מכון ויצמן למדע
WEIZMANN INSTITUTE OF SCIENCE

עבודת גמר (תזה) לתואר
דוקטור לפילוסופיה

מוגשת למועצה המדעית של
מכון ויצמן למדע
רחובות, ישראל

Thesis for the degree
Doctor of Philosophy

Submitted to the Scientific Council of the
Weizmann Institute of Science
Rehovot, Israel

מאת

לירון רוזנקרנץ

By

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חקר יחסי הגומלין בין חוש הריח להתנהגות אנושית במצבי בריאות וחולי

Investigating the interplay between olfaction and human behavior in health and disease

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January 2018

Acknowledgements:

I was extremely fortunate to have Noam and Uri as my PhD mentors. Whereas most of my PhD (90% as a-priori defined) was conducted in the lab of Prof. Noam Sobel, I feel that both labs have contributed greatly to my development and growth as a scientist and as an individual. Noam has given me unlimited space and opportunities to express myself as a researcher and a scientist. In his trust and confidence in me, he has allowed me to lead projects and mentor others, to experience and fail, to learn and grow. Whenever I needed his guidance or support, he made sure to be available, and in minutes of discussion come up with a super creative idea for an analysis or experiment that could perfectly fit the problem I encountered. I thank you for your patience, faith and for allowing me unlimited access into your innovative mind ☺.

In Prof Uri Alon's lab I learned much more than is captured by the study we published together. I received tools of meta-scientific issues, such as how to present my research in the most engaging way, how to approach PIs at a conference, etc. Above all, I had unlimited access to Uri's tool box of empowering young scientists in general, and young female scientists in particular. I thank you for listening and providing inspiring answers to difficult questions I brought up along the way.

I would like to thank my lab members, past and present: thank you for making it fun to come to work every day, for your help and unconditioned support, for listening and advising me both professionally and emotionally. For becoming true friends♡.

I would like to thank my PhD committee, Dr. Tali Kimchi and Dr. Ofer Yizhar, for supporting me along the way, for listening to my concerns and reassuring me, for seeing me for who I am, beyond the projects I presented to them, for providing guidance and advice and sharing from their own experience.

Most importantly, along with my professional development, I also grew personally; throughout five years of PhD I got married to the most supporting, accepting and caring person I could imagine, and had one beautiful, smart, sensitive and loving son, who is soon to have a baby sister. Gilad and Rotem, you are my home, my happiness, my core, my heart. Thank you for being who you are, and for allowing me to be who I am.

Last but not least, I would like to thank my family: my parents, brother and sister; for hours of deliberations, for hearing about all the difficulties, but not always about the successes, for letting go but still always being there. You are the guiding lights in my life, thank you for being a perfect role model.

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Abstract:

In my PhD I have led three projects probing the interplay between olfaction and human behavior in health and disease, two disease-related projects are conducted under the guidance of Prof. Noam Sobel, and the health-related project under the guidance of Prof. Uri Alon.

In my first project I tested whether children with autism exhibit altered olfactory processing (Rozenkrantz et al, Curr Bio, 2015), in collaboration with the Autism Center at Asaf Harofe medical center. I used the sniff response, a ten-minute non-verbal measure of respiratory response to pleasant and unpleasant odors, in two populations: children diagnosed with autism and typically-developing children. Using this objective measure, I found that children with autism display profoundly altered respiratory responses to odors, as compared with typically-developing children, and that this alteration is highly correlated with autism severity. The difference in olfactory processing between the two groups allowed for 81% correct ASD classification based on the sniff response alone.

In a second and soon-to-be submitted project, originally defined as "high-risk, high-gain", I investigated the potential role of olfactory social communication in recurrent pregnancy loss (RPL). The hypothesis rests on the Bruce effect in rodents, in which a female miscarries in response to bodily odors emitted from a male who did not father the pregnancy (Bruce effect, 1959). Here I tested the hypothesis that RPL women would display an altered olfactory profile. In RPL, which occurs in 1% of women, more than half of the women will have no identifiable cause for their losses, leaving room for alternative underlying mechanisms. I found that women with RPL display heightened social olfactory abilities, compared to age-matched control women, and that these are significantly correlated with the number of miscarriages. Physiologically, I found that women with RPL show significantly altered hormonal and physiological response to the odor of an unfamiliar man. Lastly, anatomical and functional MRI investigation implied structural differences in the olfactory bulb of the two groups and functional differences in higher processing domains.

Finally, in my third project, I conducted an experiment more close to my future research passion - the placebo effect. Under the guidance of Prof. Uri Alon, I found that a placebo effect, namely manipulation of subjects' expectations, can be used to increase creativity (Rozenkrantz et al., PLoS one, 2017). Taking advantage of the non-invasive nature of olfactory stimuli, two groups of subjects smelled an odor, while only the placebo group was told that the odor increases creativity. I found that this suggestion was enough to significantly enhance creativity scores in the placebo group, which was evident in two different tests for creativity.

תקציר

במהלך הדוקטורט שלי, הובלתי שלושה פרוייקטים הבוחנים את הממשק בין חוש הריח והתנהגות אנושית במצבי בריאות וחולי, מתוכם שני פרוייקטים הקשורים למצבי חולי תחת הנחייתו של פרופ' נעם סובל, ופרוייקט אחד הקשור להתנהגות אנושית בריאה, תחת הנחייתו של פרופ' אורי אלון.

בפרוייקט הראשון שלי בחנתי האם ילדים עם אוטיזם מפגינים פרופיל אולפקטורי שונה מאשר ילדים בעלי התפתחות תקינה (Rozenkrantz et al, Curr Bio, 2015), בשיתוף פעולה עם המרכז לאוטיזם במרכז הרפואי אסף הרופא. השתמשתי בתגובת ההרחה, מדד לא-מילולי בן 10 דקות, המודד תגובה נשימתית לריחות נעימים ולא נעימים, ובחנתי זאת בשתי אוכלוסיות: ילדים אשר אובחנו עם אוטיזם וילדים בעלי התפתחות תקינה. תוך שימוש במדד האובייקטיבי הנ"ל, מצאתי שילדים עם אוטיזם מפגינים תגובות נשימתיות שונות לחלוטין בהשוואה לילדים בעלי התפתחות תקינה, וששוני זה נמצא במתאם גבוה עם חומרת האוטיזם שלהם. השוני בעיבוד האולפקטורי בין שתי הקבוצות איפשר לסווג נכונה 81% מהילדים לקבוצותיהן המקוריות, בהסתמך על תגובת ההרחה בלבד.

בפרוייקט שני אשר בקרוב יוגש כמאמר, ואשר הוגדר במקור כבעל סיכון גבוה, אך רווח גבוה, אני חוקרת את התפקיד האפשרי של תקשורת חברתית אולפקטורית בהפלות חוזרות. ההיפותזה מבוססת על אפקט ברוס במכרסמים, בו נמצא כי נקבות מפילות את ההריון שלהן בתגובה לריחות גוף המופרשים מזכר שאינו הפרה אותן (Bruce effect, 1959). בפרוייקט זה אני בוחנת את ההשערה כי נשים החוות הפלות חוזרות יפגינו פרופיל אולפקטורי שונה משל נשות ביקורת. בהפלות חוזרות, הרווחות בקרב 1% מהנשים, יותר מחצי מהנשים לא תמצאנה סיבה כלשהי למצבן, מה שמשאיר מקום למנגנונים חלופיים. אני מצאתי שנשים החוות הפלות חוזרות מפגינות יכולות אולפרטוריות-חברתיות מוגבהות בהשוואה לנשות ביקורת התואמות להן בגיל, ושיכולות אלו הינן במתאם משמעותי סטטיסטית עם מספר ההפלות. פיזיולוגית, מצאתי שנשים החוות הפלות חוזרות מגיבות באופן שונה לריח גוף של גברים זרים, כפי שהתבטא ברמות הורמונים וכן בתגובה של עוררות אוטונומית. לבסוף, בדיקה אנטומית ופונקציונלית באמצעות MRI מרמזת כי ישנם הבדלים מיבניים בבלוטה האולפקטורית של שתי הקבוצות וכן הבדלים בפעילות המוחית באזורי עיבוד גבוהים יותר.

לבסוף, בפרוייקט השלישי שלי ביצעתי ניסוי שקרוב יותר למושא המחקר העתידי שלי – אפקט הפלצבו. תחת הנחייתו של פרופ' אורי אלון, מצאתי שאפקט הפלצבו, כלומר מניפולציה של ציפיות של נבדקים, יכול לשמש להגברת יצירתיות (Rozenkrantz et al., PLoS one, 2017). תוך רתימת האופי הלא-פולשני של גירויים אולפקטוריים, שתי קבוצות של נבדקים הריחו ריח, כאשר רק לקבוצת הפלצבו נאמר שהריח מגביר יצירתיות. מצאתי שהסוגסטיה הזו הינה מספקת להגברה משמעותית של ציוני היצירתיות של קבוצת הפלסבו, כפי שנצפה בשני מבחני יצירתיות שונים.

Introduction:

Over the last decade it has become increasingly evident that olfaction is implicated in human social communication. Most terrestrial mammals rely on their sense of smell for interpersonal social interaction[1-6]. Humans also use social chemosignals[7-10]. Sweat-bound odors may coordinate menstrual synchrony in women[11], influence human mate selection[12], convey fear[13,14], drive pronounced hormonal[15-17] and behavioral[18-20] modifications, and alter brain activity[18,21-24].

If indeed olfaction mediates social interaction in humans, one may ask what happens in individuals whose social interaction abilities are impaired – would they display altered olfaction? In my first project, I investigated the question of olfactory sampling and processing mechanism in children with autism. Using the *sniff response*, a 10-minute non-verbal olfactory measure, I found that children with autism display a completely altered response to pleasant and unpleasant odorants, and that the magnitude of this alteration is highly correlated with their autism severity score. Although I did not use social odors in this task, the altered olfactory response nonetheless reflects mechanisms at play when sampling and processing social chemosignals, and implies an olfactory contribution for the social difficulties in ASD. As a more definite evidence for this contribution, a study recently published in our lab that I did not lead but took part in further demonstrated that ASD adults display altered behavioral and physiological responses to social chemosignals. Whereas this provides significant support for the behavioral manifestation of olfaction in social disorders, my project can be seen as suggesting the underlying neural mechanism for such a behavior.

I then continued and probed for additional human behaviors that may be guided or influenced by olfactory social chemosignals. Reproduction represents perhaps the most important human social interaction. In mammals, a robust olfactory-mediated effect would cause a pregnant female to miscarry upon exposure to bodily odors from a non-stud male[25]. This effect has been mainly studied in rodents, but observed in other mammals as well, including primates. Interestingly, in humans a condition called “recurrent pregnancy loss”, apparent in 1% of women, is unexplained. In fact, about half of these women have no etiology for their losses. In this project, I found a difference in olfactory performance, responses and neural underpinnings between women experiencing recurrent pregnancy loss and women who did not have a single miscarriage. I provide assorted evidence using various behavioral, physiological and neural measures, for an altered olfactory profile in women suffering from this condition. In other words, a condition that was previously associated primarily with the womb is here for the first time associated with the brain, and more particularly, with the olfactory system.

In the appendix I detail two additional projects I led or co-led during my PhD but were not originally included in my PhD research proposal. One is another investigation of human social interaction and its role in our daily lives, where I test the hypothesis that human breast milk odor facilitates parental behavior. The second asks a more technical question regarding olfactory mechanisms: Does the sniff response reflect levels of consciousness in patients with disorders of consciousness, and can we use it to predict rehabilitation results in these patients?

Finally, in an independent project together with the Alon lab, I took advantage of the non-invasive nature of olfactory stimuli to conduct an investigation of a psychobiological phenomenon, the placebo effect. This project stemmed directly from my profound interest in the placebo effect, and specifically in exploring this effect outside the current boundaries of clinical settings, and in more daily situations. This project is not tightly linked to the other projects I'm presenting, but since I will be investigating the placebo effect in my post-doctoral training, I see it as an important connecting link in my career path.

Chapter A: Altered olfaction in children with ASD

Rozenkrantz, Zachor, Heller, Plotkin,Weissbrod, Snitz,Secundo and Sobel, current biology, 2015[26]

Autism spectrum disorders (ASD) are a cluster of neurodevelopmental disorders characterized by persistent deficits in communication and social interaction, as well as restricted and repetitive patterns of behavior, interests, or activities[27]. The prevalence of ASD is increasing, with latest estimates of 1 in 88 children in the USA, a 23% increase within two years[28]. Symptoms required for diagnosis are usually apparent by the age of three, however wide heterogeneity of the disorder and lack of biological markers for definite diagnosis result in many cases which remain unidentified until pre-school, when more symptoms emerge. Current diagnosis methods are behavioral rating scales, and though highly effective, they are time consuming. Early diagnosis is of immense importance, allowing for early intervention which markedly improves prognosis[29]. Therefore, a reliable biomarker is essential to improve screening and detection.

There are several reasons to hypothesize altered olfaction in autism. First, abnormal olfactory function in children and adults with ASD is implicated in many anecdotal observations and sensory dysfunction studies, yet only few studies have specifically considered an olfactory profile in ASD. The existing literature regarding the role of olfaction in autism is mostly conflicting, although nevertheless generates an interesting image of an understudied sensory impairment potentially related to the disorder[30-33]. Briefly, individuals with autism typically display spared odor detection, but impaired odor identification[31,34,35]. Odor identification abilities decline with age in ASD children, unlike expected in normal olfactory development[36,37]. In this respect, two studies found autistic children to have normal identification abilities, one of which reported impaired detection[30,38]. Finally, children with autism may perceive odor pleasantness differently from typically developed children, namely rating pleasant odors as less pleasant, and unpleasant odors as less unpleasant. Importantly, pleasantness is the principal perceptual dimension of olfaction[38-40]. One possible reason for the differences across these studies is the verbal and task-dependent nature of standard olfactory tests, which typically entail following verbal or written time-locked instructions, and providing verbal or written answers. This makes them susceptible to ASD-related differences in comprehension, motivation, and general task-related parameters.

In addition, when considering the neural substrates implicated in autism, the most noted substrates – Cerebellum and Amygdala – are notably olfaction-related[41,42]. In olfaction, the cerebellum may play a role in regulation of odorant-dependent sniffing, namely the sensory-motor component of olfaction[43,44], and an olfacto-cerebellar pathway is considered to be involved in odor identification

and detection[43]. The other well-noted substrate in ASD, the Amygdala, is a part of the primary olfactory cortex which receives direct input from the olfactory bulb. It takes part in olfactory processing related to odor intensity coding, and possibly valence coding as well[45,46].

Given the above, when I set out to study the olfactory profile of children with ASD, I first chose a measure which would provide insight on the olfactory processing of the participants, without involving verbal or higher cognitive abilities, and better yet – with no instructions to follow or active task to perform. This unique olfactory measure is called the *sniff response,* and is essentially a modulation of our sniffing behavior in accordance with odorant content. This modulation, namely larger sniffs for pleasant odorants and smaller sniffs for unpleasant odorants, occurs within ~160 ms of odorant delivery, and as mentioned above, is thought to be mediated via the cerebellum, which is also implicated in autism[43,47].

