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Sensitization and Desensitization to Allyl Isothiocyanate (Mustard Oil) in the Nasal Cavity

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Abstract

The aim of this study was to investigate the response, acute effects and time-course of sensitization and desensitization to allyl isothiocyanate (mustard oil) nasal stimuli in healthy subjects. Sixty subjects participated in the experiment, which employed psychophysical (intensity ratings) and psychophysiological (skin conductance response) measurements. Nasal stimuli were delivered three times with different inter-stimulus intervals. The results showed that the psychophysical and psychophysiological data were correlated and that the successive nasal stimuli after a short period of time (<2 min) produced increased intensity of irritation, whereas the stimuli delivered after >3 min produced a markedly decreased intensity of irritation. These findings are in agreement with those obtained with capsaicin, the most frequently used irritant molecule.

Introduction

Trigeminal sensitivity has been investigated for a long time. Parker (Parker, 1912) using the term 'common chemical sense', was the first to recognize that the sense of irritation produced by chemical stimuli was distinct from olfaction and gustation. Subsequently, many investigations have described several aspects and characteristics of chemical irritation (Green *et al.*, 1990). One question which remains without a definitive response concerns the differential responses produce by repetitive stimulation. Indeed, the intensity of irritation varies according to the inter-stimulus intervals (ISIs) and the nature of chemical stimuli (Cain, 1990). Repeated stimuli with odors and tastes typically show a reduction in rated stimulus intensity if the ISI is brief. In contrast, trigeminal stimuli can produce increases in rated intensity during repeated stimulation with short ISI, a phenomenon known as sensitization. Moreover, if the ISI is long the intensity markedly decreases, a phenomenon known as desensitization. Sensitization and desensitization by a chemical irritant have been principally investigated on the cutaneous receptors and the tongue. The first psychophysical evidence of such an effect in the oral cavity came from Stevens and Lawless (Stevens and Lawless, 1987), who observed that when an irritant (capsaicin or piperine) was presented twice within a short interval, the second presentation produced a more intense sensation than the first. Others (Green and Gelhard, 1989) subsequently showed that when moderate concentrations of NaCl were presented at 1 min intervals, the sensations caused by salt increased regularly over a 15 min period. The authors noted the similarity between these sequential effects and the phenomenon of sensitization that occurs when polymodal nociceptors are subjected to intense thermal stimulation or to a chemical irritant. However, sensitization cannot be produced by irritants such as ethanol, which produce a nearly constant sensation of irritation following successive stimuli, but the irritation generated by an ethanol stimulus was greatly increased following a NaCl stimulus (Green, 1990). The cross-sensitization between NaCl and ethanol suggests that the two chemicals stimulate many of the same sensory fibers. Contrasting the effect of chemical sensitization is chemical desensitization. This phenomenon was reported in the oral cavity with capsaicin when a subsequent stimulus was delivered after the initial presentation (Green, 1989). The interval between conditioning and test stimulus had to be >2.5 min but not longer than 5 min for desensitization to begin to occur. Few studies have dealt with the question of sensitization/desensitization by chemical irritants in the nasal cavity, but it would appear that the publications over the last few years have been trying to address this (Hummel, 2000).

In the nose, olfactory receptors (CN I) positioned in the upper recesses of the nasal cavity coexist with free nerve endings of the ophthalmic and maxillary divisions of the trigeminal nerve (CN V) distributed throughout the nasal mucosa and olfactory epithelium (Lang, 1989). Sensations derived from the trigeminal nerve are somatosensory and

include pain sensations, burning, stinging, itching, tickling, cooling, warming and the perception of atmospheric humidity (Proctor and Andersen, 1982; Kelly and Dodd, 1991). Two major fiber systems, C-fibers (unmyelinated) and A_{delta}-fibers (myelinated) participate in the afferent chemosensitive innervation of the nasal respiratory epithelium (Anton and Peppel, 1991; Sekizawa and Tsubone, 1994). Both fibers are activated by the intracellular accumulation of protons which modify the membrane conductance (Steen *et al.*, 1995). However, C-fibers are preferentially involved in the mediation of burning sensations and A_{delta}-fibers preferentially in stinging sensations (Mackenzie *et al.*, 1975). Moreover, it is well known that responses mediated by C-fibers and A_{delta}-fibers differ when exposed to repeated stimuli (Price, 1972; Price *et al.*, 1977). At short intervals, burning sensations increase due to a summation (central nervous summation of the successive inputs related to C-fiber afferent stimulation), whereas no such summation has been reported for stinging sensations which decrease in relation to the desensitization of A_{delta}-fibers.

