Probing the impact of gender-affirming hormone treatment on odor perception

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Abstract

Evidence suggests that women outperform men in core aspects of odor perception, and sex hormones may play a significant role in moderating this effect. The gender-affirming treatment (GAT) of transgender persons constitutes a powerful natural experiment to study the psychological and behavioral effects of high dosages of cross-sex hormone applications. Therefore, our aim was to investigate the effects of GAT on odor perception in a sample of 131 participants including female and male controls, as well as transmen and transwomen over their first four months of gender transition. The Sniffin' Sticks test battery was used to measure odor detection, discrimination and identification at baseline, as well as one and four months after the start of GAT. Plasma levels of estradiol, testosterone and sex hormone-binding globulin was analyzed for each assessment point. Results revealed no significant change of olfactory performance in the two transgender groups compared to female and male controls. There was no significant difference between groups at baseline or any other time point. Neither biological sex, nor gender identity had an influence on odor perception. Moreover, there was no significant correlation between sex hormones and odor perception and between GAT-induced changes in sex hormones and changes in odor perception. Our results indicate that effects of sex hormones on olfactory performance are subtle, if present at all. However, our results do not preclude hormonal effects on odors not included in the Sniffin' Sticks test battery, such as body odors or odors associated with sex.

Keywords: Olfactory performance, Sniffin' Sticks, testosterone, estradiol, transsex, gender dysphoria

1. Introduction

Previous studies indicate that females outperform males in odor perception. In a large sample of over 3.000 participants, Hummel et al. (2007) showed that middle aged females (between 16 and 55 years) had slightly but significantly higher scores in overall odor perception than age-matched males, whereas no significant difference was observed for younger or older samples. Other studies suggest that for some but not all odorants, women score higher than men on tests of odor detection, identification, discrimination and memory (Doty and Cameron, 2009). Given the apparent sex difference in human olfactory performance, several studies aimed at exploring a potential role of sex hormones by investigating the effects of hormone replacement therapy, the influence of menstrual cycle, intake of hormonal contraception or pregnancy on odor perception. However, study results were heterogeneous (Doty and Cameron, 2009; Doty et al., 2015).

In a previous study, we investigated the effects of sex hormones on olfactory performance in 80 females including 20 participants taking oral contraceptives (Derntl et al., 2013). The participants without intake of contraceptives were measured twice, once during the follicular, and a second time during the luteal phase using the Sniffin' Sticks test battery. Results indicated a positive correlation between olfactory performance and duration of oral contraceptive intake. Moreover, females in their luteal phase showed a lower sensitivity towards *n*-butanol compared to when measured during their follicular phase. In a recent follow-up study, Kollndorfer et al. (2016) investigated the effect of different ethinyl estradiol concentrations of oral contraceptives on olfactory performance in 42 women. Authors observed significantly higher olfactory performance in the low ethinyl estradiol group compared to participants of the high ethinyl estradiol group. Taken together, our previous results indicate that changes in sex hormone levels may affect olfactory performance. However, previous results were correlative in nature and focused on estradiol, whereas evidence on the causal role of sex hormones including estradiol and testosterone on odor perception is limited.

The gender-affirming treatment (GAT) in transgender individuals is a central component in the process of gender transition as it changes the secondary sexual characteristics from an individuals' assigned sex at birth towards the desired sex. Therefore, GAT constitutes a powerful natural experiment to study the psychological and behavioral effects of long-term high dosages of cross-sex hormone applications in humans in *vivo*. We have previously applied GAT in transgender people and postmenopausal women as a model to investigate the relationship between sex hormones and brain structure/function in several studies (Hahn et al., 2016; Kranz et al., 2018; Kranz et al., 2014b; Kranz et al., 2017; Kranz et al., 2015; Kranz et al., 2011; Seiger et al., 2016; Spies et al., 2016). In the current study, our aim was to investigate the effects of GAT on odor perception in transgender men (TM) and transgender women (TW) over their first four months of cross-sex hormone intake. As a comparison, we included a group of female and male controls (FC, MC, respectively) in this longitudinal study, who did not undergo GAT.

