Changes in olfaction quantified using the University of Pennsylvania Smell Identification Test: Influence of traumatic spinal cord injury on olfactory function in people—a pilot study.

Vidaković, Ema

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Mentor rada: dr. sc. Željka Minić

UNIVERSITY OF RIJEKA

DEPARTMENT OF BIOTECHNOLOGY

Undergraduate program

"Biotechnology and drug research"

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Bachelor's thesis

Rijeka, 2023.

Mentor: dr. sc. Željka Minić

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This bachelor's thesis was defended on the 2nd of June 2023 in front of the Committee:

1. Assistant Professor Christian Reynolds, PhD (president of the committee)

2. Associate Professor Igor Jurak, PhD (member)

3. Assistant Professor Željka Minić, PhD (member)

This thesis has 25 pages, 12 figures and 41 citations.

Sažetak

Traumatska ozljeda leđne moždine (SCI) je disruptivno neurološko stanje koje značajno utječe na kvalitetu života pojedinaca. Ozljede leđne moždine dovode do djelomičnog ili potpunog oštećenja ispod razine lezije i popraćene su kaskadom događaja koji dovode do poremećaja mnogih neuroloških puteva. Među ostalim problemima, u jednoj studiji je prethodno zabilježena korelacija između olfaktornog oštećenja i SCI-ja. Iako je miris osjet kojeg većina smatra najmanje bitnim, njegova važnost se posebno ističe nakon njegova gubitka. Gubitak osjeta mirisa može dovesti do osjećaja ranjivosti, emocionalnih i psihičkih problema, kao i do nemogućnosti prepoznavanja stvarnih opasnosti poput pokvarene hrane ili curenja plina.

Cilj ovog rada bio je testirati olfaktornu funkciju u pojedinaca koji su pretrpjeli traumatsku ozljedu leđne moždine pomoću Testa za identifikaciju mirisa Sveučilišta u Pennsylvaniji (UPSIT). UPSIT je široko primjenjivani standardizirani test od 40 pitanja s 4 ponuđena odgovora razvijen za procjenu kemosenzorne disfunkcije i temelji se na tehnologiji mikroenkapsulacije. Budući da postoje kulturno-specifične verzije istog testa, za testiranje je odabrana talijanska verzija jer nije sadržavala mirise nepoznate hrvatskoj populaciji.

20 ispitanika s traumatskom ozljedom leđne moždine testirano je neovisno o njihovom spolu, dobi, razini lezije i kompletnosti ozljede (potpuna ili nepotpuna). Dobiveni rezultati uspoređeni su s ljestvicom dijagnoze olfaktorne funkcije koja dolazi uz sam test. U 19 od 20 ispitanika (95%) uočena je smanjena olfaktorna funkcija, pri čemu je najviše ispitanika pripalo u kategoriju „blage mikrosmije”. Jedan ispitanik je tvrdio da je nakon ozljede potpuno izgubio i osjet mirisa i osjet okusa. Zanimljivo, kada ih se pitalo kakvom bi oni procijenili svoju olfaktornu funkciju, većina ispitanika ocijenila je svoj osjet mirisa vrlo dobrim ili odličnim.

U zaključku, ovim istraživanjem pokazalo se da većina ljudi koja živi s ozljedom kralježnične moždine ima smanjenu olfaktronu funkciju i da nje često nisu svjesni.

Ključne riječi

Olfaktorna funkcija

Traumatska ozljeda kralježnične moždine

UPSIT (University of Pennsylvania Smell Identification Test)

Summary

Traumatic spinal cord injury (SCI) is a disruptive neurological condition which severely affects the lives of individuals. SCI leads to partial or complete impairment of movement below the level of the lesion and is accompanied by a cascade of events resulting in the disruption of many neurological pathways. Among other complications, one study reported that people living with SCI can experience olfactory impairment. Although smell is a sense people consider non-essential, its importance is highlighted after experiencing difficulties with olfaction. Olfactory impairment can lead to feelings of vulnerability, emotional and mental problems, and an inability to detect real dangers such as spoiled food or gas leaks.

This study aimed to test olfactory function in individuals who suffered traumatic SCI using the University of Pennsylvania Smell Identification Test (UPSIT). UPSIT is a standardized 40-question multiple-choice test that utilizes the odour microencapsulation technology and is widely used for assessing chemosensory dysfunction. Since it is culture-specific, the Italian version was chosen for administration to the Croatian population since it did not contain unfamiliar odours found in other versions.

20 participants with traumatic SCI were tested regardless of their gender, age, levels of the lesion and extent of the injury (complete or incomplete). The obtained scores were compared to the diagnosis scale which comes with the test. 19 out of 20 participants (95%) had reduced olfactory function and most of them fell under the category “Mild Microsmia”. One participant claimed to have lost both a sense of smell and a sense of taste after the injury. Interestingly, when asked to assess their own olfactory function, participants evaluated it as very good or excellent.

In conclusion, this study found that a large proportion of individuals living with SCI have an olfactory impairment and that the majority of them are not aware of it.

Keywords

Olfactory function

Spinal cord injury

UPSIT (University of Pennsylvania Smell Identification Test)

Table of Contents

1. Introduction

1.1. Spinal cord injury (SCI)

Traumatic spinal cord injury (SCI) is a disruptive neurological condition which severely affects the lives of individuals (1). Earlier known as more prevalent among the younger population (2), spinal cord injury has now seen a rise in incidence over the last 30 years. This is likely due to an ageing population, which attributes to 20.6 million cases worldwide and an incidence of 0.9 million cases per year (3). Some of the major causes of SCI include falls, car accidents and explosions. The survival rate varies among the patients based on their condition after the injury itself. A cohort study encompassing 50 years of data revealed that only 47% of tetraplegic and 62% of paraplegic patients are alive 40 years after the injury (4). Traumatic SCI is most prevalent among the male population and this is likely associated with more risky lifestyle choices (3). It is estimated that those patients occupy 2-5% of beds in rehabilitation units (5) which represents a significant burden to the healthcare system especially in a context of a rapidly growing population (3).

Spinal cord injuries lead to partial or complete impairment of movement below the level of the lesion and are accompanied by a cascade of events resulting in the disruption of many neurological pathways (6). After the acute phase which is characterized by ischemia, hypoxia and haemorrhage (7), subsequent events are defined as "secondary injuries" and are characterized by neuroinflammatory changes (8). In response to oxidative stress, inflammation and release of cytokines occur during the subacute and chronic times after SCI leading to apoptosis of neurons and other non-neural cells as well as demyelination and, subsequently, the formation of glial scars (6,8). Such changes occurring at the level of the brain lead to cortical reorganisation (10) and reorganization within subcortical brain regions such as the thalamus, hippocampus (11) and areas responsible for attention and emotions (12). Furthermore, these changes have been associated with cognitive impairment and the development of neurological conditions such as dementia, anxiety and depression, but also the development of neurodegenerative diseases including Parkinson's and Alzheimer's disease (12). Importantly, up to 60% of individuals living with SCI will experience some form of cognitive decline during the chronic stages of injury. Taken together, these data suggest that understanding the changes happening in the whole CNS including the brain is crucial for unravelling pathological SCI mechanisms which lead to poor cognitive and mental health in this patient population.

1.2. Olfaction

The precondition to sensing smell is the solubility of an odorant in the mucus in the nose. That is where molecules of the odorant come in contact with the surface of the olfactory membrane, which is where olfactory cilia, which represent peripheral endings of olfactory receptor cells, are located (**Figure 1**). When the olfactory stimuli are present, olfactory receptor cells, which are first-order neurons, transmit the signal to second-order neurons in the olfactory bulb. The olfactory bulb is an enlargement in the olfactory system located above the cribriform plate and it contains glomeruli, structures which accommodate 25 000 axon endings from bipolar, olfactory neurons (12). The olfactory bulb is then further connected to the olfactory tract which enters the brain and is divided into two tracks. The first track leads to the Medial Olfactory Area, also known as the Primitive Olfactory System, which is a group of nuclei located in front of the thalamus and is responsible for primary reactions associated with olfactory sensations, i.e., production of saliva. The other tract, known as The Lateral Olfactory Area or Less Old Olfactory System, consists of the primary olfactory cortex or piriform cortex and it is connected to the amygdala and hippocampus which have a crucial role in the creation of memory and processing of emotions related to the perceived odours (13). The role of the piriform cortex is the recognition of odours while the hippocampus is the key structure related to the creation of odour memory and recall. This tract is unique since it is the only sensory area directly connected to the cortex without passing through the thalamus which is why certain scents can rapidly evoke the deepest of memories. Additionally, there is another tract discovered called The Newer Pathway which is speculated to have a crucial role in consciousness and analysis of the detected odours (12). Understanding the changes within the olfactory system/or within the olfactory neuronal network can infer alterations and pathological changes within neuronal processing in the brain.

