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# Assessment of the Human Intranasal Trigeminal System: Normative Data, Sensitivity, and Receptor-Specific Responses

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## List of Abbreviations

| | |
|---|---|
| **ANOVA** | Analysis of Variance |
| **AR** | allergic rhinitis |
| **CNO** | chronic nasal obstruction |
| **CNV** | Cranial nerve |
| **$CO_2$** | Carbon dioxide |
| **CRSsNP/wNP** | chronic rhinosinusitis without/with polyps |
| **CT** | chronic tinnitus |
| **Cz** | Central midline |
| **C3** | Left central |
| **C4** | Right central |
| **°C** | Degrees Celsius |
| **e.g.** | For example |
| **ENS** | empty nose syndrome |
| **EOG** | electro-olfactogram |
| **ERP** | electroencephalography |
| **FLIPR** | fluorometric imaging plate reader |
| **Fp2** | Frontal pole (right) |
| **Fz** | Frontal midline |
| **GLMM** | Generalized linear mixed model |
| **$H_2S$** | Hydrogen sulfide |
| **Hz** | Hertz |
| **i.e.** | that is |
| **ISI** | Interstimulus Interval |
| **L** | Left |
| **L/min** | liters per minute |
| **mA** | milliampere |
| **ml** | Milliliter |
| **mm** | millimeter |
| **mRNA** | messenger Ribonucleic Acid |
| **ms** | millisecond |
| **m/s** | meters per second |
| **NMP** | Negative Mucosal Potential |
| **OD** | Sniffin' Sticks odor discrimination |
| **OI** | Sniffin' Sticks odor identification |

| | |
|---|---|
| **OT** | Sniffin’ Sticks odor threshold |
| **PEA** | Phenylethyl alcohol |
| **pH** | Potential of Hydrogen |
| **Pz** | Parietal midline |
| **R** | Right |
| **TDI** | Sniffin’ Sticks total score |
| **tERP** | Trigeminal event-related potentials |
| **TLT** | Trigeminal lateralization test/task |
| **TRP** | Transient receptor potential |
| **VAS** | Visual Analogue Scale |
| **v/v** | Volume per volume |

## Definition of Terms

| | |
|---|---|
| Acquired anosmia | A quantitative loss or severe reduction of the sense of smell that develops after birth, to the extent that it is no longer functional in daily life. This condition differs from congenital anosmia, which is present from birth. |
| Bimodal odor | An odor that simultaneously activates both the olfactory and trigeminal systems. Also referred to as an olfactory-trigeminal mixed odor. |
| Chemosensory complaint | A reported disturbance or impairment related to the chemical senses, including smell (olfaction), taste (gustation), or chemical irritation (trigeminal sensation). |
| Olfactory dysfunction | A quantitatively impaired or qualitatively altered sense of smell. It includes reduced odor sensitivity (hyposmia), complete loss of smell (anosmia), or distortions and misperceptions (parosmia or phantosmia) |
| Psychophysical test | A method for assessing the relationship between physical stimuli and the sensations they evoke. In sensory research, these tests evaluate an individual's ability to detect, discriminate, or rate the intensity of stimuli (e.g., odors or sounds) through subjective responses to controlled presentations. |
| Somatic sensation | Sensory experiences originating from the skin, muscles, joints, and internal body structures, excluding the special senses. It includes touch, pressure, temperature, pain, vibration, and proprioception (the sense of body position and movement). |
| Trigeminal dysfunction | an impairment in the intranasal trigeminal system's ability to perform its core functions, manifesting as (1) reduced or excessive sensitivity to irritants, leading to impaired protective reflexes or abnormal responses to noxious stimuli; (2) disrupted or misinterpreted nasal breathing sensations; and (3) disruption in odor perception, including altered somatosensation and impaired olfactory-trigeminal interaction. |
| Unimodal odor | An odor that predominantly activates either the olfactory or the trigeminal system. Also referred to as a selectively olfactory odor or selectively trigeminal odor. |

## List of Figures



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## List of Published Papers

**Mai Y**, Flechsig J, Warr J, Hummel T. 2025. Responses to the activation of different intranasal trigeminal receptors: Evidence from behavioral, peripheral and central levels. Behavioural brain research 480:115371. doi:10.1016/j.bbr.2024.115371

**Mai Y**, Hernandez AK, Konstantinidis I, Haehner A, Hummel T. 2024. Normative data for the lateralization task in the assessment of intranasal trigeminal function. Rhinology 63 (1): 92-102. doi: 10.4193/Rhin24.063

**Mai Y**, Brieke B, Hummel T. 2025. Odor lateralization test is insensitive to small degrees of intranasal trigeminal activation. Eur Arch Otorhinolaryngol 282(1):241-249. doi: 10.1007/s00405-024-09016-x

# 1. Introduction

The human nasal cavities contain two closely connected sensory pathways: the olfactory and trigeminal systems, which together contribute to the global odor perception (Frasnelli & Manescu, 2017; Hummel & Frasnelli, 2019; Rombaux et al., 2023). In addition to triggering the olfactory system, most odors activate the intranasal trigeminal system, producing somatic sensations such as cooling, tingling, burning, or tickling (Doty et al., 1978; Laska et al., 1997; Viana, 2011). These somatic sensations provide information about our airborne environment, including temperature, pressure, perception of nasal airflow, and nociception (Rombaux et al., 2023). Consequently, various protective reflexes, such as sneezing, may be induced when noxious substances, like smoke or irritative gases, stimulate the trigeminal system (Rombaux et al., 2023). The intranasal trigeminal system also interacts with the olfactory system which, ultimately, provides a complete chemosensory perception of the odorous stimuli. An intact intranasal trigeminal function is thus mandatory for both the chemosensory and the somatosensory perception of the nasal mucosa, as well as the health of the upper airway (Hummel & Frasnelli, 2019; Rombaux et al., 2023). Given its vital role in sensory integration and environmental interaction, comprehensive assessment of trigeminal function is crucial for understanding both normal and pathological sensory processing. The present thesis critically evaluates the challenges associated with existing widely-used intranasal trigeminal function assessments and seeks to enhance their effectiveness.

## 1.1. Structural Basis of the Intranasal Trigeminal System

The trigeminal nerve (fifth cranial nerve, CNV) is the largest cranial nerve. It plays a crucial role in both sensory and motor functions of the face. From the trigeminal ganglion, it divides into three branches: Ophthalmic nerve (CN V1), maxillary nerve (CN V2), and mandibular nerve (CN V3). While CN V1 and CN V2 are purely sensory nerves, conveying touch, pain, temperature, and proprioception from the face, scalp, eyes, nasal cavities, and the upper part of the mouth, CN V3 contains both sensory and motor fibers. It conveys sensory information from the lower part of the mouth, and the motor branches of the mandibular nerve control the movement of the masticatory muscles and some

parts of the floor of the mouth muscles. (Singh & Singh, 2019; Suer, 2021). The nasal cavity receives sensory innervation from two of these branches: CN V1 and CN V2. The ophthalmic nerve contains the anterior ethmoidal nerve, which innervates the anterior nasal mucosa and the external skin of the nose near the tip via its external nasal branch. The maxillary nerve contains the nasopalatine nerve and posterior superior medial nasal nerve, which supply the posterior part of the nasal cavity. Additionally, some trigeminal ganglion cells with sensory endings in the nasal epithelium extend branches directly into the olfactory bulb (Finger et al., 2002). The afferent innervation of the nasal respiratory mucosa consists of two major fiber systems, unmyelinated C-fiber and myelinated Aδ-fiber, which differ in conduction speed based on axon diameter and myelination. The comparisons of the two fibers are described in Table 1 (Anton & Peppel, 1991; Hummel, 2000; Hummel, Kraetsch, et al., 1998a; Mackenzie et al., 1975).

**Table 1. Characteristics of C-fiber and Aδ-fiber**

| Fiber type | C-fiber | Aδ-fiber |
|---|---|---|
| **Diameter** | Small | Small |
| **Myelination** | Unmyelinated | Thinly myelinated |
| **Conduction speed** | Slow | Fast |
| **Primary sensory role** | Cold, warm, burning, painful perception | Stinging, sharp nociception |
| **Stimulus intensity required for activation** | Low (higher sensitivity) | High (lower sensitivity) |
| **Onset latency** | Delayed (several seconds) | Immediate |
| **Peak perception latency** | Slow | Fast |
| **Sensation decay** | Gradual | Rapid |
| **Consequences of repetitive stimulation** | Summation (increases with short intervals <3s) | Adaptation/habituation (decreases with short intervals <20s) |

## 1.2. Intranasal Trigeminal receptors

Trigeminal free nerve endings express a diverse range of chemosensitive,

mechanosensitive, and thermosensitive receptors, allowing the detection of various environmental stimuli (Hummel & Frasnelli, 2019; Viana, 2011) (See Figure 1). Among these receptors, transient receptor potential (TRP) channels play a critical role in trigeminal chemosensation and thermosensation, including TRPV1, TRPA1, TRPV3, and TRPM8 (Frasnelli, Albrecht, et al., 2011; Hummel & Frasnelli, 2019; Viana, 2011). The temperatures and chemical stimuli activating each of them is described below:

- TRPV1 responds optimally to noxious high temperatures (>43°C) (Dhaka et al., 2006; Kashio & Tominaga, 2022) and compounds like capsaicin (Caterina et al., 1997; Yang et al., 2015; Yang & Zheng, 2017), as well as to low pH conditions (Dhaka et al., 2009; Kim et al., 2012), evoking burning and painful sensations (Philip et al., 1994);
- TRPV3 reacts to innocuous warm temperatures (>33°C) (Dhaka et al., 2006; Kashio & Tominaga, 2022) and chemicals like thymol (Vogt-Eisele et al., 2007; H. Xu et al., 2006) and carvacrol (Niu et al., 2022; Vogt-Eisele et al., 2007), inducing warm sensations (Hummel & Frasnelli, 2019);
- TRPM8 responds to cool temperatures (<27°C) (Dhaka et al., 2006; Kashio & Tominaga, 2022) and chemicals like menthol (Bautista et al., 2007; McKemy et al., 2002; Yin et al., 2019) and isopulegol (G. Wang, 2021; L. Xu et al., 2020), inducing cooling and fresh sensations (Frasnelli, Albrecht, et al., 2011; Laska et al., 1997);
- TRPA1 is sensitive to noxious heat and noxious cold (<15°C) (though this remains a subject of debate) (Dhaka et al., 2006; Kashio & Tominaga, 2022) and chemicals such as allyl isothiocyanate (Cordero-Morales et al., 2011; Earley, 2012) and cinnamaldehyde (Bandell et al., 2004; Earley, 2012), evoking stinging and painful sensations (Frasnelli, Albrecht, et al., 2011; Hummel & Frasnelli, 2019). TRPA1 often co-activates with other TRP channels (Mai et al., 2025).

In addition to TRP channels, the trigeminal nerve endings also express other receptors, such as nicotinic acetylcholine receptors (Thuerauf et al., 1999), which respond to nicotine, and acid-sensing ion channels (Waldmann et al., 1997), activated by stimuli such as acetic acid. Moreover, solitary chemosensory cells (Finger et al., 2003) have been identified in the nasal epithelium, which detect bitter tastants and chemical irritants

(Lee & Cohen, 2014).

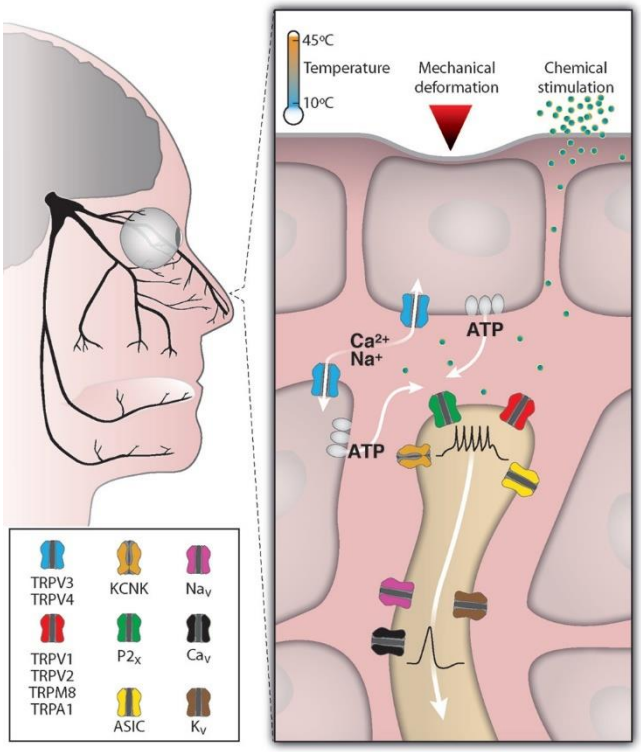



**Figure 1. Trigeminal nerve and molecular determinants of chemosensation**

Note. This Figure is from Viana F (2011). Permission has been obtained from the American Chemical Society publisher.

## 1.3. The importance of intranasal trigeminal system

### 1.3.1. Somatosensory perception

Activation of the trigeminal system mediates a variety of somatosensory percepts, described as furry, scratching, tickling, painful, sharp, warm, burning, prickling, “sneeze”, cool, pungent (Laska et al., 1997). These sensations provide essential information about the environment (Rombaux et al., 2023): ***(1) Temperature sensation***: Thermoreceptors in the nasal mucosa, such as TRPM8 (for cool sensations) allow detection of subtle temperature changes (Dhaka et al., 2006; Kashio & Tominaga, 2022); ***(2) Airflow perception***: During breathing, airflow passes through the nasal cavity, exerting pressure and causing deformation of the nasal walls, which can activate mechanoreceptors. Simultaneously, the airflow brings environmental temperature into the nasal cavity, leading to heat exchange with the body's internal temperature. This process of

conductive and evaporative heat loss can activate TRPM8 channels. (Sozansky & Houser, 2014). Activation of these receptors contributes to the perception of airflow, thereby informing the sensation of nasal patency and aiding in the detection of conditions like nasal congestion. ***(3) Nociception***: Activation of nociceptors, such as TRPV1, by noxious chemical stimuli or extreme temperatures triggers nociception, which serves as a warning signal to prevent further tissue damage (Hummel, Kraetsch, et al., 1998b; Thürauf et al., 1993).

### 1.3.2. Protective reflexes

When noxious stimuli activate the intranasal trigeminal system, the body initiates a series of protective reflexes, including sneezing to expel irritants, increased secretions (saliva, tears, and nasal mucus) to trap and clear harmful substances, reduced breathing to limit further exposure, initiation of sweating, and narrowing of the nasal passages through swelling of the turbinates (Rombaux et al., 2023). Beyond these direct effects, secondary reflexes that influence other body systems also occur. For instance, trigeminal system contributes to the nasal cycle, a physiological and periodic alternation between congestion and decongestion, by regulating the autonomic system; it also triggers reflexes that affect the eyes (leading to lacrimation and redness) and cardiovascular responses such as bradycardia and hypotension (Rombaux et al., 2023). Activation of the trigeminal nerve can also induce the release of various neuropeptides, which trigger neurogenic inflammation, partially explaining disease processes in the upper and lower airways (Lacroix & Landis, 2008).

### 1.3.3. Global chemosensory perception of odor stimuli

***(1) Integration with Olfactory Signals****:* Most natural odors are bimodal, simultaneously stimulating both the olfactory and trigeminal systems, especially at high concentrations. As an exception, gaseous $CO_2$ is considered a unimodal trigeminal stimulus; being part of inhaled and exhaled air, it has little or no smell, similar to nitrogen (Cain WS, 1976; R Fröhlich, 1851). Compounds like phenylethyl alcohol (PEA), hydrogen sulfide ($H_2S$), and vanillin have been used in studies as “selective” odorants for olfactory stimulation but might still activate the trigeminal system at high concentrations. While the olfactory

system processes the quality and identity of odors, the trigeminal component adds information about irritancy, cooling, or burning sensations, enriching the overall sensory experience (Rombaux et al., 2023). ***(2) Modulation of Odor Intensity and Quality***: The trigeminal and olfactory pathways closely interact in ways that enhance or suppress each other at multiple levels (Brand, 2006; Filiou et al., 2015; Livermore et al., 1992). On one hand, the trigeminal system can influence perceived odor intensity by signaling the presence of chemical irritants. Studies suggest that trigeminal stimulation often masks olfactory perception (Genovese et al., 2023). When subjects rated the pungency and odor of the stimulant butanol, the contribution of the odor component to the overall sensation decreased with concentration, while irritation increased (Cain WS, 1976). At the central level, both olfactory and trigeminal information converge: a mixture of $CO_2$ and PEA led to higher activations than the sum of activations of $CO_2$ and PEA presented independently (Boyle et al., 2007). On the other hand, interference from the trigeminal system often relates to decreased olfactory function. It has been observed that patients with olfactory dysfunction have lower trigeminal sensitivity compared to controls (Hummel, Futschik, et al., 2003). In acquired anosmia, there is a decreased trigeminal response at the central level but an increased trigeminal activation at the mucosal level, reflecting a mixed pattern of sensory adaptation and compensation (Frasnelli et al., 2007a).

## 1.4. Assessment of Human Intranasal trigeminal function

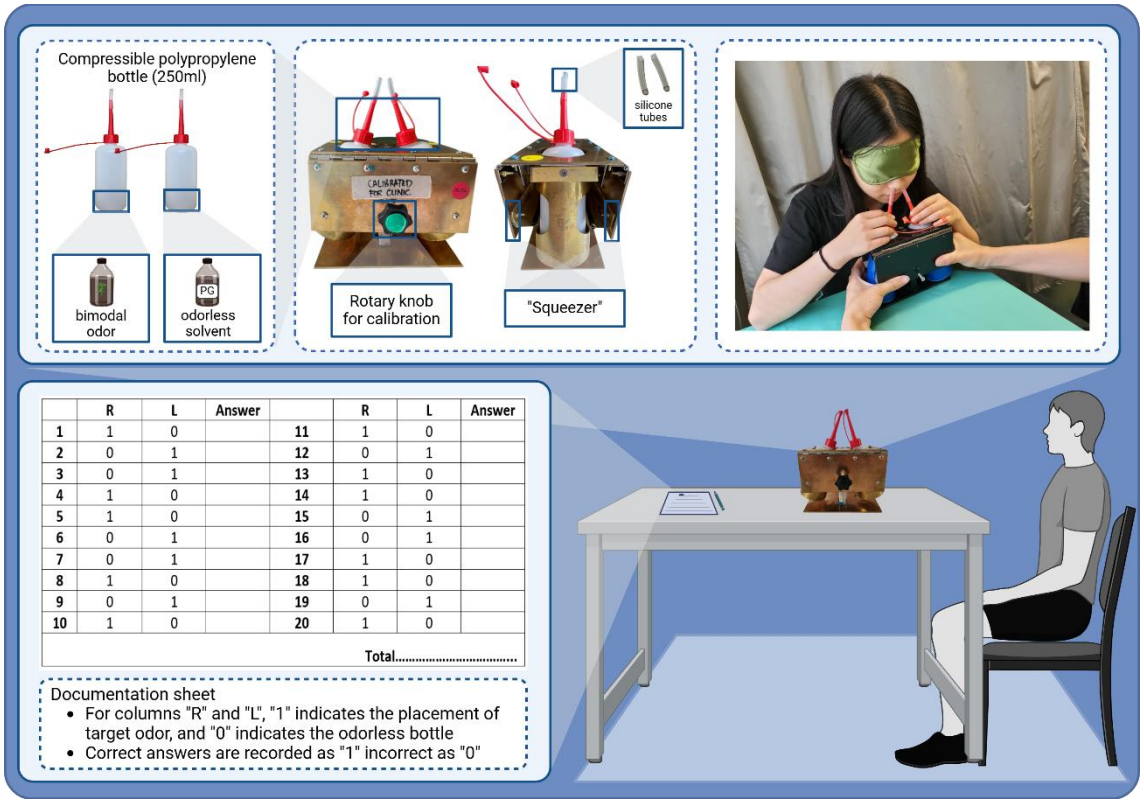
Current methods for assessing trigeminal nerve function primarily involve psychophysical tests (i.e. lateralization tests, threshold tests, and intensity ratings of suprathreshold stimuli), and electrophysiological measures (i.e., event-related potentials, and negative mucosal potentials). These techniques are relatively well-established and commonly used.

### 1.4.1. Trigeminal lateralization task

The trigeminal lateralization task (TLT) has been developed over 30 years based on Kobal et al.(1989). Since then, it has been widely used. It involves delivering a bimodal odor to one nostril while an odorless stimulus is presented to the other. Participants

must identify which nostril received the odor. This approach is based on the fact that distinguishing the side of odor presentation requires trigeminal activation, as humans generally cannot lateralize odors based on olfactory input alone (Croy et al., 2014; Kleemann et al., 2009). For example, Kobal et al.(1989) found that participants localized “pure” odorants like hydrogen sulfide or vanillin at chance level, whereas the accuracy of localizing trigeminal stimuli such as $CO_2$ or menthol was over 96%.