Human olfaction is a complex function of regions within the olfactory system, from early processing in the nasal cavity and olfactory bulb to higher order cortical brain areas. Interestingly, evidence indicates that gray matter densities within the human olfactory system are sexually dimorphic (Garcia-Falgueras et al., 2006). Recent evidence also shows differences in olfactory cortex between pre- and post-menopausal women, pointing to a role of sex hormones in olfactory gray matter volume (Kim et al., 2018). Moreover, gray matter volumes in olfactory bulb and orbitofrontal cortex are associated with odor perception in humans (Seubert et al., 2013). Androgen as well as estrogen receptors are expressed in the olfactory system including olfactory bulb and cortical regions of rodents as well as humans (Osterlund and Hurd, 2001; Simerly et al., 1990). Taken together, these studies indicate that sex hormones may affect odor perception in humans via structural changes of olfactory brain regions, mediated by nuclear hormone receptors. These changes may be observed already within the first months of GAT (Kranz et al., 2018; Seiger et al., 2016).

2. Methods

2.1. Participants

The total sample consisted of 131 participants, including 32 FC, 42 TM, 31 TW and 26 MC. Imaging data of a subsample of participants were published previously (Kranz et al., 2014a; Kranz et al., 2015). Based on our experience, we considered a potential data loss of approximately 20% and therefore aimed to recruit a sample size of 30-40 participants per group, resulting in a statistical power of about 95% based on an effect size of dz=0.64. This is consistent with our previous study on the effects of menstrual cycle phase on olfactory perception (Derntl et al., 2013), and a significance level of 0.05. Control participants were recruited via advertisement. Transgender participants were recruited from the transgender outpatient unit of the Department of Obstetrics and Gynecology, Medical University of Vienna. They were naïve to steroid hormone treatment and were seeking sex reassignment. To rule out internal medicine and neurological disorders, all participants underwent a standard medical examination including electrocardiography, routine laboratory tests and the Structured Clinical Interview (SCID) for DSM-IV disorders. Further exclusion criteria were intake of psychotropic medication within 6 months prior inclusion, past or current substance abuse or pregnancy. Diagnostic assessment of transsexualism for the transgender participants followed DSM-IV-TR and ICD-10 and was performed after several semi-structured, socio-demographic, clinical and psychiatric interviews, based on legal requirements for cross-sex hormonal treatment in Austria. Written informed consent was obtained after thorough explanation of the study and participants received monetary compensation for their participation. The study was approved by the Ethics Committee of the Medical University of Vienna and performed in accordance with the Declaration of Helsinki (1964), including current revisions and the EC-GCP guidelines.

2.2. Study Design and Steroid Hormone Treatment

After screening and inclusion in this longitudinal study, participants underwent two baseline Sniffin' Sticks Tests (within two weeks). This was done to approximate a more accurate baseline measure of olfactory perception and to reduce random variability (e.g., due to a stuffed up nose). A subsample of participants underwent a second test (18 FC, 30 TM, 23 TW, 18 MC) one month after-, and a third test (13 FC, 31 TM, 23 TW, 16 MC) four months after treatment start. GAT to change hormonal profiles towards the reference range of the individuals' gender identity followed protocols routinely implemented at the Department of Obstetrics and Gynecology, Medical University of Vienna. Briefly, TM received 1.000 mg testosterone undecanoate every 12 weeks (Nebido® 1.000mg/4ml vial, intramuscular). Two participants further received 10-15mg lynestrenol (Orgametril® 5mg, oral) daily. TW received either daily 50mg cyproterone acetate (Androcur® 50mg tablet, oral; 15 participants) or triptorelin acetate 4.12mg/month (Decapeptyl® 172mg powder for suspension for injection s.c. or i.m.; eight participants). Additionally, TW over 40 years of age received daily doses of 100µg estradiol (Estradot®/Estramon®, transdermal therapeutic system applied twice a week; three participants) while those less than 40 years of age received 4mg/day estradiol hemihydrate (Estrofem® 2mg, oral; nine participants). Alternatively, six participants received estradiol hemihydrate 0.75-1.5mg/day (Estro-Gel® 0.75mg/1.25g/day, transdermal). Because of extensive hair loss, six TW further received 2.5mg/day of the 5-alpha-reductase-inhibitor finasteride (Actavis®/Arcana®/Aurobindo® 5mg, oral).