The sniff response is the sensory-motor mechanism of olfaction, since it entails fine adjustment of a motor process (the sniff) in precise accordance with sensory input (the odor). Interestingly, an emerging theory suggests impaired sensory-motor coordination in autism[48-50], namely individuals with autism display difficulties in modulating a motor action in response to a sensory stimulus[51]. One type of brain mechanism subserving sensory-motor coordination is referred to as internal action models (IAMs). IAMs are brain templates that allow action initiation based on sensory expectations alone and ongoing refinement of motor output based on sensory input flow[52], and as such, include the sniff response. However, whether impaired IAMs occurs across sensory systems and how it relates to the major phenotype of ASD, namely impaired social communication[53], remains unclear. Thus, I set out to test the hypothesis that the sniff response will be altered in children with autism, supporting the failed IAMs theory in olfaction. Notably, the hypothesis is not for a motor impairment alone, meaning that children with autism will be unable to the sniff or have impaired sniffing, rather that the sensory-motor coordination component will be impaired, meaning children will display an inappropriate sniff given a particular odor.

EXPERIMENTAL DESIGN:

Participants: Legal guardians (all were parents) of all participants signed informed consent to procedures approved by both the Assaf Harofe Medical Center and Israeli National Helsinki Committees. Exclusion criteria for all children were organic smell disturbances or acute respiratory infection, and for TD children a Social Communication Questionnaire (SCQ) score of above 11[54]. To estimate the number of participants to enroll a power analysis was conducted based

on means and standard deviations in healthy adults (healthy controls in ref [55]). Given previous odorant-dependent changes in sniff volume from 60.65 to 55.54 ± 5 normalized volume units (nvu), at Power = 0.8 and Alpha = $p < 0.05$ power analysis implies at least 17 participants in each group. I therefore studied 18 children with ASD (17 boys, mean age = 7 ± 2.3) (this gender bias reflected the underlying population at the Autism Center) and 18 typically developing (TD) controls (17 boys, mean age = 6.7 ± 2.1). The TD and ASD groups did not significantly differ in age ($t_{34} = 0.51$, $p = 0.61$), gender (Fisher's exact test $p = 1.0$), or parental education ($t_{63} = 1.22$, $p = 0.23$). Table 1 lists all non-olfactory measures obtained. Notably, only about 1 in 4 children approached at the autism center agreed to participate. This raises a selection bias concern whereby perhaps only a specific subset of ASD (those who agreed) was tested. To address this, I obtained all the non-olfactory measures (e.g., ADOS scores, IQ, VABS, etc) from the children who were approached but not tested, and compared this to the tested group. No differences between the two groups was found ($F_{9,70} = 1.59$, $p = 0.13$).

Procedures: The child was comfortably seated in front of a computer monitor viewing a cartoon, and fitted with a custom-designed double-barreled pediatric nasal cannula that both delivered odors from a computer-controlled air-dilution olfactometer, and measured the nasal airflow of the *sniff-response* (Figure 1). The 10-minute procedure consisted of 20 trials (10 for each valence), each 1-2 seconds in duration, separated by a 30 second inter-trial-interval. I used two pairs of odorants, one mono-molecular (pleasant phenyl-ethyl alcohol, PEA, undiluted, CAS 60-12-8, Sigma-Aldrich and unpleasant butyric acid, diluted at 30% in odorless propane-1,2-diol, CAS 107-92-6, Sigma-Aldrich), and one of complex mixtures (pleasant Herbal Essence and unpleasant Rotten Fish, both from Senseale, Ramat Gan, Israel). Both pairs of odorants were presented at similar subjective intensity as rated by adult raters. The same result materialized for both odor pairs. To obtain explicit odor ratings, children sniffed the odors from jars, and rated their pleasantness using a 6-point visual analogue scale (VAS) where each point was also denoted by a "smiley", ranging from a happy face associated with pleasant to a sad face associated with unpleasant. To assess general motor performance in ASD I conducted three tasks: finger tapping test (FTT), strength of grip (SOG) and modified pegboard test (MPT)[56]. FTT - using the index finger to tap on a board-mounted manual counter as many times as possible within 10 s. The task was repeated twice with each hand, the totals from all trials were averaged for both hands combined. If the two trials were not within ±5 points, a third trial was completed, and the average of three trials was used. SOG - was measured using a hand dynamometer (NeuLog, SES Scientific Educational Systems, Israel) the subjects held in the palm of their hand and squeezed as tightly as

possible. Strength (in kilograms) was recorded in three trials for each hand, and averaged. The total SOG score was computed by combining the means of both hands. MPT - the participant was required to insert pegs in a grooved board in a specific directionality as quickly as possible using the dominant and non-dominant hand separately. The modification was in the number of pegs used (18 instead of 25) and type of pegs – two-colored wooden pegs. The score was the time required to place all 18 pegs into the holes (timing was not interrupted in the event of a dropped peg). The total MPT score was computed by combining the completion time in both hands.

Analysis: Nasal airflow was measured continuously. To account for variation across subjects stemming from such factors as cannula placement, each odorant sniff was normalized through dividing it by the average of three non-odorant nasal inhalations that preceded it. Data were then analyzed using Matlab (MathWorks, version R2013a) and STATISTICA (StatSoft, version 7). Differences in sniff response between pleasant and unpleasant odors were first estimated by conducting a t-test on every time-point of the ongoing respiratory trace (dotted black line in Figure 2). I corrected for the number of t-tests as follows: The sniff-response in adults materializes within 160 ms[47]. Thus, I down-sampled the recording to just above the relevant nyquist range, namely 16.667 Hz. Given an average sniff of about 1.5 s, this translates to 25 comparisons per sniff (16.667*1.5). Thus, I Bonferroni corrected for 25 comparisons (green line in Figure 2). Next, differences in sniff response between pleasant and unpleasant odors and specific sniff parameters as a function of group (ASD/TD) were estimated using a multivariate repeated-measures ANOVA with conditions of sniff parameter (mean airflow, airflow peak, sniff duration and sniff volume), odorant valence (pleasant or unpleasant) and group (ASD or TD). This was followed by repeated measures ANOVAs and t-tests for each sniff parameter alone. When classifying ASD and TD based on this data, each attempt to classify a subject is a Bernoulli trial with even odds of success and failure. Therefore the probability of correctly classifying 29 out of 36 subjects is given by: $p = \left(\frac{1}{2}\right)^{36} * \binom{36}{29} < 0.001$. This is therefore the statistical power of our classification result. Finally, correlation between the sniff-response and autism measures was assessed using the Spearman correlation coefficient.

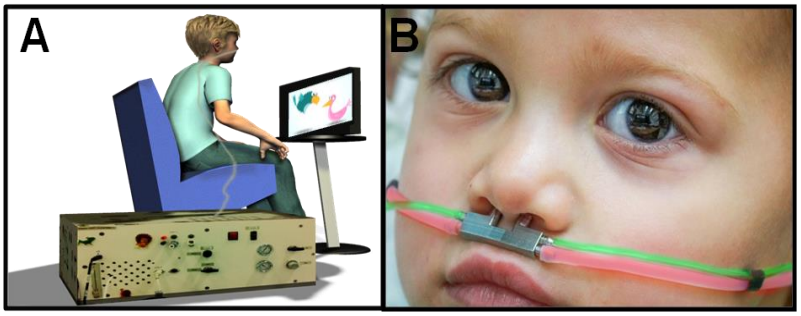
Subject	Gender	Age	IQ	VABS	ADOS			ADI			Subject	Gender	Age	SCQ
					Social	RRB	Severity	Intera.	Comm.	RRB				
ASD 1	m	7.08	- - -	82	11	5	9	4	5	4	TD 1	m	7.50	1
ASD 2	m	6.33	71$	77	15	4	8	11	12	8	TD 2	m	5.58	2
ASD 3	m	4.17	- - -	68	10	3	6	17	12	2	TD 3	m	4.58	10
ASD 4	m	10.00	90$	78	13	3	9	20	15	9	TD 4	m	9.42	10
ASD 5	m	5.58	118#	97	11	3	8	23	12	7	TD 5	m	5.25	8
ASD 6	m	6.33	104#	85	6	3	6	6	3	2	TD 6	m	7.25	0
ASD 7	m	4.92	104#	78	7	4	6	19	20	12	TD 7	m	4.33	5
ASD 8	m	6.83	94#	71	7	6	6	12	9	3	TD 8	m	7.08	1
ASD 9	m	7.33	134#	83	7	3	6	7	3	2	TD 9	m	7.33	1
ASD 10	f	9.67	73$	75	19	0	10	23	14	2	TD 10	f	7.00	2
ASD 11	m	4.92	102#	89	11	4	8	11	9	5	TD 11	m	4.58	2
ASD 12	m	4.33	130#	74	2	4	3	18	19	10	TD 12	m	4.08	6
ASD 13	m	5.92	83#	76	15	3	10	21	19	12	TD 13	m	5.67	1
ASD 14	m	11.58	40$	63	9	4	8	15	12	4	TD 14	m	10.42	1
ASD 15	m	6.17	- - -	94	9	3	7	9	3	4	TD 15	m	6.50	0
ASD 16	m	9.33	- - -	69	12	6	8	11	20	8	TD 16	m	8.83	0
ASD 17	m	11.08	106$	88	5	3	5	4	9	4	TD 17	m	10.08	6
ASD 18	m	4.92	- - -	92	6	0	3	12	4	3	TD 18	m	4.08	0
Average	94%	7.00	96.08	79.94	9.72	3.39	7.00	13.50	11.11	5.61		94%	6.65	3.11
S.D		2.33	25.57	9.52	4.17	1.58	2.06	6.26	5.98	3.45			2.05	3.46

Table 1. Non-olfactory characteristics of the ASD and TD groups. ASD measures are from the diagnostic procedure at the Autism Center, which typically includes the following: full scale IQ using the $Wechsler Scales of Intelligence (WISC-IV)[57] or #Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III)[58] (4 of the 5 missing IQ scores reflect large gaps across the IQ subscales that prevented derivation of a final score. Note that the subscale data implied average IQ for these children); Vineland Adaptive Behavior Scales (VABS) [59]; Autism Diagnosis Observation Schedule (ADOS), A semi-structured, interactive schedule designed to assess social and communicative functioning [60], to assess autism symptom severity, the standardized ADOS severity score was used [61]; and Autism Diagnostic Interview- Revised (ADI–R), A semi-structured interview administered to parents [62]. Values of severity range between 1-10, with a cut-off of 3 for inclusion in Autism spectrum disorders. All TD participants were screened for ASD using the Social Communication Questionnaire (SCQ) [54], a 40-item parent-report questionnaire for brief screening. '- - -' indicates that the test was not preformed or could not be evaluated.

RESULTS:

To measure the sniff-response in children I used a specially-built computer-controlled air-dilution olfactometer equipped with a custom-designed double-barreled pediatric nasal cannula that allowed us to simultaneously deliver odors and measure nasal airflow (Figure 1). I used this apparatus to precisely measure the sniff-response following pleasant (*rose* or *shampoo*) and unpleasant (*sour milk* or *rotten fish*) odors in 18 children with ASD (17 boys, mean age = 7 ± 2.3) and 18 age and gender matched typically developing (TD) children (17 boys, mean age = 6.7 ± 2.1) as controls (Table 1). The 10-minute procedure consisted of 20 trials (10 of each valence), each 1-2 seconds in duration, separated by a 30 second inter-trial-interval. During the paradigm, participants watched a cartoon.

Figure 1. A pediatric olfactometer delivered odors and measured sniffs



A. A subject is seated in front of a computer monitor viewing a cartoon, linked by nasal cannula to the olfactometer. **B.** A double-barreled nasal cannula delivering odorants (red) and measuring nasal airflow (green) (child is TD).

The sniff response was profoundly altered in ASD

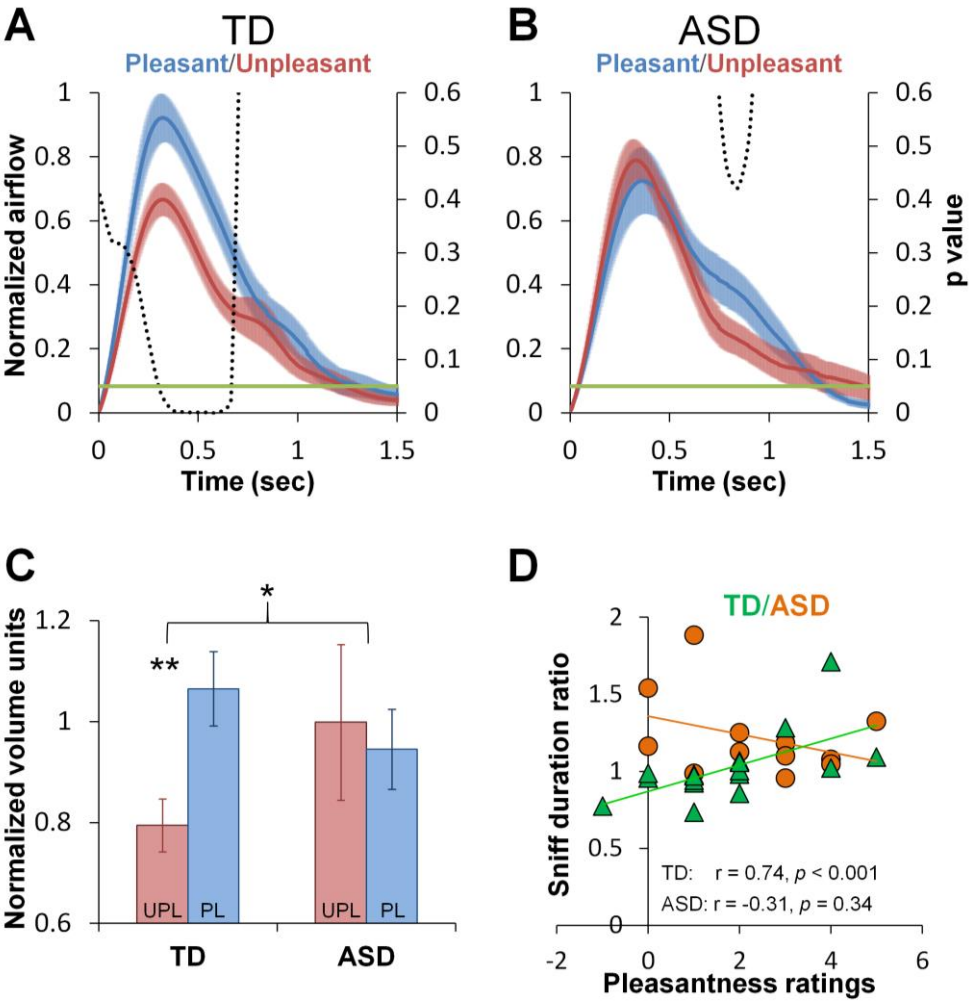
To characterize the TD and ASD sniff-responses I extracted four sniff parameters: sniff volume, peak airflow rate, mean airflow rate, and duration. A multivariate repeated-measures analysis of variance (ANOVA) applied to all parameters revealed a significant interaction between *odorant valence* (pleasant versus unpleasant) and *group* (TD versus ASD) ($F_{1,34} = 4.47$, $p < 0.05$), reflecting larger sniffs for pleasant versus unpleasant odors in TD alone. This was evident in a point-by-point comparison of the sniff traces revealing that TD children altered their sniff to account for odorant properties within 305 milliseconds of sniff onset (at 305 ms, flow pleasant = 0.918 ± 0.32 normalized flow units (nfu), flow unpleasant = 0.665 ± 0.22 nfu, $t_{17} = 3.68$, $p < 0.0019$, equivalent to $p < 0.05$ Bonferroni corrected for the multiple t-tests), and maintained this or greater difference 680 milliseconds into the sniff response (dotted line, Figure 2A). In contrast, ASD sniffs did not significantly differ by odor at any point along the sniff trace (Figure 2B).