In the nasal cavity, the most frequent molecule used in the field of sensitization/desensitization is capsaicin (Prescott, 1999), the pungent ingredient of red peppers. In humans, psychophysical studies with capsaicin have shown sensitization when a second stimulus was delivered shortly after (<1 min) the first stimulus. In contrast, when the second stimulus was delivered >3–4 min later, it produced desensitization (Sicuteri *et al.*, 1989). Some pungent substances presenting the same activation as capsaicin have been identified and one of them could be mustard oil. As with capsaicin, mustard oil (allyl isothiocyanate) is widely used as a flavoring agent in a variety of foods in numerous countries. Allyl isothiocyanate can be prepared from the seeds of mustard plants, *Brassica nigra* or *Brassica juncea* and synthetic allyl isothiocyanate has been produced commercially since 1937. Allyl isothiocyanate applied on the skin leads to a clear burning sensation (Magerl *et al.*, 1990) and has been found to activate all the cutaneous receptors and predominantly excite C-fiber afferents in the upper skin layers (Handwerker *et al.*, 1991). The toxicity of allyl isothiocyanate evaluated in animals appears to be low (Jenner *et al.*, 1964) and carcinogenic data have indicated that there is no evidence of incidence (Ioannou *et al.*, 1984).

The aim of the present work was to investigate the response, acute effects and time-course of sensitization/desensitization to mustard oil (allyl isothiocyanate) volatile nasal stimulation in healthy subjects during normal breathing. From a methodological point of view, the study tested how intensity ratings and skin conductance response (SCR) amplitudes could be modified by repetitive stimuli of the intranasal trigeminal nerve when stimuli are presented at different ISIs at the same concentration. The most widely reported method of assessing sensitization/desensitization in humans has been to use psychophysical tests (Green and Lawless, 1991). The present work added SCR recording, because it was considered to be a reactivity measure in terms of arousal and affect or basic emotion. Skin conductance response (related to the autonomic nervous system) has long been used to assess the level of arousal during specific tasks or stimuli, especially sensory stimuli, including nasal stimuli (Brand *et al.*, 1999, 2000; Brand and Millot, 2000). In this field, Brauchli *et al.* (Brauchli *et al.*, 1995) showed higher autonomic arousal in response to unpleasant versus pleasant odorants and, more recently, it has been postulated (Alaoui-Ismaïli *et al.*, 1997a) that autonomic analysis can distinguish among pleasant odorants and those with a trigeminal component. Finally, the reliability of both measures validates the subjective psychophysical estimation (Alaoui-Ismaïli *et al.*, 1997b).

Materials and methods

Subjects

A sample of 60 female volunteer students participated in this experiment. Their ages ranged from 19 to 27 years (mean age 23 years 5 months). All subjects were dextrals, non-smokers and reported normal smell and taste sensitivity; none of them had a history of nasal/sinus disease or extensive exposure to chemicals with potential olfactory or trigeminal toxicity. The study was conducted in accordance with the Declaration of Helsinki/Hong Kong.

Nasal stimuli

The nasal stimulus was allyl isothiocyanate (C_4H_5NS, mol. wt 99.15) diluted in mineral oil. The concentration used was 25%, in a suprathreshold higher than the standardized detection thresholds (Devos *et al.*, 1990). The nasal stimulus in liquid form was presented in a bottle (7.5 cm high, 1 cm in diameter at the opening) filled with 4 ml of liquid. The bottle was presented three times to the subject during a limited period of 2 s (one inspiration) at a distance of 1 cm from both nostrils using a holder to avoid any olfactory or thermic interference from the experimenter's hand.

Three groups (A, B, C) of 20 subjects were constituted. For each group, a 30 s constant ISI (± 3 s) was used between the first and the second stimuli. ISIs between the second and the third stimuli were 1 min 30 s (± 3 s) for group A, 2 min 30 s (± 3 s) for group B and 3 min 30 s (± 3 s) for group C.