2.3. Blood Sampling

Blood samples were collected prior or after olfactory assessment at each visit. The analysis of plasma levels of estradiol, testosterone and sex hormone-binding globulin (SHBG) was done by the Department of Laboratory Medicine, Medical University of Vienna, Austria (http://www.kimcl.at). The free androgen index (FAI) was computed as FAI=testosterone/SHBG*100. Likewise, a free estrogen index (FEI) was computed as FEI=estradiol/SHBG*100.

2.4. Olfactory perception tests

The Sniffin' Sticks test battery (Burghart Instruments) (Hummel et al., 1997; Kobal et al., 1996) was used to measure olfactory perception at each assessment day. The battery consists of three subtests to measure absolute odor sensitivity/detection (olfactory threshold), odor quality discrimination and cued odor identification. Odor detection was assessed in a standardized staircase three alternative forced choice procedure (Hummel et al., 1997). Briefly, participants were presented with triplets of two blank pens and one containing *n*-butanol and asked to indicate the odor-containing pen. This was repeated with decreasing dilution until participants chose the correct pen twice which triggered a reversal of the staircase. Dilution was then repeatedly increased until participants failed to indicate the correct pen which again triggered a reversal. The mean of the last four staircase reversal points of a total of seven reversals was used as the threshold estimate. Thus, the possible range for the odor sensitivity task was one-to-sixteen with higher scores indicating lower threshold i.e. higher sensitivity. In the subtest odor discrimination, participants were presented with triplets of two pens containing identical odorants and one containing a different odor (target) and had to indicate the pen which smelled different. The possible range for the odor discrimination task was zero-to-sixteen with higher scores indicating better performance. In the odor identification subtest, participants had to pick an odor label out of four alternatives which best described the quality of an odorant. 16 common odors were assessed in a fixed order, as described in Hummel et al. (1997). The possible range for the odor identification task was zero-to-sixteen with higher scores indicating better performance. Lastly, a TDI score (threshold, discrimination, identification) was calculated as the sum of the results obtained for the three subtests.

2.5. Statistical analysis

Olfactory perception was analyzed using linear mixed models analysis with group (FC, TM, TW, MC) and time (1-3) as fixed factors, and subjects as the random factor. Repeated measurement at baseline

was included as factor of no interest as done previously (Vanicek et al., 2019a; Vanicek et al., 2019b). Likewise, changes in plasma hormone levels were assessed using linear mixed models analysis. Associations between olfactory perception and plasma hormone levels at baseline were calculated using Spearman non-parametric correlations. Likewise, associations between treatment-induced changes in olfactory performance and changes in plasma hormone levels were calculated using Spearmen correlations. SPSS version 24.0 for Windows (SPSS Inc., Chicago, Illinois; www.spss.com) was used for statistical analysis. All p-values were considered significant at a significance level of p=0.05, corrected for multiple comparisons using the Holm-Bonferroni method.

3. Results

There was no difference in age between groups (p>0.1, F-test, see Table 1). Odor detection, discrimination and identification subtests, as well as TDI total scores were normally distributed for each group and time point, whereas plasma hormone levels were skewed, as assessed using Kolmogorov–Smirnov test and visual inspection. As expected, estradiol significantly increased in TW after one month (92.6 pg/mL) and after four months (115.4 pg/mL) of GAT compared to baseline (26.7 pg/mL, p<0.001). Conversely, testosterone plasma levels significantly decreased after one month (1.0 ng/mL) and after four months (0.8 ng/mL) of GAT towards the female reference range, compared to baseline (5.0 ng/mL, p<0.001). As expected in TM, testosterone treatment led to significant increases in plasma testosterone levels after one (3.7 ng/mL) and after four (5.1 ng/mL) months into GAT towards male levels, compared to baseline (0.4 ng/mL, p<0.001), (see Table 2).