As described in Figure 2 below, the test is conducted using a mechanically operated “squeezer” device (Frasnelli, Hummel, et al., 2011) with two 250 ml compressible polypropylene bottles: one containing a bimodal odor (e.g., eucalyptol) (Garefis et al., 2024; Hummel & Frasnelli, 2019) and the other containing an odorless solvent (e.g., propylene glycol) or remaining empty. The volume of both the odor and the odorless solvent in the bottles should be equal, typically ranging from 10 to 30 ml across studies. Each bottle is equipped with a spout and soft silicone tubing. During the test, participants hold the tubing in place beyond the nasal valve while the examiner delivers the stimuli by squeezing the device, an odor puff thus reaches each nostril separately but simultaneously. The volume delivered per nostril per trial is typically set at approximately 15 ml. After each stimulus, participants indicate the stimulated side. Depending on the study, 10 (Hernandez et al., 2023), 20 (Oleszkiewicz et al., 2018), or 40 (Hummel, Futschik, et al., 2003) trials (with an equal number of left- and right-sided presentations in randomized order) are administered to blindfolded participants at an interstimulus interval of 20-40s. The final score is the total number of correct responses.




| | R | L | Answer | | R | L | Answer |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 0 | | 11 | 1 | 0 | |
| 2 | 0 | 1 | | 12 | 0 | 1 | |
| 3 | 0 | 1 | | 13 | 1 | 0 | |
| 4 | 1 | 0 | | 14 | 1 | 0 | |
| 5 | 1 | 0 | | 15 | 0 | 1 | |
| 6 | 0 | 1 | | 16 | 0 | 1 | |
| 7 | 0 | 1 | | 17 | 1 | 0 | |
| 8 | 1 | 0 | | 18 | 1 | 0 | |
| 9 | 0 | 1 | | 19 | 0 | 1 | |
| 10 | 1 | 0 | | 20 | 1 | 0 | |
| | | | | | | Total..... | |

**Figure 2. Trigeminal lateralization task (TLT)**

Note. subfigure in the top-right panel has been authorized from https://creativecommons.org/licenses/by/4.0/

### 1.4.2. Threshold measurements

#### 1.4.2.1. $CO_2$ threshold measurement

Gaseous $CO_2$ is used as the model stimulus for the test because it specifically activates trigeminal afferents while having little to no smell. Due to its gaseous nature, the test is typically conducted using a computer-controlled $CO_2$ delivery device or olfactometer. Two types of principles are employed to determine the $CO_2$ threshold:

- **Fixed $CO_2$ concentration with varying stimulus duration:** Hummel and colleagues (2016) developed a method based on the idea that the trigeminal system detects the overall mass of a stimulus rather than its concentration alone. In other words, at a fixed concentration, increasing the stimulus duration has a similar effect to increasing its concentration. This method uses a portable $CO_2$ delivery device consisting of a small $CO_2$ cylinder, a pressure reducer, and a pressure regulator. A computer-controlled valve adjusts the stimulus duration, and $CO_2$ is delivered

through a standard bilateral nasal cannula at approximately 1L/min. The stimulus starts with a duration of 100-500ms and increases in 50-200ms increments. Stimuli are presented every 10s, with a warning signal appearing 3s before each stimulus. Participants press a button as soon as they experience a painful, burning sensation in the nose. Once the button is pressed, the duration is reduced until the stimulus is no longer perceived, then increased again. This alternating staircase procedure continues until the $CO_2$ threshold is determined by averaging the last four turning points. A lower stimulus duration indicates a lower $CO_2$ threshold, reflecting greater trigeminal sensitivity.

- **Fixed stimulus duration with varying $CO_2$ concentration:** $CO_2$ is delivered through an olfactometer at concentrations ranging from 30%-70%v/v in 5%v/v increments, with a typical stimulus duration of 200ms. Starting with the weakest concentration, three stimuli, including two blanks containing pure air and one containing $CO_2$, are presented in random order. Participants identify which stimulus contains $CO_2$, and their responses determine the subsequent stimulus concentration. Two consecutive correct identifications result in a weaker stimulus being presented, while an incorrect response leads to a stronger stimulus. Testing continues until seven staircase reversals are reached, and the $CO_2$ detection threshold is estimated as the average of the last four reversals (Filiz & Frasnelli, 2023; Melzner et al., 2011; Rombaux et al., 2023).

#### 1.4.2.2. Airflow perception threshold

Airflow perception, is mediated by the trigeminal system, making nasal air-puff thresholds an indicator of trigeminal sensitivity.

- **Fix stimulus duration with varying velocity**: Based on Clark et al. (1994) and Wrobel et al. (2006), custom-built air-puff delivery systems were developed, incorporating a compressed air source, pressure regulation, and pulse control to generate air jets. Testing involves presenting air jets at fixed duration (e.g., 1s per stimulus) but increasing velocities (e.g., starting at 16m/s and increasing up to 130m/s). Participants indicate when they perceive a tactile sensation, and the detection threshold is defined as the minimum velocity at which two out of three

stimuli are detected.

- **Fixed flow rate with varying stimulus duration**: Based on Yan et al. (2023), air puffs are delivered via a dedicated device consisting of a portable air compressor, pressure regulator, airflow sensor, and a nasal cannula. The computer-controlled system ensures precise stimulus delivery, the cannula is positioned approximately 2mm above the mucosal surface under endoscopic control (0° rigid nasal endoscope). Testing follows a single-staircase method: stimuli start at a subthreshold duration (e.g., 5ms) at a fixed flow rate of 2L/min, increasing in 10ms increments with a 10s interstimulus interval until the participant detects the air puff twice consecutively, triggering a reversal with decreasing durations. The process follows a 1-up, 2-down stopping rule until seven reversals are obtained, with the last four averaged to determine the threshold. To prevent interference, blindfolded participants breathe through their mouths and wear noise-canceling headphones with white noise to mask system sounds.

#### 1.4.2.3. Electrical Threshold

Electrical stimulation activates nociceptors on trigeminal free nerve endings in the nasal mucosa, making the detection threshold an indicator of intranasal trigeminal sensitivity. While testing instruments vary slightly, they generally involve a spherical electrode placed at target locations (e.g., the middle turbinate) under endoscopic guidance (Lipp et al., 2024; Poletti et al., 2017). To prevent movement, the electrode is fixed to a spectacle frame worn by the participant. Testing involves delivering electrical stimuli of fixed duration (e.g., 50ms) while gradually increasing intensity (e.g., starting at 0.05mA with 0.05mA increments) until the participant detects the stimulus. The intensity is then decreased (e.g., by 0.05mA steps) until the stimulus is no longer detected, followed by an increase until detection resumes. The turning point corresponds to the electrical detection threshold.

#### 1.4.2.4. Thermal Threshold

A trigeminal stimulator based on controlled local thermal stimulation was used (Weise et al., 2024). It consists of a heating element, a 19mm bendable rod, and a solid holder for electrical connection. The stimulator head included a polyimide substrate with gold-

coated copper tracks and a 4-ohm heating resistor. A thermistor measured temperature using a 4-point method, with control achieved by modulating pulse duration while maintaining constant current. Under endoscopic guidance, the stimulator was placed at the target site (e.g., anterior septum) and secured the placement using an external holder. Threshold testing followed a staircase model: stimulation increased until perceived, then decreased until undetected, repeated until seven inflection points were identified. The final threshold was averaged from the last four turning points.

### 1.4.3. Intensity ratings of trigeminal suprathreshold stimuli

- The intensity rating of ammonium vapor, a known stimulant of trigeminal receptors such as TRPV1 and TRPA1, has been proposed as a simple screening tool for assessing intranasal trigeminal function (Sekine et al., 2022). The stimulus is delivered using a lipstick-like pen (AmmoLa®, Devesa Dr. Reingraber GmbH, Muggensturm, Germany) containing ampoules of ammonium with traces of lavender. Participants rate the perceived irritation on a visual analogue scale (VAS) from 0 (no irritation) to 100 (extreme irritation) (Juratli et al., 2023). For screening purposes, a rating below 15% suggests potential trigeminal dysfunction, which may also be associated with olfactory dysfunction (Sekine et al., 2022). See Figure 3.
- In addition to ammonium, other trigeminal stimuli were used for subjective ratings. For example, Yan et al. (2023), delivered varying duration of air-puff at a certain flow rate, and required participants to rate the intensity from 1 (very week) to 10 (extremely strong). Paul et al. (2003), delivered $CO_2$ in various concentrations and asked participants to continuously rate the intensity by moving a slider.

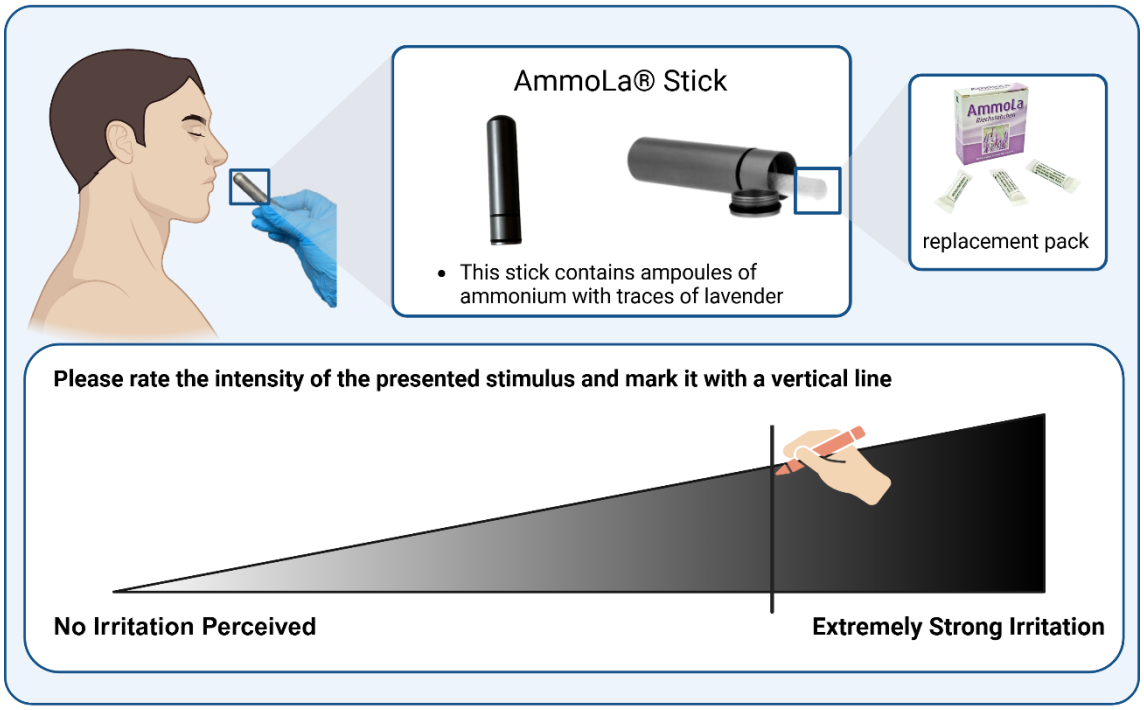



**Figure 3. Intensity ratings of ammonium vapors**

### 1.4.4. Trigeminal probes

Developed by Huart et al. (2019), this test utilizes an approach similar to the Sniffin' Sticks test but uses stimuli that activate trigeminal afferents. Stimuli were prepared using felt-tip pens (Burghart Medical Technology, Wedel, Germany) with the target odor stimuli dissolved in propylene glycol (solvent). The test comprises three parts: threshold, discrimination, and identification.

- ***Threshold Test:*** This test evaluates sensitivity to detect menthol, a prototypical bimodal odor that activates the TRPM8 receptor and induces a cooling and fresh trigeminal sensation. Ten dilutions are prepared, with a maximum concentration of 50% and diluted in a 1:2 geometric series. Following a three-alternative forced-choice procedure, in each trial, three pens are presented in random order to a blindfolded participant, with two containing only the solvent and one containing the menthol solution. Participants identify the pen with menthol. Each triplet is presented for approximately 10s with a 30s interval between trials, following an ascending staircase procedure with seven reversals. The threshold is calculated as the average of the last four reversals. Total score ranges from 1 to 10.

- ***Discrimination Test:*** This test evaluates the ability to distinguish between trigeminal and selectively olfactory stimuli. The test includes six triplets. During each trial, the participant receives three pens in random order: one containing a trigeminal stimulus (i.e., menthol, ethanol, diallylsulfide, propanol, camphor, or eucalyptol) and two containing olfactory stimuli (selected from the Sniffin' Sticks identification test). Participant identifies the pen eliciting trigeminal sensations. Each triplet is presented for 10s with a 30s interval between trials. Total score ranges from 0-6.
- ***Identification Test:*** Participants receive six pens in a randomized order, with at least 30s between presentations. After each pen, they choose the best descriptor for the sensation from a card listing five options: (1) Pungent/astringent; (2) Burning/warm; (3) Scratching/tickling/sneezing; (4) Prickling; (5) Cold/fresh. Total score ranges from 0-6.

### 1.4.5. Electrophysiology

In contrast to behavioral techniques, these measures rely less on participants' subjective response and collaboration. They therefore provide a more objective assessment of trigeminal sensitivity, even though they are still not perfect. See Figure 4.

- ***Negative Mucosal Potential (NMP)***: The NMP is recorded from the nasal mucosa and has been hypothesized to represent the summated receptor potentials of chemical nociceptors, similar to the electro-olfactogram (EOG), which represents generator potentials of olfactory receptor neurons in the olfactory epithelium (Dalton et al., 2006; Hummel, Kraetsch, et al., 1998b; Thürauf et al., 2002). Since the respiratory mucosa contains trigeminal nerves rather than olfactory nerves, NMP is relatively free from olfactory interference. Thus, NMPs represent peripheral processing of trigeminal stimulation. During repeated delivery of trigeminal stimuli (typically $CO_2$) with a relatively long inter-stimulus interval (20-40s across studies to avoid habituation or adaption), NMPs are recorded by placing an electrode on the respiratory mucosa (e.g., middle turbinate) under endoscopic control. A computer-controlled olfactometer is important for NMP recording, as it ensures the embedding of the stimulus in a constant airflow to avoid mechanical sensations, steep stimulus

onset, defined stimulus duration, and a stable temperature (36-37°C) to prevent thermoreceptor activation (Frasnelli & Manescu, 2017; Kobal, 1981). The signal is averaged based on artifact-free epochs (trials). Due to low background noise, few recordings are needed to obtain a meaningful NMP. Sometimes even a single recording may suffice for interpretation (Frasnelli & Manescu, 2017). The NMP consists of a slow negative wave (N1) with a latency of approximately 1000 to 1500ms. The largest NMP is observed at the nasal septum, with the lowest at the nasal floor and olfactory cleft (Scheibe et al., 2006, 2008).

- ***Trigeminal event-related potentials (tERP)*** tERPs are electroencephalography (EEG)-derived polyphasic signals obtained from the surface of the scalp due to the activation of cortical neurons that generate electromagnetic fields. tERPs thus provide a central nervous representation of the processing of trigeminally mediated sensations (Frasnelli & Manescu, 2017). ERPs are recorded by placing electrodes on the scalp based on the 10/20 system, with Fz, Cz, Pz, C3, and C4 being the most representative electrodes for measurement. Similar to NMP, tERPs are recorded during repetitive stimulation with relatively selective trigeminal stimuli (typically $CO_2$), with an interstimulus interval of 20-40s to avoid habituation or adaption. To obtain meaningful averages, at least 10 single responses must be recorded, resulting in ERP sessions lasting 45 minutes to 2 hours. Best signal-to-noise ratio is reached when 60-80 responses are averaged (Boesveldt et al., 2007). The features of the tERP responses are as follows: a small first positive peak (P1), typically occurring at latencies later than 200ms, followed by a major negative peak (N1) at approximately 400ms, and the late positive complex (P2 or P2/P3) at approximately 650ms. The largest amplitudes are usually obtained from the Cz and Pz electrodes (Frasnelli & Manescu, 2017).

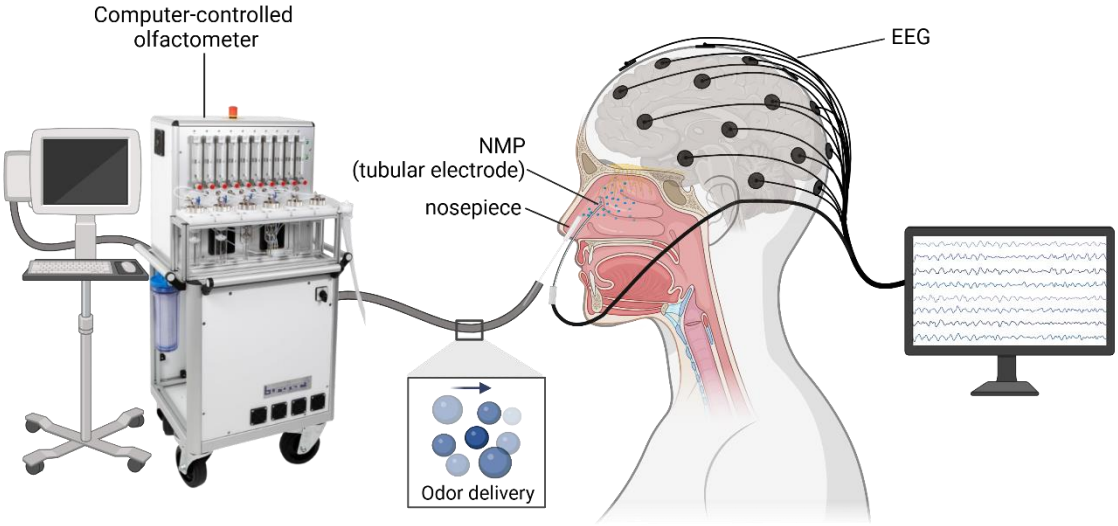



**Figure 4. Scheme of electrophysiological recording**

## 2. Context

While various methods to measure intranasal trigeminal function have been developed and adapted, intranasal trigeminal function assessment still remains less studied compared to olfactory and gustatory testing. As shown in Figure 5, olfactory and gustatory testing have been extensively studied, with over 25,000 and 2,800 publications, respectively. In contrast, research on intranasal trigeminal function assessment has only 276 publications.

Given the significance of the intranasal trigeminal system, accurate assessment is essential. However, several challenges and gaps in current intranasal trigeminal measurement methods remain to be explored. A typical example is the Trigeminal Lateralization Task (TLT), one of the most widely used tests, which evaluates an individual's ability to determine which nostril is stimulated by a trigeminal stimulus. Despite its frequent use, several fundamental questions remain regarding its interpretation, sensitivity, and limitations.

First, while some research has reported accuracy rates for different populations, no widely accepted reference score or established normative data exist for the TLT, limiting the interpretability of individual performance. Furthermore, it remains unclear whether the number of test items influences task outcome, raising concerns about methodological consistency and test reliability. Without a clear benchmark, the interpretability of TLT results remains limited.

Second, the sensitivity of the TLT in detecting trigeminal activation has not been well defined. While strong trigeminal stimuli are typically associated with high accuracy in lateralization, it remains unclear whether minimal intranasal trigeminal activation is sufficient to produce measurable behavioral effects. This question is particularly relevant for determining the threshold at which trigeminal activation becomes perceptible and how well the task reflects activation levels when encountering complex stimuli.

Lastly, current intranasal trigeminal function assessments, particularly

psychophysiological measurements like the TLT, often involve a single or limited set of stimuli. However, can conclusions drawn from a single type of stimulus truly represent overall intranasal trigeminal function? As mentioned above, multiple TRP receptors, including TRPV1, TRPV3, TRPA1, and TRPM8, respond to different chemical stimuli and mediate distinct trigeminal sensations. Understanding receptor-specific activation is crucial for improving the interpretation of trigeminal function tests and developing more refined methodologies for studying chemosensory perception.

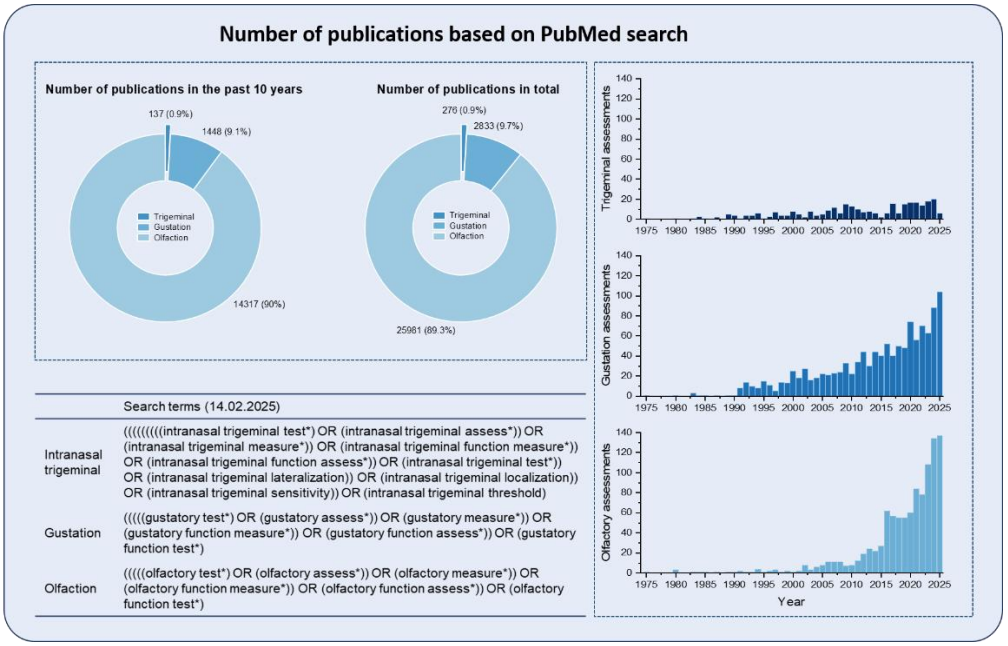



**Figure 5. PubMed search for different chemosensory assessment studies**

## 2.1. Objectives

Given the context outlined above, this thesis aims to address the following questions:

1. As the most widely used intranasal trigeminal test, what are the normative data for the TLT? Is the shorter version as useful and easy to interpret as the longer one?
2. How sensitive is the TLT to trigeminal activation? Is a minimal degree of intranasal trigeminal activation sufficient to produce measurable behavioral effects?
3. Since multiple receptors mediate trigeminal sensations, does the type of stimulation used in trigeminal function tests influence the results? Or do different stimuli elicit similar responses, making a single stimulus type sufficient to represent overall function?

# 3. Study 1: Normative data for the lateralization task in the assessment of intranasal trigeminal function

## 3.1. Relevant Publication

Mai Y, Hernandez AK, Konstantinidis I, Haehner A, Hummel T. 2024. Normative data for the lateralization task in the assessment of intranasal trigeminal function. Rhinology 63 (1): 92-102. doi: 10.4193/Rhin24.063

## 3.2. Hypothesis and objective

The trigeminal lateralization task (TLT) is one of the most widely-used measures of intranasal trigeminal function (Croy et al., 2014; Hucke et al., 2023; Kleemann et al., 2009; Kobal et al., 1989). The task typically includes 40- (Hummel, Futschik, et al., 2003), 20- (Oleszkiewicz et al., 2018), or 10 (Hernandez et al., 2023) items. Yet, the actual performance distribution within the healthy population and the level of performance that a majority of the healthy population can reach has not been reported. Given the increasing clinical significance of the trigeminal system in the field of rhinology and the widespread use of the lateralization test, establishing normative values among healthy individuals is essential. Study 1 mainly aimed to provide normative data for each version of the TLT and establish potential boundaries to distinguish between “normal” and “decreased” lateralization ability.