In addition, a three-way interaction between *sniff parameters*, *odorant valence,* and *group* ($F_{3,102} = 6.16$, $p < 0.001$) revealed the same effect materialized individually in three of the four parameters

I extracted (e.g., *Volume* = $F_{1,34} = 4.2$, $p < 0.05$, TD: normalized sniff volume: pleasant = 1.07 ± 0.3 normalized volume units (nvu); unpleasant = 0.79 ± 0.22 nvu; $t_{17} = 4.73$, $p < 0.0005$. ASD: pleasant = 0.95 ± 0.33 nvu; unpleasant, = 0.99 ± 0.64 nvu; $t_{17} = 0.36$, $p = 0.72$. Same effects for mean and peak airflow, both $F_{1,34} > 4.2$, both $p < 0.05$) (Figure 2C). No other significant main effects or interactions were found (all $p > 0.11$). In other words, consistent with our hypothesis TD children exhibited an adult-like sniff-response, yet ASD children did not activate the olfactory internal action model to adjust their sniff in accordance with odorant properties.

Figure 2. An altered sniff-response in ASD

The averaged normalized sniff trace of TD (**A**) and ASD (**B**) children (n=18) in response to pleasant (blue) versus unpleasant (red) odors. The black dotted line is the Bonferroni corrected p value of the paired t-test of airflow for pleasant vs. unpleasant; green horizontal line marks the Bonferroni corrected 0.05 significance level. **C**. The averaged normalized sniff volume in response to pleasant (blue) versus unpleasant (red) odors in ASD versus TD children. *p<0.05, **p<0.005. Error bars represent s.e.m.



A key characteristic of this approach is that it does not depend on verbal comprehension. Nevertheless, I later used a child-friendly visual-analogue scale (VAS) to obtain odorant pleasantness estimates from the participants. Whereas 17 of the 18 TD children provided such estimates directly after testing, only 3 of the 18 ASD subjects agreed to do the same. An additional 9 of the ASD children agreed to provide these estimates when approached at a later date. In TD, the sniff-response was a strong predictor of perceived explicitly reported pleasantness ($r = 0.74$, $p < 0.001$, Figure 2D, green). In turn, although there were no differences in reported pleasantness between TD and ASD ($U = 81$, $p = 0.37$), the sniff-response was unrelated to perceived explicitly reported pleasantness in ASD ($r = -0.31$, $p = 0.34$, Figure 2D, orange). The VAS reports obtained from children, both TD and ASD, are not highly reliable in our view. Nevertheless, these reports implied that both TD and ASD children perceived the pleasant and unpleasant odors as intended, yet only the TD children modulated their sniff accordingly.

The sniff-response was linked to social but not motor impairment in ASD

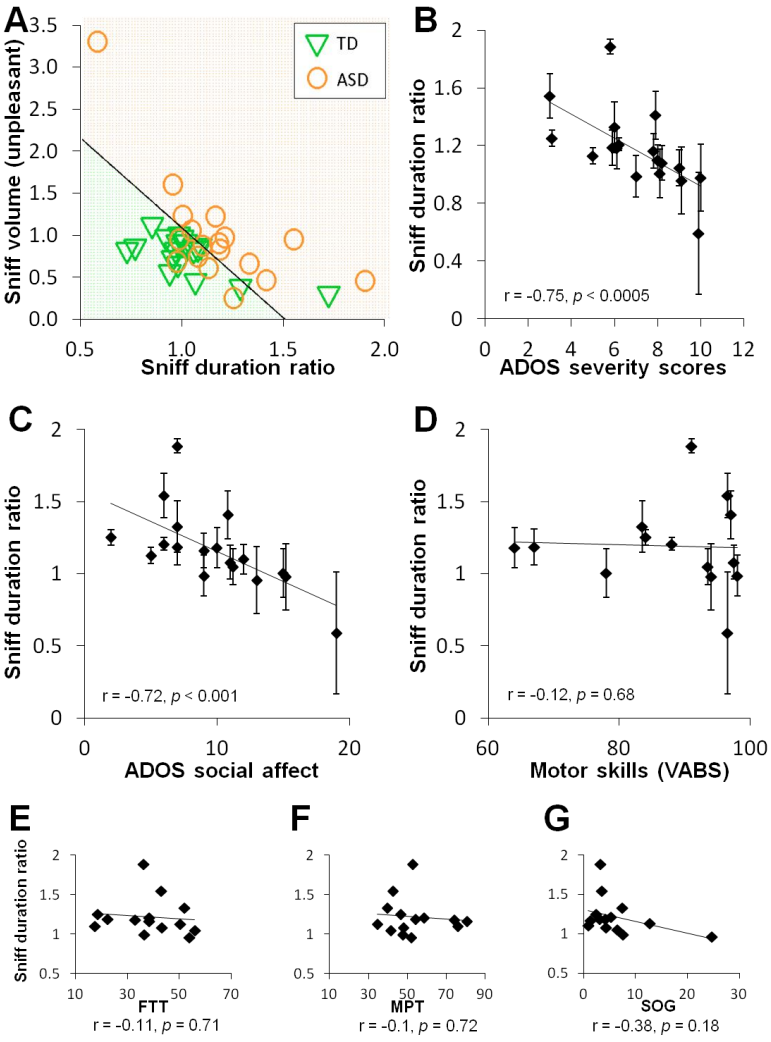
The above analyses revealed a pronounced group difference. I next tested whether the altered sniff response in ASD can differentiate ASD from TD children at a single subject level. I used a multivariate normal density classifier applied to the sniff parameters, and found that a classifier relying on the differences in pleasant vs. unpleasant sniff duration combined with the sniff volume for unpleasant odors effectively distinguished TD from ASD children. Using a leave-one-out analysis the classifier correctly identified 17 of 18 TD children as well as 12 of 18 ASD children, i.e., one false positive and six false negatives (81% accuracy, binomial $p < 0.001$) (Figure 3A). In contrast to a group-difference alone, this power at the single subject level implies that an altered sniff-response is a genuine part of ASD.

Next, to ask whether the *sniff-response* informs on ASD beyond classification alone, I correlated the *sniff-response* with independently obtained autism severity scores (ADOS)[61]. I found a strong correlation in several sniff-response parameters, most notably in sniff duration, reflecting that within the ASD group more aberrant sniffing (longer sniff durations for unpleasant vs. pleasant odors) was associated with an increase in autism severity ($r = -0.75$, $p < 0.0005$) (Figure 3B). Notably, this correlation between the sniff-dependent measure and ADOS scores is very similar to the ADOS test retest correlation[63].

To further investigate this link between autism severity and the sniff-response I looked at separate components of the non-olfactory tests I conducted. I found that the sniff response remained highly

predictive of the Social Affect component of ADOS (r = -0.72, $p < 0.001$) (Figure 3C), yet it was unrelated to the Restricted and Repetitive Behavior component of ADOS (r = 0.18, $p = 0.47$). Notably, there was a trend towards a correlation between Social Affect component of ADOS and IQ (r = -0.42, $p < 0.09$), and indeed an ensuing trend towards a correlation between the sniff-response and IQ (r = 0.55, $p < 0.06$). In other words, the sniff-response measure is reflective of the mechanism involved with the social impairment that is at the heart of ASD.

Figure 3. The sniff-response reflects social impairment in ASD



A. The results of a leave-one-out classification scheme based on sniff-response parameters (ASD in orange, TD in green). The graph reveals one false positive classification and six false negatives. **B.** Correlation of sniff duration ratio with autism severity (ADOS). **C.** Correlation of sniff duration ratio with the social affect component of the ADOS test. **D.** Correlation of sniff duration ratio with the motor skills score of the VABS test. In B-D each dot is a subject and error bars represent s.e.m. **E-G.** Correlation of sniff duration ratio with a battery of motor tests: Finger tapping test (FTT), modified pegboard test (MPT) and strength of grip (SOG).

Finally, to ask whether the sniff-response merely reflected a generalized motor impairment, I first compared it to the separately obtained motor score from the Vineland Adaptive Behavior Scale (VABS)[59], and found no relation at all (r = -0.12, $p = 0.68$) (Figure 3D). Altered sniffing was unrelated to the other VABS subscales as well (communication: r = 0.22, $p = 0.39$; daily living: r = -0.22, $p = 0.39$; social: r = 0.07, $p = 0.78$). Given that the VABS depends on parental reports

rather than direct testing, and its social score was unrelated to the ADOS social score in our (r = -0.13, p = 0.61) and several previous studies [64,65], I further assessed the relation to basic motor performance by conducting direct testing. I re-approached the children with ASD using a previously described [56] battery of simple motor tests including a finger tapping (FTT), strength of grip (SOG) and modified pegboard test (MPT). Like the VABS motor subscale, I found that performance on these tests was unrelated to the sniff-response (FTT: r = -0.11, p = 0.71; SOG: r = -0.1, p = 0.72; MPT: r = -0.38, p = 0.18, Figure 3E). In other words, the degree of alteration in the ASD sniff-response was unrelated to the level of basic motor performance.

DISCUSSION

Taken together, our results imply a pronounced alteration of olfactory perception that is evident in children with ASD, and is more pronounced with increased autism severity. Despite being a sensory-motor measure in nature, the altered sniff response in ASD was not correlated with motor deficits of the disorder, rather with the primary phenotype of ASD, namely impaired social communication. This implies that the sniff response may provide for a novel early non-verbal non-task-dependent ASD marker. That said, several limitations prevent current application of this marker: First, the current study was far in scope from a clinical trial. Second, an important open question remains whether this marker is specific to ASD or common across various developmental disorders. Third, I did not obtain full IQ scores for the TD cohort. Finally, several technical issues (such as compliance) need address before this could become a useful tool in clinics.

In turn, these findings also support an emerging theory regarding the mechanisms of ASD, and potentially link this theory to the hallmark symptom of ASD. Specifically, the impaired-IAM theory of ASD is supported here by an olfactory sensory-motor mechanism, implying a mechanistic link between the underpinnings of olfaction and ASD and directly linking an impaired IAM with impaired social abilities. Impaired IAMs subserving visual gaze and socially relevant eye fixation targets may partially underlie the social impairments in ASD[66], giving rise to an ASD-type theory of mind[67]. Finally, our results may offer a novel additional possible link between impaired sensory-motor mechanisms and the social impairment of ASD. Specifically, increasing evidence implies that social chemosignaling is a meaningful component of human social interaction[20,68]. Our lab has recently discovered that individuals with autism display altered response to social chemosignals, which may underlie part of the ASD phenotype[69]. I propose that the altered sniff-response leads to altered olfactory processing, which contributes to impaired

social communication. Consistent with this hypothesis, the degree of alteration in sniff-response was predictive of impaired social communication (Figure 3C) but not of generalized motor impairment (Figure 3D, 3E-G).

Chapter B: Human Repeated Pregnancy Loss is Associated with Altered Olfaction

Olfaction plays an important role in conveying social information. Most terrestrial mammals rely on their sense of smell for interpersonal social interaction[1-6]. Bodily odors guide mammalian behaviors ranging from simple automatic actions such as finding and suckling from a nipple[70] and onto complex behaviors related to social dominance[71] and sociosexual and aggression behavior[72]. Humans also use social chemosignals[7-10]. Sweat-bound odors may coordinate menstrual synchrony in women[11], influence human mate selection[12], convey fear[13,14], drive pronounced hormonal[15-17] and behavioral[18-20] modifications, and alter brain activity[18,21-24]. In other words, whereas species specificity is on one hand a hallmark of social chemosignaling, its mechanisms may be conserved across species.

In humans, loss of pregnancy during the first trimester occurs in more than 50-60% of total conceptions, and 2-4% of the couples that have had a spontaneous miscarriage are prone to recurring pregnancy loss. Recurrent pregnancy loss (RPL) is defined as two or more consecutive unexplained miscarriages, and occurs in about 1% of all women. Despite extensive examinations, about half of whom will have no identifiable cause for their losses[24,25]. Given this statistical backdrop, and the role of olfaction in human social communication, this study asks whether a social olfactory mechanism may explain a portion of the many unexplained human miscarriages.

The Bruce effect describes a robust odor-mediated phenomenon in rodents, in which pregnant females miscarry in response to bodily odors emitted from a male who did not father the pregnancy[25]. Initially studied in the laboratory mice, this effect was characterized as pregnancy block prior to embryo implantation[25,73,74], however it was since further established in many other rodents, at various stages of the pregnancy, including post-implantation, mid-gestational and up to 17 days of 23 days pregnancy[75-78]. Remarkably, it has also been described, although not directly tested, in lions[79,80], wild horses[81] and even primates[82-85], with the most comprehensive study describing gelada baboons females terminating 80% of pregnancies in the weeks after a dominant male is replaced, using demographic and hormonal data to establish a causal connection[86]. Such a large scope of occurrences suggests the possibility of a human analogous effect.

The most common evolutionary explanation for the Bruce effect is an adaptive strategy for a female to limit investment in offspring more likely to die near birth, for example due to infanticide following male replacement[87]. The exact mechanism by which the odor of the non-stud male causes miscarriage is unclear. Studies in rodents suggest at least two separate pathways, however both provide possible mechanisms for pregnancy block at pre-implantation stage alone. In the first

pathway, male-specific urinary pheromones bind to the female's vomeronasal organ (VNO)[88,89]. These chemical signals can trigger a downstream neuroendocrine response which is thought to cause a miscarriage by releasing dopamine, which prevent the secretion of prolactin, a crucial hormone for the maintenance of the curpus luteum and thus the implantation of the embryo[89-91]. However, if the male-specific urinary pheromones are learned by the female during mating or shortly after, a release of noradrenaline will lower the receptivity of the VNO to these pheromones, preventing pregnancy disruption[2,89]. The hormones oxytocin and vasopressin play an important role in this social memory process[92,93]. Removing the Vomeronasal organ (VNO) of females significantly reduced pregnancy block[94]. A separate pathway involves estradiol (E2), a metabolic product of testosterone. When a female is exposed to a male's urine, E2 enters the bloodstream via nasal ingestion, and travels to the uterus, which has high density of suitable receptors. Excessive estradiol prevents implantation and disrupt pregnancy[95-97]. Castrated males are incapable of terminating female pregnancies, except when these males are given testosterone[95,98].

