, no pain treatment administered at the assessment time. The study comprised two sessions: during the first, patients filled out a detailed sensory interview and smell and taste functions were evaluated (Sniffin' Sticks Extended Test for smell and Taste Strips Test for taste, Burghart, Germany). The second one took place within 2 weeks from the first session: patients completed a pain and psychological questionnaire and the experiment in interaction with smell and taste substances was conducted (see figure 8). 2 patients were hyposmic at the smell evaluation and therefore excluded, and 6 patients withdrew for personal reasons before the second session. The final study sample included 22 normosmic and normogeusic patients (18 females; age range 46-79 years; mean 63 ± 9.94). The study protocol was approved by the Ethical Committee of Verona University Hospital (Prot. no. 63032 of 2020/11/23) and patients gave their written and informed consent.

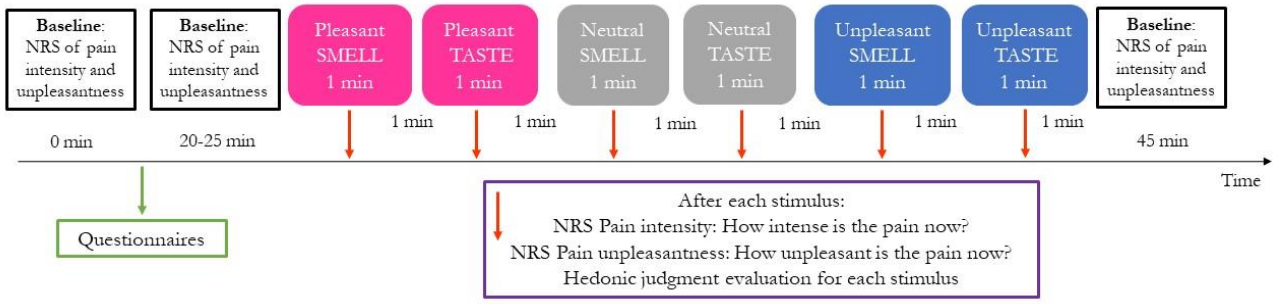

Figure 8. Experimental procedure during the second session.

Procedure

First session

An expert clinician conducted the sensory interview to collect detailed information about location, duration, main daily pain trend, qualitative and quantitative attributes, factors improving or worsening the pain.
Then, the same clinician evaluated the smell and taste perceptual status of each participant with standardized procedures (Sniffin' Sticks Extended Test for smell and Taste Strips Test for taste, Burghart, Germany; see chapter 3 for detailed description of those tests).

Second session

Oral burning pain was measured at baselines and after each stimulation of smell and taste with two NRS (pain intensity and unpleasantness). Pain intensity ("How intense was the painful stimulation?") was measured on a Numerical Rating Scale (NRS) ranging from 0 ("not at all painful") to 10 ("extremely painful"), so as pain unpleasantness ("How unpleasant was the painful stimulation?") on a NRS from 0 ("not at all unpleasant") to 10 ("extremely unpleasant").
The stimuli were counterbalanced according to a factorial design for the two senses (smell, taste) and three valences (pleasant, unpleasant, neutral), following the same design of the previous work of our research group (Riello et al., 2019). See Figure 8 for procedure. We used the same substances previously used with healthy participants: banana and fish as

odours, sucrose and quinine hydrochloride as tastes (see chapter 3). Smell and taste stimulation lasted 1 minute. Pain intensity and unpleasantness were assessed at the beginning of the session (first baseline), after questionnaire completion (second baseline), then in association with stimuli administration (six times), and finally and the end of the session (third baseline).

There was 1-minute break between each stimulation trial. Valence order (pleasant, unpleasant, neutral) as well as the order of the first chemical senses were counterbalanced between patients, but blocked for each patient (i.e., for each valence type, one participant was tested first with smell and then with taste, while another one was tested first with taste and then with smell). As for our previous projects, patients were asked to rate the hedonic valence of each substance ("How do you rate the substance?") on a Likert scale from -10 ("extremely unpleasant") to 10 ("extremely pleasant"), where 0 equals to "neutral", at the end of each stimulation.

Pain and psychological questionnaires

Given the relevant comorbidity with psychological aspects in patients with oral burning pain (Freilich et al., 2020; Galli et al., 2017; Klasser et al., 2016), and the fact that several factors (of which psychological and emotional ones) could be an important feature in the relationship between pain, smell, and taste (Krusemark et al., 2013; Beauchamp, 2016), we administered several questionnaires. We collected measure of anxiety (State Trait Anxiety Inventory, STAI – Y1 & Y2; Pedrabissi and Santinello, 1989), depression (Beck Depression Inventory, BDI-II; Ghisi et al., 2006), psychological distress (Clinical Outcomes Routine Evaluation – Outcome Measures, CORE-OM; Palmieri et al., 2009), mindfulness (Five Facts Mindfulness Questionnaire, FFMQ; Giovannini et al., 2014; Poulin et al., 2016), and alexithymia (Toronto Alexithymia Scale, TAS-20; Bressi et al., 1996).

In addition, patients filled out pain-related questionnaires: the Brief Pain Inventory (BPI; Caraceni et al., 1996), to assess intensity and interference of pain in the daily life; the Pain Catastrophizing Scale (PCS; Monticone et al., 2012), to investigate thoughts and feelings related to the pain experience; and the Italian Pain Questionnaire (QUID; De Benedittis et al., 1988), to explore the personal perception of the pain experience through different classes (Sensory, Affective, Evaluative, Miscellaneous). Finally, we included a liberal translation of the items concerning pain facial interference, a section of the BPI-facial not yet validated in Italian (Lee et al., 2010), to shed light between oral pain and facial-related activities.

The questionnaires were split in two groups, administered during the first (STAI Y-2, BPI, PCS, CORE-OM, BDI-II), and second session (FFMQ, TAS-20, STAI Y-1, QUID), respectively.

Statistical analyses

We described symmetrical and asymmetrical quantitative variables using means ± SD or median (Q1-Q3), respectively, and categorical variables using percentages. Statistical significance was set at $\alpha = 0.05$. Normality of data distribution was tested using the Shapiro-Wilk test; since it was violated for more than half of the variables, non-parametric analyses were performed, which are also robust to outliers. Friedman's test (Friedman, 1940) was employed to analyse the three baseline ratings collected during the experimental trials, in order to verify whether pain fluctuated overall during the procedure.

We normalized the data according to the same formula previously used [1].

$$\text{Normalized}_{\text{value}} = \frac{X - Bx}{Bx} \qquad [1]$$

X is the value (intensity or unpleasantness) given by a patient after one stimulation (e.g., after unpleasant taste), and *Bx* represents the mean of the three baselines (collected at the beginning, after the questionnaires, and at the end of the experiment). For sensitivity analysis, we also analysed patients' responses calculated as absolute changes in pain intensity and unpleasantness ($\text{Absolute}_{\text{value}} = X - Bx$).

We analysed factor valence (pleasant, unpleasant, neutral) separately for smell and taste substances for pain intensity and unpleasantness with the two-way non-parametric Friedman's test (Friedman, 1940). When the test resulted significant ($p < 0.05$), pairwise post-hoc comparisons were carried out with the Wilcoxon's signed rank test. *p*-values adjusted for multiple testing were computed using Benjamini-Hochberg correction (Benjamini and Hochberg, 1995). In addition, to have a more direct measure of the "effect size", we calculated the normalized values for pain intensity and pain unpleasantness in response to pleasant and unpleasant experimental stimuli corrected for the neutral stimuli (i.e., intra-individual control conditions). We also tested whether patients' responses for a given valence varied as a function of sense (smell vs taste).

To investigate whether baseline pain perception and disease duration modified patients' responses, the main analyses were repeated after stratification by median pain perception and median disease duration. Finally, since pain unpleasantness was the only pain measure modulated by smell and taste, we explored this aspect further. Using Spearman's rank correlation coefficients, we assessed the correlation of the modulation of pain unpleasantness with either the hedonic ratings assigned by the patients to the experimental substances and the psychological test scores. All statistical analyses were run using RStudio software (version 1.3.1093 © 2009-2020 RStudio, PBC).

Results

Pain features

All patients (n=28) referred a long history of oral symptoms (range: 4-192 months; median value: 22.5; Q1-Q3: 14.8-63 months). None of them was receiving any kind of treatment. The mean age was 61.4 years (range: 31-79); most of them were women (82%; see table 5). All the female patients that completed both sessions were in post-menopausal phase. When questioned about a possible trigger event, answers differed (e.g., oral diseases/oral surgery, stressful family or personal circumstances or events, medications, general surgery, or no apparent event recalled), but none of the female patients referred menopause onset to the oral pain. Generally, in none of the 28 patients, pain was present while sleeping (figure 10), but various pain patterns occurred: pain increment during the day, constant daily pain, pain perception fluctuations with large inter-individual variability (see figure 9). A wide variation of the oral pain topography was found, but at least part of the tongue hurt in almost all patients (89.3%). Generally, the pain was described as discomfort (85.7%), burning (67.9%), or mouth dryness (67.9%; see table 5). Numerous factors were reported to worsen or relief pain intensity (figure 10).