Procedure

The subjects were seated in a comfortable armchair in a quiet room (room temperature ranged from 20 to 22°C). Before the experiment, a control auditory stimulation (440 Hz, 60 dB, 1 s) was used for the dial readings and adjustment of the baseline in order to zero the GSR amplifier. Then, visual cues were excluded by a blindfold and auditory cues were excluded by a soundproof helmet. Additionally, the breathing cycle (mouth open) of the subjects was recorded with a Minigraph Lafayette instrument (Model 76107 equipped with pneumo bellows) and

monitored to present the nasal stimulus at the outset of inspiration and to check that the inspiration amplitude did not change during the experiment. Ocular and oral irritations were excluded by the fact that the eyes and the mouth were closed. There was no evidence of a cutaneous irritation in so far as the procedure excluded contact between the liquid and the skin and no subject reported irritation.

The session began with a rest period of 5 min duration. The whole session lasted ~20 min and was carried out between 10:00 a.m. and 12:00 noon.

After the experiment (so as not to disturb the SCR recordings), the subjects were asked to note the intensity of the last two stimuli on a scale ranging from 0 to 100 units (0, not perceived; 100, very high), in comparison with the first stimulus, graded 50, in order to have the same initial reference for all the subjects and the same rating either below or above this initial value.

SCR recordings

The SCR, expressed in microSiemens (μS), was recorded from the right hand with a MacLab system (GSR amplifier; ADInstruments) interfaced with a Macintosh computer. The GSR amplifier provided a low constant-voltage AC excitation (22 mV at 75 Hz). Skin preparation consisted of washing the hands in soapy water, followed by rinsing and thorough drying. The dry, bright-plated (no special electrolytes were needed) bipolar electrodes were attached with a Velcro attachment strap to the palmar surfaces of the middle phalanges of the first and second fingers of the right hand. When the electrodes were in position, the subject was told not to move and asked to relax to establish good baseline conductivity.

Data analysis

According to the classical recommendations (Fowles *et al.*, 1981), SCR data were as follows: phasic stimulus-elicited SCR amplitudes referring to the first response were ≥0.02 μS, with a minimal slope of 0.01 μS/s which occurred within an interval of 0.5–4 s after the onset of the stimulus. For each of the observed SCR following the stimulation, the compound response was scored from the inflection point to peak. If more than one response occurred in the interval (0.5–4 s), only the first was scored. The observations of a response occurring during a modified inspiration were excluded.

Data for the three groups were statistically evaluated with a computer program (Statview II) using analysis of variance (ANOVA) with repeated measures. *Post hoc* analyses following significant ANOVA effects were conducted using Scheffe tests. The arithmetic mean and the standard deviation (SD) were noted.