In humans, the existence/ functionality of the VNO is of debate[99,100]. Nevertheless, as demonstrated in other mammals such as sheep and cows, chemosignaling can occur in a mechanism involving the main olfactory epithelium rather than the VNO[101]. The hypothesis tested in the current study is a Bruce-like effect in humans, which may underlie some of the many idiopathic recurrent miscarriages. Considering the ethical limitations to causal investigation of human miscarriage, the study set out to characterize olfactory processing in RPL women and controls to probe for any circumstantial support of the hypothesis.

EXPERIMENTAL DESIGN:

Participants: I approached women arriving at the Recurring Miscarriages Unit at Sheba Medical Center (N=21, mean age 33.1 ± 6.4), and as controls women who have no known history of miscarriages and have had one child or more were recruited (N=21, mean age 34.5 ± 4). All participants signed informed consent approved by the Sheba Medical Center Helsinki Committee. Inclusion criteria for the RPL group were two or more consecutive unexplained miscarriages. Tests were conducted at a time-window where participants were not suspected to be pregnant, and were not actively treated in any way. Table 2 details subjects' age, education and mood status.

Procedures: the experiment was conducted on two separate days, for one hour each day, at the participant's house. I applied a battery of olfactory tests to assess olfactory abilities of both ordinary odors and of putative human social chemosignals. To characterize perception of ordinary odors

three tests were used: olfactory identification of 20 every-day odorants (e.g., "peanuts", "pizza" etc.) using the widely applied standardized University of Pennsylvania Smell Identification Test (UPSIT)[102], in which a subject choses one option of four that best describes the odor she smells; olfactory detection thresholds for the alliaceous odor dimethyl trisulfide (DMTS) were determined, using a maximum-likelihood adaptive staircase procedure (MLPEST), in which a subject was presented with two jars, one blank and one containing a changing concentration of DMTS, and had to choose where the odor is (forced choice); and olfactory discrimination of the musky odor muscone (P-15), in which a subject was presented with three jars, two blank and one containing the odor, and had to choose in which jar the odor is (three alternative forced choice). This task was repeated three times to gain statistical power. To characterize perception of putative human social chemosignals, I tested discrimination for the testosterone derivatives androstenone (ANN) and androstadienone (AND) and the estradiol derivative estratetraennol (EST). As described for the previous discrimination test, a three alternative forced choice paradigm was applied, with three repetitions for each odorant. An additional task included identification of spouse's body odor, which was collected using t-shirts worn for two consecutive nights, without use of deodorant. The identification was also performed in a three alternative forced choice paradigm, wherein the subject had to identify her spouse from two other t-shirts, one blank (worn by no-one) and one worn by a spouse of another woman in the study (stranger). The task was repeated four times. Shirts were presented and sampled using a designated shirt-sniffing device that we developed in our lab, details can be found in [69].

During the experiment, subjects' physiological measures were recorded, specifically breathing pattern, galvanic skin response (GSR, a measure of autonomic arousal) and pulse. In addition, saliva samples were collected before and after each experimental session, in order to test for levels of stress-related hormones, such as cortisol, and of testosterone. Due to diurnal changes in cortisol and testosterone, both meetings with every subject were carefully set to occur in the same time of day. Finally, two standardized mood questionnaires were administrated to test for stress, anxiety and depression levels in both groups, the Beck Depression Inventory (BDI)[103] and Cohen's perceived stress scale (CPSS)[104]. Subjects did not differ in mood ratings, age or education (Table 2).

Table 2. Non-olfactory characteristics of the RPL and control (CNTR) groups. The table contains information regarding subjects' age, years of education, and for the RPL group, also number of miscarriages. Scores for the two mood questionnaires, the Beck Depression Inventory (BDI)[103] and Cohen's perceived stress scale (CPSS)[104], are also displayed. --- stands for missing data.

Subject	Age	Education	Number of mis-carriages	BDI score	CPSS score	Subject	Age	Education	BDI score	CPSS score
RPL 1	29	14	4	12	19	CNTR 1	43	15	10	15
RPL 2	35	18	3	0	1	CNTR 2	41	12	14	33
RPL 3	44	16	2	9	10	CNTR 3	33	15	0	5
RPL 4	38	17	4	10	10	CNTR 4	39	15	8	28
RPL 5	42	17	3	10	26	CNTR 5	33	13	17	21
RPL 6	27	15	4	8	17	CNTR 6	41	18	0	9
RPL 7	28	16	2	7	18	CNTR 7	33	12	11	14
RPL 8	35	12	3	19	27	CNTR 8	31	15	13	14
RPL 9	22	15.5	2	1	11	CNTR 9	31	18	2	10
RPL 10	28	12	3	6	11	CNTR 10	32	22	0	8
RPL 11	40	19	3	8	16	CNTR 11	35	15	1	4
RPL 12	29	12	4	5	13	CNTR 12	34	16	17	22
RPL 13	32	12	5	13	23	CNTR 13	39	12	2	18
RPL 14	24	12	3	10	20	CNTR 14	33	18	2	8
RPL 15	31	15.5	3	19	25	CNTR 15	31	17	10	16
RPL 16	31	---	3	3	14	CNTR 16	34	21	3	16
RPL 17	39	13	6	0	14	CNTR 17	28	15	8	12
RPL 18	42	12	3	4	18	CNTR 18	31	16	6	21
RPL 19	29	16	3	3	15	CNTR 19	32	16	15	18
RPL 20	41	14	4	9	20	CNTR 20	37	15	1	16
RPL 21	30	19	3	8	20	CNTR 21	33	23	3	8
Mean	33.1	14.9	3.33	7.8	16.6		34.5	16.1	6.8	15
S.D	6.4	2.4	0.97	5.3	6.2		4	3.1	6	7.3

Analysis: Olfactory performance: three tasks of each odor type (ordinary odors: odor identification (UPSIT), threshold detection (DMTS) and P15 discrimination; and putative human social chemosignals: ANN, AND and EST discrimination, for details see procedures section above) were administered. In order to generate an average score for ordinary odor tasks and an average score for putative social chemosignal tasks, I averaged each three tasks per subject. Since performance is measured differently for each task, I first set all scores to be on the same scale, meaning between 0 and 1. Discrimination tasks inherently generate results which are in this range, so for the other tasks, namely odor identification and threshold, I normalized the results so that the maximum value of both groups would be regarded as 1, the minimum value as 0, and all other scores would accordingly be in between 0 and 1 (specific calculation per score was *(x-min)/(max-min)*). For the threshold test, instead of using the concentration at threshold for each subject, which varied

between 8*e-5 and 6*e-8, I used the –log(concentration) per subject, and then applied the above normalization. Data were then analyzed using Matlab (MathWorks, version R2013a) and STATISTICA (StatSoft, version 7). To compare these averaged ordinary and social odors scores between groups, I used a multivariate repeated-measures ANOVA with conditions of odor type (social, ordinary) and group (RPL, control). Follow up tests included t-tests for comparison between groups, first on each averaged score, and next for each of the raw scores of original tests. Correlation between performance and number of miscarriages was computed using Spearman coefficient, because data on number of miscarriages is discrete.

Hormonal data: Saliva collection was by un-stimulated passive drooling. Samples were kept in a portable minifridge until arrival at the lab, where they were stored at -20 °C, and thawed and centrifuged before testing. The saliva from each tube was assayed in duplicate wells. Tubes from a given participant were all assayed on the same plate, and tubes from different visits obtained at a given time were assayed on the same column of the 96-well plate to avoid systematic errors between conditions. For cortisol measures, the Extended Range High Sensitivity Salivary Cortisol Immunoassay kit was used and for testosterone measures, the Extended Range Salivary Testosterone Immunoassay kit was used (Salimetrics). After completion of the immunoassay, the absorbance of the fluorescent cortisol/testosterome conjugate–antibody complex in the wells were obtained at 450 nm and corrected at 490 nm with a microplate reader. Standard dilutions of cortisol (0, 0.012, 0.037, 0.111, 0.333, 1.0, 3.0 μg/dL) and testosterone (0, 6.1, 15.4, 38.4, 96, 240, 600 pg/ml) were used along a nonspecific binding well in the first two columns of the kit for calibration. Defined high and low control concentrations were used as a quality control for each column of the plate. The absolute salivary hormonal concentration was estimated from the fluorescence of the hormone conjugate–antibody complex by computing the inverse value on a four-parameter sigmoid fit obtained with the standard values. In order to compare hormonal status between the groups in the different conditions, a repeated-measures ANOVA with conditions of group (RPL, CNTR) and time (before, after) was applied for each hormone. Follow up tests included independent t-tests for comparison between groups and pair-wise dependent t-tests for comparison within each group, between the time points.

Physiological measures: Galvanic skin response (GSR), pulse and nasal respiration measurements were sampled at 1 KHz and recorded using a Power-Lab 4/35 Monitoring System (ADInstruments, Australia). Data were later displayed, and stored using LabChart 7 and 8 softwares (ADInstruments). Nasal airflow was measured using a nasal cannula (1103, Teleflex medical) placed at the nares and attached to a spirometer (Spirometer FE141 ADInstruments). GSR was

measured through two finger electrodes placed on the index and the third digit of the non-dominant hand (GSR Amp. FE116 ADInstruments). The analysis was conducted after band-pass filtering the data (0.05-35 Hz) to remove drift, and zeroing at event onset. During data analysis I found that some data files contained a constant high-frequency noise which was easily detected by applying a Fourier-transform on the data. Once detected, this noise was removed by filtering out the specific frequency. To conduct the analysis, for each subject, a 10 seconds GSR response the first exposure for each odor type - blank shirt, spouse shirt and stranger shirt - was extracted, the response to the blank shirt was deducted from the two body odor responses. Next, the two responses were normalized together using z-score within each subject to allow for inter-subjects comparison. After normalization, two parameters from each normalized GSR response (spouse, stranger) of each subject were extracted: peak and area under the curve (AUC). In order to conduct a multi-variate repeated-measures ANOVA which would include both variables, an adjustment of scales was in order. AUC range was between -40000 and 30000 normalized volume unit (nvu), whereas peak unit range between 0 and 4. For this aim, AUC units were divided by 10000, to range between -4 and 3, and a multi-variate repeated-measures ANOVA was applied to the GSR parameters (AUC, peak), with conditions of group (RPL, control) and odor (spouse, stranger). Follow up tests included univariate repeated-measures ANOVA for each GSR measure separately, independent t-tests for comparison between groups and pair-wise dependent t-tests for comparison within each group, between the conditions. Thirty-eight participants were available for this analysis, of which 31 had valid data that could be analyzed due to technical problems. In total, 17 RPL women and 14 control women from our original cohort were analyzed. Analysis was performed using Matlab (MathWorks, version R2013a) and STATISTICA (StatSoft, version 7).

Anatomical and functional magnetic resonance imaging:

Participants: subjects were recruited via social media groups or from the previous experiment. Inclusion criterion for the RPL group was at least two consecutive unexplained miscarriages, and for the control group no known miscarriages or abortions. Exclusion criteria for both groups were reported olfactory impairments or any additional reason which would prevent subjects from being scanned in the MRI (metal implants, claustrophobia etc.). All subjects signed an informed consent approved by the Ethics Committee of Wolfson Medical Center. A total of 28 RPL subjects and 45 control subjects (seven nulliparous) took part in the structural MRI scans (details below). Of whom, five subjects were excluded prior to analyses: three RPL subjects: one who miscarried one of two miscarriages due to chromosomal abnormality, thus did not fit the criterion for RPL, one who was reportedly, and evidently, hyposmic, and one who had an uterus removal; and two control

subjects: one who had an intentional abortion, and one who had an outer-uterus pregnancy which resulted in an abortion. Thus, a total of 25 RPL and 43 (seven nulliparous) control subjects were structurally analyzed. Most, but not all, subjects had the structural scans as part of the full-length experiment, which included two functional scans (details below). These included 25 of 28 RPL subjects, and 25 of 45 control subjects. Three were excluded for reasons detailed above (RPL: uterus removal; control: abortion and outer-uterus pregnancy), and one control subject was excluded from the analysis due to extensive head motion, leaving 24 RPL and 22 controls for the functional analyses (Table 3). All subjects completed a trait-anxiety questionnaire taken from the State-Trait Anxiety Inventory (STAI)[105], and a personality questionnaire (The "Big Five" Inventory[106]). Anxiety levels or personality traits did not differ between the groups (STAI: $t_{55} = 0.22$, $p = 0.82$; Big Five: all $t_{55} < 1.47$, all $p > 0.15$).

Table 3. Functional MRI subjects. The table contains information regarding 24 RPL and 22 control subjects who were included in the functional MRI analysis. Note that subjects are not matched, but sorted by age. --- stands for missing data.

Subject	Age	Number of children	Age of children	Number of mis-carriages	Anxiety STAI score	Subject	Age	Number of children	Age of children	Anxiety STAI score
RPL 1	25	2	1.8, 0.3	3	23	CNTR 1	29	0	---	46
RPL 2	27	2	5,0.6	3	50	CNTR 2	31	2	2.5,5	39
RPL 3	28	0	---	5	37	CNTR 3	31	1	1.3	34
RPL 4	29	1	0.7	3	56	CNTR 4	32	2	3.7, 1.4	31
RPL 5	29	0	---	2	34	CNTR 5	32	2	4,2	47
RPL 6	29	0	---	7	37	CNTR 6	32	2	4,2	25
RPL 7	31	0	---	3	41	CNTR 7	33	2	2.5,0.4	41
RPL 8	32	2	2.6, 0.6	3	29	CNTR 8	33	3	6,3.5,0.4	22
RPL 9	32	3	5,5,4	2	40	CNTR 9	33	1	1.5	27
RPL 10	34	2	6.5, 2	5	52	CNTR 10	34	1	1.5	39
RPL 11	35	3	6,2.5,0.5	3	31	CNTR 11	35	2	5,3	35
RPL 12	35	2	7.8, 2.8	4	34	CNTR 12	35	2	6.5, 3.5	38
RPL 13	35	3	9,5,4	9	38	CNTR 13	35	1	3	36
RPL 14	35	3	5.5, 2,2	3	37	CNTR 14	36	2	1.7,3	30
RPL 15	37	2	1.5, 3.3	4	---	CNTR 15	37	3	7.5,6,0.7	62
RPL 16	37	2	4,9	6	51	CNTR 16	39	1	5.5	46
RPL 17	39	3	8,5,2	3	52	CNTR 17	39	4	10,8,6,3	32
RPL 18	40	1	8.5	6	39	CNTR 18	39	4	9.5,7,3.5,1	28
RPL 19	43	2	14,11	4	37	CNTR 19	39	3	5,5,3	46
RPL 20	45	0	---	5	37	CNTR 20	40	2	3.1,4.8	48
RPL 21	45	1	5.9	12	38	CNTR 21	42	2	9,7	---
RPL 22	45	3	13,10,0.8	4	38	CNTR 22	47	2	16, 12	53
RPL 23	48	2	12,6.5	6	40					
RPL 24	48	1	10	3	40					

Data acquisition: MRI Scanning was performed on a 3 Tesla Trio Magnetom Siemens scanner. Functional data were collected using a T2*-weighted gradient-echo planar imaging (EPI) sequence (450 repetitions comprising 34 3×3×3.7 mm slices, TR = 2000 ms, TE = 25 ms). Anatomical images were acquired using a 3D T1-weighted magnetization prepared rapid gradient echo (MP-RAGE) sequence at high resolution (1 x 1 x 1 mm voxel, TR = 2300 ms, TE = 2.98 ms, inversion time = 900 ms, and a flip angle = 9°), using 12-channel head coil. For olfactory bulb (OB) volume and olfactory sulcus (OS) depth, a 32-channel head coil was used. The sequence included acquisition of 1.6-mm-thick T2-weighted Turbo Spin-Echo (TSE) images without inter-slice gap in the coronal plane covering the anterior and middle segments of the base of the skull (slices = 35, voxel size: 0.4 x 0.4 mm^2, TE = 85 ms, flip angle = 120°).