3.1. Olfactory performance

All participants were in the normosmic range. Linear mixed models analysis revealed neither a main effect of group or time, nor an interaction between the two factors for the DTI score or any of the

three sub-measures (all p>0.05, uncorrected, see Table 3A). Separate models controlling for smoking status, age, time of day or odor perception self-assessment (good, average, bad) did not change the insignificance of any of the main or interaction effects.

Specifically testing effects of GAT in the two transgender groups separately in an exploratory post-hoc approach revealed no significant changes in olfactory perception, neither for TDI, nor for any subscale (all p>0.05, uncorrected). In a further exploratory post-hoc analysis, we investigated differences between biological sex and gender, as well as between all diagnostic groups at baseline. Linear mixed models revealed no significant difference between the four groups at baseline in any of the studied subscales (all p>0.05, uncorrected, see Table 3B). There was no significant difference between biological sex (FC&TM versus MC&TW) in any subscale, or between female and male gender (FC&TW versus MC&TM) at baseline.

3.2. Associations between olfactory performance and plasma hormone levels

Next, we investigated the relationship between odor perception and plasma hormone levels in the entire sample and in all groups separately at baseline. There was no significant correlation between any hormone and olfactory performance for the entire sample, not even at an uncorrected level of significance (p>0.05). There was also no significant correlation when biological sex or gender was investigated separately. Finally, there was no significant correlation when all four groups were investigated separately, although at an uncorrected level of significance, there was a positive correlation between odor discrimination and the free androgen index in MC (see Table 4 for all correlation coefficients).

Finally, we investigated the relationship between changes in GAT-induced plasma hormone levels and changes in olfactory performance. There was no significant correlation for any of the plasma hormone changes with odor perception changes; neither for changes after one month nor after 4 months into

GAT (see Table 5 and Table 6 for all correlation coefficients including two significant correlations at an uncorrected p-level).

4. Discussion

In this study, we investigated the potential influence of sex hormones on odor perception in TM and TW transgender people undergoing GAT, compared to female and male controls. We were unable to replicate previous findings that females outperform males and that changes in sex hormone levels affect olfactory performance. We found no significant difference between sex, gender, or transgender on olfactory performance. Moreover, we found no significant association between estradiol or testosterone, as well as free estrogen and free androgen index and any subscale of odor perception. Finally, we found no significant association between GAT-induced changes in plasma hormone levels and changes in olfactory performance.

Given our sample size of overall 131 participants, our insignificant results indicate that sex differences and hormone influences on olfactory performance are very small, if present at all. Indeed, in a large sample of 3.282 subjects, Hummel et al. (2007) found no significant overall sex difference and only a marginal statistical difference of $0.05>p>0.04$ in a middle-aged group (between 16 and 55 years). In a more recent study, Guarneros et al. (2015) aimed at determining normative data for a Mexican population of 916 participants using the Sniffin' Sticks test. Although there was a significant decline in olfactory function with age, authors found no statistically significant difference between sexes. Negative findings regarding sex were also observed in a more recent study by the German group of T. Hummel (Reden et al., 2016). Authors assessed two olfactory test batteries in 49 patients with olfactory dysfunction and 21 controls. Although tests reliably differentiated between the two groups, there was no differences between sexes. Authors interpreted their negative results with reference to their sample size being too small to detect meaningful effects. However, the same group also observed negative findings in another study (Sorokowska et al., 2015), in which authors tested an extended

version of the Sniffin' Sticks identification test in 100 patients with olfactory dysfunction and 100 controls. In contrast to these negative studies, there are two recent publications reporting significant sex differences in olfactory performance. In a Portuguese population of 203 participants, Ribeiro et al. (2016) observed higher DTI scores in women compared to men across a wide range of age groups studied. In addition, in an U.S. American sample of 1.436 older adults, Pinto et al. (2015) observed greater age related decline in odor identification in men compared to women. Taken together, previous studies indicate that sex differences in olfactory performance are very small and only detectable with a sufficiently large sample size.