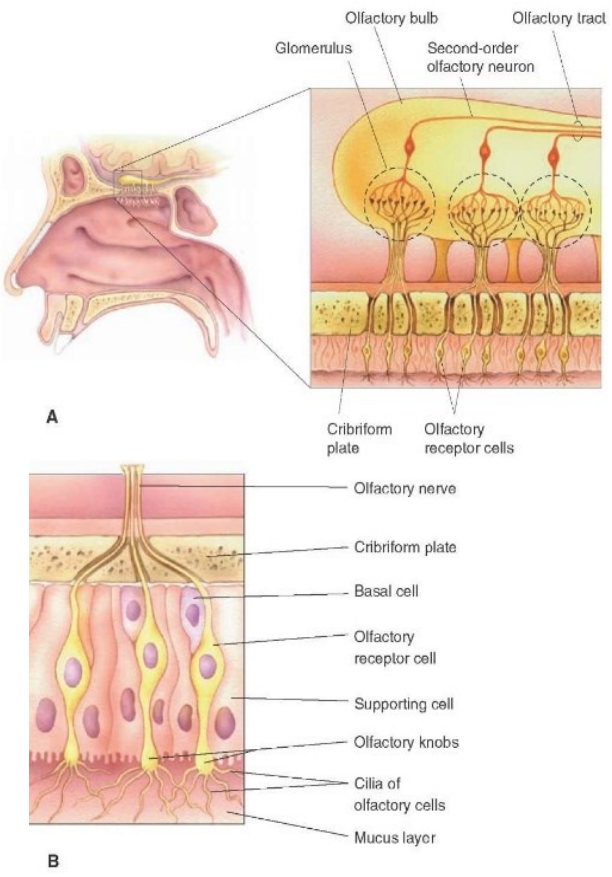

Figure 1: Olfactory system. (A) Position of the olfactory bulb and the olfactory tract in the nasal cavity. (B) Olfactory neurons transmit signals from the mucus layer to the olfactory tract passing through the glomerulus located in the olfactory bulb.

1.3. Smell impairment

Smell is the sense which most would think of as non-essential when compared to the other four senses. However, its importance is highlighted after experiencing a decreased sense of smell or complete smell loss (13). There are many challenges which individuals with olfactory impairment are facing on an everyday basis. They are more exposed to danger since they are less likely to detect leaking gas, spoiled food or smoke (5). They are also often very attentive to their personal hygiene, report lower sex drive and decreased appetite. All of this can lead to decreased social interactions, isolation and decreased quality of life (13).

Smell impairment is common and can be associated with the normal physiology of ageing. Natural processes which occur during ageing can lead to impairments in the olfactory nerve, olfactory tract or olfactory bulb (13). These changes can lead to the thickening of the cribriform plate and the degeneration of olfactory receptors (14). Approximately 50% of people older than 65 years will experience a decrease in the sense of smell and the incidence increases to 75% among people over 80 years of age (5). Although there is a 20% loss in smell sensitivity between the ages of 20 and 80, olfactory loss among the elderly is often undetected (13). Other most common causes of pathological olfactory impairment include head injuries, viral infections, substance abuse (alcohol, smoking, drugs), medication side effects or even vitamin deficiencies (5). To that end, chronic smell loss is associated with the development of anxiety, depression and dementia which suggests its very important role in everyday sensory perception (5, 13). Olfactory impairment can be emotionally devastating since it can lead to feelings of vulnerability, lack of self-confidence and loneliness. The emotional toll associated with the loss of smell is exacerbated by the lack of sympathy from both relatives and medical professionals which cannot provide any promising solutions for their condition (3). Furthermore, decreased olfactory function is considered a strong predictor of neurodegenerative diseases such as depression, Parkinson's, and Alzheimer's disease. Neurotransmitters, such as serotonin and dopamine, have a crucial role in development of the neurodegenerative diseases and are likely to influence olfactory processing within the amygdala and hippocampus (11). Together these data suggest that a certain degree of smell loss can be a consequence of physiological processes associated with ageing, however, smell loss can also infer about the pathological changes occurring in the brain. Therefore, understating olfactory processing and its changes during ageing and in certain disease states is crucial for determining alterations within neuronal processing in the brain.

1.4. Olfactory impairment following SCI

A correlation between olfactory impairment and SCI has been previously reported (10). Hirsch et al. tested olfactory functions in people living with chronic SCI and found a high incidence of hyposmia in this population. Hyposmia is defined as a decrease in the sense of smell. Although the exact mechanism is not known, olfactory impairment occurred independently of other risk factors such as smoking, drugs, medication, other diseases etc. (5). To their surprise, no correlation between olfactory impairment and the level or the severity of the injury was found. It has been long known that SCI induces changes in the brain similar to those happening after traumatic brain injury (TBI) (15) and there is well-established evidence both from animal models as well as clinical populations which links TBI and olfactory impairment. Following impact to the head, the brain moves within the skull and the neural tracts which are passing through the cribriform plate are likely to be damaged (16). To that end, the olfactory nerve (CN I) is the most affected cranial nerve by traumatic brain injuries (17). One anecdotal case dates back to 1964 when a famous chef developed anosmia (complete loss of the sense of smell) after being inflicted upon a tray of chicken which he was carrying while trying to avoid scaffolding (18). Some of the changes occurring at the level of the brain following SCI are similar to those occurring following TBI and include neuroinflammatory processes such as activation of microglia within the cerebral cortex, hippocampus, and thalamus (19). Interestingly, these brain regions which are affected by SCI are also involved in the processing of olfactory sensations. Additional changes in cellular molecular mechanisms which link both pathologies include the downregulation of BDNF (brain-derived neurotrophic factor) and NGF (nerve growth factor) (11). A decrease in hippocampal neurogenesis after SCI leads to downregulation of BDNF which is associated with the development of neurodegenerative diseases in this patient population (20). Following the robust neuroinflammatory stage which spans subacute and chronic timeframes following SCI, neuronal atrophy occurs and is associated with a reduction in the number of olfactory receptors within the olfactory bulb and poor processing of olfactory signals. Therefore, the overarching goal of this study was to investigate potential olfactory deficits in patients with SCI in Croatia.

1.5. Quantifying olfactory function in people: the UPSIT

To assess olfactory function in people with spinal cord injury we used the UPSIT (University of Pennsylvania Smell Identification Test) (21) (**Figure 2**). The UPSIT is a standardized test developed for assessing chemosensory dysfunction of the olfactory system. It is used for quantification of olfactory impairment both in laboratory and clinical settings. It consists of 4 booklets each containing 10 multiple-choice questions (40 questions in total) with 4 given answers to each question and 1 correct option. Underneath the answers which are provided, there is a brown strip containing

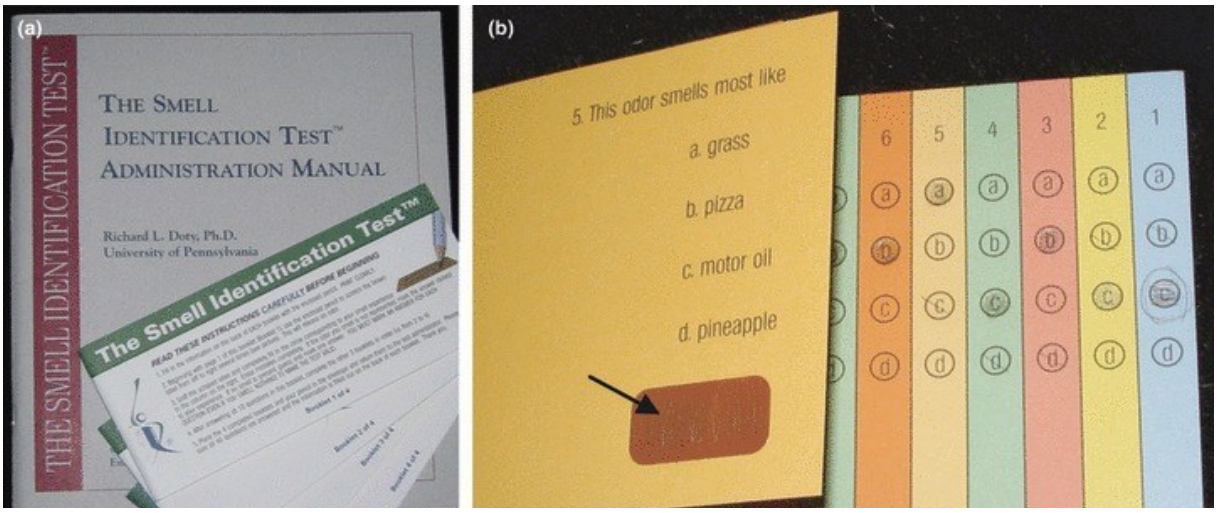

Figure 2: University of Pennsylvania Smell Identification Test (UPSIT) (a) Administration Manual and 4 booklets with 10 multiple-choice questions each. (b) The inside of the booklet with an odour strip.

microencapsulated odours (**Figure 3**) (22). The odours are released upon scratching the strip (preferably with a pencil which comes with the test). The test was developed by a group in Pennsylvania in 1984 and is based on microencapsulation technology (22), technology by which odours are contained within such strips. Microencapsulation technology dates back to the 1930s, however, it was only in the 1970s when the company 3M introduced Microfragrance™ capsules the UPSIT is based on (23). The UPSIT is culture-specific. Each version of the UPSIT is geared towards a certain geographical region and consists of a unique set of odours which are most familiar to people within that geographical area and the culture. For example, there are Italian, German, British, American and Czech versions of the tests which were recommended to us given that there is no specific test for Croatia and the southeast Mediterranean region. For that reason, it was critical to select the most appropriate version of the above-mentioned tests to be used to assess olfactory function in people with SCI in Croatia.