Results

An example of SCR amplitude recordings related to ISIs,

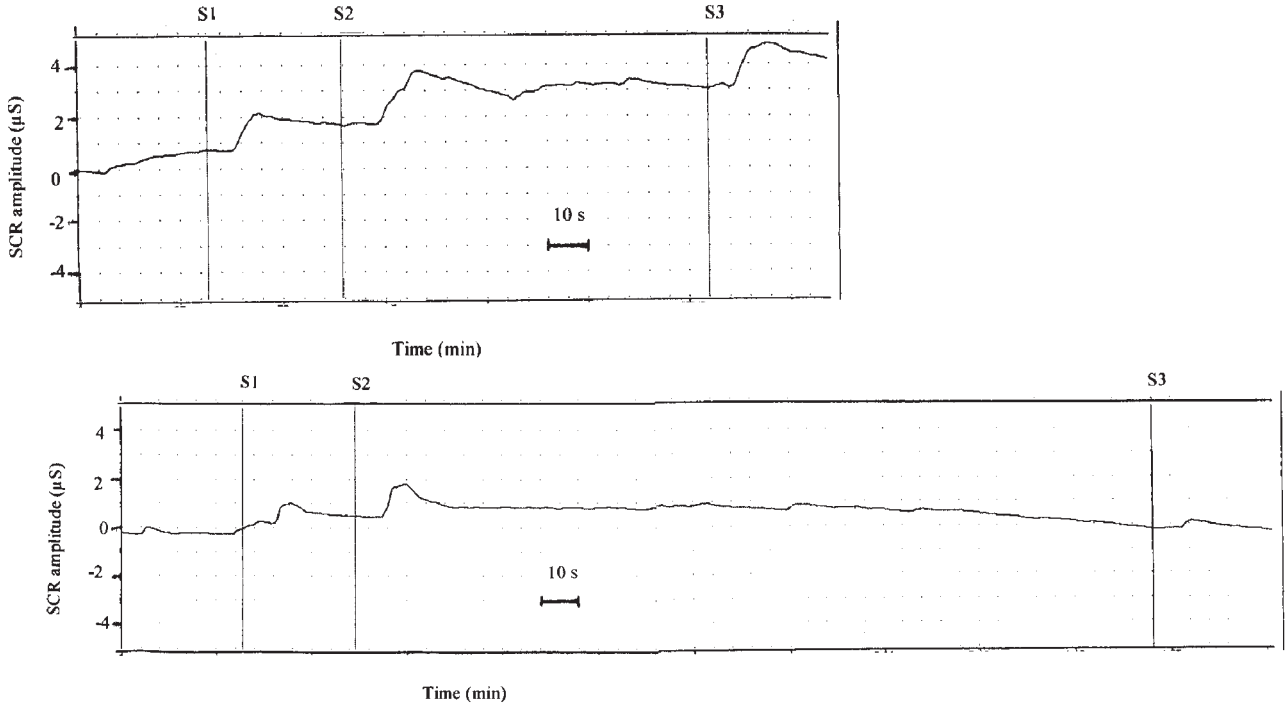

Figure 1 Sensitization and desensitization to allyl isothiocyanate. Examples of SCR amplitude recordings (in microSiemens) from two subjects in relation to ISIs (min). Above: stimulus 1 (s1), 0 min 31 s; stimulus 2 (s2), 1 min 04 s; stimulus 3 (s3), 2 min 34 s. Below: stimulus 1 (s1), 0 min 31 s; stimulus 2 (s2), 1 min 01 s; stimulus 3 (s3), 4 min 30 s.

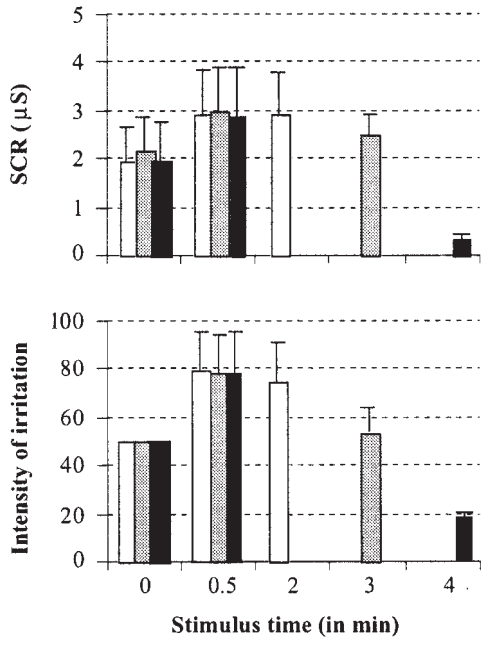

Figure 2 Mean SRC amplitude and mean intensity of irritation to allyl isothiocyanate in relation to ISI for three groups of subjects (n = 20 each). A constant ISI was used between the first and the second stimuli (30 s). ISIs between the second and the third stimuli were 1 min 30 s for group A, 2 min 30 s for group B and 3 min 30 s for group C (indicated by different shading). The intensity was scored on a scale from 0 (not perceived) to 100 (very high) in comparison to the first response, arbitrarily set to 50.

for a subject of group A and a subject of group C, is given in Figure 1 and the results of SCRs and of intensity ratings are reported in Figure 2.

For group A, the ANOVA yielded significant differences for the SCR amplitude [$F(2,18) = 22.89$, $P < 0.0001$] and for the intensity rating [$F(2,18) = 61.27$, $P < 0.0001$].

SCR amplitude

Scheffe *post hoc* tests showed that the second stimuli (mean = 2.888, SD = 1.11) produced significantly higher SCR amplitudes [$F = 16.07$, $P < 0.01$] than the first stimuli (mean = 1.946, SD = 0.94). In the same way, the third stimuli (mean = 2.856, SD = 1.04) produced significantly higher SCR amplitudes [$F = 18.21$, $P < 0.001$] than the first.