## 3.3. Methods

### 3.3.1. Data source and Participants

Data were obtained from healthy adult participants without any chemosensory complaints, including 820 participants from published studies (Frasnelli et al., 2007a, 2008; Henriette Friederike Katrin Hornstein-Schnellhardt, 2024; Hernandez et al., 2023; Hummel et al., 2007; Hummel, Futschik, et al., 2003; Joshi et al., 2021; Z. Li et al., 2022; Meusel et al., 2010; Oleszkiewicz et al., 2018; Stuck et al., 2006) and 194 from unpublished data. The 40-item version included 360 participants, the 20-item version included 284 participants, and the 10-item version included 418 participants.

### 3.3.2. Measurements

***Trigeminal lateralization task.*** As described in the *Measurement of intranasal trigeminal function* section, this test followed a methodology published by Kobal et al. (1989), and Hummel (2000). A mechanically operated “squeezer” device with two 250ml compressible polypropylene bottles was used. One bottle contained eucalyptol (99%; C80601, Merck, Darmstadt, Germany), a prototypical bimodal stimulant activating the TRPM8 receptor and inducing a cooling sensation, while the other contained air/solvent (propylene glycol). During the test, participants held the tubing in place beyond the nasal valve while the examiner delivered the stimuli separately to each nostril (approximately 15ml). After each stimulus, participants indicated the side of stimulation. Blindfolded participants underwent 40, 20, or 10 trials (half per nostril in randomized order) with an interstimulus interval of 30-40s. The score was the sum of correct responses.

***Olfactory function.*** Olfactory function was assessed using the "Sniffin’ Sticks" test (Burghart Messtechnik, Holm, Germany), which includes subtests for threshold (OT), discrimination (OD), and identification (OI) (Hummel et al., 1997; Oleszkiewicz et al., 2019). In OT, participants identify an odorous pen among odorless ones using a staircase paradigm with 16 concentrations, scoring based on the last four turning points. OD involves identifying the odd odor in 16 triplets, while OI requires matching 16 odors to descriptors on flash cards. Each subtest scores up to 16, with a composite TDI score ranging from 1 to 48. Tests were performed birhinally.

### 3.3.3. Data analysis

Data were analyzed using SPSS 29 software (SPSS Inc., Chicago, Ill., USA). All analyses were conducted separately for the 40-, 20-, and 10-trial versions. Descriptive statistics calculated the 5th, 10th, 25th, 50th, 90th, and 95th percentiles of lateralization scores to show data distribution. To determine boundary to distinguish between “normal” and “decreased” lateralization ability, two cutoff scores were involved:

(1) ***Distribution cutoff ($10^{th}$ percentile cutoff)***: the 10th percentile of the best-performing reference age group considering the absolute performance (Brumm et al., 2023; Doty RL, 2020; Kobal et al., 2000; Oleszkiewicz et al., 2019), indicating that the majority (90 percent) of healthy individuals without trigeminal-related complaints

in the reference age group can perform at least at this level.

(2) ***Above-chance cutoff (binomial cutoff)***: A score calculated using binomial statistics, where achieving or exceeding this score indicates performance statistically above chance level. Specifically, individuals scoring ≥27/40, ≥15/20, or ≥9/10 trials are considered statistically above chance.

(3) Performance was classified as “Normal” (≥ both cutoffs), “Decreased” (< both cutoffs), or “Gray area” (between cutoffs).

Additional analyses (ANOVA, independent t-tests, Pearson correlations, logistic regression) examined relationships between lateralization, age, sex, and olfaction.

## 3.4. Results

### 3.4.1. TLT score distribution

For the 40-trial version (n=360, 37.5±17.4 years, 189 female), the mean score was 35.46±4.50. The 10th percentile scores by age group were: 18-25 years (reference group): 33; 25-35 years: 31; 35-45 years: 26.4; 45-55 years: 29.7; 55-65 years: 26.8; and >65 years: 22.

For the 20-trial version (n=284, 32.6±14.1 years, 172 female), the mean score was 15.64±3.65. The 10th percentile scores were: 18-25 years (reference group): 11; 25-35 years: 11; 35-45 years: 8.4; 45-55 years: 9.1; 55-65 years: 8; and >65 years: 8.

For the 10-trial version (n=418, 42.6±15.6 years, 257 female), the mean score was 8.14±2.16. The 10th percentile scores were: 18-25 years (reference group): 6; 25-35 years: 5; 35-45 years: 5; 45-55 years: 5; 55-65 years: 4; and >65 years: 4. (See *Publication 1: Table 1, Figures 1 and 2*)

### 3.4.2. Scoring Between 10th Percentile and Binomial Cutoff

When comparing the three trial versions, 14% (n=52) of the population in the 40-trials version, 24% (n=69) of population in the 20-trial version, and 21% (n=86) of the tested population falls within this “grey zone”. See Figure 6 below.

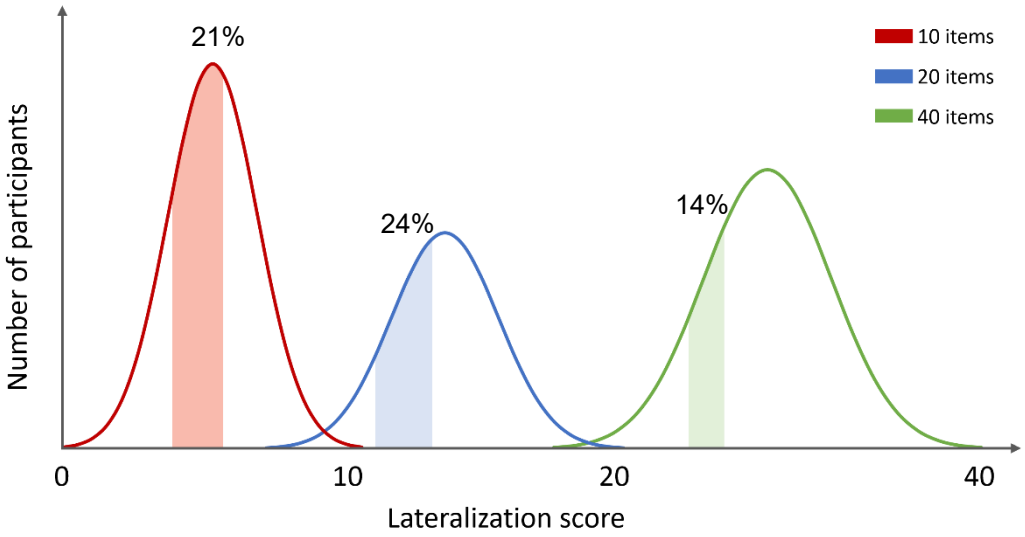



**Figure 6. Number of participants scoring between the "grey area" (10th Percentile and Binomial Cutoff)**

### 3.4.3. Relationship between TLT and age, sex and olfaction

Age effects were found in the 40- and 10-trial versions ($F=6.26$-$7.74$, $p<0.001$) and marginally significant in the 20-trial version ($F=2.23$, $p=0.052$). Age also correlated negatively with lateralization scores across all versions ($r=-0.30$ to $-0.16$, $p\leq0.028$). No significant sex differences were observed in the 40- and 10-trial versions. However, in the 20-trial version, females performed better than males in the 18-25 age group ($t=2.88$, $p=0.005$), but not in other age groups. Positive correlations were also found between lateralization and OD, OI, and TDI scores ($r=0.21$-$0.25$, $p\leq0.025$). (See *Publication 1: Table 2, Figures 3, 4*)

## 3.5. Conclusion

Study 1 provided a reference distribution of the eucalyptus TLT, showing the approximate percentile of a given score relative to the performance of healthy individuals. Considering both the distributional and above-chance cutoffs, the boundary between normal and decreased lateralization was suggested as follows:

(1) 40-trial version: Normal (≥33), "Gray area" that warrants further assessment (27-32), Decreased (<27)

(2) 20-trial version: Normal (≥15), "Gray area" that warrants further assessment (11-14), Decreased (<11)

(3) 10-trial versions: Normal (≥9), “Gray area” that warrants further assessment (6-8), Decreased (<6).

The 40-trial version is more useful than the shorter versions due to its narrower gray area, where fewer scores require further tests. It may serve as an adjunctive test for intranasal trigeminal function, though future studies are needed to determine whether these cutoff scores can reliably differentiate pathological cases from healthy individuals.

## 3.6. Publication 1 discussion

**What are the current normative data for the TLT? Is the shorter version as useful and easy to interpret as the longer version?**

This study established normative data for the TLT as a measure of intranasal trigeminal function by providing a reference distribution of eucalyptus TLT scores in a large, healthy population. Additionally, we incorporated binomial statistics to define a conservative cutoff for performance that significantly exceeds random guessing. The distribution-based cutoff (10th percentile) reflects typical performance within the population, while the binomial cutoff determines the lowest score that is significantly above chance. These two approaches together define three interpretation zones: normal, gray area (uncertain classification), and decreased function.

In the 40-trial version, the 10th percentile cutoff was 33, and the above-chance cutoff was 27. A score of ≥33 indicates normal function. Scores below this threshold fall into two categories: (1) scores <27 indicate a decreased lateralization ability, as they do not exceed chance levels nor reach the 10th percentile of the healthy population; (2) scores between 27 and 33 represent a gray area, significantly better than random guessing but still below 90% of healthy individuals. Without clinical validation from patients with trigeminal dysfunction, it remains unclear whether scores in this gray area reflect normal variability or true impairment. Additional assessments, such as trigeminal event-related potentials, or repeated assessment with the TLT may help clarify these cases.

In the shorter versions (20- and 10-trial tasks), the distribution cutoffs were 11 and 6, while the above-chance cutoffs were 15 and 9, respectively. Unlike the 40-trial version, these shorter versions had distribution cutoffs lower than their above-chance cutoffs. In these cases, scores <11 (20 trials) or <6 (10 trials) indicate decreased function. A score exceeding both cutoffs (≥15 for 20 trials, ≥9 for 10 trials) reflects normal function. However, scores falling between the distribution and above-chance cutoffs (≥11 to <15 for 20 trials, ≥6 to <9 for 10 trials) remain in a gray area. If classified solely by the distribution cutoff, they would be considered normal, but since they could still result from

random guessing, they do not reliably indicate an intact function and thus require further verification.

When comparing the shorter versions to the full 40-trial task, our findings suggest that the longer version is more precise and easier to interpret. The 40-trial task resulted in a narrower gray area (14% of participants) compared to the 20- and 10-trial versions (24% and 21%, respectively). While shorter versions may reduce testing time, they sacrifice interpretability, making the 40-trial version preferable when a robust assessment is needed. However, the clinical usefulness of the 40-tiral version still need to be validated with pathological cases

In this first study, we established reference score for the TLT to distinguish normal from decreased trigeminal function. While these thresholds clearly classify significant deficits, it remains uncertain whether the TLT can capture the subtle, fine-grained differences in trigeminal function that may be clinically relevant. Specifically, we do not yet know if even minimal trigeminal activation is sufficient to elicit a measurable behavioral effect. If the TLT is highly sensitive, even slight increases in trigeminal activation might produce observable changes in performance, thereby potentially enabling us to monitor fluctuations or early recovery in trigeminal deficits. Conversely, if not, such changes may go undetected. This motivated our next study, which aimed to evaluate the sensitivity of the lateralization task paradigm.

# 4. Study 2: Odor lateralization test is insensitive to small degrees of intranasal trigeminal activation

## 4.1. Relevant publication

**Mai Y**, Brieke B, Hummel T. 2025. Odor lateralization test is insensitive to small degrees of intranasal trigeminal activation. Eur Arch Otorhinolaryngol 282(1):241-249. doi: 10.1007/s00405-024-09016-x

## 4.2. Hypothesis and objective

The rationale for using TLT to assess trigeminal function is based on the fact that odor lateralization requires trigeminal activation (Croy et al., 2014; Kleemann et al., 2009), as humans generally cannot lateralize odors using olfactory input alone. However, the ability to localize odorants is not entirely impossible. It can be improved by training or mixing odorants with trigeminal stimuli (Negoias et al., 2013; Tremblay & Frasnelli, 2018). In a previous study, Tremblay & Frasnelli (2018) demonstrated that lateralization performance for mixtures of odorants and trigeminal stimuli at ratios of 1:1 or 2:1 was significantly better than for “pure” odorants. This finding raises an important question: how sensitive are lateralization tasks to trigeminal activation? Can even a small addition of a trigeminal stimulus to an olfactory odor significantly enhance lateralization performance? In other words, does odor lateralization follow an “all-or-none” rule, where minimal trigeminal input is sufficient to significantly improve performance? Or does it follow an “accumulative” pattern, where improvement requires a more prominent trigeminal component? Study 2 aimed to investigate whether mixing olfactory odors with varying low levels of trigeminal compounds significantly enhances lateralization performance.

## 4.3. Methods

### 4.3.1. Participants and procedure

We recruited healthy adults with a self-reported normal sense of smell, confirmed by the Sniffin’ Sticks Identification test (Hummel et al., 1997; Oleszkiewicz et al., 2019). The study included three appointments over two weeks:

- First appointment: Participants completed demographic questionnaires, olfactory tests, and TLTs with five of the twelve odors (randomized sequence). They also rated the intensity of these five odors.
- Second appointment: Participants completed a questionnaire on the importance of olfaction, underwent TLTs with another five odors (randomized sequence), and rated their intensity.
- Third appointment: Participants completed TLTs with the remaining two "olfactory" odors (randomized sequence) and rated their intensity.

### 4.3.2. Measurements

***Odor lateralization test.*** The odor lateralization task followed the same procedure as in Study 1, where target odor was delivered to on nostril and air was delivered another nostril, and participants indicated which nostril received the odor (Frasnelli, Hummel, et al., 2011; Kobal et al., 1989; Lötsch et al., 2022). For each odor condition, 20 trials were conducted (10 per nostril in a randomized order) with a 30-second interval between trials. The score was the sum of correct responses. A total of 12 odors were tested: 2 "olfactory" (O1, O2), 2 "trigeminal" (T1, T2), and 8 mixtures, where O1 and O2 were each combined with small amounts (4% and 8%) of T1 and T2. Further details are provided in *Publication 2: Table 1*.

***Additional measurements.*** (1) Odor intensity: participants rated the intensity of all tested odors using a scale from 0 (no perception) to 10 (extremely intense). (2) Olfactory function: Olfactory function was assessed using the Sniffin' Sticks odor identification (OI) test (Burghart, Holm, Germany). Scores ≥11 is considered as "normosmia" (Hummel et al., 1997; Oleszkiewicz et al., 2019). (3) The importance of olfaction: the Importance of Olfaction Questionnaire, consisting of 20 Likert-scale items, was used to assess how individuals perceive and use their sense of smell in daily life (Croy et al., 2010).

### 4.3.3. Data analysis

Data were analyzed using SPSS 29.0 software (IBM Corp., Armonk, NY, USA). Generalized linear mixed model (GLMM) was used to examine the effects of irritant concentration (0%, 4%, 8%, and 100%), odorant type (O1, O2), irritant type (T1, T2), and

their interactions on odor lateralization performance. Chi-square tests compared the percentage of participants reaching the above-chance threshold across different odor conditions. A binomial test confirmed that ≥15 correct responses out of 20 trials was the above-chance threshold (test level=0.50, p=0.041). Pearson correlation was used to examine the relationships between lateralization and other variables.

## 4.4. Results

### 4.4.1. Descriptive statistics

We recruited 81 participants (25.4±4.8 years, 51 women) with normosmic Sniffin' Sticks scores (OI≥11). Due to dropouts, 53 completed all lateralization tasks. Dropouts (n=28) and non-dropouts (n=53) showed no significant demographic differences. Missing data were handled using GLMM with the Satterthwaite method (Satterthwaite, 1946). (See *Publication 2: Descriptive results and Table 2*)

### 4.4.2. Lateralization performances across tested odors

GLMM revealed significant effects for "irritant degree" ($F=82.32$, $p<0.001$) and "odorant type" ($F=4.81$, $p=0.03$), but not interactions ($F=0.12$-$1.86$, p's>0.05).

- Irritant degree: performance was significantly higher with 100% irritants (16.47±0.19) than 0% (12.59±0.28), 4% (13.07±0.21), and 8% (12.80±0.22, $t=11.60$-$12.75$, p's<0.001), with no differences among the latter three ($t=0.60$-$1.39$, p's>0.05).
- Odorant type: performance was significantly better with odors containing O1 (13.98±0.16) than odors containing O2 (13.48±0.16; $t=2.19$, $p=0.03$).

(See *Publication 2: Figure 1*)

### 4.4.3. Percentage of participants reaching the above-chance cutoff (≥15 points)

The percentage of participants reaching the cutoff differed by irritant degree ($\chi^2=30.89$ to 47.33, p's<0.001), with a higher percentage of participants reaching this threshold in the 100% irritants condition (75%-78%) compared to the 0%, 4%, and 8% irritants conditions (28%-44%, p's<0.05). (See *Publication 2: Figure 2*)

### 4.4.4. Correlation results

There were significant correlations between lateralization performance with mixed odors and their corresponding intensity ratings ($r=0.24$-$0.31$, p's≤0.03). Lateralization scores

also correlated with odor identification score (r=-0.24-0.24, p's=0.03) and importance of olfaction (r=0.23, p=0.02). (See *Publication 2: Correlation section*)

## 4.5. Conclusion

Lateralization performance for odors mixed with small amounts of trigeminal compounds was comparable to selective "olfactory" odors but significantly worse than "trigeminal" odors. Trigeminal lateralization is more likely to follow an "accumulative" pattern rather than an "all or none" rule. A small amount of irritating (trigeminal) compounds was insufficient to significantly enhance lateralization performance, indicating that the lateralization paradigm is insensitive to low trigeminal activation. The presently used lateralization task with 20 trials may lack sensitivity in identifying odors with low degrees of trigeminal irritation among more selective olfactory odors, yet without excluding the possibility of trigeminal system activation. However, the task can still serve as a screening tool for identifying trigeminal odors with substantial irritating compounds by comparing group-level performance with that of a selective olfactory odor.

## 4.6. Publication 2 discussion

**How sensitive is the TLT to trigeminal activation? Is a very small degree of intranasal trigeminal activation sufficient to produce measurable improvement?**

The results consistently showed that adding a small amount of an irritant to a selectively olfactory odorant was insufficient to significantly improve lateralization performance. These results challenge the “all-or-none” assumption. Because if that rule were true, even a minimal level of trigeminal stimulation would have produced a significant improvement. Instead, our findings suggest that lateralization performance in response to olfactory-trigeminal mixtures follows an accumulative pattern, in which only sufficiently strong activation of the trigeminal system significantly enhances performance. In this study, the lateralization task paradigm appeared insensitive to a small degree of intranasal trigeminal activation and thus serves as a conservative measure, as measurable effects only emerged with sufficiently strong activation, at least when using 20 items.

But to what degree can the TLT detect trigeminal activation? To answer this question, a concentration-response gradient is needed to establish. In our follow-up ongoing experiment based on Publication 2, we built this gradient by testing different percentages of eucalyptol mixed with PEA using a 40-trial TLT. Our preliminary findings confirm Publication 2’s observations, showing that small amounts of trigeminal compounds added to an olfactory odor do not significantly improve performance. Furthermore, we identified a turning point at which the addition of at approximately 40% irritant produced a significant improvement in scores. See Figure 7.

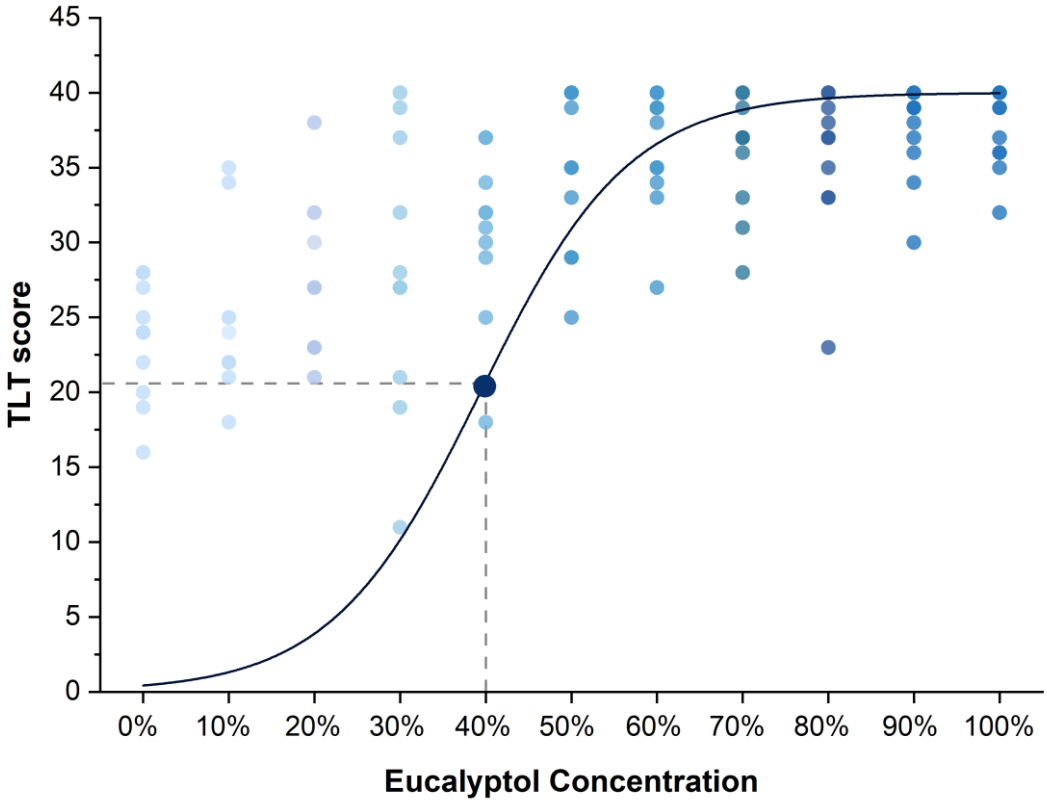



**Figure 7. Concentration-response curve of the lateralization task performance**

Note. Percentages shown on the x-axis represent the concentration of eucalyptol. For example, 0% indicates 0% eucalyptol + 100% PEA, while 10% indicates 10% eucalyptol + 90% PEA. Both eucalyptol and PEA used for mixing were in neat concentration (99%). At 40% eucalyptol, scores were significantly improved compared to 0%. The test followed a within-subject design. Sample size was ten.