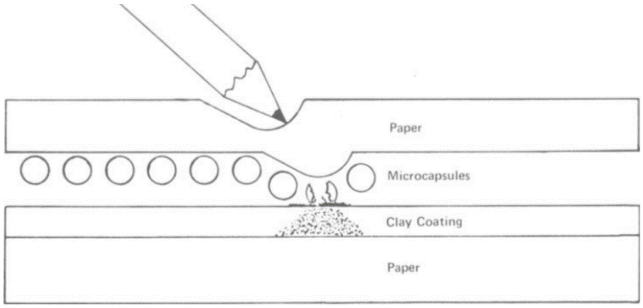

Figure 3: Microencapsulation technology. Scratching the strip with a pencil releases an odour which is contained in microcapsules.

2. Aim

The overarching aim of this study was to determine and quantify smell impairment in people with traumatic spinal cord injury (SCI) living in Croatia. The present study consisted of two specific aims:

1. The aim was to determine the optimal UPSIT version to be used for the assessment of olfactory function in people living in Croatia.
2. The aim was to determine whether chronic SCI leads to the loss of smell.

The overall goal of this study was to set the ground for further research in which specific markers connecting SCI and the olfactory system would be assessed.

3. Materials and methods

3.1. Choosing the optimal version of the UPSIT

All the odours mentioned in all versions of the tests were tabulated and the differences between different versions of the test were quantified by PCA (principal component analysis). Statistical analysis was performed using Rstudio v.4.1.2. and PCA was performed using the following packages: readxl, FactoMineR, factoextra and corrplot.

3.1.1. Survey about the familiarity of the odours

An analysis of the unfamiliarity with odorants was performed in order to determine the optimal version of the UPSIT to be used in the present study. The most optimal version was thought to be the one containing the fewest unfamiliar odours for the Croatian population.

3.1.2. Volunteer details

31 volunteers were interviewed, 7 male and 24 female Croatian nationals between the age of 17 and 69 (mean age 25 ± 13). Volunteers from the following regions of Croatia were included in the study: Istra, Kvarner, Dalmatia, Slavonia, Medimurje, Zagreb and the surrounding area. The volunteers were presented with a survey listing all odours found among 5 different versions of the UPSIT. They were asked to identify odours they are unfamiliar with or those odours they thought would be difficult to identify.

3.1.3. Data analysis

All odours mentioned in 5 versions of the test (Italian, German, British, American and Czech) were ranked based on their "unfamiliarity index" based on the data obtained from the survey. Unfamiliarity index was defined as a percentage of volunteers marking an odour as unfamiliar. The data were expressed as a percentage representing the portion of volunteers who deem each odour unfamiliar. Odours that 25% or more volunteers marked as unfamiliar were plotted based on their prevalence in different versions of the test. The prevalence was obtained based on the number of times an odour appeared in each of the tests and the data were compared among different versions of the test.

3.2. Olfactory assessment in individuals living with SCI

3.2.1. Participant details

20 participants (16, 80% male and 4, 20% female, mean age=42.4 years, SD=12.5 years, range 24 to 73 years) with traumatic spinal cord injury took part in the olfactory testing. People of all genders, ages, levels of the lesion and all extents of the injury (complete or incomplete) were included in the study regardless of their smoking habits and the medications they use.

However, we tested only those whose injuries happened as a result of a traumatic event (car accidents, explosions, falls), or in general, those whose injuries happened after birth. Other spinal cord-related diagnoses such as spina bifida, tumours, neurodegenerative disorders and other types of spinal column and vertebral pathologies were not taken into consideration.

3.2.2. Participant details questionnaire

Prior to the olfactory assessment, participants filled out a 15-point questionnaire (**Figure 4**) containing information about the type of their injury and medication history. Additionally, information about smoking was noted as it was previously found that smoking can markedly affect olfactory function (24).

Participant details
Name and surname
Address, city, and postal code
Phone number, email
Gender
Age
The level at which the injury is located (cervical, thoracal, lumbar, sacral)
The severity of the injury (complete, incomplete, unknown)
The year of the injury
Are you taking any medications at the moment, and if yes, which?
Rate your sense of smell (from 1 to 10)
Was there a head injury accompanied by a spinal cord injury, and if yes, did you fall unconscious
Have you had COVID-19 in the last 3 months
Do you smoke, and if yes, what (cigarettes/cigars/rolled tobacco/electric cigars/other (and specify)
If you smoke, how many a day do you smoke and when have you started?
Which functional recovery would be of the utmost importance to you (rate 1 to 8) – hand/palm function, strength and balance of the upper body, the function of the bladder/gut and elimination of dysreflexia, sexual function, elimination of chronic pain, normal smell perception, being able to move by walking, absence of depression and other mental problems

Figure 4: Information requested within the participant details questionnaire.

3.2.3. Olfactory testing procedure

The UPSIT was administered with the help of an examiner who explained how the test worked. The examiner read a standard explanatory script and used an example card to demonstrate how to scratch, sniff and mark the correct option. The test was subsequently self-administered by the subject if he or she was deemed by the examiner as able to understand and fully cooperate. In other cases, the test was performed with the help of the examiner who scratched the test, read the options aloud, and marked the subject's responses on the answer page. The participants were placed in a room, and they were given a booklet containing microencapsulated odours and multiple-choice questions written in the Italian language alongside the in-house translated sheet with answers written in Croatian language

(**Figure 5**). They were also given instructions that they are not allowed to return to previous questions, had to mark all of them even if they felt as if they could not decide and were allowed to take breaks. The examination, accompanied by the questionnaire, lasted 30-60min. There was no control group since the UPSIT is a standardized test taken by thousands of individuals (21).

3.2.4. Quantifying olfactory function

Normative tables were used to quantify the olfactory function of each participant based on sex and age (**Figure 6**). The normative values are available in the administration manual of the UPSIT (21). Doty et al. assigned scores into groups which represent certain diagnoses – "Normosmia" as no olfactory impairment, then hyposmia divided into three subcategories - "Mild", "Moderate" and "Severe Microsmia", followed by "Anosmia" and "Probable Malingering". Malingering is a term used to describe when individuals are feigning the answers. The normative scale is developed to expose possible cheaters since, even by picking the answers randomly, each participant should score around 10 out of 40 points.

Ime i prezime: _____ **Šifra:** _____

1. knjižica			2. knjižica		
1	A	Benzin	11	A	Čokolada
	B	Pizza		B	Banana
	C	Kikiriki		C	Luk
	D	Jorgovan		D	Voćni sok/punč
2	A	Kiseli krastavac	12	A	Koža
	B	Žvakaća guma		B	Voćni sok/punč
	C	Orah		C	Mentol
	D	Lubenica		D	Limun
3	A	Rajčica	13	A	Crni haribo/dimnjačar bomboni/sladić
	B	Crni haribo/dimnjačar bomboni/sladić		B	Ananas
	C	Jagoda		C	Sir
	D	Mentol		D	Trešnja
4	A	Pivo	14	A	Razrjeđivač
	B	Med		B	Trešnja
	C	Vanilija		C	Kokos
	D	Trešnja		D	Baby puder
5	A	Trava	15	A	Coca-cola
	B	Pizza		B	Cimet
	C	Motorno ulje		C	Bor
	D	ananas		D	Kokos
6	A	Pas	16	A	Ruža
	B	Menta		B	Limun
	C	Riba		C	Breskva
	D	Coca-cola		D	Benzin
7	A	Banana	17	A	Jagoda
	B	Češnjak		B	Kiseli krastavac
	C	Trešnja		C	Čokolada
	D	Motorno ulje		D	Cedrovina
8	A	Crni haribo/dimnjačar bomboni/sladić	18	A	Cedrovina
	B	Klinčić		B	Benzin
	C	Špageti		C	Limun
	D	Banana		D	Guma
9	A	Klinčić	19	A	Limun
	B	Jorgovan		B	Čokolada
	C	Koža		C	Guma
	D	Jabuka		D	Papar
10	A	Pas	20	A	Mentol
	B	Kokos		B	Koža
	C	Cedrovina		C	Jabuka
	D	Med		D	Baby puder

3. knjižica			4. knjižica		
21	A	Jorgovan	31	A	Lubenica
	B	Špageti		B	Kikiriki
	C	Kokos		C	Ruža
	D	Pivo		D	Razrjeđivač
22	A	Razrjeđivač	32	A	Menta
	B	Sapun		B	Jabuka
	C	Pas		C	Trava
	D	Špageti		D	Jagoda
23	A	Čokolada	33	A	Kiseli krastavac
	B	Breskva		B	Trava
	C	Koža		C	Dim
	D	Pizza		D	Riba
24	A	Guma	34	A	Bor
	B	Lubenica		B	Dim
	C	Banana		C	Jorgovan
	D	Dim		D	Naranča
25	A	Ananas	35	A	Pizza
	B	Kiseli krastavci		B	Razrjeđivač
	C	Guma		C	Klinčić
	D	Papar		D	Grožđe
26	A	Dim	36	A	Motorno ulje
	B	Pivo		B	Vanilija
	C	Ananas		C	Ruža
	D	Luk		D	Limun
27	A	Coca-cola	37	A	Sapun
	B	Češnjak		B	Papar
	C	Malina		C	Crni haribo/dimnjačar bomboni/sladić
	D	Limeta		D	Kikiriki
28	A	Baby puder	38	A	Naranča
	B	Naranča		B	Orah
	C	Žvakaća guma		C	Coca-cola
	D	Luk		D	Prirodni plin
29	A	Limeta	39	A	Limeta
	B	Orah		B	Ruža
	C	Riba		C	Menta
	D	Koža		D	Žvakaća guma
30	A	Pivo	40	A	Kikiriki
	B	Mentol		B	Limun
	C	Naranča		C	Jabuka
	D	Lubenica		D	Guma

Figure 5: List of translated multiple-choice questions from the Italian language to Croatian.