Gender	
Women (%)	23 (82%)
Men (%)	5 (18%)
Age (years)	
Range	31 – 79
Mean ± SD	61.4 ± 11.6

Work status	
Employed (%)	16 (57%)
Retired (%)	12 (43%)
Comorbidities	
Yes (%)	26 (93%)
No (%)	2 (7%)
Drug assumption (not for oral pain)	
Yes (%)	21 (75%)
No (%)	7 (25%)
Oral pain duration (months)	
Range	4 – 192
Median (Q1 – Q3)	22.5 (14.8 – 63)
Oral pain trigger event	
Yes (%)	20 (71%)
No (%)	8 (29%)
Oral pain topography	
Tongue (%)	25 (89.3%)
Hard palate (%)	9 (32.1%)
Lips (%)	5 (17.8%)
Cheek mucosa (%)	1 (3.6%)
Gums (%)	1 (3.6%)
Oropharynx (%)	1 (3.6%)
Oral pain description	
Burning (%)	19 (67.9%)
Discomfort (%)	24 (85.7%)
Dryness (%)	19 (67.9%)
Pain in other sites	
Yes (%)	25 (89%)
No (%)	3 (11%)

Table 5. Sociodemographic, clinical and pain features of patients (n=28).

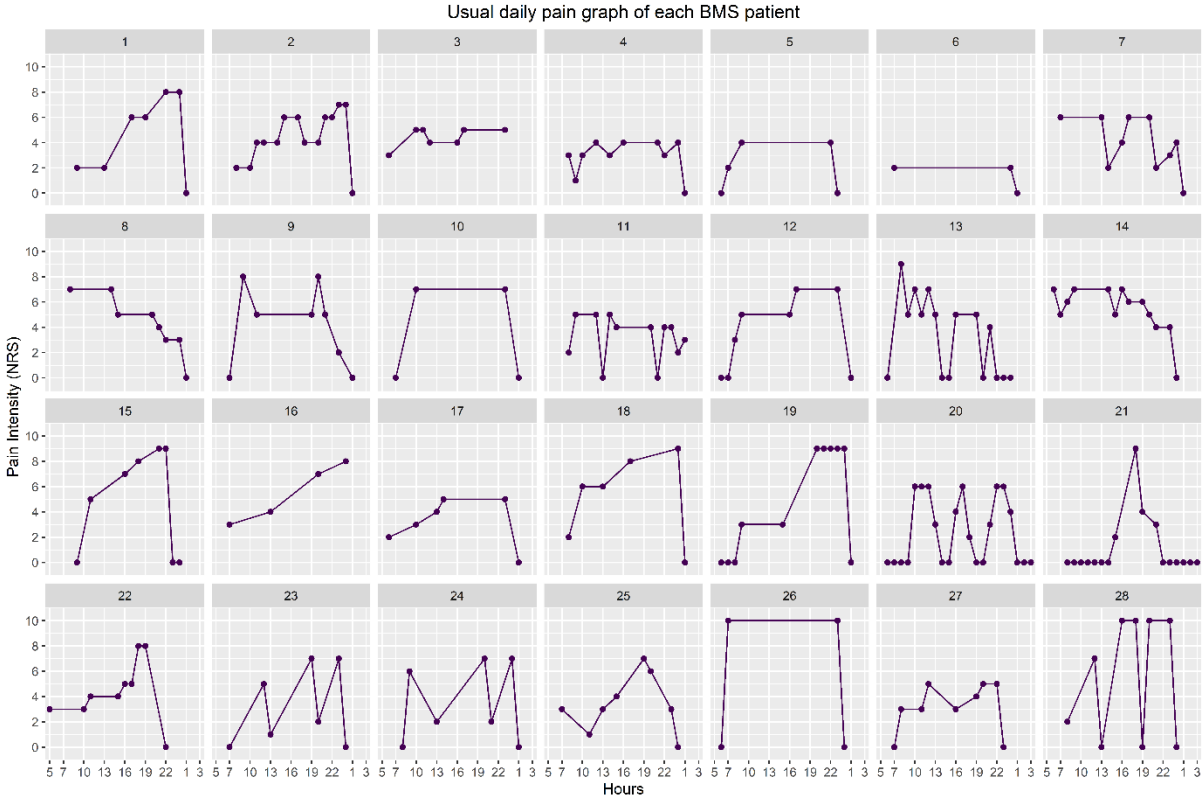

Figure 9. Daily pain graph. Graphs showing the main daily time course of pain perception for each patient (n=28), indicating pain intensity (Y axis) over time (X axis).

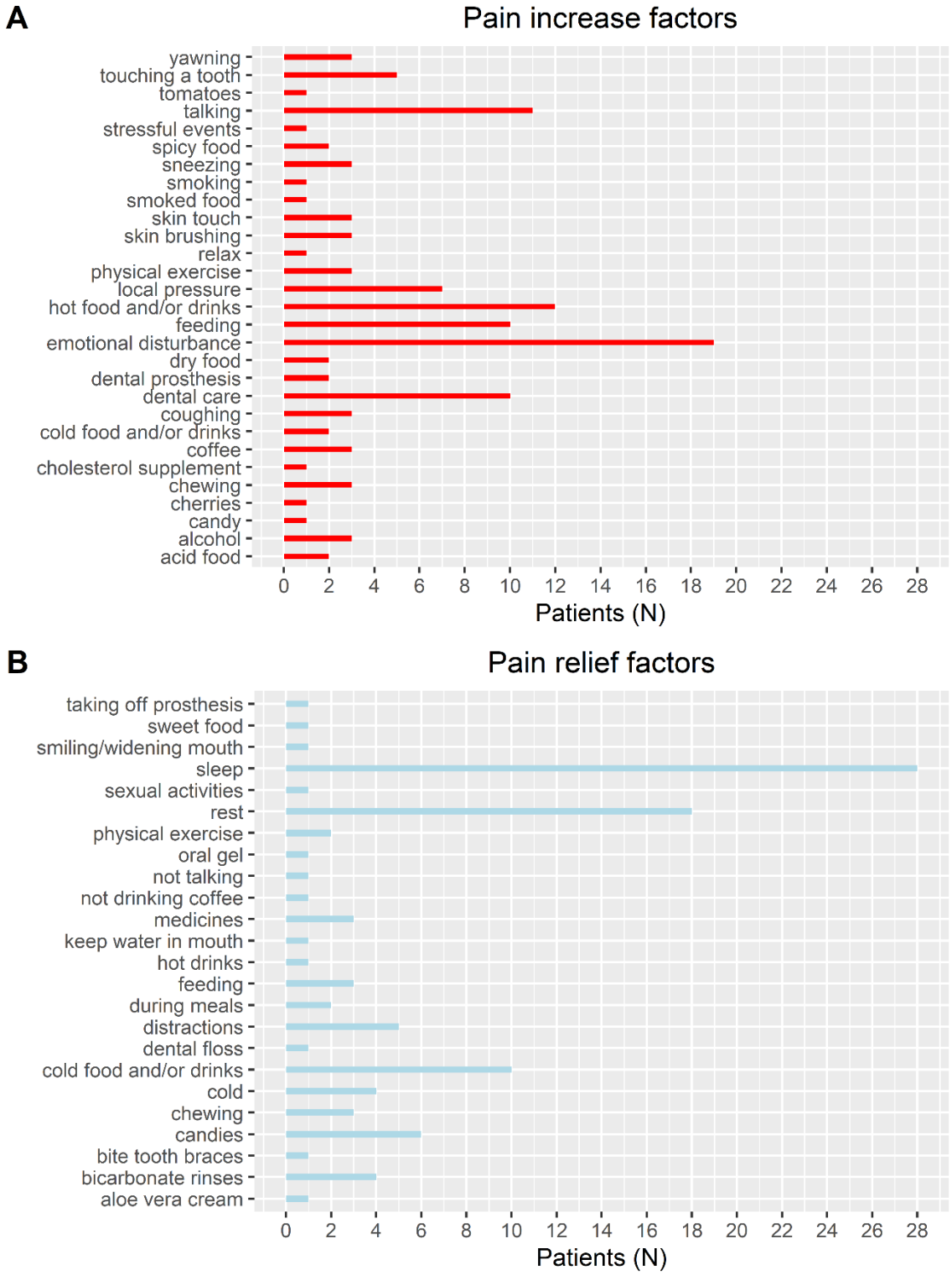

Figure 10. Factors affecting the pain. Factors worsening (A) and relieving (B) pain perception (n=28).

Experimental trials

Pain intensity perception did not fluctuate between the three baseline measurements (at beginning, after the questionnaires at 20 minutes, and at the end of the experimental session, namely at 40 minutes from the beginning; Friedman's test, $\chi^2 = 3.0545$, $df = 2$, $p = 0.22$), nor did the pain unpleasantness perception (Friedman's test, $\chi^2 = 0.15094$, $df = 2$, $p = 0.93$). Such results indicate the pain perception steadiness in the patients (table 6). Not normalized mean ratings ± SD for each experimental trials are reported in table 7.