Stimuli: Twenty scenes from 11 commercial films were chosen: "chandler by the sea", "lion", "I Daniel Blake", "Moonlight", "The Shack", "God on Trial", "My Sister's Keeper", "Legends Of The Fall", "The Fault in Our Stars", "Miss You Already", and "Forrest Gump". An important consideration in choosing the scenes was including human characters in all the scenes. All scenes were approximately 1 minute long, and were rated for emotional arousal and familiarity in a separate behavioral session, by an independent group of eleven women. On a scale of 1 to 10, the averaged familiarity of the scenes was rated 2.8 ± 1.8, and the averaged emotional arousal was rated 5.3 ± 1. The scenes were then edited into short, equalized in sound volumes, 12 sec clips, trying to condense the emotional essence of the scene into this short timeframe.

Procedure: Prior to the scan session, subjects watched the complete scenes (as described above, 1 min on average) from the commercial films. This was performed in order to equalize movie familiarity and to introduce subjects with the narrative and emotional context of the stimuli. A one hour long fMRI scan was conducted, which included two functional scan (15 min each), separated by T1-anatomical scan (4 min), and followed by OB and OS T2-anatomical scan. During the functional scans, 12-seconds video clips were presented: 20 clips of high emotional content and 20 landscape clips, in alternating order (ISI = 8-12). Following each video clip, subjects were asked to rate their emotional arousal, on a scale of 1 to 8, with 8 being highest emotional aroused. Simultaneously with each video clip presentation, an odor body odor was delivered using a computer-controlled air-dilution olfactometer that embedded the odorant pulse within a constant stream of clean air at 1.5 liter per minute. The two functional scans were identical except for the odor content, which was either blank pads or pads containing body-odor collected from twenty unfamiliar men-donors prior to the experiment (standard procedure as detailed in [107]). The two conditions (blank vs. body odor) were counterbalanced for order between subjects. Importantly,

subjects were aware of the possibility of an odor to be delivered, and also were fitted with a nasal cannula for that aim, but were not told at which part of the experiment the odor will be delivered, and in the end of the scanning, subjects reported mostly sensations of air-flow, and not a specific odor. Following the scanning, subjects watched the emotional 12-sec clips again, outside the scanner, and rated them for specific emotions (rather than general emotional arousal, as was during the scans). Emotions included: compassion, happiness, fearful, sadness, stressed and emotional. There were no significant differences between the two groups in either of the ratings (all $t_{44} < 1.56$, all $p > 0.12$).

Data preprocessing: fMRI data were analyzed using the BrainVoyager QX version 2.8 software package (Brain Innovation, Maastricht) and Matlab software (MathWorks, Natick, MA). Preprocessing of the anatomical images included inhomogeneity correction and white matter and gray matter segmentation. The first 5 images of each functional scan were discarded. Functional scan preprocessing included 3D head motion correction, slice scan time correction, and linear trend removal. Functional images were co-registered to 3D anatomical images and transformed into Talairach space (Talairach and Tournoux, 1988). Next, spatially smoothing (FWHM= 6 mm) was applied.

Statistical analysis: First-level single-subject analysis was conducted using general linear model (GLM), with conditions of clip-content (emotional, landscape) and odor type (body odor, blank) as predictors of interest, and the six motion parameters as nuisance predictors. The boxcar functions of each condition were convolved with the canonical hemodynamic response function (HRF). The averaged beta values from each subject were submitted to second level, between groups, multi-subjects random-effects analysis. The critical significant threshold was set to 0.001, and cluster-based correction was applied to correct for multiple comparisons. A cluster size that was evident in less than 0.1% of the simulated runs started at 9 contiguous functional voxels.

A random-effects GLM multi-subject analysis was used to define functional regions of interest (fROIs) that were activated by the contrast of the Emotional>Landscape clips. The most activated regions were the R fusiform and bilateral occipital cortex. Next, individual fROIs for each subject were delineated, maintaining a similar number of voxels for each region across subjects. The time course of the averaged voxels in each fROI was extracted, and subtracted by the mean of the signal time course. Finally, the area under the curve (AUC) from TR=3 to TR=8 was compared between body odor and blank for each group (RPL, control).

Emotional ratings of the video clips, during and following the scanning, were analyzed using STATISTICA (StatSoft, version 7), applying a repeated-measures ANOVA for conditions of group (RPL, control), clip type (emotional, neutral) and odor type (body odor, blank), followed by a two-tailed t-test between group using independent samples t-tests, or within each group using paired-sampled t-tests.

OB volume and OS depth measurements: The OB volume and OS depth were measured according to the standard method described by Rombaux and colleagues[108,109]. The OB volume (mm^3) was computed by plannimetric manual countering the OB surface in each coronal slice, using a costume-programmed MATLAB script. The slices were multiplied by voxel size (0.39 x 0.39 mm^2) and slice thickness (1.6 mm). The OS depth was measured using ITK-SNAP (www.itksnap.org). The coronal slice was picked by the Plane of the Posterior Tangent through the Eye-balls (PPTE), which in most individuals traverses the anterior-mid segment of the OB. In this slice, a virtual line that was tangent to the inferior border of the orbital and rectus gyri was drawn, and then perpendicular line connecting the above virtual line and the deepest part of the OS was marked. This line represents the OS depth (Figure 4).

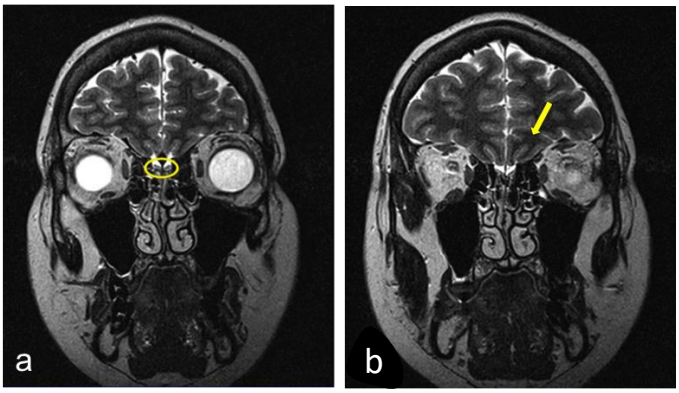


Figure 4. Olfactory bulb and sulcus measurement. a. OB in a normosmic subject (yellow circle), **b**. normal OS in normosmic subject (arrow).

Two independent raters measured the OB volume and OS depth in each of the 68 subjects. Then, a between-rater analysis of percent change was conducted. If a change of below 15% was found, the averaged measurements of the two raters were taken. If the percent change between the two rates was above 15%, a third rater judged between the two alternatives. Subjects were only excluded due to poor resolution which did not allow for reliable measure of the sulcus or bulb. This resulted in 14 excluded subjects, three RPL and 11 controls.

Finally, an analysis of bulb volume and sulcus depth was conducted between 22 RPL subjects and 22-matching controls, using independent samples t-tests. Matching of the subjects was performed in two ways: one, based on their age alone, and following the recent finding regarding the impact

of pregnancies on brain anatomy for up to two years after birth[110], the second matching was based on the number and age of children they have had, specifically the age of their youngest child (Table 4). The matching was performed before analysis of structural MRI, and included all subjects who were scanned. Thus, if any control subject who was found as a match for an RPL subject was later excluded for the reason I detailed above, the next best match was selected instead. When an RPL subject was excluded, her control match was taken out of the analysis as well, and could be used to replace excluded control subjects.

Table 4. Matching of the RPL and control (CNTR) groups for the bulb analysis. The table contains information regarding all 22 RPL subjects who were included in the structural MRI analysis. MATCH A: 22 control subjects, matched based on number and age of children, with an emphasis on the age of children below 2yo. MATCH B: 22 control subjects, matched based on age alone. Note that in light grey information regarding number and age of children is depicted, however was not used for matching. --- stands for missing data.

					MATCH A: number and age of children				**MATCH B: age of women**		
Subject	**Age**	**Number of children**	**Age of children**	**Number of mis-carriages**	**Subject**	**Age**	**Number of children**	**Age of children**	**Age**	**Number of children**	**Age of children**
RPL 1	29	0	---	7	CNTR 1	27	0	---	27	0	---
RPL 2	29	0	---	2	CNTR 2	29	0	---	29	0	---
RPL 3	31	0	---	3	CNTR 3	29	0	---	31	1	1.3
RPL 4	28	0	---	5	CNTR 4	27	0	---	27	0	---
RPL 5	29	1	0.7	3	CNTR 5	35	1	0.9	29	0	---
RPL 6	35	1	4	2	CNTR 6	35	1	3	35	1	3
RPL 7	45	1	5.9	12	CNTR 7	39	1	5.5	39	4	9.5,7,3.5,1
RPL 8	40	1	8.5	6	CNTR 8	36	2	8,4	37	3	7.5,6,0.7
RPL 9	37	2	1.5, 3.3	4	CNTR 9	36	2	1.7,3	36	1	1.8
RPL 10	25	2	1.8, 0.3	3	CNTR 10	47	2	16,12	26	0	---
RPL 11	43	2	14,11	4	CNTR 11	31	2	3.5, 1.5	39	1	5.5
RPL 12	32	2	2.6, 0.6	3	CNTR 12	35	2	6.5, 3.5	32	2	3.7, 1.4
RPL 13	37	2	4,9	6	CNTR 13	32	2	3.7, 1.4	37	4	9,6,1,1
RPL 14	27	2	5,0.6	3	CNTR 14	36	2	6,2	27	0	---
RPL 15	34	2	6.5, 2	5	CNTR 15	35	2	5,3	34	1	1.5
RPL 16	35	2	7.8, 2.8	4	CNTR 16	37	3	7.5,6,0.7	35	2	6.5, 3.5
RPL 17	45	3	13,10,0.8	4	CNTR 17	38	2	5,3	47	2	16,12
RPL 18	32	3	5,5,4	2	CNTR 18	32	2	4,2	32	2	4,2
RPL 19	35	3	5.5, 2,2	3	CNTR 19	37	4	9,6,1,1	35	2	5,3
RPL 20	35	3	6,2.5,0.5	3	CNTR 20	39	4	9.5,7,3.5,1	35	1	0.9
RPL 21	39	3	8,5,2	3	CNTR 21	32	2	3.7, 1.4	38	2	5,3
RPL 22	41	5	23,19,14, 5,4	2	CNTR 22	39	3	5,5,3	39	3	5,5,3

RESULTS

To characterize olfaction a battery of tests was applied in 21 women who experience RPL (mean age = 33.1 ± 6.4, mean number of miscarriages = 3.33) and 21 matched controls (mean age = 34.5 ± 4, never experienced miscarriage and have one child or more). Tests were conducted at a time-window where participants were not suspected to be pregnant, and were not actively treated in any way. To characterize perception of ordinary odors I tested olfactory identification of 20 every-day odorants (e.g., "peanuts", "pizza" etc.) using the widely applied standardized University of Pennsylvania Smell Identification Test (UPSIT), and olfactory detection thresholds for the alliaceous odor dimethyl trisulfide (DMTS) and discrimination of the musky odor muscone (P-15). I also characterized perception for three odorants that have been studied as putative human social chemosignals, testing discrimination of the testosterone derivatives androstenone (ANN) and androstadienone (AND) and the estradiol derivative estratetraennol (EST), which all typically smell sweaty to those who can perceive them.

Women with RPL have better olfactory abilities, specifically for social chemosignals, and these are correlated with number of miscarriages

For each participant I generated an ordinary odor composite performance score and a social chemosignaling composite performance score ranging from zero to one by combining the normalized test scores in each domain (Methods). A repeated-measures analysis of variance (ANOVA) with conditions of group (RPL and Control) and odor type (ordinary, putative social chemosignal) revealed a significant main effect of group ($F_{1,40} = 8.47$, $p = 0.0059$) reflecting that RPL women had better olfaction than controls (mean RPL = 0.7 ± 0.09, mean control = 0.58 ± 0.16, $t_{40} = 2.91$, $p = 0.0059$), and a significant interaction ($F_{1,40} = 4.58$, $p = 0.039$) reflecting that this advantage of RPL over controls reflected better performance in social chemosignals but not ordinary odors (Ordinary odors: mean RPL = 0.69 ± 0.096, mean control = 0.67 ± 0.12, $t_{40} = 0.66$, $p = 0.51$. Social chemosignals: mean RPL = 0.7 ± 0.2, mean control = 0.49 ± 0.29, $t_{40} = 2.75$, $p = 0.0089$, Figure 5A). In addition, a marginally significant main effect of odor type was observed ($F_{1,40} = 3.67$, $p = 0.063$) reflecting better performance for ordinary odors (mean ordinary = 0.68 ± 0.11, mean chemosignal = 0.6 ± 0.27, $t_{41} = 1.84$, $p = 0.074$). To further explore the sources of these differences, I also looked at the individual tests that made up the composite scores. Consistent with the composite picture I observed no group differences in odor identification (RPL: 85.3 ± 8.5% correct; control group: 81 ± 9% correct; $t_{39} = 1.57$, $p = 0.12$), detection (DMTS threshold,

RPL group: $1.42e^{-5} \pm 1.25e^{-5}$; control group: $2e^{-5} \pm 2.2e^{-5}$; $t_{36} = 1.03$, $p = 0.31$), and discrimination (P-15 success rates, RPL group: 93.7% ± 22.7; control group: 98.4% ± 7.3; $t_{40} = 0.92$, $p = 0.37$, Figure 5B). In contrast, I observed significant differences in discrimination of the putative chemosignals ANN (success rates, RPL group: 84.1% ± 22.7; control 61.9% ± 38.4; $t_{40} = 2.28$, $p = 0.028$) and EST (success rates: RPL group: 57.1% ± 36.7; control 31% ± 35.1; $t_{40} = 2.36$, $p = 0.023$) but not AND (success rates: RPL group: 69.8% ± 34.8; control group: 55.6% ± 42.6; $t_{40} = 1.2$, $p = 0.24$, Figure 5C).