Our present results further indicate that hormone effects on olfactory performance are small, if present at all. However, this assumption is in conflict with studies of similar sample size, demonstrating significant associations between menstrual cycle phase, oral contraception and pregnancy on human odor perception. Indeed, our sample size was determined by a power calculation based on these correlational studies (see above). For example, Derntl et al. observed lower sensitivity for females in the follicular phase compared to the same participants measured in the luteal phase (Derntl et al., 2013). Estradiol and progesterone are on average higher in the luteal phase so it is tempting to speculate that these hormones increase olfactory sensitivity. Indeed, odor sensitivity and estradiol plasma levels positively correlated across the menstrual cycle with a peak in both variables around the time of ovulation (Doty and Cameron, 2009). However, women taking oral contraceptives showed a similar peak in olfactory sensitivity during mid-cycle despite of invariant hormone plasma levels (Doty et al., 1982); a result that tempted authors to consider that hormones and olfactory sensitivity are not directly causally related although they are correlated. Hence, Doty and Cameron (2009) conclude that the relationship between reproductive hormones and olfactory function is rather complex.

In addition to a limited sample size that may have impeded significant hormonal influences on olfactory performance, several other factors may explain inconsistencies between studies. Factors pertaining to experimental procedures and methods may include differences in olfactory tests and tested odors, or the choice of investigated sex hormones. For example, there are known limitations in using free

androgen and free estradiol indices, i.e. there is evidence that FAI only correlates well with free testosterone in women but not in men (Rosner et al., 2007). In addition, although testosterone levels significantly decreased in TW due to GAT, levels were still slightly above the female reference range. Hence, remaining testosterone levels in TW may have hampered the administered estrogen to unfold its fullest effect on odor perception. Finally, several confounding variables have to be considered likely, such as seasonal fluctuations, attention or willingness of participants to perform the tests. Further research controlling for these and other confounders is needed that utilizes GAT to investigate the potential effects of sex hormones on odor perception.

5. Conclusion

This study is the first to investigate the effects of high-dose cross sex GAT in transgender individuals on odor perception. Despite the powerful effects of GAT on changing secondary sexual characteristics, we found no evidence for changes in olfactory performance. However, despite the advantage of GAT to investigate the causal effects of estradiol and testosterone on odor perception, our study is limited by the small sample size and the restriction to certain odors. Our data therefore do not preclude associations between sex hormones and olfactory performance, especially for other, untested odors.

Conflict of interest

All authors declare no competing financial interests in relation to the work described. Without any relevance to this work, R. Lanzenberger declares that he received conference speaker honorarium within the last three years from Shire and support from Siemens Healthcare regarding clinical research using PET/MR. He is shareholder of BM Health GmbH since 2019. G.S. Kranz received travel grants and/or conference speaker honoraria from Roche, AOP Orphan and Pfizer. U. Kaufmann reports no financial relationships with commercial interests.

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Tables

Table 1: Olfactory performance at baseline, after one month and after four months of continuous gender-affirming treatment