Taken together, Study 2 has two practical implications. First, if a group of healthy participants consistently shows better lateralization performance with an unknown odor (i.e., significantly higher scores or a higher percentage reaching the above-chance threshold compared to a purely olfactory odor), this likely indicates the presence of substantial irritating compounds. However, if an odor does not show this improvement in the same group, it does not necessarily mean that the odor is completely devoid of trigeminal properties. By comparing group-level performance with that of a selectively olfactory odor, this paradigm can serve as a screening tool to identify odors containing significant irritants. Secondly, because of its insensitivity to subtle trigeminal activation, the paradigm appears to be a conservative measure of intranasal activation.

Consequently, the TLT might lack effectiveness in monitoring fluctuations or early changes (such as initial recovery or decline) in trigeminal function.

In addition to the challenges posed by the task paradigm itself, the stimuli used in trigeminal testing are another important aspect to be explored. Therefore, the next study aimed to investigate the influence of different types of stimulation on measurable trigeminal responses.

# 5. Study 3: Responses to the activation of different intranasal trigeminal receptors: Evidence from behavioral, peripheral and central levels

## 5.1. Relevant publication

**Mai Y**, Flechsig J, Warr J, Hummel T. 2025. Responses to the activation of different intranasal trigeminal receptors: Evidence from behavioral, peripheral and central levels. Behavioural brain research 480:115371. doi:10.1016/j.bbr.2024.115371

## 5.2. Hypothesis and objective

Trigeminal sensations are diverse (e.g., burning, painful, cooling, fresh, warm, stinging) (Doty et al., 1978; Laska et al., 1997; Viana, 2011) and are mediated by specific trigeminal receptors, most of which belong to the transient receptor potential (TRP) family as described in *Introduction* section (Boonen et al., 2017; Hummel & Frasnelli, 2019). Although various types of TRP channels mediate trigeminal activity, however, existing studies often simplify this complexity by using stimuli that selectively target a single or limited number of receptor types to represent overall trigeminal activation (e.g., eucalyptol, which activates TRPM8, in lateralization tasks) (Hernandez et al., 2023; Mai et al., 2024; Migneault-Bouchard et al., 2024). This raises a critical question: Can conclusions be drawn from a single type of stimulation to truly reflect overall intranasal trigeminal function? However, there is a lack of studies that comprehensively examine and compare response patterns across different receptor activations. This study thus aimed to compare the responses elicited by odors that primarily activate different intranasal trigeminal receptors (TRPV1, TRPV3, TRPA1, and TRPM8) at peripheral (negative mucosa potential, NMP), central nervous (event-related potential, ERP), and behavioral (perceptual ratings) levels.

## 5.3. Methods

### 5.3.1. Participants and Study procedure

Healthy adult volunteers with self-reported normal olfaction participated in the study. They first completed a demographic questionnaire, olfactory and trigeminal tests, and

rated the intensity of the study odors. Participants were then trained in a breathing technique involving velopharyngeal closure to prevent nasal airflow during olfactometric stimulation, minimizing respiratory artifacts in NMP recordings (Kobal, 1981). Next, participants underwent NMP and ERP recordings while receiving five trigeminal odors (each activated different TRP channels) and one olfactory odor as control via a computer-controlled olfactometer in a block-randomized sequence. In addition to the electrophysiological recordings, ten participants completed a continuous intensity rating task to assess behavioral responses to these tested odors.

### 5.3.2. Stimulation

Stimuli were first selected based on previous studies (Akiba et al., 2008; Bassoli et al., 2009; Cattaneo et al., 2017; Genovese et al., 2023; Karunanayaka et al., 2024; Kawai et al., 2014; Kim et al., 2012; Niu et al., 2022; Saunders et al., 2013; Silver et al., 2006; Vogt-Eisele et al., 2007; G. Wang, 2021; Y. Y. Wang et al., 2010; Willis et al., 2011; L. Xu et al., 2020) and then validated with a cell-based assay method using a fluorometric imaging plate reader (FLIPR) (see *Publication 3: Supplement 1*). Results supported prior studies, but also showing that most compounds also co-activating TRPA1, suggesting TRPA1 as a potential control for trigeminal stimulation. The selected stimuli were:

- Cyclohexanone (TRPV1)
- $CO_2$ (TRPV1 + TRPA1)
- Isopulegol (TRPM8 + TRPA1)
- Carvacrol (TRPV3 + TRPA1)
- Perillaldehyde (TRPA1)
- Phenyl ethyl alcohol (olfactory control)

### 5.3.3. Measurements

***Electrophysiological recordings*** ERP and NMP data were recorded using an 8-channel EEG amplifier (Burghart, Holm, Germany). NMP was measured with a tubular Ringer-agar (1%) electrode containing a silver-chlorided wire (0.3mm diameter, 0.4mm inner, 0.8mm outer tubing diameter) (Kobal, 1981). The electrode was positioned endoscopically on the anterior middle turbinate and stabilized with a clip-mounted frame.

ERP was recorded at Fz, Cz, and Pz (10/20 system), with blink artifacts monitored at Fp2, linked earlobe references, and mastoid ground electrodes. Each trial began 500ms before stimulus onset and lasted 4000ms, sampled at 125Hz. Stimuli were presented for 500ms, with interstimulus intervals (ISI) of 27-33s to prevent habituation/adaption. Each odor was presented 12 times, totaling 72 trials in a randomized block sequence of three identical stimuli per block.

***Continuous intensity rating task.*** During the task, odor stimuli were delivered to participants' nasal cavity with the stimulation setting the same as what used for EEG/NMP recording. At the same time, participants held an adjustable lever and continuously adjusted the mechanical pressure on the lever to indicate perceived odor intensity (*Publication 3: Figure 1*).

***Additional measurements.*** (1) Odor Ratings and Descriptor Selection: Participants rated six dimensions of each odor (e.g. painfulness). Using a forced-choice paradigm they then chose four descriptors from a list of 14 (e.g. stinging, burning) (Frasnelli, Albrecht, et al., 2011; Laska et al., 1997). (2) Psychophysical chemosensory testing: Olfactory performance was assessed using the Sniffin' Stick Identification test (Hummel et al., 1997). Trigeminal sensitivity was evaluated with a 20-trial eucalyptus TLT (Kobal et al., 1989).

### 5.3.4. Data analysis

ERP/NMP data were preprocessed using Letswave 6 (https://letswave.cn/). A bandpass filter (0.2-15 Hz) was applied, and epochs were segmented from -500 to 4000 ms with baseline correction (-500 to 0 ms). Artifacts were visually inspected, and for each odor condition, only averages based on at least three artifact-free trials were included. Amplitude and latency for NMP (N1, P1N1) and ERP (N1, P2, N1P2) were extracted. Three indicators were computed for the continuous intensity rating task: peak rating (highest odor intensity rating), latency (from onset to perception), and steepness [(peak rating-baseline)/(peak latency-onset)]. GLMMs compared responses across odors, with odor type as the within-subject variable. Odor intensity ratings were included as covariates in ERP/NMP comparisons. Repeated measures correlation examined

relationships between NMP, ERP, and behavioral responses.

## 5.4. Results

The final NMP data included 24 participants (25.2±2.7 years, 17 women) and ERP data included 17 participants (26.7±3.6 years, 12 women). Ten participants (25.1±2.6 years, 6 women) completed the continuous odor intensity rating task.

### 5.4.1. NMP responses across different stimuli

A significant effect of odor type was found for NMP N1 and P1N1 amplitudes (F=13.51-21.88, p's<0.01). Post hoc tests showed:

- Cyclohexanone had greater N1 and P1N1 amplitudes than PEA, carvacrol, perillaldehyde, and isopulegol (t=3.76-7.24, p's<0.01).
- $CO_2$ showed greater N1 and/or P1N1 amplitudes than PEA, carvacrol, perillaldehyde, and isopulegol (t=3.28-7.54, p's<0.05).
- Perillaldehyde had a greater N1 amplitude than PEA (t=3.22, p=0.02).

(See *Publication 3: Table 1, Figures 2-4* for details)

### 5.4.2. ERP responses across different stimuli

A significant odor effect was found in P2 and N1P2 amplitudes at Fz, Cz, and Pz (F=3.69-12.25, p's<0.05). Post hoc tests showed:

- Cyclohexanone had greater amplitudes than PEA (N1P2: t=3.30-3.57, p's<0.05), carvacrol (P2/N1P2, t=4.06-4.10, p=0.02), and perillaldehyde (P2/N1P2, t=3.13-3.95, p's<0.05).
- $CO_2$ had greater amplitudes than carvacrol (P2/N1P2, t=3.53-4.42, p's<0.05).

(See *Publication 3: Table 1, Figures 2-3* for details)

### 5.4.3. Intensity perception across different stimuli

Significant differences were found in peak rating, latency, and steepness (F=6.15-13.86, p's<0.01). Post hoc tests showed:

- Cyclohexanone had higher peak ratings than all odors (t=3.14-7.76, p's<0.05), shorter latency than carvacrol, isopulegol, and $CO_2$ (t=3.80-5.38, p's<0.01), and steeper responses than PEA, carvacrol, and perillaldehyde (t=3.42-4.73, p's<0.05).

- $CO_2$ had higher peak ratings than carvacrol (t=3.90, p<0.01).
- Isopulegol had higher peak ratings than carvacrol and perillaldehyde (t=3.84-6.04, p's<0.01).
- PEA had higher peak ratings than carvacrol (t=3.62, p<0.01).

(See *Publication 3: Table 1, Figures 2-3* for details)

### 5.4.4. Correlation across central, peripheral, and behavioral results

- NMP and ERP: NMP N1 amplitude correlated with P2 (Fz) amplitude (r=0.22, p=0.04). NMP P1N1 amplitude correlated with P2 (Fz) and N1P2 (Fz, Pz) amplitudes (r=0.23-0.27, p's<0.05).
- NMP and Behavior: NMP N1 and P1N1 amplitudes correlated positively with peak ratings/steepness (r=0.28-0.52, p's<0.05) and negatively with latency (r=-0.38 to -0.35, p's<0.05).
- ERP and Behavior: P2 and N1P2 (Fz, Cz, Pz) amplitudes correlated with peak ratings/steepness (r=0.38-0.61, p's<0.01). P2 (Fz) amplitude correlated negatively with latency (r=-0.51, p's<0.01).

## 5.5. Conclusion

Activation of various trigeminal receptors elicits distinct responses, with patterns that are largely consistent across behavioral, peripheral, and central levels. Notably, stimuli involving TRPV1 activation (i.e., Cyclohexanone and $CO_2$), which is associated with the perception of irritation or pain, elicited overall greater behavioral, central, and peripheral neural activity compared to stimuli involving other receptors, even when controlling for stimulus intensity. This suggests the critical role of TRPV1-mediated sensation in survival adaptation. Given the difficulty of finding ideal odors that target only a single receptor and without any olfactory properties, testing a different set of odors with distinct smell characteristics would help validate these findings.

## 5.6. Publication 3 discussion

**Since multiple receptors mediate trigeminal sensations, does the type of stimulation in trigeminal function tests influence results? Or do different stimuli elicit similar responses, making a single stimulus type sufficient to represent overall function?**

Study 3 consistently indicated that activation of different TRP receptors induced distinct responses at behavioral, peripheral, and central levels. First, TRPV1 activation, either predominantly alone (e.g., cyclohexanone) or in combination with TRPA1 (e.g., $CO_2$), generally led to stronger NMP and ERP amplitudes, as well as higher peak intensity ratings compared to other receptor activations. This was expected, as TRPV1 primarily mediates irritation and pain, both of which carry significant evolutionary importance in avoiding hazardous stimuli and disease (Cervero, 2012). Strong responses and neural activity can act as signals to trigger protective reflexes like sneezing or highlight the irritating properties of certain foods before consumption (Meusel et al., 2010).

Notably, while both cyclohexanone and $CO_2$ activated TRPV1, $CO_2$ also activated TRPA1. Co-activation of TRPV1 and TRPA1 by the same chemical compounds is well-documented (Legrand et al., 2020) and may explain the complexity of our results, where both stimuli elicited similar response amplitudes, yet cyclohexanone had a shorter perceptual latency. Like $CO_2$, perillaldehyde also exhibited TRPV1 and TRPA1 activity in cell-based assays. However, due to its lower volatility and molecular mass, TRPV1 activation by perillaldehyde was unlikely to play a role in our experimental results. The fact that perillaldehyde elicited generally smaller responses than $CO_2$ and cyclohexanone further highlights the important role of TRPV1 in driving stronger behavioral and neural reactions.

Regarding TRPA1 activation, it could occur alone (e.g., perillaldehyde) or in combination with other receptors as described in the Supplement of Publication 3. The fact that TRPA1 co-activated with multiple receptors across different stimuli suggests that it may have a more general role in trigeminal processing, potentially contributing to

the detection of a wide range of chemical irritants. Although TRPA1 is often associated with pain, some of its agonists do not produce strong irritancy or pungency when applied to the skin or mucosa. Perillaldehyde appears to be one such case (Boonen et al., 2017; Viana, 2011). Its weaker neural and behavioral responses, compared to cyclohexanone and $CO_2$, suggest that response magnitude may be influenced by odor quality, with nociceptive stimulation triggering stronger reactions, highlighting the role of nociception in human sensory processing.

Isopulegol, which activates TRPM8 in addition to TRPA1, produced higher peak intensity ratings than perillaldehyde (TRPA1) and carvacrol (TRPV3+TRPA1), suggesting that TRPM8 activation may have a stronger impact on perceptual experience than TRPA1 or TRPV3 activation. This underscores TRPM8's role in modulating temperature-related perception (e.g., subjective nasal patency). However, responses to isopulegol and carvacrol were generally weaker than those elicited by stimuli involving TRPV1 activation. This may reflect TRPV1's priority in immediate protection, whereas TRPM8 and TRPV3 primarily regulate nasal temperature perception for long-term adaptation, highlighting the important role of nociception in immediate protective responses.

Not all tested odors, such as carvacrol or perillaldehyde, elicited stronger behavioral or NMP/ERP responses than PEA, an olfactory control. While previous studies suggest that trigeminal stimuli typically evoke more intense perceptions and higher ERP amplitudes than PEA (Flohr et al., 2015; Stuck et al., 2006), our results indicate that this effect may also depend on the type of receptor activation.

Taken together, since the activation of various TRP receptors leads to diverse response patterns, the type of stimulation does matter in trigeminal function testing and related studies. While different stimuli may share some underlying mechanisms, the distinct processing of each receptor suggests that a single stimulus type is not sufficient to represent the overall function of the trigeminal system. Therefore, caution is needed when generalizing findings based on one type of stimulation.

# 6. Discussion and outlook

In summary, the findings of the three studies in this thesis show the following:

(1) **Normative data serve as a reference for interpreting intranasal trigeminal function:** By applying both distribution-based and above-chance cutoffs, TLT performance can be classified as normal, abnormal (indicating potential dysfunction requiring action), or grey zone (necessitating further assessment before action).

(2) **The number of items matters for TLT in assessing intranasal trigeminal function:** The 40-item full version exhibits better error tolerance, with fewer test scores falling within the grey zone pending further assessment. It is more useful for interpreting results than the 20- and 10-item versions.

(3) **The odor lateralization paradigm is a conservative measure of intranasal trigeminal function:** Although odor lateralization requires trigeminal activation, the task paradigm itself is not sensitive to minimal trigeminal activations. Lateralization performance in response to trigeminal activation follows an accumulative pattern.

(4) **Receptor-specific effects exist in the intranasal trigeminal system:** Activation of different TRP receptors leads to distinct response patterns. Stimuli involving TRPV1 activation, which is associated with the perception of irritation or pain, elicit overall greater behavioral, central, and peripheral neural activity compared to stimuli involving other receptors. A single stimulus type is insufficient to represent the overall function of the trigeminal system.

## 6.1. Overview of Current Challenges in Intranasal Trigeminal Function Assessment

Building on the findings of this thesis and existing research, we review each type of measurement across several key aspects, including reliability, validity, sensitivity, specificity, and utility, to provide an organized overview of the current challenges in existing intranasal trigeminal function testing (see Table 2).

**Table 2. Comparison of different measures of intranasal trigeminal function**

| | Lateralization task | Threshold test | Trigeminal probes | Subjective ratings | NMP | tERP |
|---|---|---|---|---|---|---|
| **Test nature** | Semi-objective or psychophysiological | Semi-objective or psychophysiological | Semi-objective or psychophysiological | Subjective | Objective | Objective |
| **Test stimuli** | Eucalyptol, menthol, etc. | $CO_2$, air-puff, electricity, heat | Multiple bimodal odors | Ammonium, $CO_2$, etc. | $CO_2$ | $CO_2$ |
| **Reliability** | | | | | | |
| 1.Reproducibility | Partial | Partial | N/A | N/A | Partial | Partial |
| 2.Normative data | Yes | No | No | Limited | No | Limited |
| 3.Test-retest reliability | Moderate | $CO_2$&mechanical: Moderate; Others: N/A | Moderate to good | Moderate | Moderate | Moderate to good |
| **Validity** | | | | | | |
| 1.Differentiate pathological case | OD, ENS, CNO, AR, CRSwNP, CRSsNP, Acute cold | $CO_2$: OD, AR, Acute cold, CT; Electrical: CRSwNP; Others: N/A | OD, CRSwNP, CRSsNP | $CO_2$: CRSwNP; Ammonia: OD | AR, OD | AR, OD, CRSwNP |
| 2.Inter-task correlation | TRPM8 TLT: Ammonia rating, tEFP, receptor expression level, Trigeminal probes; TRPA1 TLT: Cold and heat pain threshold | $CO_2$: TRPM8 expression; Others: N/A | TLT | TLT, tERP, NMP | Intensity ratings | Intensity ratings, TLT |
| 3.adequately cover the domain | Limited (typically one receptor) | Limited (typically one receptor) | Extensive | Limited (typically one receptor) | Limited (typically one receptor) | Limited (typically one receptor) |
| **Sensitivity** | | | | | | |
| 1.identify true positives | N/A | N/A | N/A | N/A | N/A | N/A |
| 2.detect minor change | Limited | N/A | N/A | $CO_2$: Yes | Yes | Yes |
| **Specificity** | | | | | | |
| 1.identify true negatives | N/A | N/A | N/A | N/A | N/A | N/A |
| 2.Affected by olfactory properties | Yes (Squeezer) | No | Yes | Yes | No | No |
| 3.Affected by strategic | Yes (can be minimized) | Yes (can be minimized) | Yes | Yes | Less affected | Less affected |
| **utility** | | | | | | |
| 1.Price | $ | $$ | $$ | $ | $$$ | $$$ |
| 2.Time request | 40: 20-30 min; 20: 10-15 min; 10: 5-7 min; | $CO_2$: 5-15min; Air-puff: 30min; Others: N/A; | 30 min | 2-5 min | >30-45 min | >30-45 min |
| 3.test difficulty | Easy | Easy | Moderate | Easy | Requires expertise | Requires expertise |
| 4.Participants' cooperation | Minor | Minor to moderate | Minor to moderate | Minor | Moderate to high | Moderate to high |
| 5.Device requirement | Squeezer, cheap, portable | Specific device with a computer-controlled unit | Blank Sniffin' Sticks, with six sets of odors | Simple, portable | Olfactometer, EEG system | Olfactometer, EEG system |
| 6.Commercially available | No | No | Partly | Ammonia: Yes; Others: No | Yes | Yes |

Note. N/A: no data; OD: olfactory disorder; ENS: empty nose syndrome; CNO: chronic nasal obstruction; AR: allergic rhinitis; CRSsNP/wNP: chronic rhinosinusitis without/with polyps; CT: chronic tinnitus. Test-retest correlation: ≥0.9 (perfect), 0.7-0.9 (strong), 0.4-0.7 (moderate).

### 6.1.1. Reliability

Reliability refers to the extent to which results are consistent over time and an accurate representation of the total population. If the results of a study can be reproduced with a similar methodology, then the research instrument is considered to be reliable (Ahmed & Ishtiaq, 2021; Nahid Golafshani, 2003). Based on this definition, we reviewed the *reproducibility* (i.e., whether similar results can be repeated across studies or labs with similar methodology), *test-retest reliability* (correlation obtained from two different times in the same sample) and the *availability of normative data* (i.e., norms that help compare individual results to a standard population).

First, most tests, including the TLT task, demonstrate partial reproducibility. On one hand, TLT mean scores showed no significant differences across several studies, such as data obtained from Germany and Greece in Study 1 (Mai et al., 2024). However, mean scores from other studies exhibited significant differences, such as 32.95±3.69 (n=41) from Hummel et al. (2003) versus 35.94±4.87 (n=16, t=2.22, p=0.04) from Migneault-Bouchard et al. (2024). While factors such as age and sample size may partially explain these differences, how the test was applied also plays a role. For instance, Migneault-Bouchard et al. (2024) cited and followed the methodology described by Hummel et al. (2003), with both studies using the 40-item TLT, 30ml of eucalyptol in a 250ml bottle, and an odor-puff volume of 15ml per nostril. However, neither study specified the breathing pattern (e.g., regular breathing, breath-holding, or sniffing when squeezing the bottles) or the exact concentration of eucalyptol. Additionally, Hummel et al. (2003) used an ISI of approximately 30s, while Migneault-Bouchard et al. (2024) applied a slightly longer ISI of approximately 30-40s. This issue is not limited to a single case. Many studies have used the same TLT paradigm but with different parameters (e.g., concentration, odor volume), and certain test details were often overlooked (e.g., filling the odorless bottle with solvent or using an empty bottle as control, whether the ISI was timed precisely or estimated by the examiner, or how participants were instructed in term of the breathing pattern). These variations can influence the comparability and reproducibility of measurements. Some factors, such as stimulus volume (Frasnelli, Hummel, et al., 2011),

have already been identified as influencing TLT, while others, such as ISI (e.g., 10s vs. 30s) or breathing patterns (e.g., normal breathing vs. breath-holding), require further investigation. Other commonly used tasks, such as the $CO_2$ threshold test and tERP measurements, face similar issues. For example, in tERP studies, $CO_2$ concentrations have ranged from 40% to 70%, flow rates from 6-10L/min, and ISIs from 10-60s (Frasnelli et al., 2007a; Huart et al., 2012; Hummel, Barz, et al., 1998; Hummel & Kobal, 1999; Rombaux et al., 2006; Stuck et al., 2006; Tremblay et al., 2019). In other words, these intranasal trigeminal measurements lack standardization. It is important to note that good reproducibility does not mean a test is unaffected by external factors; rather, it means that results are repeatable under highly similar protocols. To improve reproducibility and comparability across studies, standardizing protocols is essential. Achieving this would likely require several efforts: (1) Identifying parameters that have been inconsistently set across studies; (2) Examining the influence of these factors using adequate sample sizes; (3) Justifying fixed values for factors that significantly affect results while allowing flexibility for irrelevant factors. (4) Publishing standardized protocols with broad expert consensus, ensuring clinical usefulness is also considered.