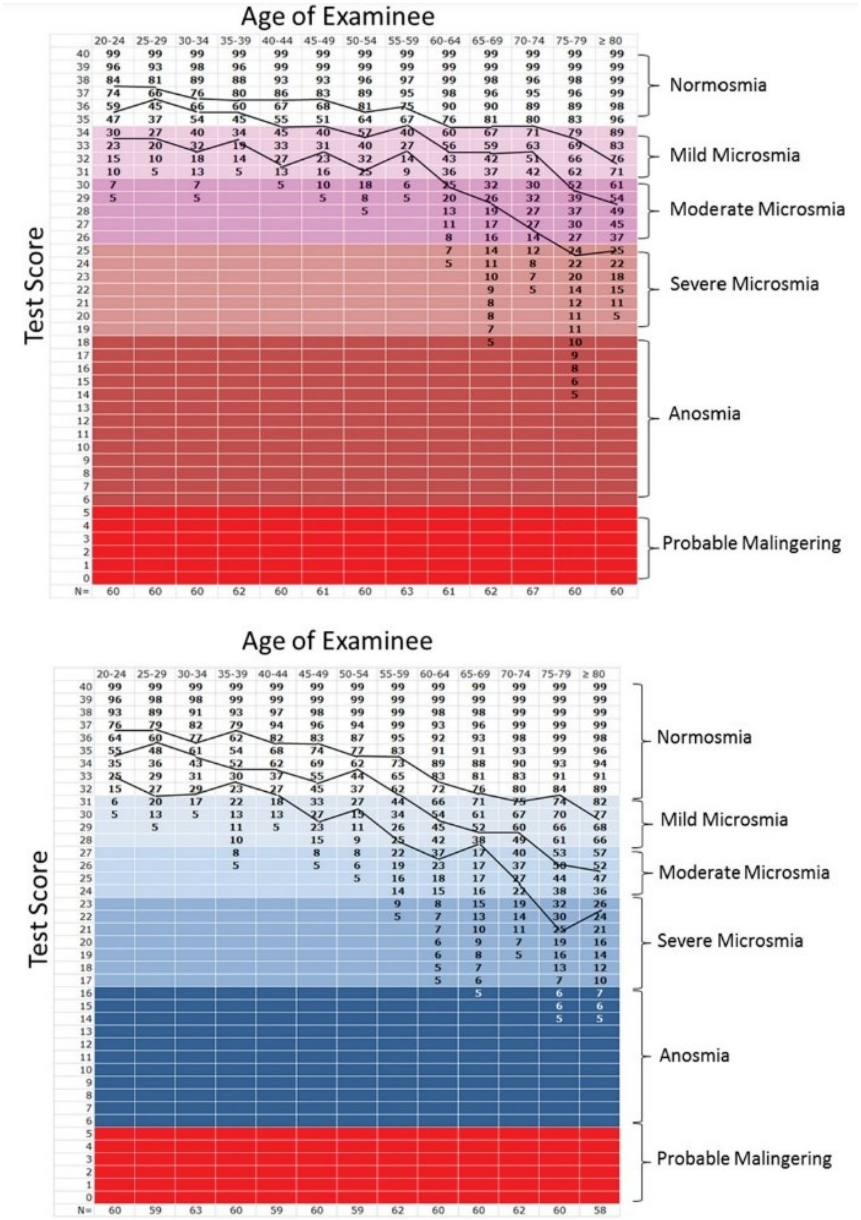

Figure 6: Normative tables used for quantifying olfactory function based on the sex and age of the participant. Under each age range, there are percentiles of the population expected to obtain a certain score.

3.3. Data analysis

Data were analysed using descriptive statistics for the analyses of proportions.

4. Results

4.1. Choosing the UPSIT

In order to investigate the variability among different versions of the test further, odours found in all five versions were counted and PCA (principal component analysis) was performed (**Figure 7**). The biplot shows that the German and Italian tests are similar to each other based on the odours which appear in the tests. The American and Czech versions of the test markedly differ from the German and Italian versions. The UK version of the test is significantly different from all the other tests. Dimension 1, which corresponds to the PCA component 1, contributed to the variability of the tests the most, however, on the variable contribution plot neither of the odours contribute majorly to the variability. In dimension 2, which corresponds to the PCA component 2, there are certain odours on the variable contribution plot that significantly contribute to the variability of the tests (Chutney, Lillac, Dill Pickle, Liniment, Nutmeg, Pepper, Rose). These odours are either the ones which are appearing only in one version of the test and are considered characteristic of that nation or are appearing significantly more times in only certain versions. Additional analyses were performed to choose the most representative test for Croatian nationals. **Figure 8** depicts the unfamiliarity index of all 64 odours mentioned in all 5 versions of the test. Unfamiliarity index was defined as a percentage of participants marking an odour as unfamiliar. The data shows that for the Croatian population liniment, chutney and musk were highly unfamiliar odours. The average unfamiliarity index for these three

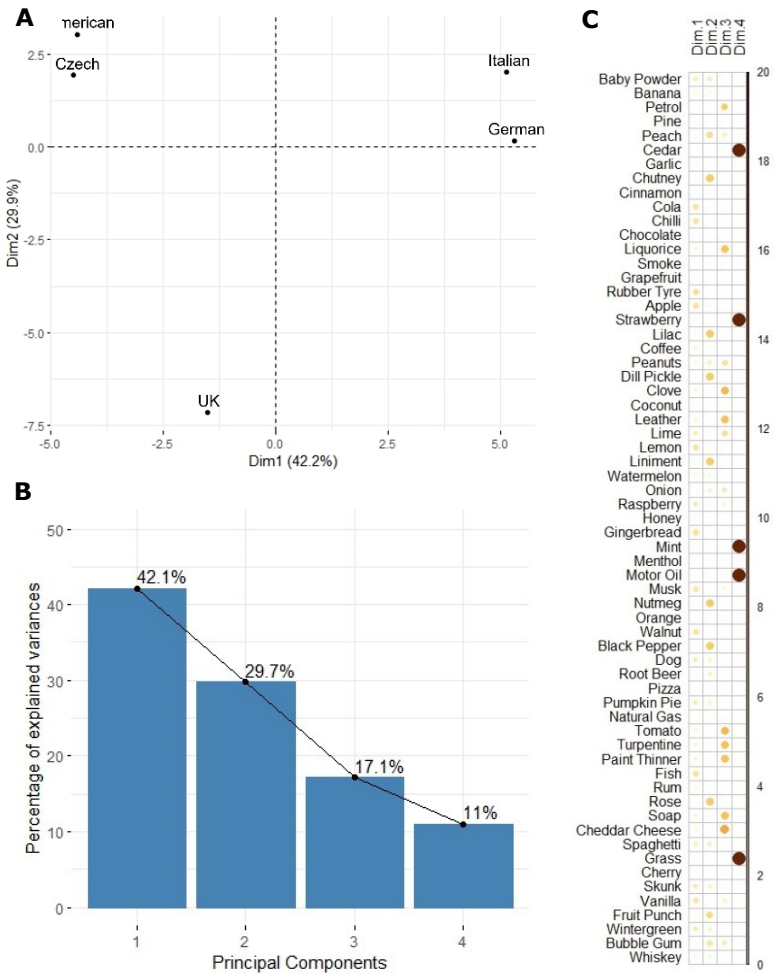

Figure 7: PCA analysis. (A) Differences between versions of the test. (B) Fraction of total variance explained by PC-1 through PC-4. (C) Factor map of individual odours contributing to PC-1 through PC-4. The contribution of odours to PC-1 through PC-4 is proportional to the size and colour of the dots.

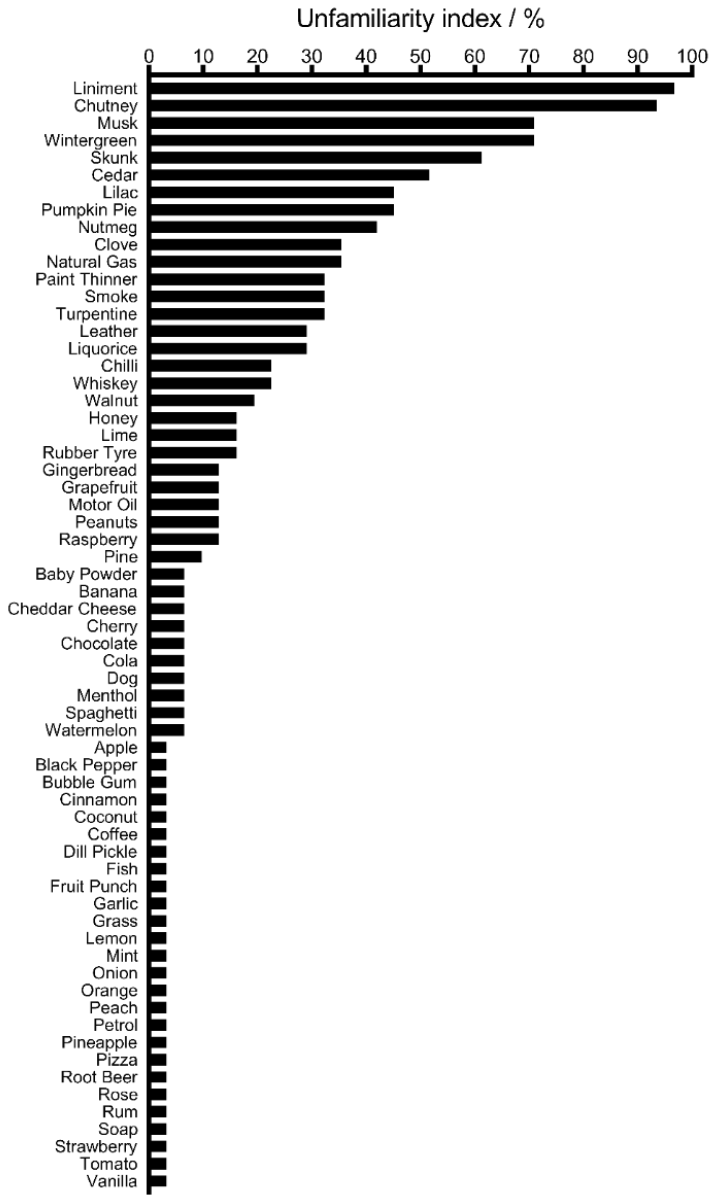

Figure 8: Unfamiliarity with the odours evaluated by the survey. 31 volunteers answered the survey. Unfamiliarity index was defined as a percentage of volunteers marking an odour as unfamiliar.

odours was 88%, which means that the majority of people found them unfamiliar. On the contrary, odours such as vanilla, tomato and strawberry were highly familiar to the participants.