Intensity rating

Scheffe *post hoc* tests showed that the intensity rating during the second stimuli (mean = 78.4, SD = 13.7) was significantly higher than the first stimuli graded 50 [$F = 76.38$, $P < 0.0001$]. In the same way, the intensity rating during the third stimuli (mean = 73.5, SD = 11.8) was significantly higher than the first [$F = 50.71$, $P < 0.0001$].

No differences were statistically significant between the second and the third stimuli for either SCR or intensity rating measures. The psychophysical and psychophysiological data were significantly correlated during the second ($r = 0.787$) and the third ($r = 0.724$) stimuli.

For group B, the ANOVA yielded significant differences for SCR amplitude [$F(2,18) = 19.87$, $P < 0.0001$] and for the intensity rating [$F(2,18) = 48.25$, $P < 0.0001$].

SCR amplitude

Scheffe *post hoc* tests showed that the second stimuli (mean = 3.025, SD = 1.18) produced a significantly higher SCR amplitude [$F = 13.556$, $P < 0.01$] than the first stimuli (mean = 2.203, SD = 0.85) and than the third stimuli [mean = 2.226, SD = 0.82; $F = 15.11$, $P < 0.01$].

Intensity rating

Scheffe *post hoc* tests showed that the intensity rating during the second stimuli (mean = 76.3, SD = 14.8) was significantly higher than the first stimuli graded 50 [$F = 54.56$, $P < 0.0001$], as well as the third stimuli [mean = 52.3, SD = 19.2; $F = 35.23$ $P < 0.0001$].

No differences were statistically significant between the first and the third stimuli for either SCR or intensity rating measures. The psychophysical and psychophysiological data were significantly correlated during the second ($r = 0.809$) and the third ($r = 0.709$) stimuli.

For group C, few subjects produced scorable SCR data during the third stimuli: 14 of the 20 subjects presented no SCR responses and the maximum SCR amplitude was 0.58 μS (mean = 0.371, SD = 0.19). In the same way, 12 of the 20 subjects noted intensity ratings as 0 for the third stimuli (mean = 16.4, SD = 5.71). Consequently, the data were not appropriate for repeated measures analysis. For SCR amplitude, a t-test between the data of the first (mean = 1.946, SD = 0.94) and the second stimuli (mean = 2.844, SD = 1.32) showed significant differences ($t = 5.95$, $P < 0.001$). For the intensity ratings, a t-test between the data of the first stimuli graded 50 and the second stimuli (mean = 76.5, SD = 15.1) showed significant differences ($t = 8.45$, $P < 0.0001$). The psychophysical and psychophysiological data were significantly correlated during the second stimuli ($r = 0.791$).

Discussion

Firstly, the results of the present study indicate that mustard oil (allyl isothiocyanate) nasal stimuli produced an increase and a decrease in both intensity ratings and SCR amplitudes in relation to the ISIs. Specifically, in the present work allyl isothiocyanate induced a response which increased when delivered repeatedly at 30 s or 1 min 30 s intervals (sensitization), but then decreased markedly following a 3 min 30 s rest period (self-desensitization). The time-course of self-sensitization and self-desensitization for allyl isothiocyanate presented similar properties to those previously established for capsaicin (Stevens and Lawless, 1987; Green, 1989; Green and Lawless, 1991), piperine (Stevens and

Lawless, 1987) and NaCl (Green and Gelhard, 1989). A recent study (Prescott and Swain-Campbell, 2000) showed a high degree of inter-subject variability in oral capsaicin irritation. As well, inter-subject variability could be further explored in nasal trigeminal sensitization. The findings of the present work are in agreement with those obtained using other techniques, such as event-related potentials (Hummel and Kobal, 1999) and functional magnetic resonance imaging (Yousem *et al.*, 1997), which showed that habituation occurred with repeated testing of olfactory nerve-mediated odorants, while differential responses occurred with repeated exposure to odors that also stimulated the trigeminal nerve in relation to ISIs.