Regarding the availability of normative data, most tests lack adequate norms for score interpretation. Notably, Study 1 contributed to address this gap for the widely-used TLT task, providing normative data for 40-, 20-, and 10-item versions based on a sufficient number of healthy participants. However, other measures still lack well-established normative data, limiting the reliability of these tests in interpreting individual scores. Although one study has tried to build normative data for tERP to $CO_2$ stimuli, the sample size was only 18 (Rombaux et al., 2006). Ammonia intensity ratings have also been normed, with ratings below the 10th percentile suggesting trigeminal dysfunction (Sekine et al., 2022), but this was based on data from OD patients, making it difficult to apply to the general population. Overall, it is essential to establish normative data for threshold tests, trigeminal probes, and tERP/NMP, while also evaluating the clinical utility of TLT and ammonia rating norms in distinguishing patients with trigeminal dysfunction (beyond OD) from healthy individuals.

When it comes to the test-retest reliability, most trigeminal tests show moderate ($0.5<r<0.8$) to good ($0.8<r<0.9$) test-retest reliability (Frasnelli & Hummel, 2003; Huart et al., 2019; Hummel et al., 2016; Hummel, Kraetsch, et al., 1998b; Welge-Lüssen et al., 2003; Yan et al., 2023). However, the correlation of each test type was based on a very limited number of studies, requiring stronger evidence.

### 6.1.2. Validity

Validity refers to the extent to which a test accurately measures what it is intended to measure. In other words, it determines whether the results or conclusions drawn from a test or research study are accurate and meaningful (Ahmed & Ishtiaq, 2021; Nahid Golafshani, 2003). Based on this definition, we justified these tests' ability to *differentiate pathological cases from healthy individuals*, its *inter-task correlation*, and whether it *comprehensively covers all aspects of intranasal trigeminal function*.

Overall, all tests appear to demonstrate at least some degree of discriminant validity, which is important for clinical applicability. TLT is the most widely used test for studying pathological cases and currently shows the strongest discrimination ability among them. It has been able to distinguish between healthy individuals and patients with empty nose syndrome, chronic sinusitis (with and without polyps), allergic rhinitis, chronic nasal obstruction, and acute cold (Frasnelli et al., 2007a; Hernandez et al., 2024; Huart et al., 2019; Hummel, Futschik, et al., 2003; Konstantinidis et al., 2017; Migneault-Bouchard et al., 2021, 2024; Saliba et al., 2016). Other tests have also shown the ability to differentiate healthy individuals from some of these clinical conditions (Burghardt et al., 2023; Danioth et al., 2020; Frasnelli et al., 2007a; Huart et al., 2019; Hummel et al., 2016; Pellegrino et al., 2017; Poletti et al., 2017; Sekine et al., 2022; Tremblay et al., 2019). However, since not all types of pathological cases have been assessed using different intranasal trigeminal measurements (e.g., no tERP or NMP measurements for ENS), further studies are needed to provide a more comprehensive understanding of discriminant validity across different tests.

Due to currently no gold standard or widely accepted definition of intranasal trigeminal

dysfunction, measuring intranasal trigeminal function is challenging. Therefore, cross-validation among different tests is essential to evaluate the validity of these tasks, and inter-task correlations can help determine whether different measures are related. Currently, eucalyptol TLT shows extensive correlations with other measures, such as ammonia intensity ratings, tERP, TRPM8 protein levels, TRPA1 mRNA expression (unpublished data, see Appendix), and trigeminal probes (Hernandez et al., 2024; Huart et al., 2019; Juratli et al., 2023; Migneault-Bouchard et al., 2024; Pellegrino et al., 2017; Stuck et al., 2006; Weise et al., 2024). However, no correlation has been found between TLT and the $CO_2$ threshold test (Hernandez et al., 2024; Pellegrino et al., 2017). The $CO_2$ threshold test was found to correlate with TRPM8 expression levels (Weise et al., 2024), but other threshold tests remain understudied. Moreover, the correlation between electrophysiological measurements and other psychophysiological tests has not been thoroughly explored, only found to be related to intensity ratings (Mai et al., 2025; Meusel et al., 2010). Future studies should aim to provide a comprehensive analysis of the correlations between all these measures.

In terms of content validity, which refers to whether a test comprehensively covers all aspects of intranasal function, only trigeminal probes test incorporates multiple types of stimuli (TRPA1, TRPV1, TRPM8, TRPV3) (Huart et al., 2019). This allows them to assess a broad spectrum of intranasal trigeminal activation. However, other tests focus on a single stimulus type. For example, the TLT test typically uses eucalyptol, which activates the TRPM8 receptor (Mai et al., 2024). In contrast, $CO_2$ threshold, NMP/tERP tests, which typically use $CO_2$ that activate primarily TRPA1 (and possibly TRPV1). Thermal, mechanical, and electrical threshold tests respectively activate their specific receptors as well. As discussed in Study 3, stimuli that activate different receptors lead to distinct response patterns, meaning that each test may reflect different aspects of intranasal trigeminal function. It remains evident that tests involving only one type of stimulation may not comprehensively capture all facets of intranasal trigeminal function, thus limiting content validity. This is a challenge because the use of multiple stimuli would lengthen testing times, increasing test burden and practical difficulties. One potential solution is

using a simple nasal swab, as done by Weise et al. (2024) and in our unpublished study (see Appendix), to collect samples from the nasal cavity and analyze the expression levels of various TRP channels. However, it is essential to first determine whether these physiological markers correlate with current behavioral and electrophysiological measurements and whether they can effectively differentiate pathological cases from healthy individuals. Another option for clinical practice is to prioritize relevant stimuli based on patients' complaints and screening-based subjective ratings with various stimuli that activate different receptors (as discussed in the clinical application section).

### 6.1.3. Sensitivity and Specificity

Sensitivity and specificity are two important concepts used to evaluate the performance of a diagnostic test. Sensitivity refers to the ability of a test to correctly identify individuals who have the condition or disease (i.e., the true positives). Specificity refers to the ability of a test to correctly identify individuals who do not have the condition (i.e., the true negatives). As mentioned earlier, there is currently no widely accepted definition of intranasal trigeminal dysfunction, and lack of formally diagnosed with this condition. Consequently, there is still a lack of evidence regarding the diagnostic sensitivity and specificity of these tests.

In relation to these concepts, sensitivity is also linked to a test's ability to detect subtle changes or variations, while specificity is concerned with whether the test results reflect only the targeted function (e.g., trigeminal function) and are not confounded by other factors, such as cognitive strategies or olfactory sensations.

Among all types of measurements, NMP appears to exhibit good sensitivity in detecting small differences in trigeminal activation. Thurauf et al. (2002) investigated NMP sensitivity to small $CO_2$ concentration increments (3% v/v) and reported significant differences in NMP amplitudes between 62% v/v, 65% v/v, and 68% v/v. Frasnelli et al. (2003) observed a linear increase in tERP amplitudes with $CO_2$ concentrations (45%, 50%, 55%, 60%, 65%) but did not provide statistical comparisons between conditions. Interestingly, $CO_2$ intensity ratings also showed good sensitivity in detecting small

concentration changes, though this was influenced by stimulation duration (Hummel, Mohammadian, et al., 2003): At 200ms (50%v/v $CO_2$), the just noticeable difference was 2%v/v, while at 1600ms, the same concentration had a just noticeable difference of 3%v/v $CO_2$. Given the low just noticeable difference of CO2 reported by Hummel, Mohammadian, et al., (2003) and the small increments (concentration or duration) used, the $CO_2$ threshold test is likely to show similar sensitivity, though this has not been formally examined.

Regarding the TLT task, Study 2 contributed to its sensitivity assessment, showing that the TLT paradigm is insensitive to very low degrees of trigeminal activation. The measured score followed an accumulative pattern, with significant improvement occurring only after sufficient activation. A concentration-response curve in follow-up Study 2 also showed a similar trend, with significant measurable improvement occurring only at concentrations of 40% and higher. This suggests that TLT may be ineffective in monitoring fluctuations or subtle changes in trigeminal function, e.g., initial recovery or decline of trigeminal sensitivity, following an injury. However, whether its sensitivity would improve with $CO_2$ as the target stimulus remains to be investigated. Future research is also needed to assess the sensitivity of other psychophysiological tests.

Regarding whether test results reflect only the targeted trigeminal function without task-specific confounds, it is clear that methods like the TLT, trigeminal probes, and intensity ratings using non-$CO_2$ stimuli are influenced by olfactory properties. Conversely, threshold testing with $CO_2$, a nearly odorless stimulus, helps isolate trigeminal responses. However, as discussed in Study 3 and the content validity section, $CO_2$ primarily activates TRPA1, meaning that relying solely on $CO_2$ may not capture the full spectrum of trigeminal function. This raises a dilemma: should we sacrifice content validity to improve specificity by separating olfaction from trigeminal function? On one hand, using bimodal odors may run the risk of “overestimation” of trigeminal function, as the odor component can serve as a cue and the olfactory-trigeminal interaction may amplify the overall perception. On the other hand, most natural odors are inherently bimodal, and

assessing them may better reflect real-world sensory processing. In fact, the integration of olfactory and trigeminal inputs is an essential part of normal trigeminal function. Moreover, despite the potential for overestimating trigeminal function, tests using bimodal odors (e.g., eucalyptol TLT) have successfully discriminated patients with trigeminal pathology such as empty nose syndrome (Kanjanawasee et al., 2022) from healthy controls (Konstantinidis et al., 2017; C. Li et al., 2018). From this perspective, evaluating trigeminal function with bimodal odors may offer a more ecologically valid assessment that mirrors real-world experiences.

In addition to the influence of olfaction, test results may also be confounded by cognitive strategies, potentially leading to overestimation. Tasks that rely heavily on participants' responses, such as stimulus intensity ratings and identification tasks in the trigeminal probes test, are particularly susceptible to this issue. In contrast, tasks like NMP and tERP are less affected, as they rely more on objective physiological measurements rather than participant's responses. While TLT and threshold tests may also be influenced by cognitive strategies, their susceptibility can be minimized. For instance, during the TLT, participants might identify the target based on visual or tactile differences between bottles, such as variations in the cut-edge of the nosepiece or differences in weight. However, these biases can be reduced through blindfolding, randomization, and improving consistency in device preparation.

### 6.1.4. Utility

The utility of different trigeminal function tests varies significantly in terms of cost, time efficiency, ease of administration, participant cooperation, and equipment requirements. Most psychophysiological measurements and subjective intensity rating tasks are relatively easy to conduct and impose minimal burden on participants, with administration times ranging from approximately 5 to 30 minutes, depending on the protocol. However, a major challenge is that these tests are primarily used for research purposes and are not yet commercially available for widespread clinical implementation. Additionally, threshold tests typically require a specialized computer-controlled unit to precisely regulate flow rate and stimulus duration (Hummel et al., 2016), making them more

expensive than TLT and subjective ratings. Trigeminal probes, which involve preparing empty Sniffin' Sticks filled with multiple odors at different dilutions (Huart et al., 2019), are also slightly more costly than TLT and subjective ratings. On the other hand, electrophysiological recordings, while well-established in clinical settings and commercially available, are the most expensive and complex to administer. These tests also require a certain level of participant cooperation, including maintaining attention, controlling eye blinks, minimizing head and muscle movement, and adjusting breathing patterns. Each test has its own advantages and limitations in terms of utility, making the choice highly dependent on the specific research or clinical purpose. Given that none of these methods are entirely comprehensive, an important question arises of whether there is a need to develop new methods that can offer improved reliability, accessibility, and practicality.

## 6.2. Despite Limitations: Optimizing the Application of Existing Trigeminal Testing

Researchers and clinicians may be interested in applying existing approaches despite limitations that cannot be fully addressed immediately. This does not imply disregarding these challenges, but rather understanding the limitations, maximizing each test's strengths, and leveraging them according to their specific purposes. In practice, the measurement of intranasal trigeminal function serves two main purposes: research studies and clinical diagnosis.

For research purpose, ensuring accuracy, reproducibility of results, and the ability to generalize conclusions to the target population are essential, while utility may not be as critical. Depending on the specific research question, the priority of method selection varies. For instance, if the goal is to explore the broad characteristics of the trigeminal system, employing different types of stimuli is crucial regardless of the tests used. This ensures that the conclusions reflect the full spectrum of trigeminal function, taking into account the receptor-specific effects highlighted by Study 3. On the other hand, if the research focuses on investigating fluctuations in trigeminal function, it is important to

choose more sensitive methods. In such cases, using the TLT, which may have limited sensitivity in detecting subtle changes, might not be ideal. Instead, NMP or threshold tests would be more suitable for capturing these fluctuations. If the goal is to completely isolate olfactory influences on trigeminal function, it is crucial to use tests that rely purely on trigeminal stimuli. Methods such as $CO_2$, electrical, or thermal threshold tests, tERP/NMP would be most appropriate. Alternatively, including participants with congenital anosmia (Frasnelli et al., 2007b) could help further isolate trigeminal function by minimizing potential olfactory influences. If TLT is used to study this topic, employing different sets of odors that activate the same receptors might help. Comparing the results of these different odors or using advanced statistical methods to extract common effects could partially reduce or justify the influence of olfaction. For studies investigating the effect of trigeminal function on airflow perception, using TLT with eucalyptol (a TRPM8 activator), thermal threshold (using cold temperatures), or airflow threshold test could provide valuable insights (Garefis & Konstantinidis, 2023; Sozansky & Houser, 2014). If the focus is on studying the detection of noxious odors in the environment, methods such as $CO_2$ threshold testing or TLT with odors activating TRPA1/TRPV1 would be beneficial. Additionally, incorporating self-rating of trigeminal stimuli in the study can always be beneficial, as it is a time-efficient method. However, when giving instructions to participants, it is essential to emphasize that their task is to assess the somatosensations caused by the odor itself, rather than the odor's smell, to ensure the focus remains on trigeminal sensations.

For clinical purposes, the priority shifts to practicality, clinical and ecological validity. As discussed earlier, establishing a clear definition of "intranasal trigeminal dysfunction" is the first step in diagnosis. Fundamentally, the intranasal trigeminal system serves three main functions: (1) detecting irritation and triggering protective reflexes; (2) mediating the perception of airflow; and (3) contributing to overall odor perception. Intranasal trigeminal dysfunction could therefore be defined, subject to refinement through expert consensus, as *an impairment in the intranasal trigeminal system's ability to perform its core functions, manifesting as (1) reduced or excessive sensitivity to irritants, leading to impaired*

*protective reflexes or abnormal responses to noxious stimuli; (2) disrupted or misinterpreted nasal breathing sensations; and (3) disruption in odor perception, including altered somatosensation and impaired olfactory-trigeminal interaction.*

Based on this definition, measurements should focus on these core functionalities. Drawing from clinical assessments of olfaction (Whitcroft et al., 2023), a structured testing framework can be applied to evaluate trigeminal function (See Figure 8):

(1) ***Test Selection*****:** Since multiple tests covering all trigeminal receptors can be time-consuming, it is important to prioritize relevant tasks. This selection can be guided by both patients' complaints and broad stimulus intensity ratings. Patient-reported symptoms serve as primary indicators for selecting the appropriate tests. For individuals experiencing disruptions in airflow perception, tests such as eucalyptol TLT or thermal threshold assessments (using cold temperatures) targeting TRPM8 may be most relevant. This is supported by studies that nasal patency is associated with TRPM8 TLT, but not with TRPA1 TLT (Migneault-Bouchard et al., 2024). For patients reporting difficulty detecting irritants (e.g., gas leaks or hazardous chemical vapors), $CO_2$ threshold testing is more relevant, as $CO_2$ primarily activates TRPA1/TRPV1, which mediate painful and irritating sensations typically associated with these noxious stimuli (Hummel & Frasnelli, 2019). Similarly, individuals experiencing persistent burning sensations may benefit from $CO_2$ testing, as they are typical sensations medicated by TRPA1 or TRPV1. Additionally, subjective intensity ratings of multiple receptor-specific stimuli serve as a secondary screening tool to identify overlooked aspects that may not directly correspond to subjective complaints.

(2) ***Psychophysical Assessments and Interpretation of Initial Results:*** based on patient-reported symptoms and VAS screening outcomes, corresponding psychophysical tests (e.g., TLT, threshold tests, trigeminal probes) should be conducted. If using eucalyptol TLT, the 40-item version is the most useful. However, for clinical practice, an adapted procedure could be used:

- If, within the first 10 items, patients score 9-10, the task stops and is categorized as "normal." If they score 0-5, the task stops and is categorized as "decreased

function." If the score is between 6-8, the test continues until 20 repetitions.

- If, within the first 20 repetitions, the score is 15-20 (normal) or 0-10 (decreased), the test stops. If they score 11-14 (grey zone), the full 40 items should be completed.

(3) ***Confirmation Testing:*** In the diagnosis of olfactory disorders within Smell and Taste Clinics, electrophysiological recordings serve as valuable tools to confirm whether individuals who score within the anosmic range on psychophysical tests still process olfactory information at the cortical level. Thus, employing these electrophysiological methods (tERP, NMP) with various trigeminal stimuli can help validate whether trigeminal function is reduced or nearly absent in patients whose psychophysical results are abnormal or inconclusive (falling within a diagnostic "grey area").

(4) ***Diagnosis:*** Intranasal trigeminal function is classified as normal, reduced, or abnormal based on results, guiding appropriate clinical decision-making, including referrals for medical management, surgery, or other treatments.

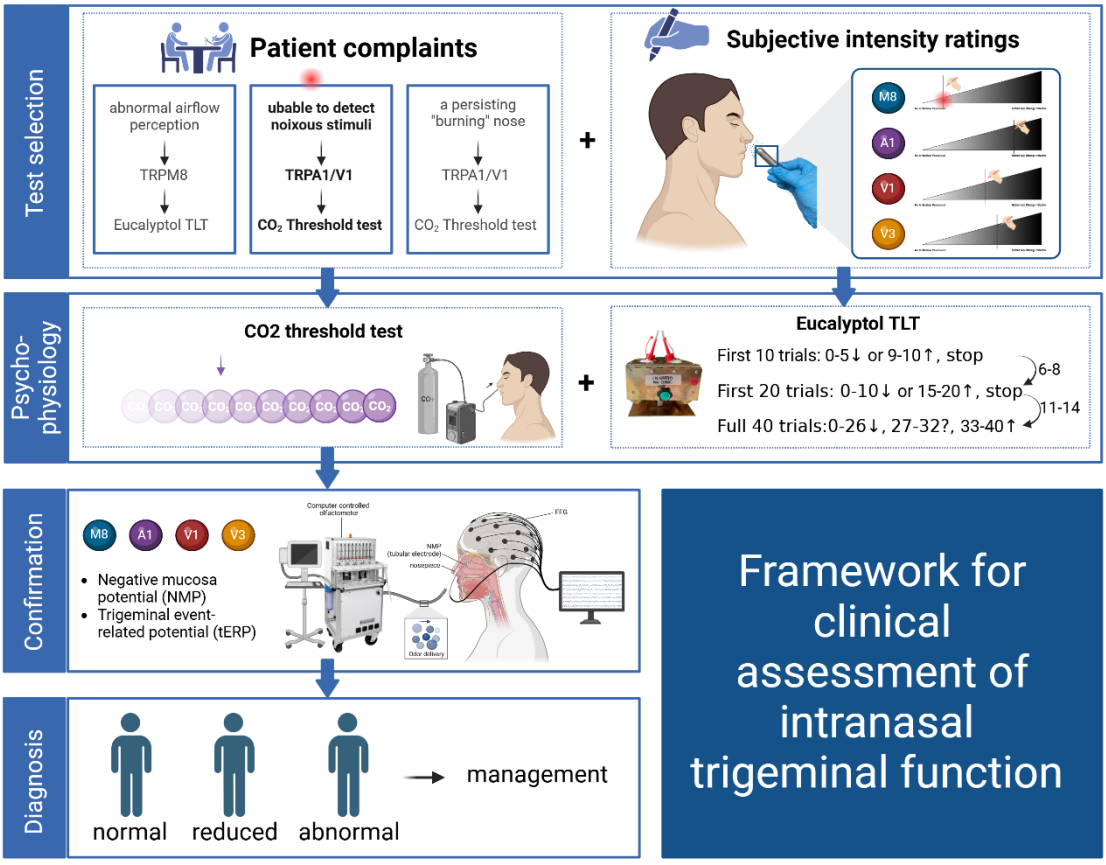



**Figure 8. A potential testing framework for intranasal trigeminal function**

## 6.3. Limitations

The primary limitation across all three studies is sample size. In Study 1, some age groups had limited sample, which requires larger samples to confirm the stability of the cutoff values. Similarly, Studies 2 and 3 would benefit from larger samples to strengthen the robustness of the results.

Another common limitation concerns the influence of olfactory properties and receptor co-activation. In Study 1, the normative data were based on eucalyptol, which activates TRPM8 but also has a strong olfactory component. Comparing these cutoff values with those from other TRPM8 activators, such as menthol, could provide a clearer understanding of intranasal trigeminal function. Study 2 faced similar challenges. Although this is difficult to fully separate, repeating the study with different odors would help confirm the findings, as addressed in our follow-up study (see *Publication 2 Discussion*). In Study 3, all stimuli except $CO_2$ activated both olfactory and trigeminal systems, with receptor co-activation observed. Future research should employ stimuli that selectively target individual receptors or include patients with anosmia to minimize such influences.