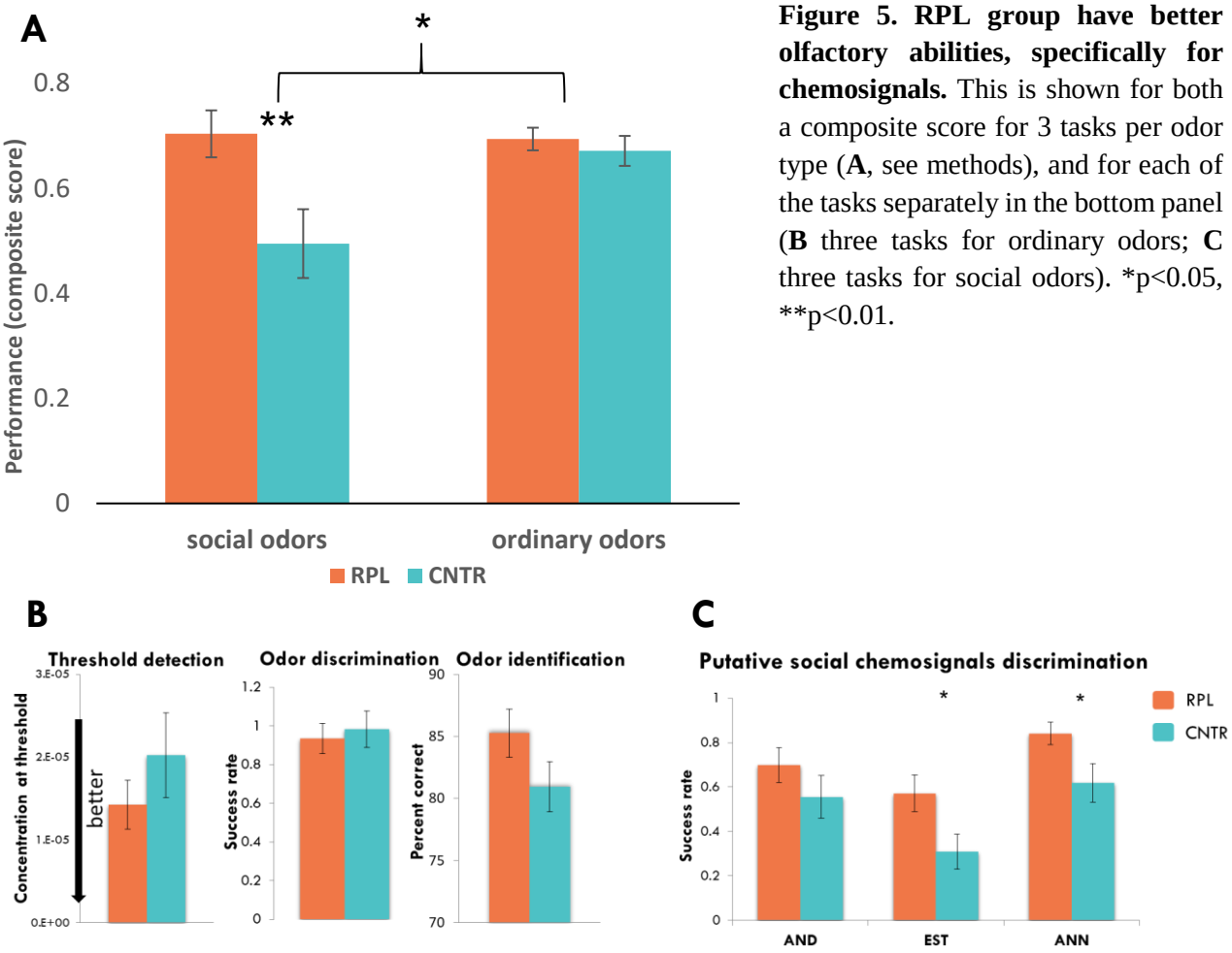
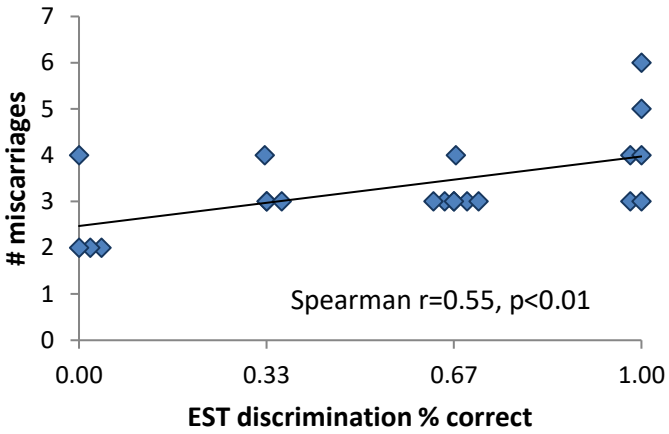


Figure 5. RPL group have better olfactory abilities, specifically for chemosignals. This is shown for both a composite score for 3 tasks per odor type (**A**, see methods), and for each of the tasks separately in the bottom panel (**B** three tasks for ordinary odors; **C** three tasks for social odors). *p<0.05, **p<0.01.

Having found that RPL women have better olfaction than controls and that this difference is most pronounced for the two putative social chemosignals ANN and EST, I asked whether this advantage is related to their condition. I tested for a correlation between discrimination of ANN and EST and number of experienced miscarriages. Remarkably, a significant correlation whereby better detection of EST was associated with an increased number of miscarriages was observed (Spearman r = 0.55, p = 0.0096; Bonferroni correction for multiple comparisons set the a-priori p value to be 0.05/2 = 0.025. Figure 6). This correlation implies a meaningful link between social olfaction abilities and miscarriage in this cohort.

Figure 6. Ability to detect EST is correlated with number of miscarriages.
Discrimination values were jittered to prevent overlay.



To ask whether these differences in performance using synthesized chemosignals at non-biological concentrations translated to differences in response to realistic stimuli, I tested real body-odors. Each woman smelled three unmarked samples: spouse, stranger, and blank. In contrast to human olfactory kin recognition[111], olfactory spouse recognition is poor[112]. Consistent with this record, neither group was significantly above chance at discriminating spouse from stranger (success rates: RPL group: 46.3% ± 40.8, $t_{19} = 1.45$, $p = 0.16$; control group: 31.3% ± 30.2; $t_{19} = 0.26$, $p = 0.8$; direct comparison between the groups: $t_{38} = 1.32$, $p = 0.19$). Moreover, perceptual ratings of pleasantness, intensity, familiarity and sexual attraction applied to the body-odors were similar in RPL and control women (repeated measurers ANOVA of odor parameter (pleasantness, intensity, familiarity, sexual attraction), shirt type (spouse, stranger, blank), and group (RPL, control) yielded no significant effects or interactions (all $F < 1.01$, all $p > 0.45$), and also could not serve to separate spouse from stranger (same repeated measurers ANOVA, but for two shirts: all $F < 1.01$, all $p > 0.42$).

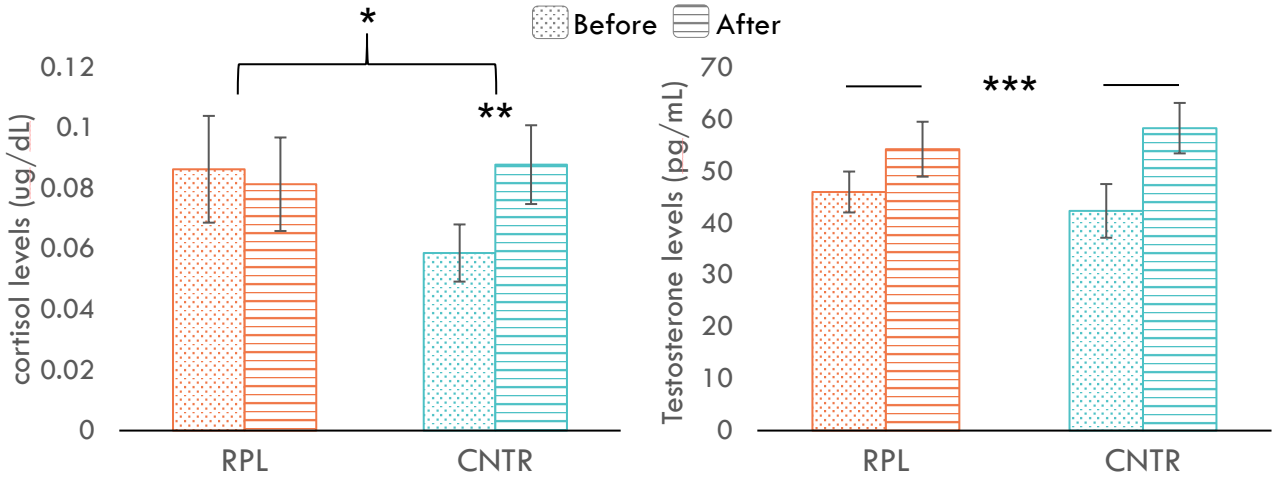
Women with RPL have altered physiological responses to body odors, specifically to odors of non-spouse men

I next tested the impact of these real body odors on physiological measures of arousal. First, I looked at the impact on salivary hormones as a result of exposure to body odors. I measured levels

of cortisol and testosterone before and after the exposure to body odors. Using a repeated measure ANOVA of group (RPL, CNTR) and time (before, after), I observed a significant interaction between time and group for cortisol levels, reflecting higher cortisol levels in the control group following exposure to body odors, but not in the RPL group ($F_{1,35}$ = 5.8, p = 0.02, Figure 7, left panel). Follow-up pair-wise analyses within each group confirmed that the RPL group displayed no elevation in cortisol levels (before: 0.086 ± 0.073 pg/ml, after: 0.081 ± 0.064 pg/ml; t_{17} = 0.47, p = 0.65), whereas the control group's cortisol level increased significantly (before: 0.059 ± 0.04 pg/ml, after: 0.088 ± 0.055 pg/ml; t_{18} = -3.1, p = 0.0066). Please note that despite seemingly different baseline cortisol levels between the two groups, no main group effect was observed ($F_{1,35}$ = 0.35, p = 0.56), nor when directly comparing the two groups' cortisol levels before exposure (t_{35} = 1.44, p = 0.16).

The same repeated-measures ANOVA was applied for testosterone levels, yielding a significant main effect of time ($F_{1,35}$ = 31, p = 0.000003, Figure 7, right panel), reflecting higher testosterone levels following exposure to body odors in both groups, and a trend for significant interaction between time and group ($F_{1,35}$ = 3.15, p = 0.085), implying that the control group displayed higher testosterone elevation than the RPL group.

Figure 7. Changes in cortisol (left panel) and testosterone (right panel) levels following exposure to men's body odors. The control group displayed elevated cortisol levels following exposure to body odors (dotted pattern is before exposure and line pattern is after), whereas the RPL group shows no such elevation (left panel). Testosterone levels increased in both groups following exposure to body odors (right panel). *p<0.05, **p<0.01, ***p<0.001.



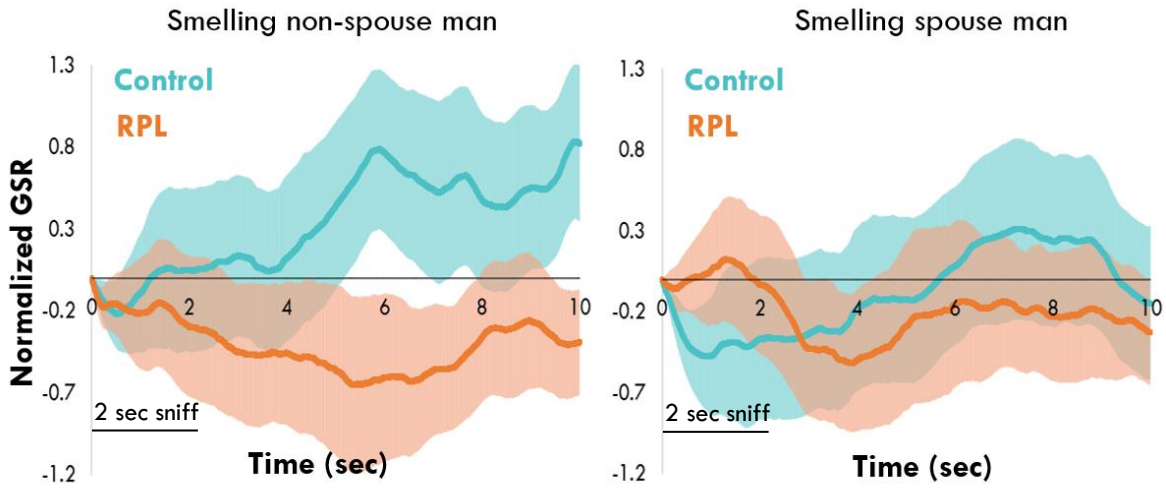
I then looked at the impact on salivary hormones as a result of exposure synthetic putative chemosignals (and not real body odors), using the same repeated-measures ANOVA on cortisol

and testosterone levels. Both ANOVAs yielded no significant main effects and no significant interactions (cortisol: all $F<1.46$, all $p>0.23$; testosterone: all $F<2.8$, all $p>0.103$).

Since hormonal measures were taken at two time points, the precise event causing cortisol level elevations in the control group but not the RPL group cannot be detected using this method. To further gauge the physiological response, I monitored the galvanic skin response (GSR) throughout performance of the tasks. GSR is a measure reflecting autonomic arousal and is modulated within seconds. Review of the event-related responses in this study revealed that I neglected to plan experimental procedures with this analysis in mind. Event-related GSR responses achieve their full course of response and return to baseline over 10 seconds or more, yet here I had inter-stimulus-intervals that were variable, and sometimes as short as 5 seconds. I thus returned to the subjects and re-ran the experiment with increased ISIs. For each subject, a 10 seconds GSR response of the first exposure for each odor type - blank shirt, spouse shirt and stranger shirt - was extracted, normalized as detailed in the methods section, and two parameters from the normalized GSR response were extracted: peak and area under the curve (AUC).