	Baseline 1 (Mean ± SD)	Baseline 2 (Mean ± SD)	Baseline 3 (Mean ± SD)	Baseline all (Mean ± SD)	Friedman's Test
Intensity (NRS 0 – 10)	5.7 ± 2.6	5.4 ± 2.8	6.2 ± 2.4	5.6 ± 2.4	$\chi^2 = 3.0545$, $df = 2$, $p = 0.22$
Unpleasantness (NRS 0 – 10)	5.6 ± 3.2	5.6 ± 3.2	6.0 ± 3.0	5.7 ± 2.9	$\chi^2 = 0.15094$, $df = 2$ $p = 0.93$

Table 6. Baseline values (mean ± SD) for intensity and unpleasantness at three time points: at beginning (1), after the questionnaires (2), and at the end of the experiment (3). Baseline all represents the mean of the three baselines.

	Smell (Mean ± SD)			Taste (Mean ± SD)		
	Banana	Fish	Neutral	Sucrose	Quinine H	Neutral
Intensity (NRS 0 – 10)	5.2 ± 3.1	6.5 ± 2.8	5.3 ± 3.0	4.7 ± 2.9	6.0 ± 3.1	5.4 ± 3.3
Unpleasantness (NRS 0 – 10)	5.0 ± 3.7	7.1 ± 3.4	5.3 ± 3.6	4.4 ± 3.4	6.8 ± 3.3	5.1 ± 3.6

Table 7. Mean values (± SD) of pain intensity and pain unpleasantness for each smell and taste stimulus.

Effect of smell on pain perception

A significant factor valence (pleasant, unpleasant, neutral) was found through Friedman's test on the normalized data in the smell-pain trials for the NRS unpleasantness scores ($\chi^2 = 10.292$, $df = 2$, $p = 0.006$; figure 11A). Benjamini-Hochberg correction was applied to the pairwise comparisons, showing a significant effect of unpleasant vs. pleasant odours (Wilcoxon test, $Z = 6.5$, $p = 0.007$), and unpleasant vs. neutral odours (Wilcoxon test, $Z = 59$, $p = 0.023$), but not in the pleasant vs. neutral odours (Wilcoxon test, $Z = 19$, $p = 0.414$). Patients' percentage that referred an increment in pain unpleasantness were 41% and 18% for the unpleasant and neutral conditions, respectively (table 8). Such results show a significant increase in pain unpleasantness perception in the unpleasant smell trial in comparison to the pleasant and neutral smell conditions. No significant effect was found between valences (pleasant, unpleasant, neutral) and NRS pain intensity normalized ratings (Friedman's test, $p > 0.05$; figure 12A).

Effect of taste on pain perception

Friedman's test on the normalized data of the taste-pain trials discovered a significant factor valence (pleasant, unpleasant, neutral) for the NRS unpleasantness scores ($\chi^2 = 14.583$, $df = 2$, $p < 0.001$; see figure 11B). Pairwise comparisons after the Benjamini-Hochberg correction showed a significant effect of unpleasant vs. pleasant gustatory substances (Wilcoxon test, $Z = 20.5$, $p = 0.008$) and unpleasant vs neutral ones (Wilcoxon test, $Z = 140$, $p = 0.019$). No effect was found for the pleasant vs. neutral taste comparison (Wilcoxon test, $Z = 54.5$, $p = 0.309$). Patients' percentage of increment reports in pain unpleasantness were 55% and 18% for the unpleasant and neutral conditions, respectively (table 8). These results show a significant increase in pain unpleasantness perception in the unpleasant taste condition in comparison to the pleasant and neutral taste trials.
A significant factor valence (pleasant, unpleasant, neutral) was found at the taste-pain interaction also for NRS pain intensity (Friedman's test, $\chi^2 = 8.3934$, $df = 2$, $p = 0.015$; see figure 12B). Post-hoc comparison indicated a trend in the pleasant vs. unpleasant taste trials (Wilcoxon test, $Z = 12.5$, $p = 0.023$), not significant after the Benjamini-Hochberg correction.

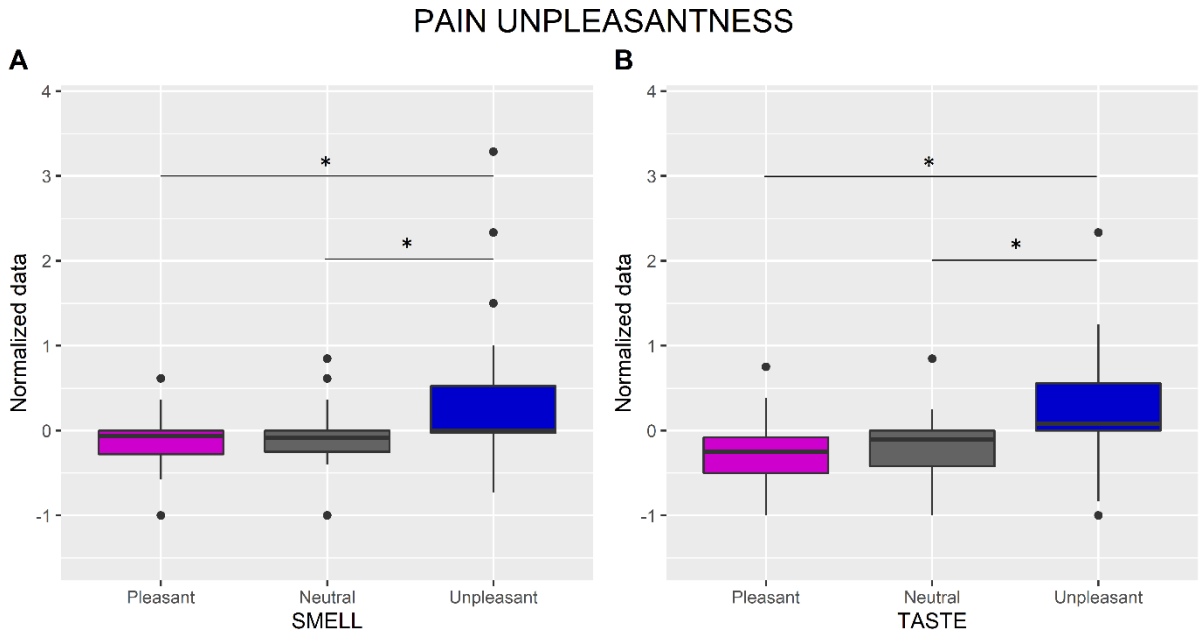

Figure 11. Box plots of pain unpleasantness ratings for related to pleasant (magenta), neutral (dark grey), unpleasant (blue) stimuli, for the smell (A) and taste stimulation trials (B). The horizontal black lines represent median values. Ratings were significantly higher in the unpleasant compared to the pleasant and neutral conditions, both for smell and taste. Data are presented as normalized scores relative to baseline (mean of three baseline values). * = $p < 0.05$ after application of Benjamini-Hochberg correction.

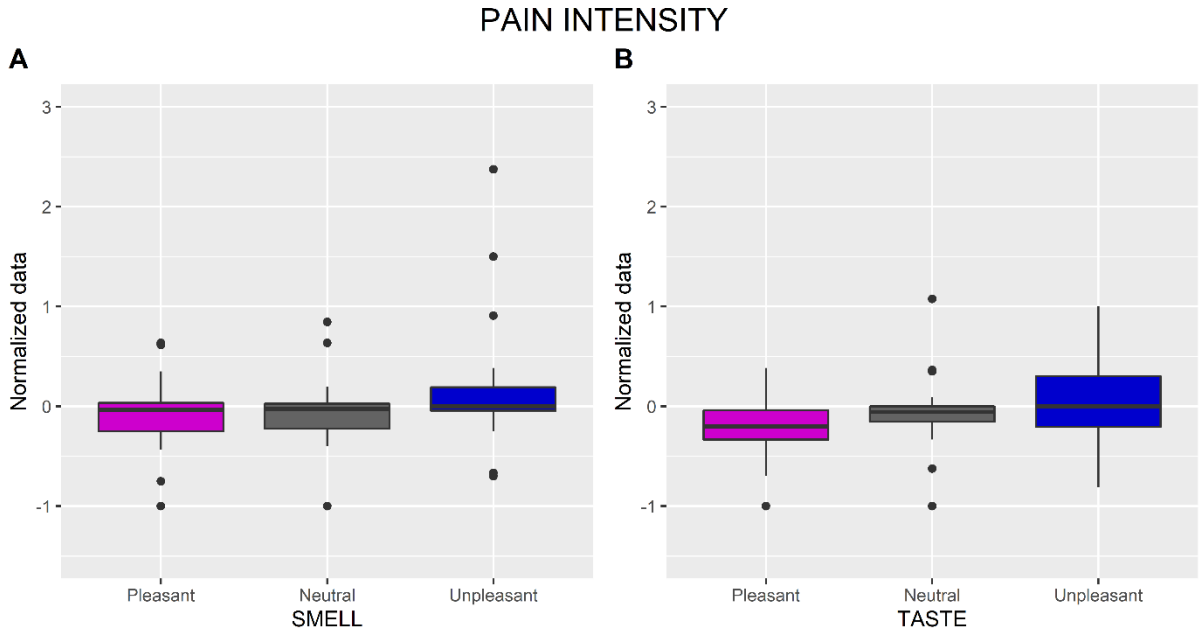

Figure 12. Box plots of pain intensity ratings for pleasant (magenta), neutral (dark grey), unpleasant (blue) stimuli, for the smell (A) and taste stimulation trials (B). The horizontal black lines represent median values. No differences were found between conditions, both for smell and taste. Data are presented as normalized scores relative to baseline (mean of three baseline values).