The findings of this study are relevant because, as with capsaicin (Green, 1989) or nicotine (Hummel *et al.*, 1992; Greiff *et al.*, 1993), allyl isothiocyanate evokes a burning sensation. This suggest that, from a neurophysiological point of view, mustard oil, as with other irritants such as capsaicin, could strongly activate C-fibers (preferentially involved in the mediation of burning sensations) in so far as most A_{delta}-afferents adapt during sustained painful stimulation (Adriaensen *et al.*, 1983). A neurophysiological explanation of the afferent nerve fiber response activation is known for capsaicin (Holzer, 1991; Wang *et al.*, 1998), but it has not yet been established for allyl isothiocyanate. Further research dealing with this question is needed to understand the mechanisms of sensitization/desensitization with allyl isothiocyanate and, more particularly, cross-sensitization and cross-desensitization. For example, in the human nasal cavity it has been demonstrated that desensitization by capsaicin decreased irritation by citric acid (Gepetti *et al.*, 1993). In this field, different irritant stimuli could be used to delineate the role of molecular receptors and different cellular mechanisms related to sensitization and desensitization with allyl isothiocyanate in relation to the ISI.

Secondly, the results of the present study showed that the psychophysical and psychophysiological data were significantly correlated in each experimental condition. This fact could be typical of trigeminal activation in the nasal cavity. Indeed, in a first experiment Brand *et al.* (Brand *et al.*, 2000) showed differences in the SCR amplitude in relation to the odorant, i.e. higher amplitude in response to unpleasant versus pleasant odorant, whereas the intensity was similar for both odorants. More recent work (Brand *et al.*, 2002) showed differences in the SCR amplitude in relation to the trigeminal component of the nasal stimulus, i.e. higher amplitude in response to nasal stimulus with high trigeminal stimulation versus nasal stimulus with low trigeminal stimulation, whereas (in the birhinal condition) the intensity was similar for both stimuli. The correlation between intensity self-rating and level of autonomic activation could be explored further in relation to the specificities of the intranasal trigeminal system, which mediates a relatively limited spectrum of sensations (compared to the large number of different odors mediated by the olfactory system) and projects differently into the cortical pathways. More particularly, the olfactory system projects directly into the limbic system and it is well known that the perception (including intensity perception) is strongly linked to the emotional treatment of olfactory information (Alaoui-Ismaïli *et al.*, 1997a,b). In the same way as autonomic analysis can distinguish between pleasant/unpleasant odorants, it can be postulated that autonomic analysis can distinguish among nasal stimuli which preferentially stimulate the olfactory or the trigeminal system.

Thirdly, from a methodological point of view, these findings are relevant because general studies in olfaction have frequently used odorants which stimulate both the olfactory nerve and the trigeminal nerve in the nasal cavity (Cain, 1976; Doty *et al.*, 1978; Cain and Murphy, 1980; Brand and Jacquot, 2001). Usually, the recording procedures are based on averaging the reactions to repeated stimuli. Moreover, even most volatile organic compounds can elicit odor sensation at low concentrations and pungency at higher concentrations that represent the recruitment of chemesthesis via the stimulation of the trigeminal nerve. Thus, the ISI used in experimental sessions could be adjusted in relation to the trigeminal component of the intrasal stimulus.

References

Adriaensen, H., Gybels, J., Handwerker, H.O. and **Van Hees, J.** (1983) *Response properties of thin myelinated (A-) fibers in human skin nerves*. J. Neurophysiol., 49, 111–122.

Alaoui-Ismaïli, O., Vernet-Maury, E., Dittmar, A., Delhomme, G. and **Chanel, J.** (1997a) *Odor hedonics: connection with emotional response estimated by autonomic parameters*. Chem. Senses, 22, 237–248.

Alaoui-Ismaïli, O., Robin, O., Rada, H., Dittmar, A. and **Vernet-Maury, E.** (1997b) *Basic emotions evoked by odorants: comparison between autonomic responses and self-evaluation*. Physiol. Behav., 62, 713–720.

Anton, F. and **Peppel, P.** (1991) *Central projections of trigeminal primary afferents innervating the nasal mucosa: a horseradish peroxidase study in the rat*. Neuroscience, 41, 617–628.

Brand, G. and **Millot, J.L.** (2000) *Comparison between olfactory and trigeminal stimulations in bilateral electrodermal activity*. J. Psychophysiol., 14(Suppl.), 36.