Odours with an "unfamiliarity index" of 25% or higher were plotted by their prevalence in different versions of the test. The prevalence was obtained considering the number of times an odour appeared in each of the versions of the test compared to the number of times other odours (the remaining odours with an "unfamiliarity index" less than 25%) appeared in the test. Given that some odours were found to be more unfamiliar than others, the next step was to determine which test offered the fewest unfamiliar odours.

Figure 9 depicts the incidence of all unfamiliar odours across different versions of the tests. American (USA), Czech (CZE) and British (UK) tests had the highest incidence of unfamiliar odors compared to the German (GER) or the Italian (ITA) version. In the US tests, unfamiliar odours were provided as answers 19.4% of the time while this value reached only 11.9% in the Italian version. Based on the rate of the occurrence of the unfamiliar odours in different versions of the tests, the American,

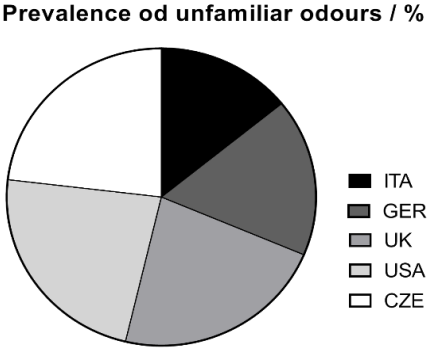

Figure 9: Prevalence of "unfamiliar odours" in different versions of the test. (25% of all odours mentioned in all versions of the UPSIT test ranked as unfamiliar by the Croatian population).

UK and Czech versions of the test were excluded. The German version was excluded since it contained the odour "Musk" which was ranked high on the unfamiliarity scale among the Croatian population. Italian test did not contain any of the highest-ranked unfamiliar odours and was the one with the least number of unfamiliar odours overall. Because of these reasons, the Italian version of the test was selected for use in our research.

4.2. Olfactory testing using the Italian version of the UPSIT

4.2.1. Information about the participants

A total of 20 participants (16, 80% male and 4, 20% female, mean age=42.4 years, SD=12.5 years, range 24 to 73 years) with traumatic spinal cord injury took the UPSIT. Only participants who happened to have had their injury after the birth were selected (average number of years living with injury=17.0 years, SD=11.5). 50% of participants reported their injury was complete, 35% reported it was incomplete and for the remaining 15% of the participants the completeness of the injury was unknown. 55% of participants reported they are currently taking some medication 20% are smokers and 25% are former smokers. At the time of an injury of the spine, 30% of the participants had their head injured and 40% have lost consciousness. Only one participant reported to have had COVID-19 during the past 3 months. 17% of the participants had a lesion at the cervical level, 58% at the thoracal level, 21% at the lumbar level and 4% at the sacral level. Since some participants had their lesions at different sites (e.g., both at the thoracic and the lumbar level), in the table from **Figure 11** it might seem as if there were more than 20 participants when considering only lesion levels. In the questionnaire which preceded the UPSIT testing participants were asked to rank their sense of smell from 1 to 10 (1 being "I can hardly smell anything", 10 being "my sense of smell is excellent"). The average score for the self-assessment of the sense of smell was 7.6, SD=2.3, while the average for male participants was 7.6 and for female participants 7.3. There was one participant who claimed to have lost both a sense of smell and a sense of taste after the injury and, therefore, ranked his sense of smell with 1. If his data would be disregarded, the average overall self-assessment score would be 7.9, SD=1.7, and for male participants 8.1. Finally, the results of the self-assessment of the functional recovery priorities are shown in **Figure 10**. "Being able to move by walking" and "the function of the bladder/gut and elimination of dysreflexia"

Ranking functional recovery
1. Being able to move by walking
2. The function of the bladder/gut and elimination of dysreflexia
3. Strength and balance of the upper body
4. Sexual function
5. Elimination of chronic pain
6. Normal smell perception
7. Hand/palm function
8. Absence of depression and other mental problems

Figure 10: Factors of functional recovery ranked by importance.

were ranked as most important, while "normal smell" was ranked more important than "hand/palm function" and "absence of depression and other mental problems".

4.2.2. The UPSIT results

The average score on the UPSIT was 27.6 points out of 40 (SD=7.6, range 0-34 points) which corresponds to the diagnosis of "Moderate Microsmia" when compared to the tables which come with the test (**Figure 11**). The minimum score of 0 was from a participant who claimed to have completely lost his sense of smell and taste after the injury. Without taking him into consideration, the average UPSIT score for both genders was 29.1 (SD=4.3, range 21-34 points). His score, therefore, is not included in any of the further UPSIT score analyses. The average score for male participants was 28.6 (SD=4.4) which corresponds to "Moderate Microsmia" and the average score for women was 30.8 (SD=3.7) which corresponds to "Mild Microsmia". Only one participant fell under the "Normosmia" category with a score of 34. The lowest score was 21 which corresponded to the diagnosis of "Severe

	Normosmia	Mild Microsmia	Moderate Microsmia	Severe Microsmia	Anosmia	Probable Malingering	Total
Number	1	11	1	6	0	1	**20**
Age (average)	53.0 ± 0	39.5 ± 10.9	28.0 ± 0	48.3 ± 14.0	0	42.0 ± 0	
Years lived with SCI	31.0 ± 0	18.0 ± 12.2	4.0 ± 0	15.3 ± 10.1	0	15.0 ± 0	
Sex							
Male	1	8	1	5	0	1	**16**
Female	0	3	0	1	0	0	**4**
Smoking status							
Smoker	0	3	0	1	0	0	**4**
Non-smoker	1	8	1	5	0	1	**16**
Former smoker	1	1	0	3	0	0	**5**
Lesion Level							
Cervical	0	4	0	0	0	0	**4**
Thoracic	0	8	1	4	0	1	**14**
Lumbar	1	1	1	2	0	0	**5**
Sacral	1	0	0	0	0	0	**1**
Lesion completeness							
Complete	0	6	0	3	0	1	**10**
Incomplete	1	3	1	2	0	0	**7**
Unknown	0	2	0	1	0	0	**3**
Taking medication							
Yes	1	4	1	5	0	0	**11**
No	0	7	0	1	0	1	**9**
Head injury							
Yes	0	4	0	1	0	1	**6**
No	1	7	1	5	0	0	**14**
Loss of consciousness							
Yes	0	5	0	2	0	1	**8**
No	1	6	1	4	0	0	**12**
COVID-19							
Yes	0	1	0	0	0	0	**1**
No	1	10	1	6	0	1	**19**
Self-assessment							
1	0	0	0	0	0	1	**1**
5	0	0	0	2	0	0	**2**
6	0	2	0	1	0	0	**3**
7	0	3	0	0	0	0	**3**
8	1	2	1	0	0	0	**4**
9	0	0	0	1	0	0	**1**
10	0	4	0	2	0	0	**6**

Figure 11: UPSIT scores. Corresponding scores were linked to the "olfactory diagnosis" based on the tables shown in Figure 5 which were provided with the test.

Microsmia". Based on the completeness of the injury, the average UPSIT score for participants whose injury was complete was 29.6, for participants with incomplete injury was 28.7 and 28.3 for participants with unknown injury completeness. Participants who are smokers scored worse on the UPSIT than non-smokers with an average score of 28.0 compared to 29.3 for non-smokers. The average UPSIT score for those participants who had a head injury along with a spine injury was 31.4 and for those who did not the score was 28.2. Among participants who lost their consciousness when the injury happened, the average UPSIT score was 30.3 while for those who did not lose their consciousness the score was 28.3. The participant who reported having had COVID in the past 3 months had an UPSIT score of 31 points which was even higher than the average male score. **Figure 12** ranks all 40 odours, which were correct answers, based on how many times participants answered incorrectly to them. The participants had the most difficulties identifying odours such as lemon, petrol, dill pickle and cinnamon.