Group	Age mean±SD	Sniffin' Sticks test	Baseline mean±SD	1 month mean±SD	4 months mean±SD
FC	25.1±5.3	**Sensitivity**	7.7±2.1	7.9±2.2	7.6±2.1
		Discrimination	12.6±2.1	12.7±2.1	12.6±2.8
		Identification	13.0±1.6	13.3±1.5	13.5±1.8
		TDI	33.3±3.5	33.9±3.8	33.8±5.0
			N=32	N=18	N=13
TM	26.3±6.3	**Sensitivity**	8.1±2.2	8.1±2.3	7.6±2.3
		Discrimination	12.9±2.1	13.0±1.8	13.2±1.6
		Identification	12.6±1.7	13.0±1.5	12.7±1.6
		TDI	33.5±4.4	34.1±3.2	33.4±3.0
			N=42	N=30	N=31
TW	29.8±8.5	**Sensitivity**	7.7±2.1	8.0±1.7	7.8±1.9
		Discrimination	12.8±2.0	12.7±1.6	12.0±2.7
		Identification	13.3±1.6	13.1±1.8	13.2±2.5
		TDI	33.7±3.8	33.8±3.6	33.1±5.0
			N=31	N=23	N=23
MC	28.2±5.9	**Sensitivity**	8.0±2.4	6.7±2.4	8.1±2.5
		Discrimination	12.5±2.2	12.3±2.7	13.3±1.5
		Identification	13.1±1.6	13.1±1.7	13.1±1.8
		TDI	33.7±4.7	32.0±5.2	34.5±3.9
			N=26	N=18	N=16

FC, female controls; TM, transmen; TW, transwomen; MC, male controls; TDI, sum score of sensitivity (threshold), discrimination and identification scores. Baseline levels are calculated as the mean value of the first two assessments.

Table 2: Sex hormone levels at baseline, after one month and after four months of continuous gender-affirming treatment

Group	Hormone	Baseline mean±SD & 25/50/75 percentile	1 month mean±SD & 25/50/75 percentile	4 months mean±SD & 25/50/75 percentile	P value
FC	**Estradiol**	96.1±110.8	141.8±125.2	140.3±81.4	0.687
	(pg/mL)	23.5/55.0/130.3	49.8/80.5/265.8	80.0/126.0/219.0	
	Testosterone	0.3±0.1	0.3±0.1	0.4±0.1	0.952
	(ng/mL)	0.18/0.27/0.38	0.2/0.3/0.5	0.3/0.4/0.5	
	SHBG	96.5±75.4	77.1±46.4	78.1±45.5	0.962
	(nmol/L)	51.4/73.7/107.4	43.1/69.2/102.7	43.1/61.2/106.3	
	FEI	150.3±181.0	231.9±228.2	223.6±154.1	0.328
		36.7/89.7/173.3	82.4/153.7/294.1	65.4/231.8/288.6	
	FAI	0.5±0.5	0.7±0.7	0.7±0.5	0.209
		0.2/0.4/0.6	0.2/0.4/0.9	0.4/0.5/1.2	
TM	**Estradiol**	109.5±84.4	112.1±124.8	78.9±75.4	0.206
	(pg/mL)	44.0/77.5/140.8	31.3/64.5/136.5	45.8/55.0/78.3	
	Testosterone	0.4±0.2	3.7±2.3	5.1±2.7	<0.001
	(ng/mL)	0.3/0.4/0.6	1.9/2.8/5.7	3.5/4.6/6.3	
	SHBG	60.8±26.7	40.1±16.8	38.6±14.2	<0.001
	(nmol/L)	42.1/57.1/74.9	30.0/36.6/51.3	28.2/35.9/46.5	
	FEI	192.7±143.0	298.4±330.5	202.0±149.8	0.091
		87.6/163.9/230.7	111.2/183.8/323.3	100.0/175.1/218.2	
	FAI	0.9±0.9	11.3±9.9	14.4±7.1	<0.001
		0.3/0.6/1.1	5.2/8.7/14.5	10.5/13.0/18.2	
TW	**Estradiol**	26.7±11.0	92.6±47.4	115.4±84.1	<0.001
	(pg/mL)	19.0/26.5/32.8	57.0/82.0/133.0	57.5/111.0/143.8	
	Testosterone	5.0±1.9	1.0±1.8	0.8±1.8	<0.001
	(ng/mL)	3.8/4.8/6.0	0.1/0.2/0.6	0.1/0.2/0.3	
	SHBG	39.7±17.6	54.2±29.3	68.6±48.2	<0.001
	(nmol/L)	29.8/35.9/44.3	40.5/47.9/70.6	34.5/61.1/84.9	
	FEI	78.6±45.0	187.5±94.3	240.6±225.0	<0.001
		48.0/65.3/90.5	108.3/182.7/265.3	95.7/171.0/268.8	
	FAI	13.7±5.3	1.9±3.0	1.8±4.2	<0.001
		10.1/13.3/16.4	0.2/0.5/1.6	0.1/0.3/1.2	
MC	**Estradiol**	25.8±11.1	26.0±13.6	24.8±10.6	0.963
	(pg/mL)	19.0/24.5/32.0	14.8/23.5/41.0	21.6/26.5/31.8	
	Testosterone	5.0±1.6	5.9±2.0	5.5±2.2	0.453
	(ng/mL)	3.7/5.0/6.1	4.5/5.8/7.6	21.3/26.5/31.8	
	SHBG	34.6±10.6	37.3±13.1	33.8±8.0	0.919
	(nmol/L)	26.7/34.7/41.3	27.5/35.0/48.4	29.6/31.3/43.0	
	FEI	80.2±41.1	74.7±36.7	73.7±37.1	0.817
		55.9/68.7/95.9	50.9/70.4/98.5	49.7/77.4/100.5	
	FAI	15.0±4.3	16.2±3.4	16.3±5.8	0.808
		12.1/14.7/18.0	13.9/17.4/18.5	12.0/16.9/20.9	

