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Modulation of the human nociceptive flexion reflex by pleasant and unpleasant odors

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ABSTRACT

The nociceptive withdrawal reflex (NWR), a defensive response that allows withdrawal from a noxious stimulus, is a reliable index of spinal nociception in humans. It has been shown that various kinds of stimuli (emotional, visual, auditory) can modulate the transmission and perception of pain. The aim of the present study was to evaluate, by means of the NWR, the modulatory effect on the spinal circuitry of olfactory stimuli with different emotional valence. The magnitude of the NWR elicited by electrical stimulation of the sural nerve was measured while 18 subjects (9 women, 9 men) smelled pleasant, unpleasant, or neutral odors. The NWR was conditioned by odor probe with interstimulus intervals (ISIs) of 500 ms and 1,500 ms. The magnitude of NWR was significantly greater after the unpleasant odor probe ($P < .001$) and reduced following the pleasant odor probe ($P < .001$) at both ISIs. A significant effect of olfactory stimuli on subjective pain ratings were found at both ISIs for pleasant vs unpleasant odors ($P < .000$), and for both pleasant and unpleasant odors vs neutral and basal conditions ($P < .000$). No statistical differences in subjective pain ratings at different ISIs were found. Consistent with the notion that NWR magnitude and pain perception can be modulated by stimuli with different emotional valence, these results show that olfactory stimuli, too, can modulate spinal nociception in humans.

1. Introduction

Although there exists evidence suggesting that odor substances can modulate pain perception in humans [46–48], the neurophysiological mechanism mediating this effect is far from clear.

It is known that exposure to odors perceived as pleasant or unpleasant can induce emotional responses, functionally equivalent to natural mood states, and evoke memories with a significant emotional component [2,10,11,15,38].

Odor-induced emotional responses occur rapidly [3,4,10] and, compared with other emotions evoked by other sensory experiences (e.g., visual inputs), require little or no symbolic transformation or complex cognitive mediation [10]. Interestingly, the effect of odors has been associated with altered brain activity in limbic structures (such as the amygdala, orbitofrontal cortex, and anterior cingulate cortex) [46,51], which are also the anatomical structures involved in emotional processing and pain perception [5,30–33,40]. Thus, an interference effect of odor stimuli at this level is plausible. It would be particularly interesting to know whether this potential effect of odors is also diffusely exerted at the level of the spinal cord and, if present, how long it lasts. Consistent with our hypothesis that emotion determines descending modulation of spinal cord neurons, it has been demonstrated that both the magnitude of the nociceptive withdrawal reflex (NWR) and subjective

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pain are modified after exposure to emotionally evocative pictures [35–37]. In particular, the NWR and pain-related perception are inhibited during the viewing of pleasant pictures and enhanced during viewing of unpleasant pictures. It can be hypothesized that a similar mechanism, i.e., descending control of spinal cord neurons, may also be triggered by odor stimuli. Confirmation of this hypothesis could provide the rationale for using odors as analgesics (aromatherapy) in combination with other pain-relieving modalities.

To evaluate whether odors exert actions on spinal circuits, we investigated their effect on the NWR, which is one of the most reliable tools for assessing treatment efficacy and spinal nociception in humans [1,8,41] and is widely influenced by emotion-related descending pathways [41].

2. Materials and methods

2.1. Subjects

Twenty-one healthy volunteers (10 women and 11 men) participated in the study. At enrolment, the subjects' eligibility and health statuses were assessed using a brief clinical interview. The exclusion criteria were age $\leqslant$18 years; self-reported history of bronchopulmonary, neurological, cardiovascular and/or circulatory diseases; chronic pain; pregnancy or breastfeeding; current cold or allergy symptoms; smoking; allergy to perfume; current use of analgesic medications, including non-prescription drugs; olfactory screening test failures (see section 2.4), and inability to tolerate the electrical stimulation during the pre-scanning session.

All participants gave their written informed consent to participate in the study.

The study was conducted in accordance with the revised version of Declaration of Helsinki, and all procedures in the study protocol were fully approved by the local ethics committee.

2.2. Odor stimulator device

The odor stimulator was a simplified version of previously described devices [17–19]. Briefly, in one circuit clean air flowed at a steady rate of 8 L/min, with 80% relative humidity, achieved by sending the airflow through deionized water. A thermistor kept the water at a constant temperature (33–36°C), close to nostril temperature. In a second circuit, odor solution flowed through plastic tubing connected to the airflow circuit and ending just inside the nostril. At each stimulus presentation, a solenoid valve opened for 200 ms and odor solution flowed into the main airflow for the same time and vaporized into the nostril. The switching valves were acoustically isolated and a constant flow rate into the nostril was maintained at all times during electrophysiological data collection.