			Pain unpleasantness		*Pain intensity*	
Sense	*Valence*	*Substance*	*Any increment relative to baseline* ($\Delta x_{normalized} > 0$)	*>50% increment relative to baseline* ($\Delta x_{normalized} > 0.50$)	*Any increment relative to baseline* ($\Delta x_{rnormalized} > 0$)	*>50% increment relative to baseline* ($\Delta x_{normalized} > 0.50$)
Smell	*Pleasant*	*Banana*	18%	4%	27%	9%
	Neutral (control condition)	*Odourless air*	18%	9%	27%	9%
	Unpleasant	*Fish*	41%	27%	41%	14%
Taste	*Pleasant*	*Sucrose*	14%	4%	14%	0%
	Neutral (control condition)	*Natural water*	18%	4%	23%	4%
	Unpleasant	*Quinine H*	55%	27%	41%	18%

Table 8. Percentage of patients who had an increment in pain unpleasantness or intensity after the stimulus administration.

Supplementary analyses

Consistent results were found with the absolute values' analyses (figure 13), and on patients' responses estimated as a difference from the neutral condition (figures 14 and 15), both for smell and taste.

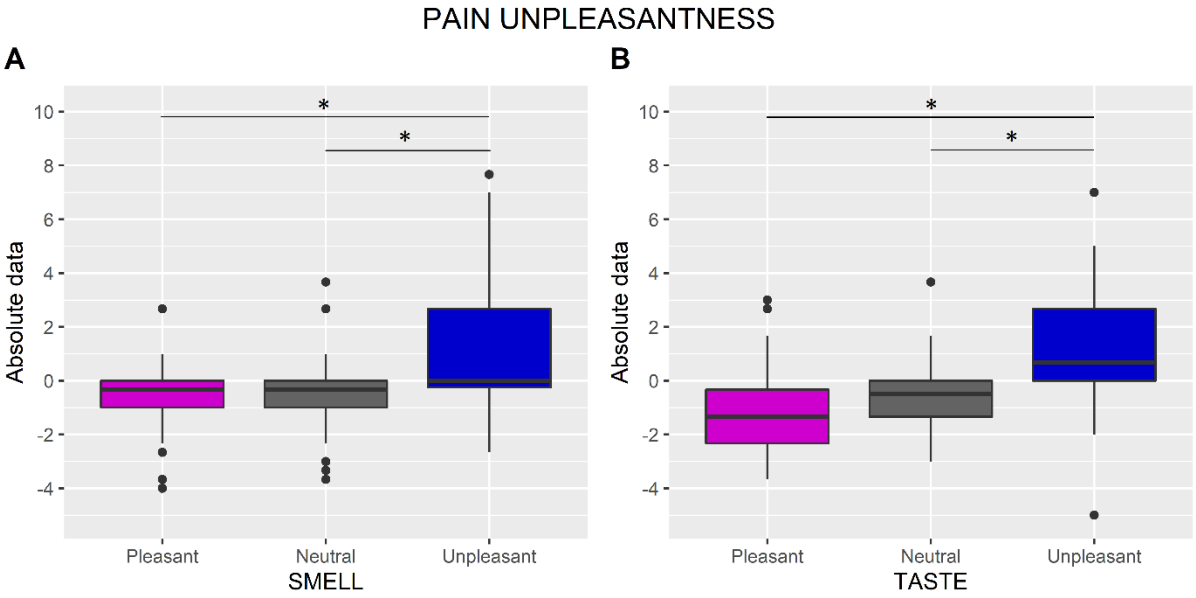

Figure 13. Box plots of pain unpleasantness ratings for related to pleasant (magenta), neutral (dark grey), unpleasant (blue) stimuli, for the smell (A) and taste stimulation trials (B). The horizontal black lines represent median values. Ratings were significantly higher in the unpleasant compared to the pleasant and neutral conditions, both for smell and taste. Data are presented as normalized absolute scores relative to baseline (mean of three baseline values). * = $p < 0.05$ after application of Benjamini-Hochberg correction.

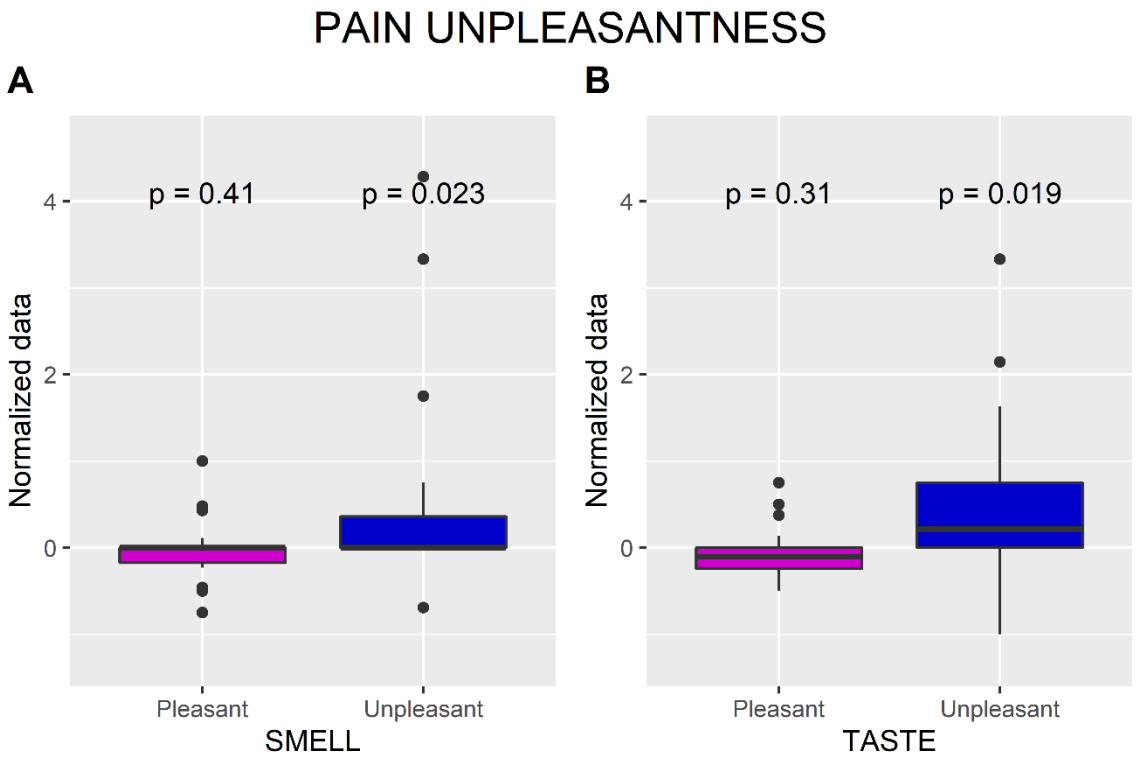

Figure 14. Normalized pain unpleasantness ratings after administration of smell (A) and taste (B) experimental stimuli, calculated as a difference from the neutral condition, and *p*-values obtained using One-sample Wilcoxon's signed-rank tests for the null hypothesis that changes are equal to zero.

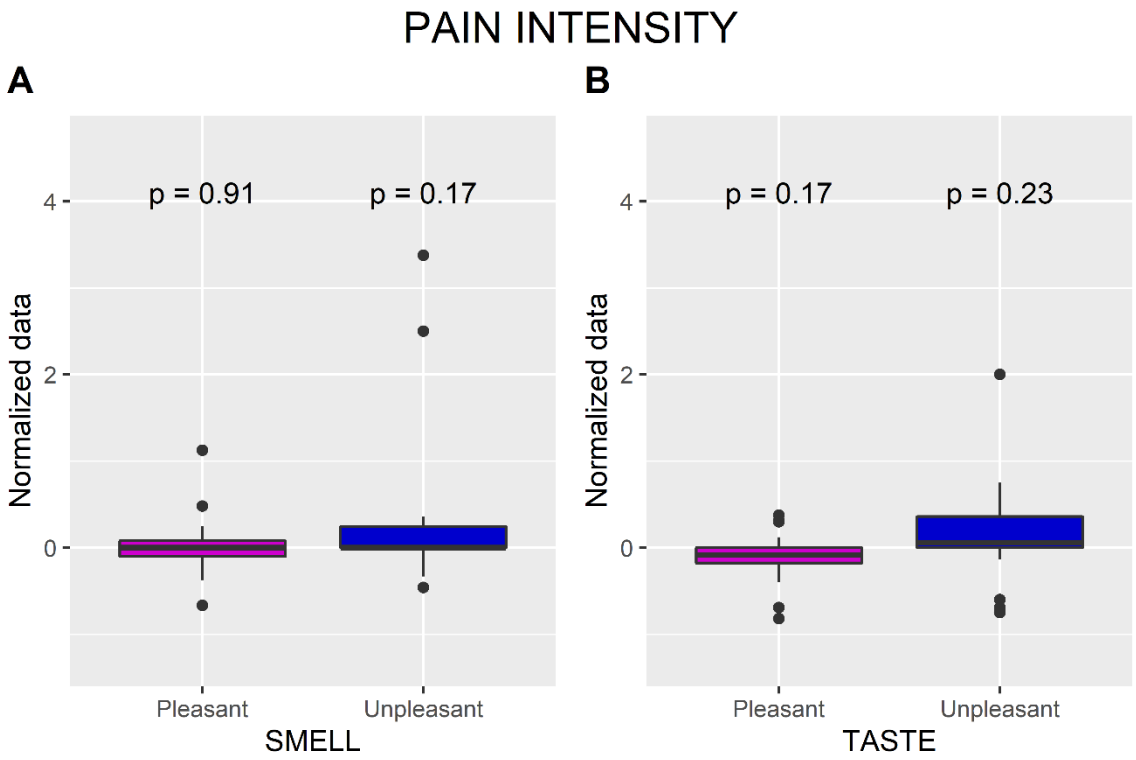

Figure 15. Normalized pain intensity ratings after administration of smell (A) and taste (B) experimental stimuli, calculated as a difference from the neutral condition, and *p*-values obtained using One-sample Wilcoxon's signed-rank tests for the null hypothesis that changes are equal to zero.