Brand, G. and **Jacquot, L.** (2001) *Quality of odor and olfactory lateralization processes in humans*. Neurosci. Lett., 316, 91–94.

Brand, G., Millot, J.L. and **Henquell, D.** (1999) *Olfaction and hemispheric asymmetry: unilateral stimulation and bilateral electrodermal recordings*. Neuropsychobiology, 39, 160–164.

Brand, G., Millot, J.L. and **Biju, C.** (2000) *Comparison between monorhinal and birhinal stimulations in bilateral electrodermal recordings*. C. R. Acad. Sci. III, 323, 959–965.

Brand, G., Millot, J.L., Saffaux, M. and **Morand-Villeneuve, N.** (2002) *Lateralization in human nasal chemoreception: differences in bilateral electrodermal responses related to olfactory and trigeminal stimuli*. Behav. Brain Res., 133, 205–210.

Brauchli, P., Rüegg, P.B., Etzweiler, F. and **Zeier, H.** (1995) *Electrocortical and autonomic alteration by administration of a pleasant and unpleasant odor*. Chem. Senses, 20, 505–515.

Cain, W.S. (1976) *Olfaction and the common chemical sense: some psychophysical contrasts*. Sensory Proc., 1, 57–67.

Cain, W.S. (1990) *Perceptual characteristics of nasal irritation*. In Green, B.G., Mason, J.R. and Kare, M.R. (eds), Chemical Senses. II. Irritation. Marcel Dekker, New York, pp. 43–58.

Cain, W.S. and **Murphy, C.** (1980) *Interaction between chemoreceptive modalities of odour and irritation*. Nature, 284, 255–257.

Devos, M., Patte, F., Rouault, J. and **Lafford, P.** (1990) Standardized Human Olfactory Thresholds. University Press, Oxford.

Doty, R.L., Brugger, W.E., Jurs, P.C., Orndoff, M.A., Snyder, P.J. and **Lowry, L.D.** (1978) *Intranasal trigeminal stimulation from odorous volatiles: psychometric responses from anosmic and normal humans*. Physiol. Behav., 20, 175–185.

Fowles, D.C., Christie, M.J., Edelberg, R., Grings, W.W., Lykken, D.T. and **Venables, P.H.** (1981) *Committee report: publication recommendations for electrodermal measurement*. Psychophysiology, 18, 232–239.

Geppetti, P., Tramontana, M., Delbianco, E. and **Fusco, B.M.** (1993) *Capsaicin desensitization to the human nasal mucosa selectively reduces pain evoked by citric acid*. Br. J. Clin. Pharmacol., 35, 178–183.

Green, B.G. (1989) *Capsaicin sensitization and desensitization on the tongue produced by brief exposures to a low concentration*. Neurosci. Lett., 107, 173–178.

Green, B.G. (1990) *Effects of thermal, mechanical and chemical stimulation on the perception of oral irritation*. In Green, B.G., Mason, J.R. and Kare, M.R. (eds), Chemical Senses. II. Irritation. Marcel Dekker, New York, pp. 171–195.

Green, B.G. and **Gelhard, B.** (1989) *Salt as an oral irritant*. Chem. Senses, 14, 259–271.

Green, B.G. and **Lawless, H.T.** (1991) *The psychophysics of somatosensory chemoreception in the nose and mouth*. In Getchell, T.V., Doty, R.L., Bartoshuk, L.M. and Snow, J.B. (eds), Smell and Taste in Health and Disease. Raven, New York, pp. 235–253.

Green, B.G., Mason, J.R. and **Kare, M.R.** (1990) Chemical Senses. II. Irritation. Marcel Dekker, New York.

Greiff, L., Wollmer, P., Andersson, M., Pipkorn, U. and **Persson, C.G.A.** (1993) *Effects of nicotine on the human nasal mucosa*. Thorax, 48, 651–655.

Handwerker, H.O., Forster, C. and **Kirchhoff, C.** (1991) *Discharge patterns of human C-fibers induced by itching and burning stimuli*. J. Neurophysiol., 66, 307–315.

Holzer, P. (1991) *Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons*. Pharmacol. Rev., 43, 143–201.

Hummel, T. (2000) *Assessment of intranasal trigeminal function*. Int. J. Psychophysiol., 36, 147–155.