In order to verify that the odor stimulator really did stimulate the olfactory mucosa, olfactory event-related potentials were recorded in 5 subjects not included in the study.

2.3. Hedonic stimulation: odor stimuli

The odor stimuli used in this study consisted of naturally occurring or synthesized substances. The pleasant odor was vanillin (International Flavors and Fragrances, NY, USA), 2 g in 100 ml pure distilled water. The unpleasant odor was N-valeric acid (1% in pure distilled water) (Sigma-Aldrich Canada, Ontario, Canada). Pure distilled water was used as the neutral odor.

2.4. Olfactory screening

In order to detect gross olfactory dysfunction, olfactory screening was performed adapting a previously described paradigm [47]. Participants were required to sniff pleasant (vanillin, diluted in distilled water [1:10 until 1:1])-, unpleasant (valeric acid diluted in distilled water [1:10 until 1:1])-, and neutral-smelling substances (pure distilled water). The stimuli were presented using a 2-alternative force choice paradigm (vanillin vs distilled water, valeric acid vs distilled water). Participants sniffed two bottles in random order 20 times, corresponding to the different dilutions of each odorous substance. They were asked to indicate the presence or absence ("yes" or "no") of odor substances. Only those who made at least 4 consecutive correct choices were enrolled in the study. Participants were also required to indicate how each smell made them feel on a scale from –5 through 0 to +5 [16]. "Extremely unpleasant", "extremely pleasant", and "neutral" (no particular feeling) were scored –5, +5, and 0, respectively. The points between these two extremes and the midpoint were rated "slightly" (1 and 2) and "moderately" (3 and 4) in both the pleasant (+) and unpleasant (−) directions.

2.5. NWR measurement

The NWR from the right lower limb was recorded according to a previously validated method [41,50]. The subjects were seated in a comfortable chair in a quiet room at a constant temperature (23 ± 2°C). Their lower limbs were positioned with their knees flexed at 130° and ankles at 90°, to obtain complete muscle relaxation. The sural nerve was stimulated percutaneously via a pair of standard surface electrodes (silver [Ag]/silver chloride [AgCl]) applied to degreased skin behind the right lateral malleolus. A common ground circle electrode was placed around the leg mid-way between the recording and stimulating electrodes. The transcutaneous electrical stimulus consisted of a constant current pulse train of five individual 1-ms rectangular pulses delivered at 200 Hz (equal to an interstimulus interval of 4 ms). In order to avoid the habituation effect, and to allow elimination of the odorant from the nostril, trains were elicited randomly at intervals ranging from 60 to 90 s [26]. Electromyographic reflex responses were recorded from the capitis brevis of the biceps femoris via surface electrodes (Ag/AgCl). The filter bandpass setting was between 3 Hz and 3 kHz. The analysis time was 300 ms, with the sensitivity set at 300 μV. Each response was full-wave rectified and integrated in the 80–150 ms post-stimulus interval [41].

A staircase method [50] was used to evaluate the NWR threshold (NWR-Th) defined as the stimulation intensity generating a stable reflex response (in 80% of trials) with an amplitude exceeding 30 μV for more than 10 ms in the reflex time window after 3 series of ascending and descending stimuli.

The basal NWR was obtained with a stimulation intensity fixed at 1.2 × NWR-Th. Six stimuli were delivered at this intensity to evoke reflex responses in each session. The first recording of each session was discarded in an attempt to reduce the influence of the startle reaction. Thus, 5 reflex responses were used to measure the reflex magnitude (area under the curve) for each subject in either baseline, conditions and ISI.

The odor stimulator device and electromyograph were both triggered by a BM ST6 digital stimulator (Biomedica Mangoni, Pisa, Italy).

2.6. Stimulus conditioning

To examine the effect of olfactory conditioning (odor conditioning) on the NWR, electrical stimuli (at 1.2 × NWR-Th) were randomly delivered at 500 ms and 1,500 ms interstimulus intervals (ISIs) with the odor stimuli conditioning the electrical stimuli. The ISI times, chosen in order to limit the number of recordings, were based on the findings of a preliminary investigation conducted in 5 participants, not included into the study, in whom the maximum effect of odor stimuli on NWR magnitude was found