The responses for a given valence did not change as a function of sense ($p > 0.05$).
The increasing in pain unpleasantness after the unpleasant stimuli was found in both patients with a lower (smell, $p = 0.023$; taste, $p = 0.020$) and higher (smell, $p = 0.047$; taste, $p = 0.031$) baseline pain unpleasantness, although responses were quite more noticeable for the former group (figure 16). Responses to experimental trials were also comparable for patients with a shorter (smell and taste, $p > 0.05$) and longer (smell, $p = 0.02$; taste, $p < 0.001$) disease duration, although responses were quite more noticeable in patients with longer disease duration (figure 17).

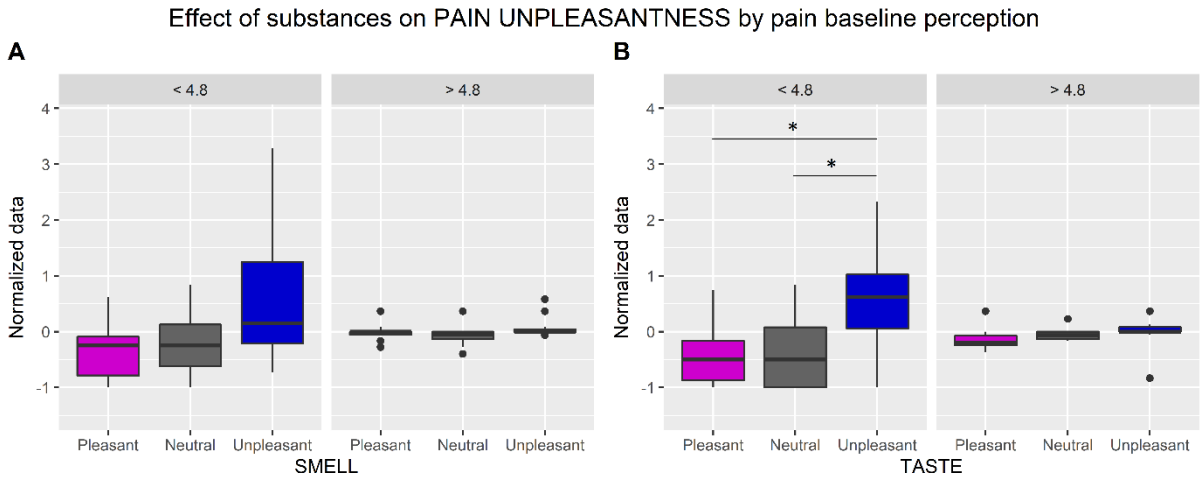

Figure 16. Normalized relative pain unpleasantness ratings after administration of smell (A) and taste (B) experimental stimuli for patients with a baseline pain perception lower (n=11) and higher (n=11) than the median score of 4.8.

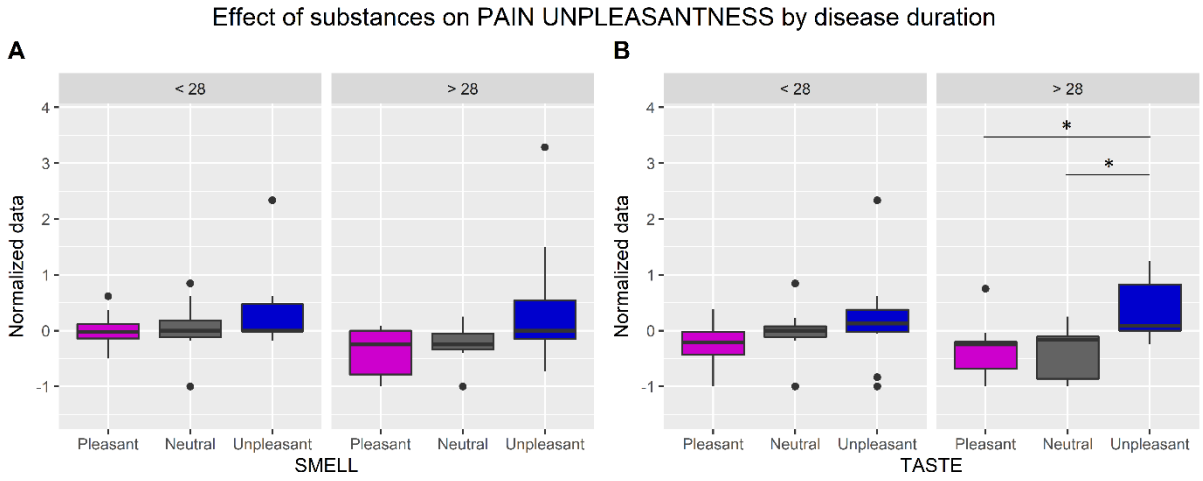

Figure 17. Normalized pain unpleasantness ratings after administration of smell (A) and taste (B) experimental stimuli for patients with a disease duration shorted (n=11) and longer (n=11) than the median of 28 months. Oral pain duration range: 7-192 months, median value: 28, interquartile range: 17.25 – 58.75 months.

Correlational analyses

We investigated possible correlations with pain unpleasantness, the only outcome significantly modulated by the substances. To evaluate if such emerged modulation was associated with the given substances' hedonic ratings (pleasant smell/taste, unpleasant

smell/taste), Spearman's correlations were run. A significant negative correlation was observed between the hedonic ratings for fish odour and the normalized value of pain unpleasantness after fish odour stimulation (R = -0.53, p = 0.011; figure 18A). Furthermore, a significant negative correlation was found between the hedonic ratings for banana odour and the normalized value of pain unpleasantness after administration of the banana odour (R = -0.58, p = 0.0057, see figure 18B). Those correlations show that the pain unpleasantness modulation correlates with the odour individual pleasantness perception: the more the odour (banana or fish) was evaluated as unpleasant, the more the pain unpleasantness increased; vice versa, the more the odour was evaluated as pleasant, the more the pain unpleasantness reduced. No correlations emerged between the hedonic ratings for the taste substances and the pain unpleasantness modulation (all p > 0.05).

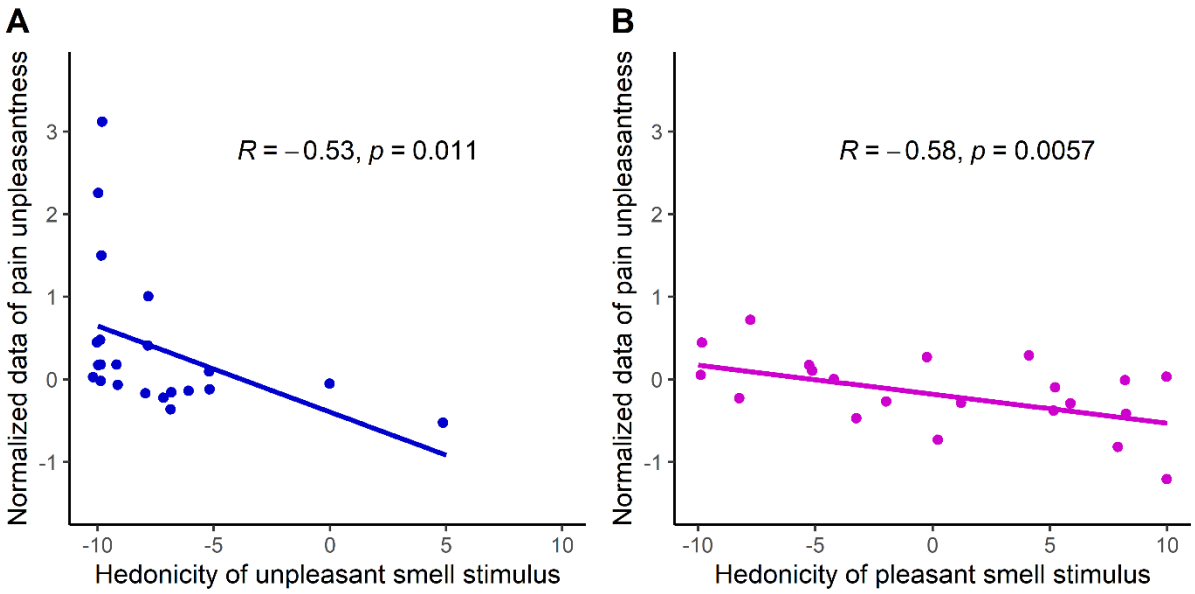

Figure 18. Scatterplots with linear interpolation and Spearman's rank coefficients for the correlation between hedonicity of smell stimulus and normalized pain unpleasantness ratings, for the unpleasant odour (A) and the pleasant odour (B). Likert scale of hedonic ratings is represented on x axis, from -10 (very unpleasant) to +10 (very pleasant), with 0 = neutral.