Hummel, T. and **Kobal, G.** (1999) *Chemosensory event-related potentials to trigeminal stimuli change in relation to the interval between repetitive stimulation of the nasal mucosa*. Eur. Arch. Otorhinolaryngol., 256, 16–21.

Hummel, T., Livermore, A., Hummel, C. and **Kobal, G.** (1992) *Chemosensory event-related potentials in man: relation to olfactory and painful sensations elicited by nicotine*. EEG Clin. Neurophysiol., 84, 192–195.

Ioannou, Y.M., Burka, L.T. and **Matthews, H.B.** (1984) *Allyl isothiocyanate: comparative disposition in rats and mice*. Toxicol. Appl. Pharmacol., 75, 173–181.

Jenner, P.M., Hagan, E.C., Taylor, J.M., Cook, E.L. and **Fitzhugh, O.G.** (1964) *Food flavourings and compounds of related structure. I. Acute oral toxicity*. Food Cosmet. Toxicol., 2, 327–343.

Kelly, J.P. and **Dodd, J.** (1991) *Trigeminal system*. In Kandel, E.R., Schwartz, J.H. and Jessel, T.M. (eds), Principles of Neural Science. Elsevier, New York, pp. 701–710.

Lang, J. (1989) Clinical Anatomy of the Nose, Nasal Cavity and Paranasal Sinuses. Thieme, Stuttgart.

Mackenzie, R.A., Burke, D., Skuse, N.F. and **Lethlean, A.K.** (1975) *Fiber function and perception during cutaneous nerve block*. J. Neurol. Neurosurg. Psychiatry, 38, 865–873.

Magerl, W., Grämer, G. and **Handwerker, H.O.** (1990) *Sensations and local inflammatory responses induced by application of carbachol, dopamine, 5-HT, histamine and mustard oil to the skin in humans*. Pflügers Arch., 415, 107.

Parker, G.H. (1912) *The relation of smell, taste and the common chemical sense in vertebrates*. J. Acad. Natl Sci., 15, 221–234.

Prescott, J. (1999) *The generalizability of capsaicin sensitization and desensitization*. Physiol. Behav., 66, 741–749.

Prescott, J. and **Swain-Campbell, N.** (2000) *Responses to repeated oral irritation by capsaicin, cinnamaldehyde and ethanol in PROP tasters and non-tasters*. Chem. Senses, 25, 239–246.

Price, D.D. (1972) *Characteristics of second pain and flexion reflexes indicative of prolonged central summation*. Exp. Neurol., 37, 371–387.

Price, D.D., Hu, J.W., Dubner, R. and **Gracely, R.** (1977) *Peripheral suppression of first pain and central summation of second pain evoked by noxious heat pulses*. Pain, 3, 57–68.

Proctor, D.F. and **Andersen, I.** (1982) The Nose: Upper Airway Physiology and the Atmospheric Environment. Elsevier, Amsterdam.

Sekizawa, S.I. and **Tsubone, H.** (1994) *Nasal receptors responding to noxious chemical irritants*. Respir. Physiol., 96, 37–48.

Sicuteri, F., Fusco, B.M. and **Marabini, S.** (1989) *Beneficial effect of capsaicin application to the nasal mucosa in cluster headache*. J. Clin. Pain, 5, 49–53.

Steen, K.H., Wegner, H., Kreysel, H. and **Reeh, P.** (1995) *The pH-release of rat cutaneous nociceptors correlates with extracellular [Na+] and is increased under amiloride,* in vitro. Soc. Neurosci. Abs., 21, 648.

Stevens, D.A. and **Lawless, H.T.** (1987) *Enhancement of responses to sequential presentation of oral chemical irritants*. Physiol. Behav., 39, 63–65.

Wang, H.W., Wang, S.H. and **Wang, J.Y.** (1998) *Effects of capsaicin on human nasal mucosa*. Otorhinolaryngology, 60, 143–146.

Yousem, D.M., Williams, S.C.R., Howard, R.O., Andrew, C., Simmons, A., Allin, M., Geckle, R.J., Suskind, D., Bullmore, E.T., Brammer, M.J. and **Doty, R.L.** (1997) *Functional MR imaging during odor stimulation: preliminary data*. Radiology, 204, 833–838.

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