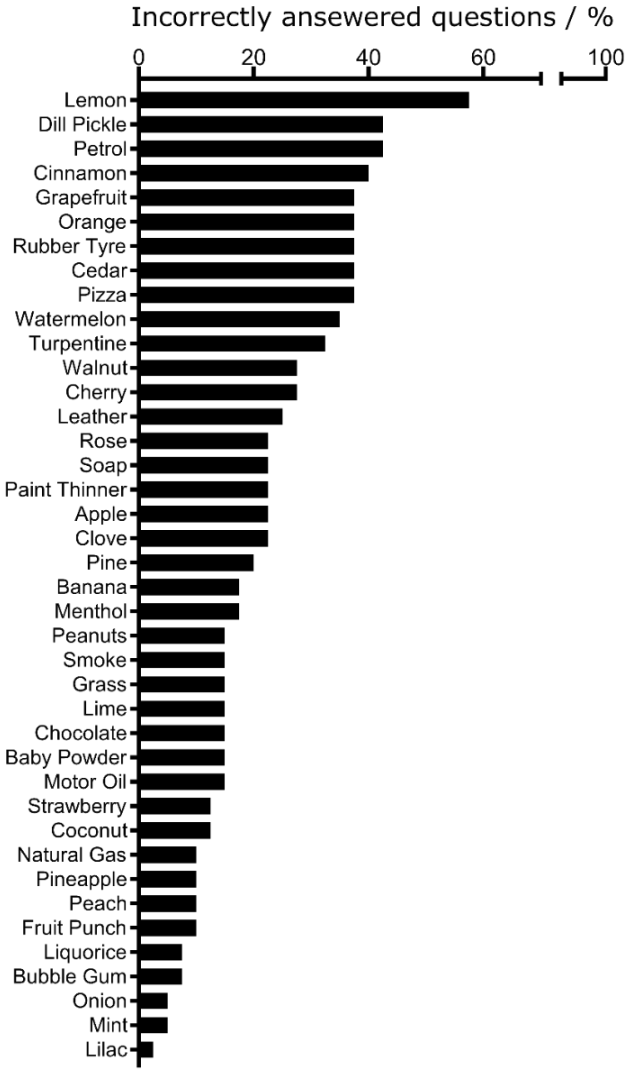

Figure 12: Incorrectly answered questions. % of participants answering incorrectly to given odours.

5. Discussion

The major goal of this study was to assess and quantify olfactory function in people living with SCI in Croatia. Olfactory function was assessed using the University of Pennsylvania Smell Identification Test (UPSIT). We found that 95% of participants had olfactory impairment up to some degree. Olfactory dysfunction after SCI can lead to other difficulties such as eating problems or mental and emotional troubles which altogether further decrease the quality of those people's lives.

SCI is not a rare condition (3) and it impacts the lives of individuals to a great degree. Such injuries lead to partial or complete impairment of movement below the level of the injury but can also cause many other difficulties such as physical pain, problems with bladder and bowel functions, sexual dysfunction, and many other emotional problems (25). One of the reported consequences of SCI is an impairment of the sense of smell (5). The olfactory nerve, which is important for transmitting olfactory signals to the brain, is the most easily damaged nerve following traumatic brain injury (TBI) (17). TBI and SCI have a lot in common since after such injuries inflammation happens in the whole central nervous system (CNS). Moreover, a direct link between SCI and changes in the olfactory tract has been found in animal models (11). Following SCI, primary damages, which involve physical damage to neural structures, and secondary injuries causing neuroinflammation, apoptosis, microglial activation, and downregulation of BDNF and NGF, also cause changes in the olfactory tract. This is possible due to the structural link between important neural tracts. The olfactory pathway directly intervenes with serotonin and dopamine tracks on their way through the hippocampus, amygdala and striatum. Interestingly, dopamine is directly connected to Alzheimer's and Parkinson's disease. Subsequently, SCI can be linked to conditions like subjective cognitive decline which is further linked to the development of mild cognitive impairment which is considered a risk factor for Parkinson's and Alzheimer's disease, stroke and major depressive disorder. The olfactory bulb and the hippocampus happen to be sites in which neural stem cells are found so, after such injuries, neurogenesis is impaired. Based on their results, olfactory impairment in rats can be considered a neurodegeneration marker.

In the present study, the olfactory function was assessed using the UPSIT (University of Pennsylvania Smell Identification Test). It is a test based on the technology of the microencapsulation of odours. It contains 40 forced-answer questions with 4 given alternative answers. Since the test has been translated into 30 languages (26) and 12 cultural adaptations have been made (27), we had to choose the most optimal version for the Croatian population among the 5 versions recommended to us: Italian, American,

German, Czech and UK. There were noticeable differences among the versions. In some cases, only the alternative answers differed but sometimes also the correct answer for a particular question was not the same among all versions. Therefore, a survey was conducted prior to olfactory testing among Croatian nationals to find out which odours are widely perceived as unfamiliar. Eventually, the Italian version was the one chosen for the conducting of the experiment since it did not contain any of the odours which the volunteers ranked as highly unfamiliar and in general it contained the least number of other odours perceived as unfamiliar. Other studies encountered similar problems with the cultural specificity of the test and as a result, more cultural adaptations were made such as the Brazilian (28) or the Arabic (29) version. One Iranian study which was conducted on 60 healthy individuals found that the average score fell under the category "Severe Microsmia" and that only 16 out of 40 odours were answered correctly by 70% of the participants (30). A similar Turkish study reported that the average UPSIT score from their healthy participants was 21/40 which also corresponds to "Severe Microsmia" (31). Both studies argued that the UPSIT was not appropriate for their population and that an appropriate culture-specific test should be made. It is not impossible that the same situation happened in our experiment which is why the UPSIT should be tested on healthy individuals from the Croatian population as a control group even though this is a standardized test taken by thousands of individuals. Moreover, Sensonics International, the company which produces the UPSIT, just released an international version of the test (Smell Identification Test Revised™) which we will be considering in our further studies. Administering the UPSIT on healthy individuals or, even better, developing a Croatian or Mediterranean version of the UPSIT would have given us the most relevant data. Only then we could be sure that the potential unfamiliarity of the odours had no impact on our results.

Other than UPSIT there are many olfactory tests utilized to test different aspects of olfaction. In general, they can be divided into four categories: odour identification tests, odour threshold tests, odour discrimination tests and odour intensity tests (26). Namely, the UPSIT is based on the idea of the recognition of different types of odours while the lower detection limit, which is dependable on the concentration of an odour, is not being tested (23). Along with the UPSIT, other popular odour identification tests include the Sniffin' Sticks test, Alberta Smell Test (16), and some shortened UPSIT versions such as CC-SIT (12-item Cross-Cultural Smell Identification Test), the Pocket Smell Test, the Brief Smell Identification Test (M-SIT) and the Quick Smell Identification Test (Q-SIT) (26). Snap & Sniff test is a popular threshold detection method which uses "smell wands" (26), while Connecticut Olfactory Test (32) and I-Smell (33) are both identification and smell threshold tests. Odour threshold tests are carried out by giving different concentrations of the odours to the subjects either in a descending

or a random manner. Odour discrimination tests are based only on the ability to differentiate between different odours (not identify them) and odour intensity tests are based on the number and frequency of neurons firing after smell stimulation. The latter two are not that widely used in the clinical settings. However, in 2017 two new olfactory tests, a threshold (sensitivity) test "SMELL-S" and a discrimination (resolution) test "SMELL-R" have been developed (34). They are made up of a mixture of monomolecular components which make up an unfamiliar scent so that the "familiarity" component, present in all other odour identification tests, can be eliminated. Since it is a relatively recent test, it is yet to be shown how effective using unfamiliar odours in olfactory testing is compared to the other well-established tests. Overall, the most comprehensive olfactory assessment would involve testing all aspects of olfaction including identification, threshold, and intensity detection as well as discrimination between similar scents even if it requires using several different tests.

Prior to the olfactory testing, participant details were collected. One goal was to assess other potential aspects which are known to have an impact on olfaction such as smoking. Also, the olfactory function can be altered either in a negative or even a positive manner due to taking some medications (35,36). The medications our patients reported to be using the most include analgesics, cardiovascular agents (especially ones for lowering blood pressure), psychopharmacologic agents and genitourinary tract agents. Among all the medications our participants reported to be taking, there was only one which appears on the list of medicines which are reported to impair olfactory function. Only one participant was taking this medication for lowering blood pressure and his UPSIT score of 26 points was somewhat lower than the average male score of 28.6 points. As a part of the questionnaire, participants were also asked to self-assess their perceived sense of smell and rank it from 1-10. Interestingly, female participants rated their sense of smell lower than the male participants while they overall scored better on the actual test. As a last question, participants were given a table to rank what kind of potential improvement to their condition would have the greatest impact on the improvement of the quality of their life. Although "being able to move by walking" and "the function of the bladder/gut and elimination of dysreflexia" were ranked as most important, "normal smell" was still ranked higher up than "hand/palm function" and "absence of depression and other mental problems". This could be due to our participants not having problems with the two latter issues or potentially not wanting to address some of them since there is still a stigma around mental issues among the Croatian population. "Hand/palm function" was overall ranked as the least important possibly because most of our participants were paraplegics since it was ranked as the number one issue by our tetraplegic participant. Similar findings were observed in studies involving a larger number of SCI individuals where tetraplegics

ranked "arm/hand function" by far as most important while paraplegics ranked "sexual function" as their main priority. "Blader and bowel" function was ranked high among both tetraplegics and paraplegics (37,38). Interestingly, one participant who reported having lost both the sense of smell and the sense of taste after the injury, put "normal smell perception" as the second most important function right after "being able to move by walking".