Pain and psychological questionnaires

The referred oral pain deepen affects the patients' daily life, as described through the BPI and QUID questionnaires (see figures 19 and 20). At the sensory interview, 22 patients (78.6%) reported anxiety, though such perception did not emerge at the STAI Y1 & Y2, and 6 of them (21.4%) reported having suffered from depression, albeit not always clinically diagnosed. At BDI-II, 4 patients (14%) reported high depressive symptoms, compared to the normal population (Ghisi et al., 2006).
Regarding the CORE-OM, only 3 patients (10.7%) out of 28 had high general psychological distress. 7 of them (25%) had high scores in the symptoms' subscale, in comparison to the normal population (Palmieri et al., 2009). At FFMQ none of the patients had lower scores compared to the normal population (Poulin et al., 2016). 7 out of 22 (31.8%) scored as borderline or alexithymic at the TAS-20 (Bressi et al., 1996).
8 patients (28.6%) had higher thoughts and feelings of fear and catastrophizing in comparison to the normal population at the PCS, and 14 (50%) had high scores on the rumination subscale (Monticone et al., 2012).

Although we predicted more comorbidity with psychological aspects, our sample did not differ from the normal population, on average. Such results could derive from both the small sample size, and also from a gender effect, given that psychological factors appear to be associated in this condition in a different way based on the patient's gender (Yoo et al., 2018). However, noticing a trend in alexithymia scores and psychological distress, we investigated the relationship between those variables and pain unpleasantness after unpleasant stimuli administration. Spearman's correlations were run with an emphasis on the affective pain component and the unpleasant stimuli that emerged as key modulatory elements at the experiment. Given the exploratory quality of those correlations, analyses were not corrected.

A positive correlation between pain unpleasantness modulation after unpleasant taste administration and life functioning CORE-OM subscale emerged ($R = 0.45$, $p = 0.036$), referring a general dysfunction (in both personal and social relationships as well) correlated with the increment of pain unpleasantness after unpleasant taste. Another positive correlation was found between pain unpleasantness modulation after unpleasant taste administration and the describe feelings TAS-20 subscale ($R = 0.49$, $p = 0.020$), reporting higher difficulty in describing their own feelings correlated with the increment of pain unpleasantness after unpleasant taste. Moreover, a negative correlation between pain unpleasantness modulation after unpleasant odour administration and the external thinking TAS-20 subscale (R

= -0.47, $p = 0.029$) was found: a low externally oriented thinking (patients were consequently more internally oriented) correlated with the increasing of the pain unpleasantness after unpleasant odour. Other correlations were not significant (all $p > 0.05$).

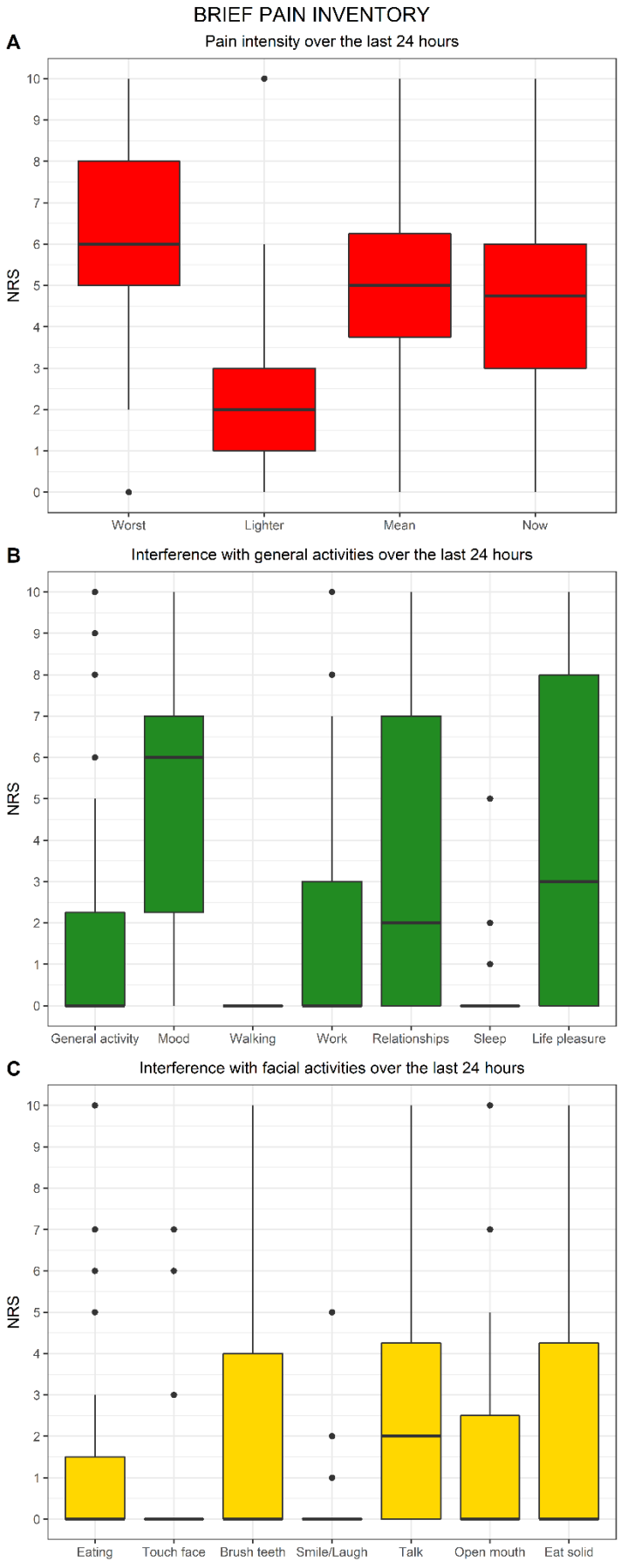
BRIEF PAIN INVENTORY
A
Pain intensity over the last 24 hours
NRS
Worst
Lighter
Mean
Now
B
Interference with general activities over the last 24 hours
NRS
General activity
Mood
Walking
Work
Relationships
Sleep
Life pleasure
C
Interference with facial activities over the last 24 hours
NRS
Eating
Touch face
Brush teeth
Smile/Laugh
Talk
Open mouth
Eat solid

Figure 19. Brief Pain Inventory. (A) Box plot representing median values (horizontal black lines) of pain intensity ratings related to the latter 24 hours at its worst, lighter, mean, and at the time of testing. (B) Box plot representing median values (horizontal black lines) of pain interference with general activities in the latter 24 hours: general activity, mood, walking, work, relation with others, sleep, life pleasure. (C) Box plot representing median values (horizontal black values) of pain interference with facial activities in the latter 24 hours: eating a meal, touching face, brushing teeth, smiling/laughing, talking, wide mouth opening, eating solid food like an apple (n=28).

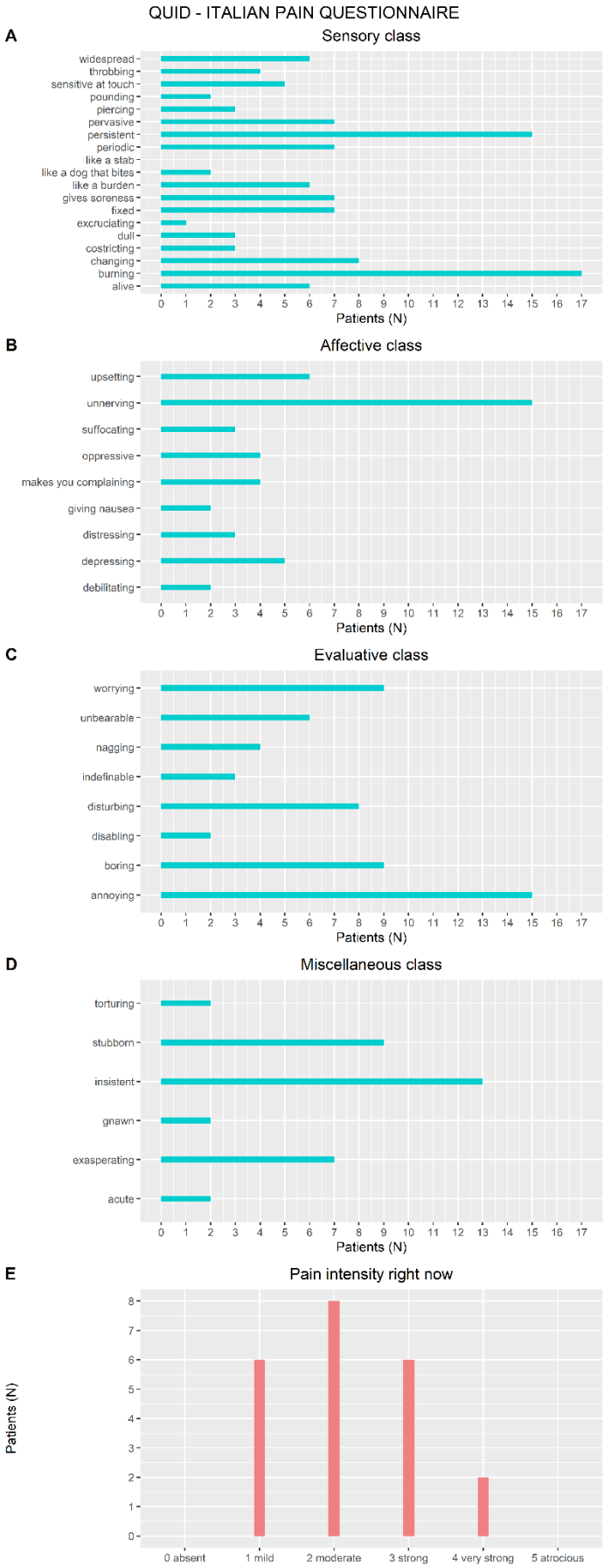
QUID - ITALIAN PAIN QUESTIONNAIRE
A
Sensory class
widespread
throbbing
sensitive at touch
pounding
piercing
pervasive
persistent
periodic
like a stab
like a dog that bites
like a burden
gives soreness
fixed
excruciating
dull
constricting
changing
burning
alive
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Patients (N)
B
Affective class
upsetting
unnerving
suffocating
oppressive
makes you complaining
giving nausea
distressing
depressing
debilitating
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Patients (N)
C
Evaluative class
worrying
unbearable
nagging
indefinable
disturbing
disabling
boring
annoying
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Patients (N)
D
Miscellaneous class
torturing
stubborn
insistent
gnawn
exasperating
acute
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Patients (N)
E
Pain intensity right now
Patients (N)
0 1 2 3 4 5 6 7 8
0 absent
1 mild
2 moderate
3 strong
4 very strong
5 atrocious