FC, female controls; TM, transmen; TW, transwomen; MC, male controls; SHBG, sex hormone-binding globulin; FEI, free estradiol index; FAI, free androgen index (FEI and FAI are calculated via division by SHBG). Baseline levels depict the mean value of the first two measurements. P-values indicate the significance of changes over the three time points (i.e., main effect of time)

Table 3: Summary of test statistics

Linear mixed models analysis statistics	Sniffin' Sticks test			
	Sensitivity	**Discrimination**	**Identification**	**TDI**
A				
ME of group	F=0.057, p=0.982	F=0.784, p=0.505	F=1.675, p=0.175	F=0.143, p=0.934
ME of time	F=0.122, p=0.885	F=0.023, p=0.977	F=0.351, p=0.705	F=0.041, p=0.960
Interaction	F=1.622, p=0.141	F=1.673, p=0.128	F=0.720, p=0.634	F=1.646, p=0.135
B				
BL differences	F=0.407, p=0.748	F=0.306, p=0.821	F=1.747, p=0.161	F=0.046, p=0.987
FC&TM vs. MC&TW at BL	F=0.090, p=0.765	F=0.079, p=0.779	F=2.939, p=0.89	F=0.137, p=0.712
FC&TW vs. MC&TM at BL	F=1.192, p=0.277	F=0.041, p=0.841	F=2.470, p=0.118	F=0, p=0.996

A: Test statistics derived from linear mixed models analyses using group (FC, TM, TW, MC) and time (1-3) as fixed factors. B: test statistics for models investigating the four groups at baseline, as well as between biological sex and gender at baseline. ME: main effect; BL: baseline; FC: female controls; TM: transmen; MC: male controls; TW: transwomen; TDI, sum score of sensitivity (threshold), discrimination and identification scores.

Table 4: Association between olfactory perception and hormone plasma levels at baseline

Group	Sniffin' Sticks test	Estradiol	Testosterone	FEI	FAI
FC	**Sensitivity**	-0.300	0.128	-0.219	0.078
	Discrimination	-0.255	0.005	-0.338	0.074
	Identification	0.004	0.194	-0.132	-0.150
	TDI	-0.325	0.172	-0.356	0.023
TM	**Sensitivity**	-0.054	-0.128	0.083	0.095
	Discrimination	-0.141	-0.019	0.015	0.028
	Identification	-0.059	-0.187	0.001	-0.209
	TDI	-0.114	-0.135	0.048	-0.012
TW	**Sensitivity**	0.290	0.262	0.144	0.093
	Discrimination	0.016	0.084	0.187	0.208
	Identification	-0.032	0.032	-0.098	-0.061
	TDI	0.158	0.350	0.134	0.132
MC	**Sensitivity**	-0.028	-0.103	0.083	0.148
	Discrimination	-0.434	0.244	-0.175	0.655*
	Identification	0.033	0.001	0.483	0.537
	TDI	-0.201	0.055	0.139	0.571