The main finding of our study was that UPSIT results suggest that there is olfactory impairment present among the majority (95%) of SCI patients with the average male score of 26.8 out of 40 points which corresponds to "Moderate Microsmia" and the average female score, 30.8, which corresponds to "Mild Microsmia". Our results were analysed using only descriptive statistics since this is a pilot study and culture-specificity of the test should be assessed prior to further result analyses. The UPSIT score from one participant was excluded from all UPSIT score calculations due to his special case of not being able to smell or taste anything since after the injury. Therefore, His UPSIT score was 0 which would greatly impact our calculations. Furthermore, the one person whose score corresponded to the olfactory diagnosis "Normosmia" was also the person whose lesion was the lowest. Since only one of our participants has had COVID-19 in the past 3 months (and his UPSIT result was even better than the average male result) we would say our data most likely isn't influenced by the loss of the sense of smell due to the above-mentioned viral infection. Considering the self-estimation of the participants' olfactory abilities in the questionnaire before the UPSIT testing, overall low test scores were not expected. However, a similar study investigated the effect of TBI on olfactory impairment using 2 smell tests (UPSIT and Alberta Smell Test) (16). They found that 40-44% of participants who had some levels of olfactory impairment were not aware of it which coincides with our findings.

There was only one other study which looked at olfactory impairment after SCI back in 1998, Hirsch et al. (5). They used two olfactory tests – UPSIT and Chicago Smell Test (CST), a 3-odour detection test with normal scores 4-6 which they developed themselves. Unfortunately, there is no additional information available about the Chicago Smell Test in the literature. Their results are in line with our findings. They found that 93% of their participants (26 out of 28) turned out to have some kind of olfactory impairment measured by the UPSIT, compared to our findings of 95% of participants. Their results using the Chicago Smell Test showed that 39% of the participants had some impairment but they argued that the difference in impairment percentages between the tests was not statistically significant. They also found no correlation between the test scores and the age, race, used medication, level and completeness of the injury nor with the fact that some suffered a head injury and others did not. Unlike us, they

did not record whether their participants lost their consciousness during the injury. However, loss of consciousness following the injury for longer time periods has been linked to olfactory impairment (39). Since Hirsch et al. conducted their study only on male subjects, our study was the first such study conducted on female participants. As expected, we found that our female participants overall scored better than our male participants. The authors of the UPSIT were aware of this gender difference in olfaction and adjusted the score scales accordingly (21). This is a well-known phenomenon and has been recorded both in practice as well as in a meta-analysis of different olfactory testing methods which included the UPSIT (40).

Our participants found some odours more difficult to identify than others. Lemon was an odour that 57.5% of participants failed to identify, which is in contrast with the results from the survey conducted prior to the study in which lemon was ranked as a highly familiar scent. Some guesses could be that our participants were confused with the given alternative answers (in this case those were "Motor Oil", "Rose" and "Vanilla") or that the odour itself did not smell similar to their perception of the way lemon smells. Findings from a UK study using a US UPSIT also showed that their participants more often incorrectly identified certain odours. In their case, those included cheddar cheese, root beer, lime, dill pickle and turpentine (65.9%). They argued that the fact that some questions are answered incorrectly more frequently than others (assuming that all questions are equally difficult) might be due to cultural differences and the overall "synthetic" smell of the strips. The UPSIT study done on the Iranian population tackled this problem by asking their participants to rank each odour's familiarity so that a direct correlation with correct answers could be made (30). This is a good approach before making a culture-specific version of the test.

There were several limitations to this study. Although we have found some correlation between SCI and olfactory impairment, we cannot say with certainty that this olfactory impairment is strictly a consequence of SCI. Additionally, other problems affecting people with SCI could account for olfactory deficits. For example, vitamin deficiencies can cause olfactory deficits which make food less appetizing and eating less only further induces the deficiency (41). Moreover, as with many other studies assessing the olfactory function, the mutual limitation is the lack of information about the state of olfactory abilities of the participants before the injury (13). Another limitation is the number of participants included in the study; however, bigger samples could be obtained in the future based on the started collaborations.

6. Conclusion

Olfactory impairment has been found among individuals who suffered traumatic spinal cord injury (SCI). The olfactory function has been assessed using the University of Pennsylvania Smell Identification Test (UPSIT) in people of different sex, age, years lived with the injury, smoking status, level, and completeness of the lesion. We cannot say with certainty that found olfactory deficits are a direct consequence of the SCI and not due to the cultural misfit of the test and other confounding factors. However, there was one participant who lost both his sense of smell and taste after the injury which suggests that the observed deficits could be related to the SCI. Olfactory deficits usually go unrecognized which goes along with the popular opinion that the sense of smell is the least important of the 5 senses. However, the loss of this sense can greatly affect the quality of life in individuals. These findings are important for understanding all aspects of difficulties that traumatic SCI cause and are important to raise awareness about all difficulties olfactory deficits bring such as feelings of vulnerability which arise from always having to rely on somebody else or not being able to detect potential dangers such as spoiled food or leaking gas. Such individuals are unable to enjoy food and are more prone to developing a variety of emotional and mental problems. Further studies on more subjects which would test all aspects of olfaction and take cultural specificities into account are needed in order to draw some conclusions about certain aspects of the injuries (such as the level and completeness of the lesion) and olfactory scores. We believe that olfactory function in people with spinal cord injuries should be routinely tested and that they should be accordingly informed of the hazards they may encounter due to their potential olfactory deficit. This would also help reduce the alienation which can occur due to all their problems and could reduce the general apathy which is present due to a lack of understanding of the problems surrounding olfactory deficits.

7. Literature

1. Alizadeh A, Dyck SM, Karimi-Abdolrezaee S. Traumatic spinal cord injury: An overview of pathophysiology, models and acute injury mechanisms. Front Neurol. 2019;10(March):1–25.

2. Devivo MJ, Chen Y. Trends in new injuries, prevalent cases, and aging with spinal cord injury. Arch Phys Med Rehabil [Internet]. 2011;92(3):332–8. Available from: http://dx.doi.org/10.1016/j.apmr.2010.08.031

3. Ding W, Hu S, Wang P, Kang H, Peng R, Dong Y, et al. Spinal Cord Injury: The Global Incidence, Prevalence, and Disability From the Global Burden of Disease Study 2019. Spine (Phila Pa 1976). 2022;47(21):1532–40.

4. Middleton JW, Dayton A, Walsh J, Rutkowski SB, Leong G, Duong S. Life expectancy after spinal cord injury: A 50-year study. Spinal Cord. 2012;50(11):803–11.

5. Hirsch AR, Cleveland LB. Olfaction and chronic spinal cord injury. Neurorehabil Neural Repair. 1998;12(3):101–4.

6. Leemhuis E, Giuffrida V, De Martino ML, Forte G, Pecchinenda A, De Gennaro L, et al. Rethinking the Body in the Brain after Spinal Cord Injury. J Clin Med. 2022;11(2).

7. Hulsebosch CE. Recent advances in pathophysiology and treatment of spinal cord injury. Am J Physiol - Adv Physiol Educ. 2002;26(1–4):238–55.

8. Grabher P, Blaiotta C, Ashburner J, Freund P. Relationship between brainstem neurodegeneration and clinical impairment in traumatic spinal cord injury. NeuroImage Clin [Internet]. 2017;15(December 2016):494–501. Available from: http://dx.doi.org/10.1016/j.nicl.2017.05.026

9. Baek A, Cho SR, Kim SH. Elucidation of gene expression patterns in the brain after spinal cord injury. Cell Transplant. 2017;26(7):1286–300.

10. Henderson LA, Gustin SM, Macey PM, Wrigley PJ, Siddall PJ. Functional reorganization of the brain in humans following spinal cord injury: Evidence for underlying changes in cortical anatomy. J Neurosci. 2011;31(7):2630–7.

11. Lin MS, Chiu IH, Lin CC. Ultrarapid Inflammation of the Olfactory Bulb After Spinal Cord Injury: Protective Effects of the Granulocyte Colony-Stimulating Factor on Early Neurodegeneration in the Brain. Front Aging Neurosci. 2021;13(June):1–14.

12. Jure I, Labombarda F. Spinal cord injury drives chronic brain changes. Neural Regen Res. 2017;12(7):1044–7.

13. Van Toller S. Assessing the impact of anosmia: Review of a questionnaire's findings. Chem Senses. 1999;24(6):705–12.

14. The-Crankshaft Publishing's. Organization of the human olfactory system. [Internet]. http://what-when-how.com/. 2012. Available from: http://what-when-how.com/wp-content/uploads/2012/04/tmp15F79.jpg

15. Wu J, Zhao Z, Sabirzhanov B, Stoica BA, Kumar A, Luo T, et al. Spinal cord injury causes brain inflammation associated with cognitive and affective changes: Role of cell cycle pathways. J Neurosci. 2014;34(33):10989–1006.

16. Fortin A, Lefebvre MB, Ptito M. Traumatic brain injury and olfactory deficits: The tale of two smell tests! Brain Inj. 2010;24(1):27–33.

17. Coello AF, Canals AG, Gonzalez JM, Martín JJA. Cranial nerve injury after minor head trauma: Clinical article. J Neurosurg. 2010;113(3):547–55.

18. Sumner D. 107 post-traumatic anosmia. 1936;(1933):107–20.

19. Methodology UF, Planck M, Cybernetics B, Society MP, Methodology UF, Planck M, et al. Metabolic changes in the Hippocampus after Spinal Cord Injury is associated with Memory Function. 2023;(0):23–4.