Figure 20. QUID. Italian Pain Questionnaire. Each graph (panel A-D) shows the pain perceived through various pain semantic descriptors exploring different classes (Sensory, Affective, Evaluative, Miscellaneous). Panel E shows the pain intensity perceived (mild, moderate, strong, very strong, atrocious) (n=22).

Discussion

The exploratory experiment presented here delve into the modulatory role of olfactory and gustatory substances in chronic oral burning pain, a disabling condition that is a challenge to deal and tend to (Klein et al., 2020). Two main results emerged: a) chemosensory stimuli modulated pain unpleasantness, but not pain intensity; b) only the unpleasant stimuli (fish odour and bitter taste) increased pain perception, whereas others (banana odour and sucrose taste) did not modulate it. With regard to olfactory substances, such modulation appears to be associated to the subjective stimulus perception. Associations with psychological distress (CORE-OM and TAS-20 scores) and clinical aspects were also noticed.

In this experiment, the unpleasant chemosensory stimuli modulated pain unpleasantness without altering pain intensity perception, highlighting the dissociation between the sensory and the affective dimensions of pain in this kind of chronic oral pain, similarly to our findings in healthy participants (chapter 3).

In this context, a pleasant stimulus might not be adequate to exert an impact on pain, while the unpleasant stimulus in conjunction with the negative pain condition might provoke an additive effect. It has been found that an unpleasant odour increased the perception of pain intensity and unpleasantness in a patient with neuropathic pain, and such report was linked to an increment in neural activation (fMRI) after exposure to the unpleasant odour, suggesting an underlying neural substrate linked to the odour effect (Villemure et al., 2006). It has been observed that, in patients affected by oral burning pain, there seems to be a change at the central level of pain processing, found through MRI studies which have proved variations in cerebral blood flow and gray matter volume in pain-related brain regions (e.g., the anterior cingulate cortex, medial orbitofrontal cortex, pars orbitalis, insula, thalamus; Albuquerque et al., 2006; Wada et al., 2017; Su et al., 2020).

In summary, only the unpleasant stimuli had an influence on patients' pain perception. The pathophysiology of chronic oral burning pain is yet to be fully mastered. In fact, in previous studies on the matter, only Hirsch and collaborators (2011) reported an instant analgesic effect on pain intensity in three patients with burning mouth syndrome after the administration of sucralose, an artificial sweetener 600 times sweeter than sucrose. In our experiment, sucrose did not modulate neither pain intensity nor pain unpleasantness. In our sample, the lack of influence exerted by the pleasant gustatory substance might be due to the fact that it could have been not sweet enough. Notably, the three patients in Hirsch's study had a compromised perception of sweet (sucrose) substances at a preliminary evaluation, while our sample was normogeusic.

A recent and promising clinical study on chronic low back pain found higher pain thresholds for cutaneous stimuli after an olfactory training with pleasant odours for 4 weeks, useful for pain control too (Gossrau et al., 2020). In our patients, the banana olfactory stimulus did not have any impact, probably due to the perceived valence of the odour itself. Correlation analyses revealed that the more the odours (banana and fish) were evaluated as unpleasant, the more an increment was observed in the pain unpleasantness; equally, the more pleasant they were evaluated, the less pain unpleasantness was rated. Given that odours are strongly interconnected to the affective domain (Krusemark et al., 2013), they might impact the individual perception differently. The modulatory effect of pleasant odours might have been masked: we could account for such a variability by selecting the substances evaluated as most pleasant/unpleasant by each participant at a preliminary evaluation, for instance, as previously done (Villemure et al., 2003, 2006, 2009; Gossrau et al., 2020). Moreover, odours are defined by their pleasantness (Yeshurun and Sobel, 2010), and the saliency of a stimulus is defined by how much such stimulus contrasts with past experiences (Iannetti & Mouraux, 2010). Those peculiarities could explain how differently stimuli produced an effect depending on their perceived valence.

A link rose between pain perception, unpleasant chemosensory stimuli, and emotional aspects related to distress (CORE-OM) and emotions awareness (TAS-20) at the correlations. However, given the exploratory type of the analyses and the small sample size, such results require future investigations. We know that pain unpleasantness is more strongly affected by emotions than pain intensity (Rainville et al., 2005). Recently it has been found out that a personal belief in the ability to deal with or adapt to chronic oral burning pain

plays a significant role in the global pain experience as well. Such experience is related to catastrophizing, pain self-efficacy, and acceptance in those patients (Chana et al., 2021). Therefore, the catastrophizing and rumination emerged from the PCS responses (meaning, a negative orientation towards the pain experience) might have had a role in pointing the attention to the unpleasant stimulus.

Indeed, what we feel about one stimulus may carry over and bias what we feel about another stimulus (Spence & Gallace, 2011), both positively and negatively. For instance, in patients with lower back pain the sound of a creaking door synchronized with the bending of the lower back had a negative effect on affective ratings (Stanton et al., 2017). In healthy subjects, the perceived sexiness of another's touch was modulated by the "sexiness" of the background music (Fritz et al., 2017), highlighting once again the importance of the context in which we experience a particular stimulus. Such cross-modal effect is often referred to as the phenomenon of "sensation transference" (Spence & Gallace, 2011).

Regarding pain, its chronicity has a negative impact on general well-being (Sardella et al., 2006), and the peculiar facets of the oral chronic painful condition may be the key element to explore. Notably, substances had a more pronounced effect on patients with a longer disease duration. Oral pain was referred to be persistent during the day, confirmed both during the sensory interview and through the baseline ratings during the experiment. It has also been described by the patients with almost all the possible adjectives from the QUID questionnaire, underlining the complexity of such experience. Sir Thomas Lewis wrote in the preface to his monograph entitled "Pain":

> « I am so far from being able to define pain that the attempt could serve no useful purpose. »
> (Lewis ST, "Pain", New York: Macmillan, 1942)

In our attempt to better define this type of pain, it is worth remembering that the oral cavity is one of the most deeply innervated anatomical areas in the body, where a broad range of signals arises there (tactile, nociceptive, thermic, chemosensory). Accordingly, oral somatosensory awareness is a highly complex matter (Haggard and de Boer, 2014). When associated to unpleasant feelings (e.g., distress, fear, etc.), pain unpleasantness, described under the term "secondary pain effect" (Price, 2000), may raise the interference of an unpleasant stimulus in the modulation of pain perception. The more patients reported distress and struggle in dealing with emotions, the more the unpleasant stimuli increased their pain unpleasantness perception. It seems that patients with oral burning pain presented different

functional brain connectivity patterns in relation to perceived pain. It has been found that higher brain functional activity in affective-motivational neural circuits is associated to depression and anxiety, suggesting the connection between psychogenic components and chronic oral burning pain (Khan et al., 2014).

In the sensory interview, many patients reported suffering from anxiety or depression. They also referred that, as reported elsewhere (Grushka, 1987; Grushka et al., 2006; Klasser et al., 2016), emotional disturbances, as well as talking, dental care, and hot food or drinks, exacerbated their oral pain. Pain, typically influenced by emotional states, might be aggravated by an unpleasant chemosensory stimulus that usually expresses threatening cues (Beauchamp, 2016; Stevenson, 2010). Indeed, the olfactory perception is strongly linked with physiological and behavioural responses to emotionally arousing events (Jo et al., 2021; Wudarczyk et al., 2016; Zald and Pardo, 1997). Pain can modulate an emotional state but, on the other hand, an emotional state has a considerable impact on pain processing too, indicating that positive or negative mood can differently modulate the perceived pain sensitivity and brain activity in the pain pathway (Villemure et al., 2003, 2009; Bartolo et al., 2013; Jo et al., 2021).