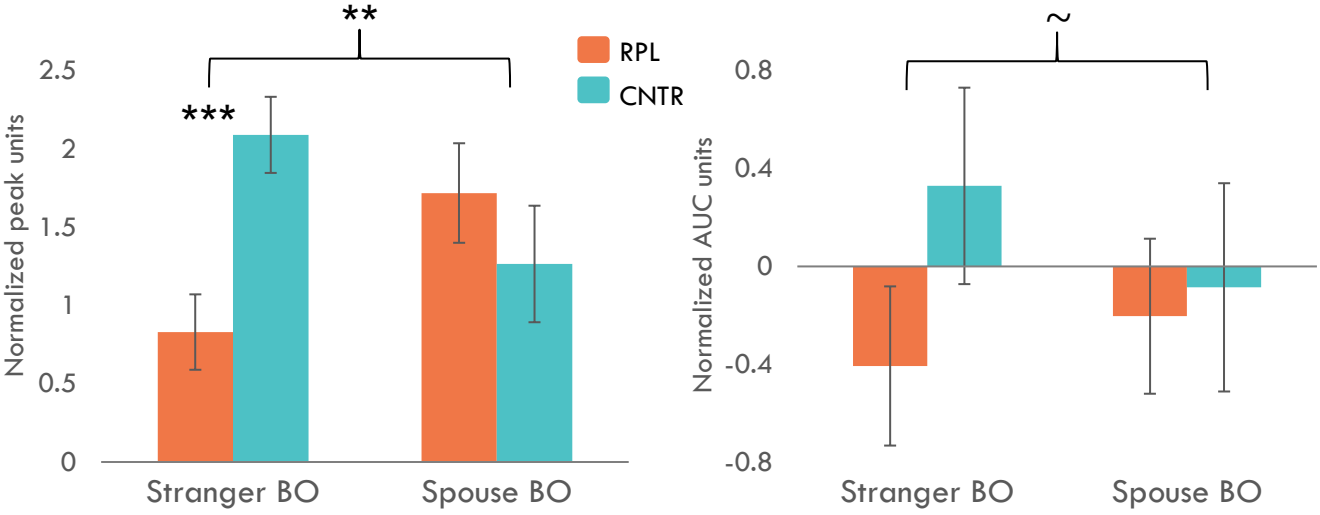
A multivariate repeated-measures ANOVA applied to the GSR parameters (AUC, peak) with conditions of group (RPL, control) and odor (spouse, stranger) revealed a significant main effect of parameter, reflecting different ranges (AUC: -0.11±1.37, peak 1.46±1.2, $F_{1,29} = 198$, $p < 0.001$) and a significant interaction between group and odor ($F_{1,29} = 8.95$, $p = 0.0056$), indicating that whereas for the spouse body odor both groups responded similarly, the response for the stranger's body odor was higher in the control group (Figure 8).

Figure 8. Increased autonomic arousal following exposure a stranger's body odor in the control, but not RPL, group (left panel). Exposure to spouse body odor yields similar responses in both groups (right panel).



Following a significant 3-way interaction between group, odor and GSR parameters ($F_{1,29}$ = 11.3, p = 0.002), I performed a repeated-measures ANOVA for each parameter separately. Both AUC and peak showed the same pattern of higher response for the stranger's body odor in the control group only (peak: $F_{1,29}$ = 12.3, p = 0.0015), yet this effect was only marginally significant for AUC ($F_{1,29}$ = 3.25, p = 0.08, Figure 9). Further comparisons between groups for each odor type confirmed that the two groups did not differ in their responses to the spouse body odor (AUC: t_{29} = -0.23, p = 0.82; peak: t_{29} = 0.96, p = 0.34), yet differed in their responses to the stranger's body odor, with the control group displaying higher arousal (AUC: t_{29} = -1.5, p = 0.147; peak: t_{29} = -3.77, p = 0.0007).

Figure 9. Increased autonomic arousal following exposure a stranger's body odor in the control, but not RPL, group. This was statistically significant for the normalized peak of the GSR response (left panel), and marginally significant for the normalized area under the curve of the GSR response (right panel). **p<0.01, ***p<0.001, ~p<0.1.



Spouses of women with RPL smell different than spouses of control women

Taken together, I observed dissociated responses in RPL and control women to the body odor of their spouses. These differences can stem from two sources: one is the difference in olfaction across RPL and control women that I indeed observed in response to synthetic social odors, but a second is a possible actual real difference between the body odor of RPL and control men. To address this possibility, I recruited a separate cohort of 35 nulliparous heterosexual women (average age 24.8 ± 2.6), and asked then to smell and rate 17 shirts of spouses from the control group and 15 shirts of spouses from the RPL group. In order to prevent olfactory variability along the menstrual cycle and maintain hormonal balance, all subjects in this experiment were taking

birth control pills but were not off cycle. Subjects were asked to smell and rate the shirts along five different parameters: pleasantness, intensity, familiarity, sexual attraction and fertility.

I first verified valence attributes are not affected by odor intensity or familiarity (t_{30} = 0.174, p = 0.86, t_{30} = 1.04, p = 0.31). I next tested ratings of pleasantness, sexual arousal and fertility using a multivariate repeated-measures ANOVA with conditions of group (RPL men, control men) and rating (pleasantness, sexual arousal, fertility). I found a significant main effect of rating ($F_{2,29}$ = 31, p < 0.001), reflecting differences between ratings of the descriptors across groups, and a marginally significant main effect of group ($F_{1,30}$ = 4, p = 0.054, Figure 10), reflecting differences in body odor perception of men from the two groups by the women raters. Follow-up pair-wise comparisons revealed that RPL men smelled significantly more fertile (t_{30} = 2.39, p = 0.023), and marginally significantly more pleasant (t_{30} = 1.9, p = 0.067) and sexually attractive (t_{30} = 1.66, p = 0.107) than men in the control group. In other words, men in RPL relationships may smell different (better) than men in control relationships.

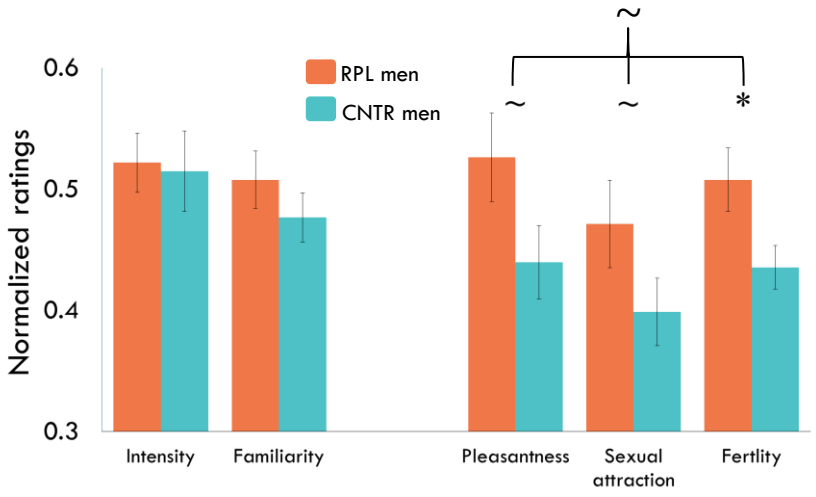


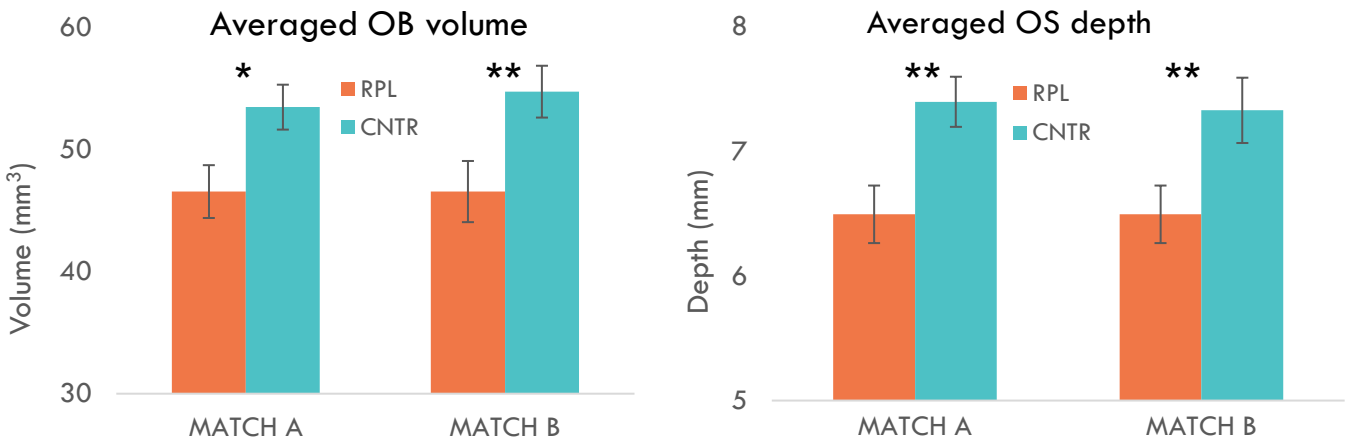
Figure 10. RPL men's body odor is perceived as more fertile, as rated by 35 women. Thirty five women who were not a part of the control or RPL group rated body odors of men from the RPL group as significantly more fertile marginally significantly more attractive and pleasant than body odor of men from the control group. *p<0.05, ~p<0.1

RPL women have different olfactory-related brain anatomy than that of control women

Women experiencing recurrent pregnancy loss show better olfactory abilities, specifically for social odors, yet fail to respond physiologically to these cues, as opposed to women in the control group. In order to shed additional light on the olfactory differences between RPL and control women, we recruited 25 RPL women and 43 matched-control women for anatomical scans to measure the olfactory bulb (OB) volume and olfactory sulcus (OS) depth. The human OB is a highly plastic structure whose volume reflects changes in olfactory sensitivity[113,114]. After applying exclusion criteria (detailed in methods), a total of 22 RPL subjects and 22 age-matched controls were compared for OB volume and OS depth. The subjects were matched in two ways: one, based

on their age, and the second based on the number and age of children they have had, specifically the age of their youngest child (Table 4, methods). The latter is following a recent finding regarding the impact of pregnancies on brain anatomy for up to two years after birth[110]. For both OB and OS, these two matches yielded significant results: the average OB of RPL women was found to be significantly smaller than that of control women (match by age: RPL: 46.6 ± 9.9 mm^3, control: 53.3 ± 8.6 mm^3, t_{42} = 2.42, p = 0.02; match by number and age of children: RPL: 46.6 ± 9.9 mm^3, control: 54.6 ± 8.3 mm^3, t_{42} = 2.92, p = 0.0057, Figure 11, left panel), and the OS of RPL women was found to be significantly shorter than that of control women (match by age: RPL: 6.5 ± 1 mm, control: 7.4 ± 0.9 mm, t_{42} = 2.78, p = 0.008; match by number and age of children: RPL: 6.5 ± 1 mm, control: 7.3 ± 1.1 mm, t_{42} = 2.85, p = 0.007, Figure 11, right panel). These results remained significant for left and right OBs separately (match by age: right OB: RPL: 46.7 ± 11.5 mm^3, control: 54.1 ± 9.8 mm^3, t_{42} =2.29, p = 0.027; left OB: RPL: 46.4 ± 9.1 mm^3, control: 52.6 ± 9 mm^3, t_{42} =2.26, p = 0.029. Match by number and age of children: right OB: RPL: 46.7 ± 11.5 mm^3, control: 54.6 ± 9.9 mm^3, t_{42} =2.45, p = 0.019; left OB: RPL: 46.4 ± 9.1 mm^3, control: 54.6 ± 7.9 mm^3, t_{42} =3.18, p = 0.003), and for left and right sulci separately (match by age: right OS: RPL: 6.6 ± 1 mm, control: 7.5 ± 1.1 mm, t_{42} = 2.51, p = 0.016; left OS: RPL: 6.4 ± 1.2 mm, control: 7.3 ± 1 mm, t_{42} =2.57, p = 0.014. Match by number and age of children: right OS: RPL: 6.6 ± 1 mm, control: 7.4 ± 1.3 mm, t_{42} =2.37, p = 0.022; left OS: RPL: 6.4 ± 1.2 mm, control: 7.2 ± 1.2 mm, t_{42} =2.88, p = 0.006).

Figure 11. RPL group has smaller olfactory bulb (OB, left panel) and sulcus (OS, right panel). RPL and control groups are matched either by age (MATCH A) or by number and age of children (MATCH B), see methods. *p<0.05, **p<0.01.



RPL women display reduced activation in orbitofrontal cortex in response to emotional video clips

In an fMRI experiment that we conducted, consisting of two functional scans, the subjects viewed and rated 20 emotional and 20 landscape video clips for their emotional arousal. Simultaneously with each video clip presentation, an odor was delivered, which was either body odor or blank, counterbalanced for order. Overall, the two functional scans were completely identical except for odorant identity. One control subject was excluded from the analysis due to extensive head motion, so a total of 46 subjects were included: 24 RPL and 22 control (Table 4, methods). We chose movie clips because naturalistic stimuli may better reflect the nature of emotional experience in real life. Furthermore, movies clips are known to elicit emotions with greater intensity than still images[115].

Subjects' ratings of emotional arousal throughout the experiment were submitted to a repeated-measures ANOVA for conditions of group (RPL, control), clip type (emotional, landscape) and odor type (body odor, blank), which revealed a main effect of movie type ($F_{1,44} = 206.4$, $p < 0.001$), indicating that emotional clips were rated as significantly more emotional arousing than the landscape clips (on a scale of 1 to 8: neutral clips: 2.3 ± 1.4, emotional clips: 5.6 ± 1, $t_{90} = 13.3$, $p < 0.001$). In addition, a trend towards a main group effect was observed ($F_{1,44} = 3.2$, $p = 0.08$), indicating that the RPL group had higher emotional ratings than the control group, regardless of movie type or odor (on a scale of 1 to 8: RPL: 4.2 ± 0.8, control: 3.7 ± 1, $t_{44} = 1.8$, $p = 0.08$). We thus performed a repeated-measures ANOVA of group (RPL, control) and odor (body odor, blank) for each movie type separately, but found no significant main effects or interactions (all $F_{1,44} < 2.66$, all $p > 0.11$).

Functional MRI Results: The random-effect ANOVA uncovered a main between-groups effect in the right orbitofrontal cortex (OFC) in response to emotional clips presentation. The RPL group displayed significantly lower neural activity in the OFC, compared to the control group (Figure 12).

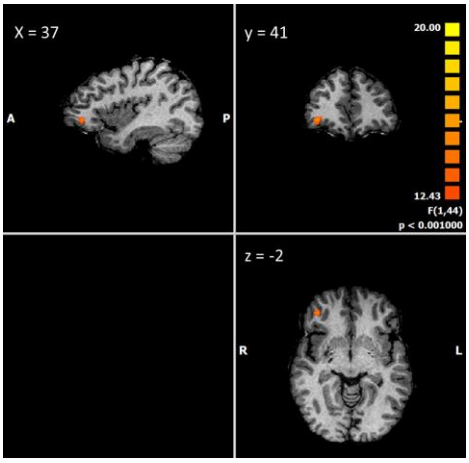


Figure 12. The right OFC BOLD signal is reduced in RPL women in response to emotional video clips. Random-effects group analysis showing significant group main effect in BOLD signal in the right OFC (RPL: n=24, control: n=22). The Talairach coordinates (x,y,z) are reported. $p < 0.001$, cluster size of above 9 contiguous functional voxels.