The representativeness of the stimuli also requires further validation. While Study 3 demonstrated receptor-specific trigeminal responses, Studies 1 and 2 focused on a single type of trigeminal stimulus. Future studies should include irritants that activate other trigeminal receptors to provide a broader understanding of intranasal trigeminal function. For example, using $CO_2$ in Study 2 could clarify whether the lateralization test is more sensitive in detecting minor activation, while Study 1 could examine whether odors activating other receptors yield different normative data.

Several methodological aspects also require improvement. In Study 1, the lateralization test should be repeated using various devices, such as an olfactometer, to assess the repeatability of results. Study 2 would benefit from a concentration gradient to better characterize the dose-response relationship, as discussed in our ongoing follow-up study (see *Publication 2 Discussion*). In Study 3, verifying receptor-specific effects with the

same stimuli using other methods, such as the TLT, could further strengthen the findings.

# 7. Conclusions

This thesis focuses on understanding the limitations and refining the current assessment methods of human intranasal trigeminal function, a relatively under-studied chemosensory system. Through three independent studies, we established normative values for the most widely used intranasal trigeminal task, the eucalyptol TLT, which help interpret individual scores and categorize functionality into three groups: normal (33-40 out of 40; 15-20 out of 20; 9-10 out of 10), decreased function (0-26 out of 40; 0-10 out of 20; 0-5 out of 10), and inconclusive (27-32 out of 40; 11-14 out of 20; 6-8 out of 10), which require additional assessment. Our findings indicate that the number of repetitions used in the TLT impacts its interpretability, with the 40-item version appearing to be the most effective. A significant challenge identified with the TLT is its limited sensitivity in detecting minor trigeminal activation, and thus might not be effective in monitoring fluctuations or early changes, such as initial recovery or decline, in trigeminal function. Additionally, we identified receptor-specific effects in the trigeminal system, which were consistently observed at the central, peripheral, and behavioral levels, underscoring the complexity of intranasal trigeminal activation and the limitation of relying on a single stimulus to capture its full scope. Lastly, the existing challenges and potential improvement strategies for widely used trigeminal function tests were summarized and discussed. Based on these insights, a structured measurement framework for clinical practice was proposed, which hopefully would be refined and applied in the near future.

# SUMMARY


### Introduction

The intranasal trigeminal system plays a crucial role in a complete chemosensory perception, detecting noxious irritants and triggering protective reflexes, and regulating airflow perception. An accurate measurement is essential, yet it remains less studied and developed than other chemosensory systems, with challenges and gaps overlooked. This thesis critically evaluates these challenges and aims to enhance the effectiveness of current assessment methods.

### Hypothesis

In Study 1, we established normative data for each TLT item version and defined potential boundaries to distinguish between “normal” and “decreased” lateralization ability. We hypothesized that a majority of the healthy population (90%) would perform significantly better than chance levels in the 10-, 20-, and 40-trial versions.

In Study 2, we investigated whether combining selective odorants with low levels of trigeminal compounds enhances lateralization performance. We hypothesized that odor lateralization would follow either an “all-or-none” rule, where minimal trigeminal input significantly improves performance, or an “accumulative” pattern, where improvement only occurs with a stronger trigeminal component.

In Study 3, we explored whether odors that activate different intranasal trigeminal receptors (TRPV1, TRPV3, TRPA1, and TRPM8) result in distinct patterns of response. We hypothesized that receptor-specific effects exist, with stimuli activating TRPV1 or TRPA1 (which induce irritating or painful sensations) eliciting greater responses than those that mediate warmth or coolness, due to the evolutionary significance of pain as a warning signal.

### Methodology

In Study 1, we collected eucalyptol TLT scores from healthy adults: 360 participants for the 40-trial version, 284 for the 20-trial version, and 418 for the 10-trial version. We calculated the percentiles of the TLT scores to show data distribution and combined the 10th percentile with an above-chance cutoff based on binomial statistics to define boundaries for categorizing lateralization ability into “normal” (higher than both cutoffs), “decreased” (lower than both cutoffs), and a “grey area requiring additional tests” (between cutoffs).

In Study 2, 81 healthy participants completed the TLT with various olfactory-trigeminal mixtures, including 12 odors: 2 “olfactory,” 2 “trigeminal,” and 8 mixtures, where the olfactory odors were each combined with small amounts (4% and 8%) of trigeminal compounds. GLMM and Chi-square tests were used to compare TLT scores across different odor mixture conditions.

In Study 3, NMPs were recorded from 24 participants and ERPs from 17 participants during exposure to five trigeminal odors activating different TRP channels and one olfactory control. Additionally, 10 participants completed a continuous odor intensity rating task. GLMM with odor intensity as a control was used to compare responses across stimuli conditions, and repeated measures correlation was applied to explore correlations across behavioral, peripheral, and central levels.

**Results**

In Study 1, scores of 33-40/40, 15-20/20, or 9-10/10 were categorized as “normal” function; scores of 27-32/40, 11-14/20, or 6-8/10 were categorized as the “grey area”; and scores of 0-26/40, 0-10/20, or 0-5/10 were categorized as “decreased” ability. Only in the 40-item version, a majority of participants performed above chance level, with the 90th percentile surpassing the above-chance cutoff. While the two shorter versions showed the opposite pattern. The 40-trial version also had a narrower grey area (14%) compared to the 20- (24%) and 10-trial (21%) versions.

In Study 2, GLMM revealed a significant effect of irritant degrees, with TLT scores significantly higher for the 100% irritant compared to the 0%, 4%, and 8% concentrations, with no differences among the latter three. Chi-square tests also showed a higher percentage of participants reaching the above-chance cutoff in the 100% irritant group compared to the lower trigeminal degrees, with no differences among the 0%, 4%, and 8% groups.

In Study 3, GLMM showed significantly different response patterns across stimuli. $CO_2$ and cyclohexanone, which activate TRPV1 (and possibly TRPA1), elicited the strongest responses across NMP, ERP, and continuous rating results, while carvacrol, which activates TRPV3 (and TRPA1), showed the weakest responses, even compared to the olfactory control.

**Conclusions**

Study 1 established a reference distribution for the eucalyptol lateralization task, providing percentiles of scores relative to healthy individuals. The 40-trial version was more effective than shorter versions, due to its narrower gray area, where fewer scores required further testing. This version could serve as an adjunctive test for intranasal trigeminal function. Future studies should evaluate the clinical utility of these norms in distinguishing pathological trigeminal function from normal function.

Study 2 suggested that trigeminal lateralization follows an “accumulative” pattern rather than an “all-or-none” rule. A small amount of trigeminal compound was insufficient to significantly enhance lateralization performance, indicating that the task is insensitive to low trigeminal activation. The 20-trial version may lack sensitivity in identifying odors with low trigeminal irritation, though it doesn't exclude trigeminal activation.

Study 3 found that activation of different trigeminal receptors elicited distinct responses, with patterns consistent across behavioral, peripheral, and central levels. Stimuli involving TRPV1 activation (Cyclohexanone and $CO_2$), associated with irritation or pain, triggered greater responses across all levels compared to stimuli activating other

receptors. This underscores the importance of TRPV1-mediated sensations in survival. Given the diverse response patterns from different TRP receptors, caution is needed when generalizing findings based on a single type of stimulation.

# ZUSAMMENFASSUNG


**Einleitung**

Das intranasale trigeminale System spielt eine entscheidende Rolle in der vollständigen chemosensorischen Wahrnehmung, indem es potentiell schädliche Stoffe erkennt, Schutzreflexe auslöst und die Wahrnehmung des Luftstroms reguliert. Eine genaue Messung der trigeminalen Sensitivität erscheint nützlich, bleibt jedoch weniger erforscht und entwickelt als bei anderen chemosensorischen Systemen, wobei die besonderen Schwierigkeiten und potenziellen Lücken weitgehend unbeachtet bleiben. Diese Dissertation bewertet diese Probleme und versucht letztlich, die Effektivität aktueller Bewertungsmethoden zu erhöhen.

**Hypothese**

In Studie 1 wurden normative Daten für verschiedene TLT-Versionen gesammelt und potenzielle Grenzen definiert, um zwischen „normaler“ und „verminderter“ Lateralisierungsfähigkeit unterscheiden zu können. Dabei wurde angenommen, dass die Mehrheit der gesunden Bevölkerung (90%) in den 10er-, 20er- und 40er-Versionen signifikant besser als Zufall abschneiden würde.

In Studie 2 untersuchten wir, ob die Kombination olfaktorisch spezifischer Düfte mit trigeminalen Duftstoffen in niedrigen Konzentrationen die Lateralisierungsleistung verbessert. Die Duftstoff-Lateralisierung sollte entweder einem „Alles-oder-nichts“-Prinzip folgen, bei dem eine geringe Menge eines trigeminalen Duftstoffes die Leistung signifikant verbessert, oder einem „kumulativen“ Muster, bei dem Verbesserungen graduell mit der Zunahme der trigeminalen Duftstoffe auftreten.

In Studie 3 untersuchten wir, ob Düfte, die verschiedene intranasale trigeminale Rezeptoren (TRPV1, TRPV3, TRPA1 und TRPM8) aktivieren, zu unterschiedlichen Reaktionsmustern führen. Dabei sollten rezeptorspezifische Effekte auftreten, wobei trigeminale Reize, die über TRPV1 oder TRPA1 wirken (die reizende oder schmerzhafte Empfindungen hervorrufen), stärkere Reaktionen auslösen als solche,

die Wärme oder Kühle vermitteln, aufgrund der elementarem Bedeutung von Schmerz als Warnsignal.

**Methodologie**

In Studie 1 sammelten wir die Eucalyptol TLT-Werte von gesunden Erwachsenen: n=360 für die 40er-Version, n=284 für die 20er-Version und n=418 für die 10er-Version. Wir berechneten die Perzentilen der TLT-Werte zur Darstellung der Datenverteilung und kombinierten das 10. Perzentil mit einem über dem Zufall liegenden Grenzwert (basierend auf binomialer Statistik), um Grenzen zur Kategorisierung der Lateralisierungsfähigkeit in „normal“ (höher als beide Cutoffs), „vermindert“ (niedriger als beide Cutoffs) und „Grauzone, die zusätzliche Tests erfordert“ (zwischen den Cutoffs) zu definieren.

Bei Studie 2 nahmen 81 gesunde Teilnehmer an der TLT mit verschiedenen olfaktorisch-trigeminalen Mischungen teil. Sie wurden mit 12 Düfte getestet: 2 „olfaktorischen“, 2 „trigeminalen“ und 8 Mischungen, bei denen die olfaktorischen Düfte jeweils mit kleinen Mengen (4% und 8%) trigeminaler Verbindungen kombiniert wurden. GLMM und Chi-Quadrat-Tests wurden verwendet, um TLT-Werte über die verschiedenen Duftmischungsbedingungen hinweg zu vergleichen.

In Studie 3 wurden NMPs von 24 Teilnehmern und ERPs von 17 Teilnehmern während der Exposition gegenüber fünf trigeminalen Düften, die verschiedene TRP-Kanäle aktivieren, und einem olfaktorischen Kontrollreiz aufgezeichnet. Zusätzlich führten 10 Teilnehmer eine kontinuierliche Duftintensitätsbewertung durch. GLMM mit Duftintensität als Kontrolle wurde verwendet, um die Reaktionen über verschiedene Reizbedingungen hinweg zu vergleichen, und eine wiederholte Messkorrelation wurde angewendet, um Korrelationen zwischen Verhaltensmessungen, sowie auf peripheren und zentralen Verarbeitungsebenen zu untersuchen.

**Ergebnisse**

In Studie 1 wurden Werte von 33-40/40, 15-20/20 oder 9-10/10 als „normale“ Funktion kategorisiert; Werte von 27-32/40, 11-14/20 oder 6-8/10 als „Grauzone“; und Werte von 0-26/40, 0-10/20 oder 0-5/10 als „verminderte“ Fähigkeit. Nur in der 40-Item-Version schnitt die Mehrheit der Teilnehmer besser als der Zufall ab, wobei das 90. Perzentil den zufällig erreichbaren Grenzwert überschritt, während die beiden kürzeren Versionen das entgegengesetzte Muster zeigten. Die 40er -Version hatte auch eine engere Grauzone (14%) im Vergleich zur 20er- (24%) und 10er- (21%) Version.

In Studie 2 zeigte GLMM einen signifikanten Effekt der Reizgrad-Konzentrationen, wobei die TLT-Werte für den 100%-Reiz signifikant höher waren als für 0%, 4% und 8%, ohne Unterschiede zwischen den letzten drei Konzentrationen. Chi-Quadrat-Tests zeigten auch, dass ein höherer Prozentsatz von Teilnehmern den überzufälligen Grenzwert in der 100%-Reizgruppe erreichte im Vergleich zu den niedrigeren trigeminalen Konzentrationen, ohne Unterschiede zwischen den Gruppen 0%, 4% und 8%.

In Studie 3 zeigte GLMM signifikant unterschiedliche Reaktionsmuster über die Stimuli hinweg. $CO_2$ und Cyclohexanon, die TRPV1 (und möglicherweise TRPA1) aktivieren, lösten die stärksten Reaktionen über NMP, ERP und kontinuierliche Bewertungen aus, während Carvacrol, das TRPV3 (und TRPA1) aktiviert, die schwächsten Reaktionen zeigte, selbst im Vergleich zur olfaktorischen Kontrolle.

**Schlussfolgerungen**

Studie 1 erbrachte Normwerte für den Eucalyptol-Lateralisierungstest. Die 40er-Version war aufgrund ihrer engeren Grauzone effektiver als kürzere Versionen, bei denen niedrigere Testergebnisse weitere Tests erfordern. Diese Version könnte als Test für die intranasale trigeminale Funktion angewendet werden, obwohl die klinische Brauchbarkeit in zukünftigen Studien weiter untersucht werden muss.

Studie 2 zeigte, dass die trigeminale Lateralisierung eher einem „kumulativen“ Muster folgt als einem „Alles-oder-nichts“-Prinzip. Eine kleine Menge trigeminaler Reize war

nicht ausreichend, um die Lateralisierungsleistung signifikant zu verbessern, was darauf hindeutet, dass der Test bei niedriger trigeminaler Aktivierung unempfindlich ist. Die 20er-Version könnte auß0erdem unempfindlich gegenüber der Identifikation von Gerüchen mit geringer trigeminaler Reizung sein, schließt jedoch trigeminale Aktivierung nicht aus.

Studie 3 zeigte, dass die Aktivierung verschiedener trigeminaler Rezeptoren unterschiedliche Reaktionen hervorruft, mit Mustern, die über Verhaltensmessungen sowie periphere und zentrale Verarbeitungsebenen hinweg konsistent waren. Reize, die TRPV1 aktivieren (Cyclohexanon und $CO_2$), also mit Irritation oder Schmerz in Verbindung stehen, lösten stärkere Reaktionen auf allen Ebenen aus als Reize, die andere Rezeptoren aktivieren. Dies unterstreicht die Bedeutung der TRPV1-vermittelten Empfindungen im Kontext des Überlebens. Angesichts der unterschiedlichen Reaktionsmuster von verschiedenen TRP-Rezeptoren ist Vorsicht geboten, wenn Ergebnisse basierend auf nur einem Stimulus-Typ verallgemeinert werden.

## Publication Data

(according to Journal Citation Reports, as of April 2025, https://jcr.clarivate.com/jcr/home)

**Rhinology**

"Rhinology is the official Journal of the International Rhinologic Society and one of the journals of the European Rhinologic Society…[and] provides a platform for the dissemination
of rhinologic research and reviews, as well as position papers, task force reports and guidelines, amongst an international scientific audience."

2023 Journal Metrics

Journal Impact Factor: 4.8

5-year Impact Factor: 4.2

Immediacy Index: 1.8

Eigenfactor Score: 0.00248

Normalized Eigenfactor: 0.54497

Article Influence Score: 1.005

Rank (Otorhinolaryngology): 3 / 66

**Behavioural brain research**

"Behavioural Brain Research is an international, interdisciplinary journal dedicated to the publication of articles in the field of behavioural neuroscience, broadly defined. Contributions from the entire range of disciplines that comprise the neurosciences, behavioural sciences or cognitive sciences are appropriate, as long as the goal is to delineate the neural mechanisms underlying behaviour."

2023 Journal Metrics

Journal Impact Factor: 2.6

5-year Impact Factor: 2.8

Immediacy Index: 0.5

Eigenfactor Score: 0.01627

Normalized Eigenfactor: 3.57523

Article Influence Score: 0.742

Rank (Behavioral science): 15/55

**European archives of oto-rhino-laryngology**

“European Archives of Oto-Rhino-Laryngology is an international scientific journal covering

the broad variety of head and neck diseases with an inherent focus on clinical and translational research in all specialties of Oto-Rhino-Laryngology and Head & Neck.”

2023 Journal Metrics

Journal Impact Factor: 1.9

5-year Impact Factor: 2.2

Immediacy Index: 0.4

Eigenfactor Score: 0.0127

Normalized Eigenfactor: 2.79201

Article Influence Score: 0.569

Rank (Otorhinolaryngology): 19 / 66

## Contributions in the Publications

Study 1: Conceptualization, Investigation, data analysis and interpretation, manuscript drafting and revision, visualization, and final approval of the manuscript

Study 2: Investigation, data analysis and interpretation, manuscript drafting and revision, visualization, and final approval of the manuscript

Study 3: Investigation, data analysis and interpretation, visualization, manuscript drafting and revision, and final approval of the manuscript

## Other Publications

(* indicates corresponding author; _ indicates first or co-first author)

**Mai, Y.,*** Rosbach, M. C., & Hummel, T. (2023). Variations of olfactory function with circadian timing and chronotype. Rhinology, 61(5), 456–469. https://doi.org/10.4193/Rhin23.150

**Mai, Y.,*** Burghardt, G. K. L., & Hummel, T. (2025). Olfactory Stimulation Enhances Trigeminal Responses at the Mucosal Level. *The Laryngoscope*, 10.1002/lary.32173. Advance online publication. https://doi.org/10.1002/lary.32173

**Mai, Y.,*** Vogel, C., Thiele, J., Hölscher, T., & Hummel, T. (2023). Abnormal visual and olfactory sensations during radiation therapy: a prospective study. *Strahlentherapie und Onkologie*, *199*(10), 936–949. https://doi.org/10.1007/s00066-023-02095-5

**Mai, Y.,*** Klockow, M., Haehner, A., & Hummel, T. (2023). Self-assessment of olfactory function using the "Sniffin' Sticks". *European archives of oto-rhino-laryngology*, *280*(8), 3673–3685. https://doi.org/10.1007/s00405-023-07872-7

**Mai, Y.,*** Menzel, S., Cuevas, M., Haehner, A., & Hummel, T. (2022). Well-being in patients with olfactory dysfunction. *Physiology & behavior*, *254*, 113899. https://doi.org/10.1016/j.physbeh.2022.113899

Joshi, A., **Mai, Y.,*** Füssel, S., Hummel, T. (2025). Relationship between nasal trigeminal receptor expression and trigeminal sensitivity. (Under review)

## Conferences and Presentations

**April, 2022** **"Well-being in patients with olfactory dysfunction"**
Association of Chemoreception Sciences XLIV Conference
Bonita Springs, Florida, The United States of America

**September, 2022** **"Self-assessment of olfactory function using the 'Sniffin' Sticks' "**
European Chemoreception Research Organization XXXII Conference
Berlin, Germany

**April, 2023** **"Abnormal visual and olfactory sensations during radiation therapy: a prospective study"**
Association of Chemoreception Sciences XLV Conference
Bonita Springs, Florida, The United States of America

**September, 2023** **"Exploring the Mechanism of Phantosmia: Induction of odor phantoms through brain stimulation during radiotherapy"**
European Chemoreception Research Organization XXXIII Conference
Berlin, Germany

**June, 2024** **"ERP and NMP responses to the activation of different trigeminal receptors"**
19th International symposium on olfaction and taste
Reykjavik, Iceland

**April, 2025** **Trigeminal function and its role in flavour**
FLAVOURsome advanced training school
Dresden, Germany

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# Reference


Ahmed, I., & Ishtiaq, S. (2021). Reliability and validity: Importance in Medical Research. *JPMA. The Journal of the Pakistan Medical Association*, *71*(10), 2401–2406. https://doi.org/10.47391/JPMA.06-861

Akiba, Y., Mizumori, M., Kuo, M., Ham, M., Guth, P. H., Engel, E., & Kaunitz, J. D. (2008). CO2 chemosensing in rat oesophagus. *Gut*, *57*(12), 1654–1664. https://doi.org/10.1136/GUT.2007.144378

Anton, F., & Peppel, P. (1991). Central projections of trigeminal primary afferents innervating the nasal mucosa: A horseradish peroxidase study in the rat. *Neuroscience*, *41*(2–3), 617–628. https://doi.org/10.1016/0306-4522(91)90354-Q

Bandell, M., Story, G. M., Hwang, S. W., Viswanath, V., Eid, S. R., Petrus, M. J., Earley, T. J., & Patapoutian, A. (2004). Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron*, *41*(6), 849–857. https://doi.org/10.1016/S0896-6273(04)00150-3

Bassoli, A., Borgonovo, G., Caimi, S., Scaglioni, L., Morini, G., Moriello, A. S., Marzo, V. Di, & Petrocellis, L. De. (2009). Taste-guided identification of high potency TRPA1 agonists from Perilla frutescens. *Bioorganic & Medicinal Chemistry*, *17*(4), 1636–1639. https://doi.org/10.1016/J.BMC.2008.12.057

Bautista, D. M., Siemens, J., Glazer, J. M., Tsuruda, P. R., Basbaum, A. I., Stucky, C. L., Jordt, S. E., & Julius, D. (2007). The menthol receptor TRPM8 is the principal detector of environmental cold. *Nature 2007 448:7150*, *448*(7150), 204–208. https://doi.org/10.1038/nature05910