Depicted are nonparametric correlation coefficients (Spearman's rho); *p<0.05, uncorrected; FC, female controls; TM, transmen; TW, transwomen; MC, male controls; TDI, sum score of sensitivity (threshold), discrimination and identification scores; FEI, free estradiol index; FAI, free androgen index (FEI and FAI are calculated via division by SHBG).

Table 5: Association between changes in olfactory perception and hormone plasma levels after one month of treatment

Group	Sniffin' Sticks test	Δ Estradiol	Δ Testosterone	Δ FEI	Δ FAI
FC	**Δ Sensitivity**	-0.128	-0.064	-0.340	0.055
	Δ Discrimination	-0.568	-0.490	-0.483	-0.365
	Δ Identification	-0.232	0.093	0.070	0.210
	Δ TDI	-0.411	-0.229	-0.470	-0.085
TM	**Δ Sensitivity**	0.124	0.236	0.082	0.217
	Δ Discrimination	-0.041	0.001	0.031	-0.032
	Δ Identification	0.227	0.148	0.291	0.150
	Δ TDI	-0.069	0.139	0.028	0.169
TW	**Δ Sensitivity**	0.237	-0.190	0.077	0.274
	Δ Discrimination	-0.081	0.170	0.275	0.411
	Δ Identification	-0.356	0.294	-0.266	0.101
	Δ TDI	0.024	0.011	0.131	0.393
MC	**Δ Sensitivity**	-0.024	0.286	-0.008	0.109
	Δ Discrimination	-0.474	-0.331	-0.540	-0.608
	Δ Identification	-0.329	0.074	-0.180	0.282
	Δ TDI	-0.158	0.030	-0.083	-0.033

Depicted are nonparametric correlation coefficients (Spearman's rho); FC, female controls; TM, transmen; TW, transwomen; MC, male controls; TDI, sum score of sensitivity (threshold), discrimination and identification scores; FEI, free estradiol index; FAI, free androgen index (FEI and FAI are calculated via division by SHBG).

Table 6: Association between changes in olfactory perception and hormone plasma levels after four months of treatment

Group	Sniffin' Sticks test	Δ Estradiol	Δ Testosterone	Δ FEI	Δ FAI
FC	**Δ Sensitivity**	-0.289	-0.183	0.346	-0.486
	Δ Discrimination	-0.028	-0.665	-0.201	-0.699
	Δ Identification	-0.138	0.498	-0.097	-0.003
	Δ TDI	-0.287	-0.183	0.076	-0.594
TM	**Δ Sensitivity**	0.186	-0.160	0.441	-0.409
	Δ Discrimination	-0.048	0.210	-0.007	0.347
	Δ Identification	0.348	-0.413	0.013	-0.396
	Δ TDI	0.179	-0.052	0.117	-0.047
TW	**Δ Sensitivity**	0.089	0.220	0.085	0.489
	Δ Discrimination	0.346	-0.008	0.435	0.023
	Δ Identification	-0.380	0.597*	0.068	0.285
	Δ TDI	-0.039	0.636*	0.394	0.539
MC	**Δ Sensitivity**	0.548	-0.097	0.246	-0.211
	Δ Discrimination	0.437	0.186	0.166	0..044
	Δ Identification	0.323	0.432	0.436	0.438
	Δ TDI	0.660	0.213	0.402	0.081

Depicted are nonparametric correlation coefficients (Spearman's rho); *p<0.05, uncorrected;); FC, female controls; TM, transmen; TW, transwomen; MC, male controls; TDI, sum score of sensitivity (threshold), discrimination and identification scores; FEI, free estradiol index; FAI, free androgen index (FEI and FAI are calculated via division by SHBG).