Finally, patients indicated that sleep and resting, cold food and drinks were pain-relieving factors, as much as distractions. Villemure and Bushnell (2009) demonstrated through fMRI lower activity in the thalamocortical pain pathway in relation to diminished pain perception during distraction. In the future, it will be necessary to unravel the role of the facets that relief pain, such as attention and emotions.

5: GENERAL DISCUSSION

This work tries to dive into how such a complex experience as pain might be influenced by the chemical senses. As pain can be highly influenced both by the environment and the physiological and inner mental state of each individual, the results here presented can be summarized as follow:

-in the current literature, the pain-smell-taste interaction has been studied in healthy adult participants, with promising results for gustatory substances to modulate the quantitative measures of pain (threshold and tolerance). On the other hand, olfactory substances influence the qualitative measures of pain (unpleasantness and intensity). In clinical pain conditions, results are still preliminary, but encouraging attempts have been made, especially with smell substances;

-in healthy individuals, with a method employed to mime a sort of neuropathic pain (capsaicin cream), we found that a pleasant odour seems to reduce unpleasantness pain perception only, namely the affective pain component;

-in a clinical pain population suffering from chronic oral burning pain, we found that unpleasant substances (both olfactory and gustatory) increased pain unpleasantness perception, especially in patients with longer disease duration. The effect of olfactory substances was related to the individual hedonic judgement (regardless of the substance, if perceived as unpleasant, it tended to increase the painful unpleasant perception; if perceived as pleasant, it decreased the painful unpleasant perception); a potential role of emotions and psychological factors emerged as well.

Our results in healthy subjects fit well in our literature review: pain is composed by two distinctive pain dimensions (Price, 2000), that can be affected differently.

Moreover, no one before had employed capsaicin cream as a tonic method to induce pain, as others usually apply faster and more certain ways to assure pain perception (temperature, electric, pression, etc.). However, the results found strengthen the rationale to implement this methodology as a model that mimics neuropathic pain (van Amerongen et al., 2016). Only the affective dimension of pain happens to be influenced by chemosensory stimuli with different valences in patients with oral burning pain and in healthy subjects. In

patients we found an increment in pain perception after the unpleasant stimuli, while with the neuropathic pain model we found only a tendency between the neutral and the pleasant conditions. This different pain modulation found in clinical vs experimental pain could depend by the different stimulus type and the context: controllable vs uncontrollable pain involves different connections between brain areas (Bräscher et al., 2016), and pain perception is influenced by natural variations in brain activations related to different factors (Atlas et al., 2014). Individual pain experience (also in relation to the past) also represents an important aspect which needs to be taken into account in relation to the differential effect of the substances. The unpleasant substances in patients with chronic pain could exacerbate the pre-existing pain, having a marked effect concomitantly to pain-related thoughts and bad mood. On the other hand, for healthy subjects the pain experience is a novel unexpected situation confined to the experiment itself. We could expect that chronic neuropathic pain is perceived as uncontrollable, and therefore associated to higher sensitization. Though healthy participants in our experiment did not have any control over the pain, knowing that the experience is limited to the duration of the experiment might have established different mechanisms underlying pain modulation. In patients, this lack of control over the pain perception leads to helplessness and depression, but improving pain management through the use of different substances could help in perceiving more control over the situation. Future studies, for instance, could tackle more directly the placebo effect (i.e., by saying for instance "this substance will reduce your painful perception"), or by attempting to invest the patient with control over the perceived pain. Furthermore, it is worth remembering that translating experimental basic research to the clinical one, especially in the algology field, is a complex matter not free of possible surprises, such as the partially unexpected results here described. It is therefore necessary to verify through the clinical practice what has been experimentally observed. Our results show the complexity of the scientific research in this field.

Moreover, odours are deeply connected to emotion, context, and personal likeability; therefore, it is coherent to find a stronger effect for the affective pain component. Olfactory processing involves default mode network deactivation (Karunanayaka et al., 2017): perception of odours may drive cognitive, attentional and memory resources, to work on the feature characterization of odour stimuli, allowing for top-down modulations. In the experiment with healthy participants, we found no effect of taste substances on the dis-

criminative component. Such results need to be further explored and confirmed, to disentangle the specific role of chemosensory substances on tonic pain in their relationship with the discriminative pain component. Besides, no results were found in the clinical pain experiment, given indirect support for the use of capsaicin cream as model for neuropathic pain. Capsaicin can induce both peripheral and central hypersensitivity, as well as trigger pain-sensing afferents and stimulate central sensitization. It has been used as a pain model of excitatory and inhibitory effects in animals and humans (O'Neill et al., 2012; Valeriani et al., 2003, 2005). In addition, it has been proved that stressful life events determined an increment in pain perception in the surrounding area of capsaicin application site (secondary hyperalgesia), suggesting a potential link between external factors (the context) and the pain hypersensitivity (You et al., 2016).

Another limitation in the studies that we conducted could have been the a priori choice of substances, which does not consider individual likeability and therefore might have affected our results. Saliency is defined by how much the stimulus contrasts with past experiences (Iannetti and Mouraux, 2010), and that could explain how differently the stimuli had an effect depending on the perceived valence. A pilot study where substances' preference is assessed in advance should be methodologically preferable, both in healthy and clinical participants, although that procedure would reduce the "saliency effect". As a first step into this research field, and given the difficulty in producing the needed substances, we chose only two substances per sense, to assess an opposite valence effect.

Our results should also be confirmed and hopefully enriched through the adoption of neurophysiological techniques that could help objectively evaluate the nociceptive pathways activity. For instance, it could be interesting to investigate the effect of olfactory and gustatory stimulation on laser evoked potentials (LEP) evoked by cutaneous painful laser stimulation. The laser stimulation would allow to explore the brain activity, evoking specific cerebral responses (LEP) with high temporal resolutions, allowing a better understanding of the timing of the interaction between the olfactory and gustatory stimuli and the cortical processing of the nociceptive input.

Finally, in the initial neuromatrix concept, Melzack stated that pain experience could emerge from a general network of neurons, conveying information that ultimately produces a sense of self (Melzack, 1989, 2005). Therefore, there would not be a specific cortical area that exclusively encodes pain, but a saliency network that encodes relevant stimuli for the

self (Iannetti and Mouraux, 2010). A nociceptive stimulus activates a broad-spread brain network, supporting the idea that pain is the result of complex cortical and subcortical processing of various sources of sensory and emotional information (Jo et al. 2021). Multisensory interaction starts on a behavioural level, given that we are part of the environment and always in relationship to multiple stimuli that interact with each other. Pain, smell and taste are all relevant salient stimuli for our survival, and they could be all conveyed through the same network, as initial evidence pointed out (Jo et al., 2021; Villemure and Bushnell, 2009; Kakeda et al., 2010). Further and more integrated experiments are required.

6: CONCLUSIONS

Pain perception can be the consequence of many experiences: it can be the result of an accident, our body hitting something, a broken bone, an open wound. It can also be the feeling of a broken heart when we lose someone (literally or figuratively). Pain might be temporary or endure and last for a long time: when the pain becomes endless, it affects the whole perception of self, and the relationship one has with the world. Clearly, in the definition of pain sits both the sensory perception coming from our body and the affective aspects which are intangible but still definitely present, including all the emotions connected to them. Moreover, when we experience pain, we are deeply connected to the context and the situation that caused it or somehow exacerbated it.

As such, as pain produces an effect on how we perceive ourselves and interact with the outside world (on levels that go from internal to external), at the same time the outside world can influence our perception, and pain can be modulated by many factors, both environmental and within ourselves. A calm environment could relax us, and thus we might perceive less pain. Also, our thoughts may be helpful in distracting us from the pain, as much as engaging in other activities. The focus of this thesis was to explore the relationship of pain perception and other different sensorial modalities as ways to modulate pain perception; being pain a feeling coming from within our body, this work explores how it can be modulated by other senses, and in particular chemical senses: smell and taste.

Here three works have been presented: the state of the art, developed in a review, one experimental work on healthy participants, and one experimental work on a clinical population. From those works we can conclude that smell and taste could and should play a bigger role in pain perception, if we want to deepen the future implementation of some alternative pain management, prior with a perceptual assessment through validated tests. Given also the partial conflicting data emerged between the experimental and the clinical results that only represents the complexity of this research field, it is vital to explore ways that might not have a side effect for patients, as opposed to pharmaceutical drugs which often have, also with the use of neurophysiological techniques that could help disentangle the timing of those interactions.

A recent study (Jo et al., 2021) on pain and smell in fMRI proposed that the functional connectivity emerged with the right supramarginal gyrus (rSMG) and left superior-frontal

gyrus (lSFG) is more related to the subjective experience of pain with respect to the sensory processing per se, and that the discovered functional coupling with the amygdala would reveal the role of emotions (represented by emotionally salient odours) into influence the pain experience (Jo et al., 2021). Future studies should not only try to connect pain and chemical senses, but also explore how emotions and attention have a role in such a multisensory interaction (Villemure and Bushnell, 2010). Moreover, a comparison between a neuropathic pain model and another chronic clinical pain could help shedding light on such interactions, hopefully involving longer manipulations to study potential long-term beneficial effects (a constant exposure similar to Gossrau et al., 2020).

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