Boesveldt, S., Haehner, A., Berendse, H. W., & Hummel, T. (2007). Signal-to-noise ratio of chemosensory event-related potentials. *Clinical Neurophysiology*, *118*(3), 690–695. https://doi.org/10.1016/J.CLINPH.2006.11.004

Boonen, B., Startek, J. B., & Talavera, K. (2017). Chemical activation of sensory TRP channels. *Topics in Medicinal Chemistry*, *23*, 73–114. https://doi.org/10.1007/7355_2015_98

Boyle, J. A., Frasnelli, J., Gerber, J., Heinke, M., & Hummel, T. (2007). Cross-modal integration of intranasal stimuli: A functional magnetic resonance imaging study. *Neuroscience*, *149*(1), 223–231. https://doi.org/10.1016/J.NEUROSCIENCE.2007.06.045

Brand, G. (2006). Olfactory/trigeminal interactions in nasal chemoreception. *Neuroscience & Biobehavioral Reviews*, *30*(7), 908–917. https://doi.org/10.1016/J.NEUBIOREV.2006.01.002

Brumm, M. C., Pierz, K. A., Lafontant, D. E., Caspell-Garcia, C., Coffey, C. S., Siderowf, A., & Marek, K. (2023). Updated Percentiles for the University of Pennsylvania Smell Identification Test in Adults 50 Years of Age and Older. *Neurology*, *100*(16), E1691–E1701. https://doi.org/10.1212/WNL.0000000000207077/ASSET/3E760763-BE66-4D1E-9216-F78972405A71/ASSETS/IMAGES/LARGE/10TTU1.JPG

Burghardt, G. K. L., Cuevas, M., Sekine, R., & Hummel, T. (2023). Trigeminal Sensitivity in Patients With Allergic Rhinitis and Chronic Rhinosinusitis. *The Laryngoscope*, *133*(3), 654–660. https://doi.org/10.1002/LARY.30512

Cain WS. (1976). Olfaction and the common chemical sense: some psychophysical contrasts. *Sensory Processes*, *1*, 57–67. https://europepmc.org/article/med/1029077

Caterina, M. J., Schumacher, M. A., Tominaga, M., Rosen, T. A., Levine, J. D., & Julius, D. (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature 1997 389:6653*, *389*(6653), 816–824. https://doi.org/10.1038/39807

Cattaneo, A. M., Bobkov, Y. V, Corey, E. A., Borgonovo, G., & Bassoli, A. (2017). Perilla Derived Compounds Mediate Human TRPA1 Channel Activity. *MEDICINAL & AROMATIC PLANTS*, *6*(1), 1–8. https://www.semanticscholar.org/reader/96061cd6e4559aef6db4ce7bd6617d360957509b

Cervero, F. (2012). *Understanding pain: exploring the perception of pain*. https://doi.org/10.7551/mitpress/8940.001.0001

Clarke, R. W., & Jones, A. S. (1994). The distribution of nasal airflow sensitivity in normal subjects. *The Journal of Laryngology & Otology*, *108*(12), 1045–1047. https://doi.org/10.1017/S0022215100128853

Cordero-Morales, J. F., Gracheva, E. O., & Julius, D. (2011). Cytoplasmic ankyrin repeats of transient receptor potential A1 (TRPA1) dictate sensitivity to thermal and chemical stimuli. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(46), E1184. https://doi.org/10.1073/PNAS.1114124108

Croy, I., Buschhüter, D., Seo, H. S., Negoias, S., & Hummel, T. (2010). Individual significance of olfaction: Development of a questionnaire. *European Archives of Oto-Rhino-Laryngology*, *267*(1), 67–71. https://doi.org/10.1007/S00405-009-1054-0/FIGURES/2

Croy, I., Schulz, M., Blumrich, A., Hummel, C., Gerber, J., & Hummel, T. (2014). Human olfactory lateralization requires trigeminal activation. *NeuroImage*, *98*, 289–295. https://doi.org/10.1016/J.NEUROIMAGE.2014.05.004

Dalton, P., Dilks, D., & Hummel, T. (2006). Effects of long-term exposure to volatile irritants on sensory thresholds, negative mucosal potentials, and event-related potentials. *Behavioral Neuroscience*, *120*(1), 180–187. https://doi.org/10.1037/0735-7044.120.1.180

Danioth, L., Brotschi, G., Croy, I., Friedrich, H., Caversaccio, M. D., & Negoias, S. (2020). Multisensory environmental sensitivity in patients with chronic tinnitus. *Journal of Psychosomatic Research*, *135*. https://doi.org/10.1016/J.JPSYCHORES.2020.110155

Dhaka, A., Uzzell, V., Dubin, A. E., Mathur, J., Petrus, M., Bandell, M., & Patapoutian, A. (2009). TRPV1 Is Activated by Both Acidic and Basic pH. *Journal of Neuroscience*, *29*(1), 153–158. https://doi.org/10.1523/JNEUROSCI.4901-08.2009

Dhaka, A., Viswanath, V., & Patapoutian, A. (2006). TRP ion channels and temperature sensation. *Annual Review of Neuroscience*, *29*(Volume 29, 2006), 135–161. https://doi.org/10.1146/ANNUREV.NEURO.29.051605.112958

Doty, R. L., Brugger, W. E., Jurs, P. C., Orndorff, M. A., Snyder, P. J., & Lowry, L. D. (1978). Intranasal trigeminal stimulation from odorous volatiles: Psychometric responses from anosmic and normal humans. *Physiology and Behavior*, *20*(2), 175–185. https://doi.org/10.1016/0031-9384(78)90070-7

Doty RL. (2020). Self-Administered Waterless Empirical Taste Test (SA-WETT) Administration Manual. In *sensonics*. https://scholar.google.com/scholar?hl=zh-

CN&as_sdt=0%2C5&scioq=Intranasale+trigeminale+Sensitivit%C3%A4t++bei+Kindern+und+Erwachsenen&q=Self%E2%80%90Administered+Waterless+Empirical+Taste+Test+%28SA%E2%80%90WETT%29+Administration+Manual.&btnG=

Earley, S. (2012). TRPA1 channels in the vasculature. *British Journal of Pharmacology*, *167*(1), 13–22. https://doi.org/10.1111/J.1476-5381.2012.02018.X

Filiou, R. P., Lepore, F., Bryant, B., Lundström, J. N., & Frasnelli, J. (2015). Perception of Trigeminal Mixtures. *Chemical Senses*, *40*(1), 61–69. https://doi.org/10.1093/CHEMSE/BJU064

Filiz, G., & Frasnelli, J. (2023). Different Methods to Assess the Trigeminal System. In *Basic Protocols on Emotions, Senses, and Foods* (pp. 3–12). Springer, New York, NY. https://doi.org/10.1007/978-1-0716-2934-5_1

Finger, T. E., Böttger, B., Hansen, A., Anderson, K. T., Alimohammadi, H., & Silver, W. L. (2003). Solitary chemoreceptor cells in the nasal cavity serve as sentinels of respiration. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(15), 8981–8986. https://doi.org/10.1073/PNAS.1531172100/SUPPL_FILE/1172FIG6.PDF

Finger, T. E., Böttger, B., Schaefer, M. L., & Silver, W. L. (2002). Trigeminal collaterals in the nasal epithelium and olfactory bulb: A potential route for direct modulation of olfactory information by trigeminal stimuli. *Journal of Comparative Neurology*, *444*(3), 221–226. https://doi.org/10.1002/CNE.10143

Flohr, E. L. R., Boesveldt, S., Haehner, A., Iannilli, E., Sinding, C., & Hummel, T. (2015). Time-course of trigeminal versus olfactory stimulation: Evidence from chemosensory evoked potentials. *International Journal of Psychophysiology*, *95*(3), 388–394. https://doi.org/10.1016/J.IJPSYCHO.2015.01.014

Frasnelli, J., Albrecht, J., Bryant, B., & Lundström, J. N. (2011). Perception of specific trigeminal chemosensory agonists. *Neuroscience*, *189*, 377–383. https://doi.org/10.1016/J.NEUROSCIENCE.2011.04.065

Frasnelli, J., & Hummel, T. (2003). Age-related decline of intranasal trigeminal sensitivity: is it a peripheral event? *Brain Research*, *987*(2), 201–206. https://doi.org/10.1016/S0006-8993(03)03336-5

Frasnelli, J., Hummel, T., Berg, J., Huang, G., & Doty, R. L. (2011). Intranasal Localizability of Odorants: Influence of Stimulus Volume. *Chemical Senses*, *36*(4), 405–410. https://doi.org/10.1093/CHEMSE/BJR001

Frasnelli, J., Lötsch, J., & Hummel, T. (2003). Event-related potentials to intranasal trigeminal stimuli change in relation to stimulus concentration and stimulus duration. *Journal of Clinical Neurophysiology : Official Publication of the American Electroencephalographic Society*, *20*(1), 80–86. https://doi.org/10.1097/00004691-200302000-00011

Frasnelli, J., & Manescu, S. (2017). The Intranasal Trigeminal System. *Springer Handbooks*, 113–114. https://doi.org/10.1007/978-3-319-26932-0_46/FIGURES/5

Frasnelli, J., Schuster, B., & Hummel, T. (2007a). Interactions between Olfaction and the Trigeminal System: What Can Be Learned from Olfactory Loss. *Cerebral Cortex*, *17*(10), 2268–2275. https://doi.org/10.1093/CERCOR/BHL135

Frasnelli, J., Schuster, B., & Hummel, T. (2007b). Subjects with congenital anosmia have larger peripheral but similar central trigeminal responses. *Cerebral Cortex*, *17*(2), 370–

377. https://doi.org/10.1093/CERCOR/BHJ154

Frasnelli, J., Ungermann, M., & Hummel, T. (2008). Ortho- and retronasal presentation of olfactory stimuli modulates odor percepts. *Chemosensory Perception*, *1*(1), 9–15. https://doi.org/10.1007/S12078-007-9000-2

Garefis, K., & Konstantinidis, I. (2023). The Human Perception of Breathing: How Do We Perceive Breathing and Why Surgery Cannot Always Resolve Nasal Congestion. *Current Otorhinolaryngology Reports*, *11*(3), 245–251. https://doi.org/10.1007/S40136-023-00476-Z/FIGURES/1

Garefis, K., Markou, D., Chatziavramidis, A., Nikolaidis, V., Markou, K., & Konstantinidis, I. (2024). Assessment of intranasal function of the trigeminal nerve in daily clinical practice. *ORL*. https://doi.org/10.1159/000536645/4167008/000536645.PDF

Genovese, F., Xu, J., Tizzano, M., & Reisert, J. (2023). Quantifying Peripheral Modulation of Olfaction by Trigeminal Agonists. *Journal of Neuroscience*, *43*(47), 7958–7966. https://doi.org/10.1523/JNEUROSCI.0489-23.2023

Henriette Friederike Katrin Hornstein-Schnellhardt. (2024). Unterschiede zwischen mit Wohlbefinden assoziierten und anderen Gerüchen–eine fMRT-Studie. In *uniklinikum-dresden.deHFK Hornsteinuniklinikum-dresden.de*. https://www.uniklinikum-dresden.de/de/das-klinikum/kliniken-polikliniken-institute/hno/forschung/interdisziplinaeres-zentrum-fuer-riechen-und-schmecken/downloads/doktorarbeiten/Hornstein_Henriette_2023.pdf/@@download/file/Hornstein_Henriette_2023.pdf

Hernandez, A. K., Uhl, C., Haehner, A., Cuevas, M., & Hummel, T. (2024). Objective nasal airflow measures in relation to subjective nasal obstruction, trigeminal function, and olfaction in patients with chronic rhinosinusitis. *Rhinology Journal*, *62*(4), 394–402. https://doi.org/10.4193/RHIN23.270

Hernandez, A. K., Walke, A., Haehner, A., Cuevas, M., & Hummel, T. (2023). Correlations between gustatory, trigeminal, and olfactory functions and nasal airflow. *European Archives of Oto-Rhino-Laryngology*, *1*, 1–9. https://doi.org/10.1007/s00405-023-07962-6

Huart, C., Hummel, T., Kaehling, C., Konstantinidis, I., Hox, V., Mouraux, A., & Rombaux, P. (2019). Development of a new psychophysical method to assess intranasal trigeminal chemosensory function. *Rhinology*, *57*(5), 375–384. https://doi.org/10.4193/RHIN19.024

Huart, C., Legrain, V., Hummel, T., Rombaux, P., & Mouraux, A. (2012). Time-Frequency Analysis of Chemosensory Event-Related Potentials to Characterize the Cortical Representation of Odors in Humans. *PLOS ONE*, *7*(3), e33221. https://doi.org/10.1371/JOURNAL.PONE.0033221

Hucke, C. I., Heinen, R. M., Wascher, E., & van Thriel, C. (2023). Trigeminal stimulation is required for neural representations of bimodal odor localization: A time-resolved multivariate EEG and fNIRS study. *NeuroImage*, *269*, 119903. https://doi.org/10.1016/J.NEUROIMAGE.2023.119903

Hummel, T. (2000). Assessment of intranasal trigeminal function. *International Journal of Psychophysiology*, *36*(2), 147–155. https://doi.org/10.1016/S0167-8760(99)00108-7

Hummel, T., Barz, S., Pauli, E., & Kobal, G. (1998). Chemosensory event-related potentials

change with age. *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, *108*(2), 208–217. https://doi.org/10.1016/S0168-5597(97)00074-9

Hummel, T., & Frasnelli, J. (2019). The intranasal trigeminal system. *Handbook of Clinical Neurology*, *164*, 119–134. https://doi.org/10.1016/B978-0-444-63855-7.00008-3

Hummel, T., Futschik, T., Frasnelli, J., & Hüttenbrink, K. B. (2003). Effects of olfactory function, age, and gender on trigeminally mediated sensations: A study based on the lateralization of chemosensory stimuli. *Toxicology Letters*, *140–141*, 273–280. https://doi.org/10.1016/S0378-4274(03)00078-X

Hummel, T., Kaehling, C., & Grosse, F. (2016). Automated assessment of intranasal trigeminal function. *Rhinology*, *54*(1), 27–31. https://doi.org/10.4193/RHINO15.002

Hummel, T., & Kobal, G. (1999). Chemosensory event-related potentials to trigeminal stimuli change in relation to the interval between repetitive stimulation of the nasal mucosa. *European Archives of Oto-Rhino-Laryngology*, *256*(1), 16–21. https://doi.org/10.1007/s004050050115

Hummel, T., Kraetsch, H. G., Pauli, E., & Kobal, G. (1998a). Responses to nasal irritation obtained from the human nasal mucosa. *Rhinology*, *36*(4), 168–172. https://europepmc.org/article/MED/9923059

Hummel, T., Kraetsch, H. G., Pauli, E., & Kobal, G. (1998b). Responses to nasal irritation obtained from the human nasal mucosa. *Rhinology*, *36*(4), 168–172. https://europepmc.org/article/med/9923059

Hummel, T., Mohammadian, P., Marchl, R., Kobal, G., & Lötsch, J. (2003). Pain in the trigeminal system: irritation of the nasal mucosa using short- and long-lasting stimuli. *International Journal of Psychophysiology*, *47*(2), 147–158. https://doi.org/10.1016/S0167-8760(02)00150-2

Hummel, T., Roudnitzky, N., Kempter, W., & Laing, D. G. (2007). Intranasal trigeminal function in children. *Developmental Medicine & Child Neurology*, *49*(11), 849–853. https://doi.org/10.1111/J.1469-8749.2007.00849.X

Hummel, T., Sekinger, B., Wolf, S. R., Pauli, E., & Kobal, G. (1997). 'Sniffin' Sticks': Olfactory Performance Assessed by the Combined Testing of Odor Identification, Odor Discrimination and Olfactory Threshold. *Chemical Senses*, *22*(1), 39–52. https://doi.org/10.1093/CHEMSE/22.1.39

Joshi, A., Thaploo, D., Yan, X., Zang, Y., Warr, J., & Hummel, T. (2021). Habitual Exposure to Trigeminal Stimuli and Its Effects on the Processing of Chemosensory Stimuli. *Neuroscience*, *470*, 70–77. https://doi.org/10.1016/J.NEUROSCIENCE.2021.07.006

Juratli, J. H., Garefis, K., Konstantinidis, I., & Hummel, T. (2023). Trigeminal function in patients with COVID-associated olfactory loss. *European Archives of Oto-Rhino-Laryngology*, *1*, 1–9. https://doi.org/10.1007/S00405-023-08391-1/FIGURES/4

Kanjanawasee, D., Campbell, R. G., Rimmer, J., Alvarado, R., Kanjanaumporn, J., Snidvongs, K., Kalish, L., Harvey, R. J., & Sacks, R. (2022). Empty Nose Syndrome Pathophysiology: A Systematic Review. *Otolaryngology–Head and Neck Surgery*, *167*(3), 434–451. https://doi.org/10.1177/01945998211052919

Karunanayaka, P. R., Lu, J., Elyan, R., Yang, Q. X., Sathian, K., & Prasanna Karunanayaka, C. R. (2024). Olfactory–trigeminal integration in the primary olfactory cortex. *Human Brain Mapping*, *45*(10), e26772. https://doi.org/10.1002/HBM.26772

Kashio, M., & Tominaga, M. (2022). TRP channels in thermosensation. *Current Opinion in Neurobiology*, *75*, 102591. https://doi.org/10.1016/J.CONB.2022.102591

Kawai, M., Zhao, J., Ishiguro, H., & Takeyama, H. (2014). Carbon dioxide-pneumoperitoneum in rats reduces ischemia/reperfusion-induced hepatic apoptosis and inflammatory responses by stimulating sensory neurons. *Molecular Medicine Reports*, *10*(3), 1303–1308. https://doi.org/10.3892/MMR.2014.2329

Kim, K. S., Yoo, H. Y., Park, K. S., Kim, J. K., Zhang, Y. H., & Kim, S. J. (2012). Differential effects of acute hypoxia on the activation of TRPV1 by capsaicin and acidic pH. *Journal of Physiological Sciences*, *62*(2), 93–103. https://doi.org/10.1007/S12576-011-0185-4

Kleemann, A. M., Albrecht, J., Schöpf, V., Haegler, K., Kopietz, R., Hempel, J. M., Linn, J., Flanagin, V. L., Fesl, G., & Wiesmann, M. (2009). Trigeminal perception is necessary to localize odors. *Physiology & Behavior*, *97*(3–4), 401–405. https://doi.org/10.1016/J.PHYSBEH.2009.03.013

Kobal, G. (1981). *Elektrophysiologische Untersuchung des menschlichen Geruchssinns*. https://scholar.google.com/scholar?cluster=5063728553798660357&hl=zh-CN&as_sdt=2005&sciodt=0,5

Kobal, G., Klimek, L., Wolfensberger, M., Gudziol, H., Temmel, A., Owen, C. M., Seeber, H., Pauli, E., & Hummel, T. (2000). Multicenter investigation of 1,036 subjects using a standardized method for the assessment of olfactory function combining tests of odor identification, odor discrimination, and olfactory thresholds. *European Archives of Oto-Rhino-Laryngology : Official Journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : Affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery*, *257*(4), 205–211. https://doi.org/10.1007/S004050050223

Kobal, G., Van Toller, S., & Hummel, T. (1989). Is there directional smelling? *Experientia*, *45*(2), 130–132. https://doi.org/10.1007/BF01954845/METRICS

Konstantinidis, I., Tsakiropoulou, E., Chatziavramidis, A., Ikonomidis, C., & Markou, K. (2017). Intranasal trigeminal function in patients with empty nose syndrome. *The Laryngoscope*, *127*(6), 1263–1267. https://doi.org/10.1002/LARY.26491

Lacroix, J. S., & Landis, B. N. (2008). Neurogenic inflammation of the upper airway mucosa. *Rhinology*, *46*(3), 163–165. https://europepmc.org/article/MED/18853864

Laska, M., Distel, H., & Hudson, R. (1997). Trigeminal Perception of Odorant Quality in Congenitally Anosmic Subjects. *Chemical Senses*, *22*(4), 447–456. https://doi.org/10.1093/CHEMSE/22.4.447

Lee, R. J., & Cohen, N. A. (2014). Sinonasal solitary chemosensory cells “taste” the upper respiratory environment to regulate innate immunity. *American Journal of Rhinology and Allergy*, *28*(5), 366–373. https://doi.org/10.2500/AJRA.2014.28.4077/ASSET/EDE5D860-42D4-4ACB-927E-D7E813862127/ASSETS/IMAGES/LARGE/10.2500_AJRA.2014.28.4077-FIG3.JPG

Legrand, C., Merlini, J. M., de Senarclens-Bezençon, C., & Michlig, S. (2020). New natural agonists of the transient receptor potential Ankyrin 1 (TRPA1) channel. *Scientific Reports 2020 10:1*, *10*(1), 1–10. https://doi.org/10.1038/s41598-020-68013-2

Li, C., Farag, A. A., Maza, G., McGhee, S., Ciccone, M. A., Deshpande, B., Pribitkin, E. A., Otto, B. A., & Zhao, K. (2018). Investigation of the abnormal nasal aerodynamics and

trigeminal functions among empty nose syndrome patients. *International Forum of Allergy & Rhinology*, *8*(3), 444–452. https://doi.org/10.1002/ALR.22045

Li, Z., Stolper, S., Draf, J., Haehner, A., & Hummel, T. (2022). Smell, taste and trigeminal function: similarities and differences between results from home tests and examinations in the clinic. *Rhinology*, *60*(4), 293–300. https://doi.org/10.4193/RHIN21.430

Lipp, C., Glushkov, E., Stanley, H. B., Ferdenzi, C., Fieux, M., Bertsch, A., Brugger, J., & Bensafi, M. (2024). Stimulation of the Nasal Cavity Using Flexible PCB Electrodes. *Proceedings 2024, Vol. 97, Page 231*, *97*(1), 231. https://doi.org/10.3390/PROCEEDINGS2024097231

Livermore, A., Hummel, T., & Kobal, G. (1992). Chemosensory event-related potentials in the investigation of interactions between the olfactory and the somatosensory (trigeminal) systems. *Electroencephalography and Clinical Neurophysiology*, *83*(3), 201–210. https://doi.org/10.1016/0013-4694(92)90145-8