20. Bathina S, Das UN. Brain-derived neurotrophic factor and its clinical Implications. Arch Med Sci. 2015;11(6):1164–78.

21. Doty RL, Shaman P, Dann M, Doty RL, Shaman P, Development MD. Development of the University of Pennsylvania Smell Identification Test: A Standardized Microencapsulated Test of Olfactory Function. Vol. 32, Physiology & Behavior.

22. Fanger GO. J. E. Vandegaer (ed.),. Microencapsulation. 1974;1–20.

23. Doty RL, Shaman P, Kimmelman CP, Dann MS. University of pennsylvania smell identification test: A rapid quantitative olfactory function test for the clinic. Laryngoscope. 1984;94(2):176–8.

24. Peatfield RC, Turner JAMM, Sillett RW, McNicol MW. The effect of anosmia on smoking habits. Postgrad Med J. 1981;57(663):1–3.

25. Westgren N, Levi R. Quality of life and traumatic spinal cord injury. Arch Phys Med Rehabil. 1998;79(11):1433–9.

26. Doty RL. Psychophysical testing of smell and taste function. 1st ed. Vol. 164, Handbook of Clinical Neurology. Elsevier B.V.; 2019. 229–246 p.

27. Muirhead N, Benjamin E, Saleh H. Is the University of Pennsylvania Smell Identification Test (UPSIT) valid for the UK population? Otorhinolaryngologist. 2013;6(2):99–103.

28. Fornazieri MA, Dos Santos CA, Bezerra TFP, De Rezende Pinna F, Voegels RL, Doty RL. Development of normative data for the Brazilian adaptation of the university of pennsylvania smell identification test. Chem Senses. 2015;40(2):141–9.

29. Abd-Elhafez TA, Kasemy ZAA, Hamdan AM. The validity of the Arabic version of the University of Pennsylvania smell identification test in Egyptian population. Egypt J Ear, Nose, Throat Allied Sci. 2020;21(3):186–91.

30. Kamrava SK, Farhadi M, Jalessi M, Khosravian B, Pousti B, Amin Tehran E, et al. University of Pennsylvania Smell Identification on Iranian population. Iran Red Crescent Med J. 2014;16(1):18–20.

31. Yücepur C, Ozücer B, Değirmenci N, Yildirim Y, Veyseller B, Ozturan O. University of Pennsylvania smell identification test: application to Turkish population. Kulak Burun Bogaz Ihtis Derg. 2012;22(2):77–80.

32. Veyseller B, Ozucer B, Karaaltin AB, Yildirim Y, Degirmenci N, Aksoy F, et al. Connecticut (CCCRC) Olfactory Test: Normative Values in 426 Healthy Volunteers. Indian J Otolaryngol Head Neck Surg. 2014;66(1):31–4.

33. Gupta N, Singh PP, Goyal A, Bhatia D. Assessment of Olfaction Using the 'I-Smell' Test in an Indian Population: A Pilot Study. Indian J Otolaryngol Head Neck Surg. 2013;65(1):6–11.

34. Hsieh JW, Keller A, Wong M, Jiang RS, Vosshall LB. SMELL-S and SMELL-R: Olfactory tests not influenced by odor-specific insensitivity or prior olfactory experience. Proc Natl Acad Sci U S A. 2017 Oct;114(43):11275–84.

35. Schiffman SS. Influence of medications on taste and smell. World J Otorhinolaryngol - Head Neck Surg [Internet]. 2018;4(1):84–91. Available from: https://doi.org/10.1016/j.wjorl.2018.02.005

36. Henkin R. Treatment of Sensory Receptor Dysfunction Drug-Induced Taste and Smell Disorders. 1994;11(5):318–77.

37. French JS, Anderson-Erisman KD, Sutter M. What do spinal cord injury consumers want? A review of spinal cord injury consumer priorities and neuroprosthesis from the 2008 neural interfaces conference. Neuromodulation. 2010;13(3):229–31.

38. Anderson KD. Targeting recovery: Priorities of the spinal cord-injured population. J Neurotrauma. 2004;21(10):1371–83.

39. Gudziol V, Hoenck I, Landis B, Podlesek D, Bayn M, Hummel T. The impact and prospect of traumatic brain injury on olfactory function: A cross-sectional and prospective study. Eur Arch Oto-Rhino-Laryngology. 2014;271(6):1533–40.

40. Sorokowski P, Karwowski M, Misiak M, Marczak MK, Dziekan M,

Hummel T, et al. Sex differences in human olfaction: A meta-analysis. Front Psychol. 2019;10(FEB):1–9.

41. Bauman WA, Zhong YG, Schwartz E. Vitamin D deficiency in veterans with chronic spinal cord injury. Metabolism. 1995;44(12):1612–6.

8. Curriculum Vitae

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Address: Vatroslava Lisinskog 9, 32100, Vinkovci, Croatia (Home)

About me:
Biotechnology and drug research student in Rijeka, Croatia.
Organized, proactive and always willing to gain new knowledge.

WORK EXPERIENCE

2022 – CURRENT Rijeka
INTERN AT THE DIVISION FOR MOLECULAR AND SYSTEMS BIOMEDICINE UNIVERSITY OF RIJEKA

Currently working on my Bachelor's thesis in the field of neuroscience.

11/2022 – 12/2022
STUDENT DEMONSTRATOR - COURSE: MICROBIOLOGY UNIVERSITY OF RIJEKA

10/2022 – 10/2022
MANDATORY INTERNSHIP RUĐER BOŠKOVIĆ INSTITUTE, INSTITUTE FOR MEDICAL RESEARCH AND OCCUPATIONAL HEALTH

2020 – CURRENT
PRACTICAL LABORATORY WORK WITHIN THE BACHELOR'S PROGRAMME UNIVERSITY OF RIJEKA

07/2021 – 08/2021
WAREHOUSE WORKER PEKAR D.O.O., VINKOVCI, CROATIA

EDUCATION AND TRAINING

10/2020 – CURRENT Rijeka, Croatia
BACHELOR OF BIOTECHNOLOGY AND DRUG RESEARCH Undergraduate programe at the Department of Biotechnology, University of Rijeka

Biochemistry, Pharmacology, Microbiology, Physiology, Immunology, Bioassays

Address Rijeka, Croatia | **Field of study** Biotechnology

09/2016 – 07/2020
GENERAL GRAMMAR SCHOOL Gimazija Matije Antuna Reljkovića, Vinkovci

09/2008 – 07/2020
MUSIC THEORY Josip Runjanin Music school, Vinkovci

LANGUAGE SKILLS

Mother tongue(s): **CROATIAN**

Other language(s):

	UNDERSTANDING		SPEAKING		WRITING
	Listening	Reading	Spoken production	Spoken interaction	
ENGLISH	C1	C1	C1	C1	C1

Levels: A1 and A2: Basic user; B1 and B2: Independent user; C1 and C2: Proficient user

DIGITAL SKILLS

Microsoft Office | Microsoft Word | Microsoft Excel | Microsoft Powerpoint | Outlook | Google Drive | Zoom | Power Point | Social Media | Google Docs | Internet user

ADDITIONAL INFORMATION

CONFERENCES AND SEMINARS

14/10/2022 – 16/10/2022 – Faculty of Pharmacy and Biochemistry
CPSA - Croatian Pharmaceutical Students' Association Congress

08/10/2022 – 08/10/2022 – Faculty of Medicine and Faculty of Sciences, Zagreb
Brain-Gut Axis Conference, Zagreb

29/08/2022 – 02/09/2022 – Mediterranean Institute for Life Sciences, Split, Croatia
MedILS Summer school in Bioinformatics

30/06/2022 – 03/07/2022 – University of Rijeka, Croatia
ALS Rijeka 2022. Symposium

21/06/2022 – 22/06/2022 – Faculty of Health, Medicine and Life Sciences, Maastricht University, Netherlands
MOSA Conference 2022.

14/06/2022 – University of Rijeka
Biotechnology students symposium "PosteRi" active participant, poster: "Spinal cory injury causes neurodegeneration in the brain"

22/04/2022 – 24/04/2022 – Faculty of Medicine, Rijeka
NeuRi 2022. - Student congress in neuroscience

03/12/2021 – 04/12/2021 – Online, Indian conference
Darwin 2021.

MANAGEMENT AND LEADERSHIP SKILLS

Secretary of the "Brain-Gut axis Conference" monitored and coordinated work of organization depatments

Project manager of "Students-mentors", Association of Biotechnology Students Leader of the project which assigns mentors to freshmen and helps them adapt to their student life

Year Representative of the generation

ORGANISATIONAL SKILLS

Member of the Organization committee, "Brain-Gut axis Conference" coordination and arranging the lectures, workshops and the social programme of the conference

Member of the Student's union at the Department of Biotechnology

Quality management and promotion board of the Department of Biotehnology Student representative

COMMUNICATION AND INTERPERSONAL SKILLS

MedILS Summer school in Science Communication

VOLUNTEERING

10/2020 – CURRENT
Volounteer at USBRi - Association of Biotechnology Students, Rijeka projects: "Future and perspective" Conference, Biotech Journal, SOBRI Summer school

03/05/2022
Department of Biotechnology open day

SCHOLARSHIPS

2022 – CURRENT
Scholarship for excellence, University of Rijeka

2021 – 2022
STEM scholarship, Ministry of Science and Education, Croatia