Lötsch, J., Huster, A., & Hummel, T. (2022). Sorting of Odor Dilutions Is a Meaningful Addition to Assessments of Olfactory Function as Suggested by Machine-Learning-Based Analyses. *Journal of Clinical Medicine*, *11*(14). https://doi.org/10.3390/jcm11144012

Mackenzie, R. A., Burke, D., Skuse, N. F., & Lethlean, A. K. (1975). Fibre function and perception during cutaneous nerve block. *Journal of Neurology, Neurosurgery & Psychiatry*, *38*(9), 865–873. https://doi.org/10.1136/JNNP.38.9.865

Mai, Y., Flechsig, J., Warr, J., & Hummel, T. (2025). Responses to the activation of different intranasal trigeminal receptors: Evidence from behavioral, peripheral and central levels. *Behavioural Brain Research*, *480*, 115371. https://doi.org/10.1016/J.BBR.2024.115371

Mai, Y., Hernandez, A. K., Konstantinidis, I., Haehner, A., & Hummel, T. (2024). Normative data for the lateralization task in the assessment of intranasal trigeminal function. *Rhinology*, *0*(0), 0–0. https://doi.org/10.4193/RHIN24.063

McKemy, D. D., Neuhausser, W. M., & Julius, D. (2002). Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature 2002 416:6876*, *416*(6876), 52–58. https://doi.org/10.1038/nature719

Melzner, J., Bitter, T., Guntinas-Lichius, O., Gottschall, R., Walther, M., & Gudziol, H. (2011). Comparison of the Orthonasal and Retronasal Detection Thresholds for Carbon Dioxide in Humans. *Chemical Senses*, *36*(5), 435–441. https://doi.org/10.1093/CHEMSE/BJR013

Meusel, T., Negoias, S., Scheibe, M., & Hummel, T. (2010). Topographical differences in distribution and responsiveness of trigeminal sensitivity within the human nasal mucosa. *PAIN®*, *151*(2), 516–521. https://doi.org/10.1016/J.PAIN.2010.08.013

Migneault-Bouchard, C., Boselie, F. J. M., Hugentobler, M., Landis, B. N., & Frasnelli, J. (2021). Trigeminal impairment in treatment-refractory chronic nasal obstruction. *Rhinology*, *59*(3), 312–318. https://doi.org/10.4193/RHIN20.510

Migneault-Bouchard, C., Lagueux, K., Hsieh, J. W., Cyr, M., Landis, B. N., & Frasnelli, J. (2024). Trigeminal cold receptors and airflow perception are altered in chronic rhinosinusitis. *Rhinology*, *62*(1), 63–70. https://www.rhinologyjournal.com/Rhinology_issues/manuscript_3116.pdf

Nahid Golafshani. (2003). Understanding reliability and validity in qualitative research. *The Qualitative Report*, *8*, 597–607.

https://www.brown.uk.com/teaching/HEST5001/golafshani.pdf

Negoias, S., Aszmann, O., Croy, I., & Hummel, T. (2013). Localization of Odors Can Be Learned. *Chemical Senses*, *38*(7), 553–562. https://doi.org/10.1093/CHEMSE/BJT026

Niu, C., Sun, X., Hu, F., Tang, X., & Wang, K. W. (2022). Molecular determinants for the chemical activation of the warmth-sensitive TRPV3 channel by the natural monoterpenoid carvacrol. *The Journal of Biological Chemistry*, *298*(3), 101706. https://doi.org/10.1016/J.JBC.2022.101706

Oleszkiewicz, A., Schriever, V. A., Croy, I., Hähner, A., & Hummel, T. (2019). Updated Sniffin' Sticks normative data based on an extended sample of 9139 subjects. *European Archives of Oto-Rhino-Laryngology*, *276*(3), 719–728. https://doi.org/10.1007/s00405-018-5248-1

Oleszkiewicz, A., Schultheiss, T., Schriever, V. A., Linke, J., Cuevas, M., Hähner, A., & Hummel, T. (2018). Effects of "trigeminal training" on trigeminal sensitivity and self-rated nasal patency. *European Archives of Oto-Rhino-Laryngology*, *275*(7), 1783–1788. https://doi.org/10.1007/S00405-018-4993-5/FIGURES/3

Pellegrino, R., Walliczek-Dworschak, U., Winter, G., Hull, D., & Hummel, T. (2017). Investigation of chemosensitivity during and after an acute cold. *International Forum of Allergy & Rhinology*, *7*(2), 185–191. https://doi.org/10.1002/ALR.21869

Philip, G., Baroody, F. M., Proud, D., Naclerio, R. M., & Togias, A. G. (1994). The human nasal response to capsaicin. *Journal of Allergy and Clinical Immunology*, *94*(6), 1035–1045. https://doi.org/10.1016/0091-6749(94)90122-8

Poletti, S. C., Cuevas, M., Weile, S., & Hummel, T. (2017). Trigeminal sensitivity in chronic rhinosinusitis: topographical differences and the effect of surgery. *Rhinology*, *55*(1), 70–74. https://doi.org/10.4193/RHIN16.194

R Fröhlich. (1851). *Ueber einige modificationen des geruchsinnes*. https://scholar.google.com/scholar?hl=zh-CN&as_sdt=0%2C5&q=Ueber+einige+modificationen+des+geruchsinnes&btnG=

Rombaux, P., Huart, C., Landis, B., & Hummel, T. (2023). Intranasal trigeminal perception. In *Nasal Physiology and Pathophysiology of Nasal Disorders* (pp. 193–204). Springer International Publishing. https://doi.org/10.1007/978-3-031-12386-3_17/FIGURES/3

Rombaux, P., Mouraux, A., Bertrand, B., Guerit, J., & Hummel, T. (2006). Assessment of olfactory and trigeminal function using chemosensory event-related potentials. *Neurophysiologie Clinique/Clinical Neurophysiology*, *36*(2), 53–62. https://doi.org/10.1016/J.NEUCLI.2006.03.005

Saliba, J., Fnais, N., Tomaszewski, M., Carriere, J. S., Frenkiel, S., Frasnelli, J., & Tewfik, M. A. (2016). The role of trigeminal function in the sensation of nasal obstruction in chronic rhinosinusitis. *The Laryngoscope*, *126*(5), E174–E178. https://doi.org/10.1002/LARY.25952

Satterthwaite, F. E. (1946). An Approximate Distribution of Estimates of Variance Components. *Biometrics Bulletin*, *2*(6), 110. https://doi.org/10.2307/3002019

Saunders, C., Li, W. Y., Patel, T. D., Muday, J. A., & Silver, W. L. (2013). Dissecting the role of TRPV1 in detecting multiple trigeminal irritants in three behavioral assays for sensory irritation. *F1000Research*, *2*, 74. https://doi.org/10.12688/F1000RESEARCH.2-74.V1

Scheibe, M., Van Thriel, C., & Hummel, T. (2008). Responses to Trigeminal Irritants at

Different Locations of the Human Nasal Mucosa. *The Laryngoscope*, *118*(1), 152–155. https://doi.org/10.1097/MLG.0B013E318156599D

Scheibe, M., Zahnert, T., & Hummel, T. (2006). Topographical differences in the trigeminal sensitivity of the human nasal mucosa. *NeuroReport*, *17*(13), 1417–1420. https://doi.org/10.1097/01.WNR.0000224764.61959.E8

Sekine, R., Hähner, A., Laudien, M., Mori, E., & Hummel, T. (2022). Ratings of trigeminal stimulation in patients with olfactory loss. *Rhinology*, *60*(4), 313–315. https://doi.org/10.4193/RHIN21.483

Silver, W. L., Clapp, T. R., Stone, L. M., & Kinnamon, S. C. (2006). TRPV1 Receptors and Nasal Trigeminal Chemesthesis. *Chemical Senses*, *31*(9), 807–812. https://doi.org/10.1093/CHEMSE/BJL022

Singh, G. P., & Singh, G. P. (2019). Anatomy of Trigeminal Nerve. *Handbook of Trigeminal Neuralgia*, 11–22. https://doi.org/10.1007/978-981-13-2333-1_2

Sozansky, J., & Houser, S. M. (2014). The physiological mechanism for sensing nasal airflow: A literature review. *International Forum of Allergy & Rhinology*, *4*(10), 834–838. https://doi.org/10.1002/ALR.21368

Stuck, B. A., Frey, S., Freiburg, C., Hörmann, K., Zahnert, T., & Hummel, T. (2006). Chemosensory event-related potentials in relation to side of stimulation, age, sex, and stimulus concentration. *Clinical Neurophysiology*, *117*(6), 1367–1375. https://doi.org/10.1016/J.CLINPH.2006.03.004

Suer, M. (2021). Anatomy of the Trigeminal Nerve. *Trigeminal Nerve Pain*, 5–16. https://doi.org/10.1007/978-3-030-60687-9_2

Thuerauf, N., Kaegler, M., Dietz, R., Barocka, A., & Kobal, G. (1999). Dose-dependent stereoselective activation of the trigeminal sensory system by nicotine in man. *Psychopharmacology*, *142*(3), 236–243. https://doi.org/10.1007/S002130050885/METRICS

Thürauf, N., Günther, M., Pauli, E., & Kobal, G. (2002). Sensitivity of the negative mucosal potential to the trigeminal target stimulus CO2. *Brain Research*, *942*(1–2), 79–86. https://doi.org/10.1016/S0006-8993(02)02697-5

Thürauf, N., Hummel, T., Kettenmann, B., & Kobal, G. (1993). Nociceptive and reflexive responses recorded from the human nasal mucosa. *Brain Research*, *629*(2), 293–299. https://doi.org/10.1016/0006-8993(93)91333-N

Tremblay, C., Emrich, R., Cavazzana, A., Klingelhoefer, L., Brandt, M. D., Hummel, T., Haehner, A., & Frasnelli, J. (2019). Specific intranasal and central trigeminal electrophysiological responses in Parkinson's disease. *Journal of Neurology*, *266*(12), 2942–2951. https://doi.org/10.1007/S00415-019-09517-4/FIGURES/4

Tremblay, C., & Frasnelli, J. (2018). Olfactory and Trigeminal Systems Interact in the Periphery. *Chemical Senses*, *43*(8), 611–616. https://doi.org/10.1093/CHEMSE/BJY049

Viana, F. (2011). Chemosensory properties of the trigeminal system. *ACS Chemical Neuroscience*, *2*(1), 38–50. https://doi.org/10.1021/cn100102c

Vogt-Eisele, A. K., Weber, K., Sherkheli, M. A., Vielhaber, G., Panten, J., Gisselmann, G., & Hatt, H. (2007). Monoterpenoid agonists of TRPV3. *British Journal of Pharmacology*, *151*(4), 530–540. https://doi.org/10.1038/SJ.BJP.0707245

Waldmann, R., Champigny, G., Bassilana, F., Heurteaux, C., & Lazdunski, M. (1997). A proton-gated cation channel involved in acid-sensing. *Nature*, *386*(6621), 173–177. https://doi.org/10.1038/386173A0

Wang, G. (2021). Ligand-stereoselective allosteric activation of cold-sensing TRPM8 channels by an H-bonded homochiral menthol dimer with head-to-head or head-to-tail. *Chirality*, *33*(11), 783–796. https://doi.org/10.1002/CHIR.23364

Wang, Y. Y., Chang, R. B., & Liman, E. R. (2010). TRPA1 Is a Component of the Nociceptive Response to CO2. *Journal of Neuroscience*, *30*(39), 12958–12963. https://doi.org/10.1523/JNEUROSCI.2715-10.2010

Weise, S., Hanslik, P., Mignot, C., Glushkov, E., Bertsch, A., Dubreuil, R., Bensafi, M., Fuessel, S., & Hummel, T. (2024). Hot topic: Mapping of the human intranasal mucosal thermal sensitivity: A clinical study on thermal threshold and trigeminal receptors. *PloS One*, *19*(8). https://doi.org/10.1371/JOURNAL.PONE.0304874

Welge-Lüssen, A., Wille, C., Renner, B., & Kobal, G. (2003). Test-retest reliability of chemosensory evoked potentials. *Journal of Clinical Neurophysiology*, *20*(2). https://doi.org/10.1097/00004691-200304000-00008

Whitcroft, K. L., Altundag, A., Balungwe, P., Boscolo-Rizzo, P., Douglas, R., Enecilla, M. L. B., Fjaeldstad, A. W., Fornazieri, M. A., Frasnelli, J., Gane, S., Gudziol, H., Gupta, N., Haehner, A., Hernandez, A. K., Holbrook, E. H., Hopkins, C., Hsieh, J. W., Huart, C., Husain, S., … Hummel, T. (2023). Position paper on olfactory dysfunction: 2023. *Rhinology*. https://doi.org/10.4193/RHIN22.483

Willis, D. N., Liu, B., Ha, M. A., Jordt, S., & Morris, J. B. (2011). Menthol attenuates respiratory irritation responses to multiple cigarette smoke irritants. *The FASEB Journal*, *25*(12), 4434. https://doi.org/10.1096/FJ.11-188383

Wise, P. M., Wysocki, C. J., & Radil, T. (2003). Time–Intensity Ratings of Nasal Irritation from Carbon Dioxide. *Chemical Senses*, *28*(9), 751–760. https://doi.org/10.1093/CHEMSE/BJG065

Wrobel, B. B., Bien, A. G., Holbrook, E. H., Meyer, G. E., Bratney, N. A., Mesa, J., & Leopold, D. A. (2006). Decreased nasal mucosal sensitivity in older subjects. *American Journal of Rhinology*, *20*(3), 364–368. https://doi.org/10.2500/AJR.2006.20.2862

Xu, H., Delling, M., Jun, J. C., & Clapham, D. E. (2006). Oregano, thyme and clove-derived flavors and skin sensitizers activate specific TRP channels. *Nature Neuroscience 2006 9:5*, *9*(5), 628–635. https://doi.org/10.1038/nn1692

Xu, L., Han, Y., Chen, X., Aierken, A., Wen, H., Zheng, W., Wang, H., Lu, X., Zhao, Z., Ma, C., Liang, P., Yang, W., Yang, S., & Yang, F. (2020). Molecular mechanisms underlying menthol binding and activation of TRPM8 ion channel. *Nature Communications 2020 11:1*, *11*(1), 1–13. https://doi.org/10.1038/s41467-020-17582-x

Yan, X., Menzel, S., Zhao, K., Kim, K., & Hummel, T. (2023). Intranasal trigeminal sensitivity to mechanical stimuli is associated with the perception of nasal patency. *European Archives of Oto-Rhino-Laryngology*, *280*(12), 5391–5399. https://doi.org/10.1007/S00405-023-08126-2/FIGURES/5

Yang, F., Xiao, X., Cheng, W., Yang, W., Yu, P., Song, Z., Yarov-Yarovoy, V., & Zheng, J. (2015). Structural mechanism underlying capsaicin binding and activation of the TRPV1 ion channel. *Nature Chemical Biology 2015 11:7*, *11*(7), 518–524.

https://doi.org/10.1038/nchembio.1835

Yang, F., & Zheng, J. (2017). Understand spiciness: mechanism of TRPV1 channel activation by capsaicin. *Protein and Cell*, *8*(3), 169–177. https://doi.org/10.1007/S13238-016-0353-7

Yin, Y., Le, S. C., Hsu, A. L., Borgnia, M. J., Yang, H., & Lee, S. Y. (2019). Structural basis of cooling agent and lipid sensing by the cold-activated TRPM8 channel. *Science*, *363*(6430). https://doi.org/10.1126/SCIENCE.AAV9334

# Appendix

## Relationship Between Nasal Trigeminal Receptor Expression and Trigeminal Sensitivity

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**Dear Editor:**

Trigeminal receptors, primarily found in the nasal and oral cavities, respond to chemical stimuli that elicit somatosensory experiences such as cooling, burning, or tingling. These receptors are particularly important for nasal airflow perception, many of them belong to the transient receptor potential (TRP) family, including TRPA1, TRPM8, and TRPV1 (1). TRPA1 is activated by pungent compounds found in mustard like allylisothiocyanate and environmental irritants such as cigarette smoke. TRPM8 responds to cooling agents like eucalyptol, while TRPV1 is sensitive to capsaicin, the burning component in chilis (1,2).TRPV1 expressing neurons also co-express TRPA1 and TRPM8 (3).

To quantify intranasal trigeminal sensitivity, the lateralization task (TLT) is used. It assesses an individual's ability to localize stimuli presented unilaterally. Higher lateralization scores are related to increased neural processing of trigeminal stimuli (4), suggesting a potential increased receptor engagement. This study investigates whether individuals with higher lateralization scores exhibit increased expression of TRP receptors.

Thirty-six healthy adults (25.0±2.8 years, 23 women) with self-reported normal olfaction were recruited and confirmed as normosmic using the “Sniffin’ Sticks” extended test. Participants then underwent the TLT with 20 trials of eucalyptol stimulation. Nasal swabs were collected for RNA extraction. TRPA1, TRPV1, and TRPM8 mRNA expression levels were analyzed by qPCR and normalized to the reference genes PPIA/TBP using the 2-ΔΔCT method. Pearson correlation and independent t-tests were used for statistical analyses (descriptive statistics in Supplement).

TLT scores did not significantly correlate with TRPM8, TRPV1, or TRPA1 expression (normalized to TBP: r=-0.18 to 0.17, p’s>0.29; normalized to PPIA: r=-0.21 to 0.16 p’s>0.22). However, when participants were divided into high- (n=14) and low-performing (n=10) TLT groups based on the scores’ upper and lower terciles, differences occurred in TRPA1 expression, with low lateralization group exhibiting reduced TRPA1/TBP ($3.3\times10^{-5}\pm9.7\times10^{-5}$ vs. $3.0\times10^{-4}\pm3.9\times10^{-4}$; t=2.51, p=0.024, Hedges’ g=0.86) and TRPA1/PPIA ($1.1\times10^{-6}\pm3.2\times10^{-6}$ vs. $1.0\times10^{-5}\pm1.4\times10^{-5}$; t=2.45, p=0.027, Hedges’ g=0.84) compared to the high lateralization group, while TRPM8 and TRPV1 remained insignificant (p’s>0.05) (Figure 1).

The most notable finding was that individuals in the low-TLT group exhibited decreased TRPA1 expression levels compared to those in the high-TLT tercile, linking behavioral performance to receptor density. Given that the TLT used eucalyptus that activates TRPM8, it was initially hypothesized that TRPM8 expression would differ between the two groups. However, the observed differences occurred in TRPA1 expression. On one hand, most odors commonly co-activate TRPA1 (but not TRPV1) alongside other TRP channels (5), including eucalyptol that has been shown to activate TRPM8(6). This widespread co-activation may explain the group differences. On the other hand, rodent studies suggest TRPA1, alongside TRPM8, contributes to both innocuous and noxious cold sensations, serving as a complementary or synergistic cold transduction system (7), highlighting TRPA1’s broader role beyond its well-established involvement in nociception. Thus, TRPA1 expression may associate with eucalyptus TLT performance either directly through sensory transduction or indirectly via interactions with other

TRP channels. One possible explanation for the lack of correlation between eucalyptol TLT and TRPM8 expression is that nasal swab primarily samples the superficial mucosa, whereas TRPM8 may be more densely localized in deeper mucosal layers (8), as a biopsy study found correlations between TRPM8 levels and eucalyptol TLT (9). Limited variability among healthy individuals might explain the absent correlation in the total sample, with significant differences only between the upper and lower tercile. Moreover, behavioral performance likely does not scale linearly with TRP channel density, as it is influenced by factors beyond receptor expression alone, such as cognitive aspects.

## Conclusion

Despite its limitations, this study provides promising evidence linking TRP expression to psychophysiological measures, supporting nasal swabs as a simple, biologically objective tool for assessing intranasal trigeminal function.

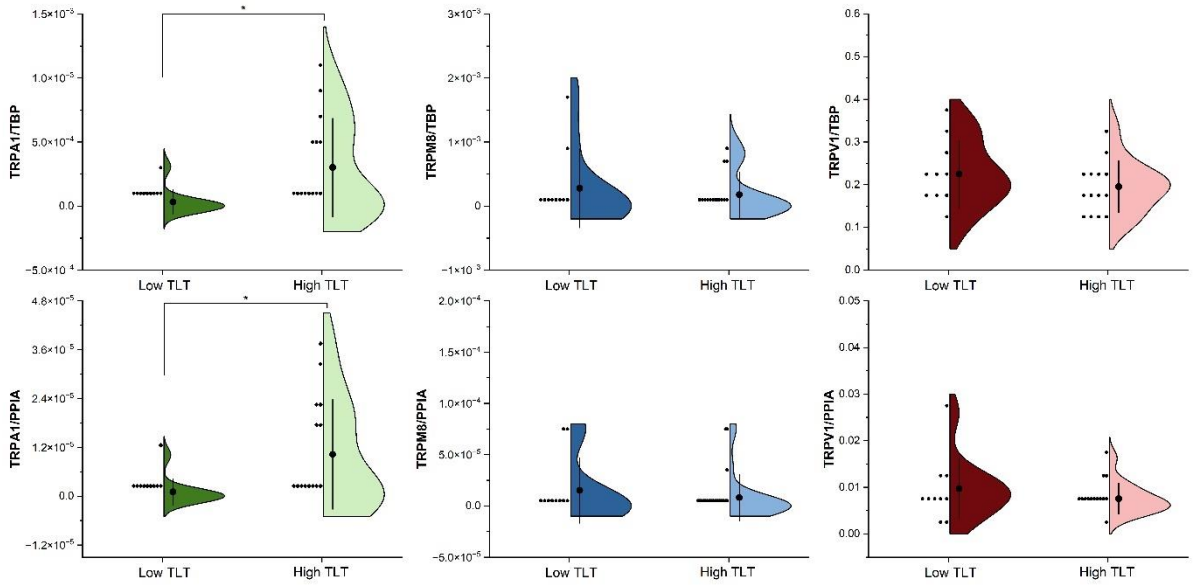


**Figure 1. TRP Expression Levels in Low- and High-Performing Trigeminal Lateralization Groups.**

Note. Violin plots indicate relative expression of TRPA1, TRPM8, and TRPV1 normalized to TBP and PPIA. Each dot represents an individual data point, with black markers indicating the mean and error bars representing standard deviation. Asterisks (*) indicate statistically significant differences.