Dissociated activity in response to body odors in the right fusiform cortex

To test whether body odor alters brain activity in RPL for emotional stimuli, a random-effects GLM multi-subject analysis was used to define functional regions of interest (fROIs) that were activated by the contrast of the Emotional>Landscape clips. The most activated regions were the right fusiform and bilateral extrastriate cortex (Brodmann areas 18 and 19, Figure 13).

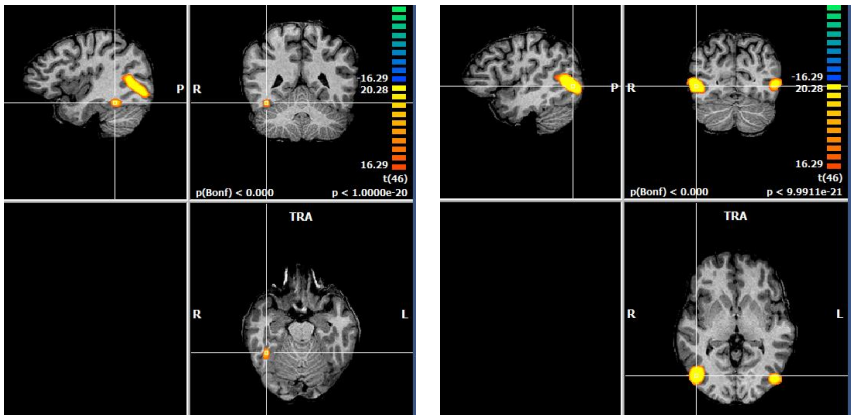


Figure 13. Group activation map (n = 24) for the contrast emotional > landscape video clips. The right fusiform and bilateral extrastriate cortex are activated at high threshold ($p < e^{-20}$).

Next, individual fROIs for these regions each subject were delineated, based on individual contrasts (emotional > landscape clips). The AUC between TR=3 to TR=8 of each individual was measured and submitted to repeated-measures ANOVA with levels of group (RPL, control) and odor (body odor, blank). This analysis revealed a significant interaction in the right fusiform ($F_{1,44} = 7.3$, $p = 0.01$, Figure 14), reflecting a trend towards higher neural activation in response to body odor in the RPL group (body odor: 34.7 ± 10.6, blank: 32.3 ± 12.1; $t_{23} = 1.86$, $p = 0.077$), yet a trend toward lower activation in response to body odor in the control group (body odor: 31.9 ± 8.6, blank: 34.3 ± 9; $t_{21} = 1.98$, $p = 0.06$). No significant interactions or main effects were found for the right or left extrastriate cortex.

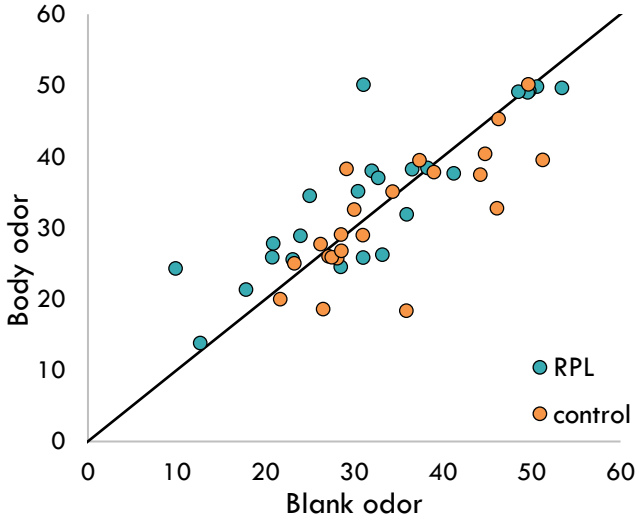


Figure 14. RPL and control groups display altered activation in fusiform cortex in response to body odors. Each point reflects the AUC of each subject during the emotional movies, while exposed to blank and body odors. The diagonal line is the unit slope line. The points mostly fall above the line for RPL and under the line for control.

DISCUSSION

In this study, I set out to test the hypothesis that women experiencing unexplained recurrent pregnancy loss (RPL) would display an altered olfactory profile. Given an olfactory mechanism to miscarriages in non-human mammals, the Bruce effect[25], the hypothesis was specifically aimed towards altered responses of RPL women to social odors, either synthetic or natural.

The evidence I obtained here portray a somewhat counter-intuitive picture: RPL women showed better olfaction abilities than control women in general, and this effect was highest specifically regarding social chemosignals. In addition, it was correlated with number of miscarriages. While detecting social cues better than the control group, physiologically RPL women fail to produce the same physiological response following exposure to body odors, as does the control group. This was generally displayed in cortisol levels following exposure to body odors, and using the more fine-tuned galvanic skin response, this difference was observed specifically following exposure to a stranger's body odor. In addition, using structural MRI, the RPL group was found to have a significantly smaller olfactory bulb and sulcus than that of the control group. This finding is specifically perplexing because evidence ties larger olfactory bulb volume to better olfactory performance[113,114], however tests of olfactory performance in these studies were for ordinary odors, and did not include social odors, and thus leave room to speculate that perhaps performance in social odors tasks has different anatomical traces. An interesting option is that the olfactory bulb ties between (social) olfactory input and its downstream physiological impact, and thus smaller bulb volume can explain the decreased physiological response in RPL women, despite better olfactory performance. In rodents – pheromonal input to the accessory olfactory bulb activates downstream neuroendocrine pathway which is considered one of the mechanisms underlying the Bruce effect[89-91]. Functional MRI comparison of the two groups' responses to body odors versus blank in emotional context revealed a significant main group effect in the right orbitofrontal cortex (OFC): regardless of odor presentation, the RPL group displayed decreased activation in the OFC following emotional video clips. The human OFC is specifically implicated in emotion regulation, and OFC lesions cause overly sensitive responses to emotional stimuli[116,117]. The lower OFC response in the RPL group falls in place with the trend towards higher emotional ratings of the movies. In addition, although no interaction with odor type was found, the OFC is key in olfactory information processing and integration[46,118], and thus its differential group activity in this aspect may be of interest. In a further fMRI investigation, a dissociative activation in the right fusiform cortex in response to body odors was found: whereas the RPL group displayed higher neural activity in response to body odor versus blank, the control group displayed an opposite trend, with

lower neural activity in response to body odor versus blank. The fusiform is mostly activated in response to social stimuli (faces), and has been specifically investigated in the visual domain in this regard[119]. Interestingly, in olfaction right fusiform activation has been demonstrated in several chemosignaling studies, following exposure to body odors[17,120,121], and it has been suggested that it may play a role in the processing of social signals, independent of the stimulus modality[120]. In this study we find right fusiform activation following exposure to body odors only in the RPL group. This effect may be complementary to their general heightened olfactory abilities, which are specific to social chemosignals. The body odor we used was undetectable, evident by subjects' reports following the experiment, thus it could be that only the RPL group was able to detect it and subsequently display a neural response to it. However, ample evidence have shown that conscious awareness is not indicative nor necessary for body odor processing[7,8,20], thus it could also be that this activation is the neural reflection of their increased olfactory abilities. In other words, the RPL group may display altered neural processing of olfactory social stimuli which mediates their increased behavioral abilities.

Finally, testing the perception of body odors of men from the two groups, I found that men in RPL relationships smell better than men in control relationships. This was established by 35 nulliparous women who were not a part of the original experiment, who rated RPL men as significantly more fertile, and marginally more pleasant and sexually attractive. This finding adds additional complexity since it implies a male factor in olfactory-related RPL. In RPL research, the importance of the male factor has increasing interest and evidence, and it is getting clearer that men genetic factors or other factors as semen quality contribute to RPL[122]. Although somewhat controversial, several evidence converge to suggest that genetic load is reflected in human body odor[12,123]. In this context, it is interesting to speculate that any genetic factor which may contribute to RPL is somehow reflected, and perceived by other women, in these men's body odor.

To conclude, this project was originally defined as "high-risk, high-gain" since even if RPL women do have altered olfactory responses which may explain their condition, for obvious ethical considerations they could not be tested for these responses while pregnant. So whether this altered olfactory profile would manifest in non-pregnant periods was unclear, as is still unclear to what extent can it reflect on olfactory responses during pregnancy. Having said that, I presented here evidence for assorted differences related to olfaction in RPL. These findings imply altered olfactory processing in RPL, specifically in response to body odors, which may be accompanied with a male factor of altered body odor. In this I provide support for the hypothesis that RPL

women have different olfactory profile than that of women who experienced no miscarriages, and circumstantial support for what may be a Bruce-like effect in humans.

Chapter C: Odorant-induced placebo effect can enhance creativity

Rozenkrantz, Mayo, Ilan, Hart, Noy and Alon, PLoS one, 2017[124].

The placebo effect is a fascinating phenomenon describing improvement in condition which is not due to active treatment, but rather induced by the subject's beliefs or expectations regarding the treatment[125,126]. This psychobiological phenomenon can be induced by expectation, verbal suggestions and classical conditioning[127-129 130,131]. Most studies of placebo so far have been in clinical settings with the goal of decreasing negative symptoms such as pain, depression and anxiety. These studies suggest several neurobiological pathways for placebo, which can be differentially activated in different contexts[132]. Analgesia placebo, the best understood placebo to date, is characterized by activation of endogenous opioids and dopamine to reduce spinal nociceptive responses[133]. This pathway provides evidence of how high-order processes - such as expectation - can regulate immediate peripheral sensations such as pain[134]. Placebo has also been widely studied in the treatment of Parkinson's disease. Here, placebo involves the dorsal striatum, which plays a role in motor control, and dopamine release in the ventral striatum, which is part of the reward system[135]. Activation of the reward system has also been shown to affect immune states in mice[136].

There have been much fewer studies on using placebo outside of the clinic in order to enhance positive aspects of performance or cognition. Several studies showed that placebo can improve sports performance (reviewed in [134,137]). Most of these studies were on professional athletes and used an inert substance or treatment together with suggestions. The athletes were told they were receiving an ergogenic aid (anabolic steroids, caffeine etc.), when in fact they received a placebo. They were then tested for their endurance or strength in the relevant field. Some studies administered an active substance alongside the placebo and some administered only placebo but manipulated expectations regarding its effect. In diverse athletic fields ranging from anaerobic sprint runs and weightlifting to long-range aerobic endurance cycling, placebo extended performance in an expectation-dependent manner. For example, if subjects expected a higher dose of caffeine, they had higher performances, and if they expected negative effects of the substance, performance worsened[138-141]. Pre-conditioning strengthened the placebo effect. For example, subjects received a placebo said to be caffeine, and then were tested for lifting a weight which was reduced without their knowledge. Then they received the same placebo-caffeine, and this time tested on the original weight. Performance was improved relative to a group which received placebo-caffeine in one session only[142,143].

Several other studies tested the ability of placebo to enhance cognitive performance. In these studies there was no comparison to an active substance. Instead, the independent variable was subjects' expectations manipulated by means of suggestion: a group told that a sham drug or intervention will improve performance (placebo group) is compared to a group going through the same procedure with no mention of improvement (control group). For example, Parker and colleagues showed that an inert substance presented as a drug that acts as a cognitive enhancer increased performance on a prospective memory task, compared with a group which received the same substance and was told it was an inactive control. Prospective memory improvement was at the expense of response times in an ongoing task performed in parallel, indicating increased cognitive effort[144]. Oken and colleagues showed that an inert pill, which was said to be a cognitive enhancer, improved various cognitive abilities in healthy seniors. The control group were same-age subjects which went through the same procedures but were not given the pills. Regression analysis indicated that expectancy, self-efficacy and perceived stress were significant predictors for placebo-related improvement[145].

Placebo was also found to enhance performance in subconscious cognitive tasks. For example, performance on the Stroop effect, a classical response-time test in cognitive psychology, was improved by a sham EEG[146] or by verbal suggestion[147]. More specifically, in the former study, de Gama et al used a within-subjects design that compared performance on the Stroop task at baseline versus -performance during sham EEG said to modulate participants' visual ability to perceive colors: either to enhance it or to decrease it. Participants exhibited less or more interference from written color words in accordance with the corresponding suggestion[146]. In another subconscious implicit learning trial, Colagiuri et al told subjects that an odor influence performance, either positively (placebo group 1), negatively (placebo group 2), or not at all (control). Subjects completed the task in alternating blocks in which odor was presented or not presented. The study found that reaction times on cued trials were faster or slower according to the placebo suggestion[148]. Finally, Weger et al. used a sham subliminal priming procedure which was said to unconsciously enhance subjects' knowledge. Performance on a general knowledge test was enhanced compared to a control group[149]. Based on these encouraging findings, there is scope to explore placebo for improving additional positive aspects of human performance.

Here, I ask whether placebo can enhance creativity. Creativity is the ability to generate ideas, solutions or insights that are new and potentially useful[150-152]. Creativity is often viewed as a trait characteristic of a person; however, creativity can also be viewed as a *state*, affected by expectation and motivation[153-155]. Motivation appears to be a central factor in creative performance[150,156-158], a

finding which is hopeful because motivation can be bolstered, for example, by enhancing belief in one's own competence[159,160]. In this regard, Green et al found that a suggestion to be more creative increased novelty scores in a word association task, and several studies indicated that conditions that reduce inhibitions can enhance performance on creativity tests[153,161-164].

There is much current interest in finding ways to enhance the creativity of individuals and groups[165-167]. One obstacle in the study of creativity is the lack of experimental paradigms that allow automated and multi-dimensional views of the creative process. Current paradigms, such as the alternate uses test (AUT) and the Torrance test of creative thinking (TTCT), require laborious manual coding, and do not allow access to the process or intermediate steps by which solutions are reached. A recent advance presented an automated test for creativity called the creative foraging game (CFG[168]). The CFG is a computer game in which participants search for interesting and beautiful shapes in a well-defined geometric space. This test allows measurement of multiple aspects of the search including fluency, uniqueness of solutions and the length and timing of the paths taken to reach the solutions.

Here, I hypothesize that a placebo that combines an inert substance with a verbal suggestion aimed at increasing creativity and reducing inhibitions will increase subjects' creativity. I used an odorant as an inert placebo substance, which is less invasive than pills or injections and hence appropriate for non-clinical settings. I tested creativity with two standard manual tests, AUT and TTCT, and with the automated creative foraging game to compare aspects of the creative search between groups that smelled the odorant with and without the suggestion.

EXPERIMENTAL DESIGN:

Participants: Participants were recruited between March 2014 and June 2015 via social media groups dedicated to recruiting subjects for experiments, and were mostly students from nearby universities. Compensation was 40 NIS (about 10$) per hour. Ninety-six participants took part in the experiment. Before analysis, I removed three participants who were subject to another suggestive experiment directly before the current one, two participants with abnormally short games and one participant who had previous knowledge of the research hypothesis. Data from ninety participants entered the analysis. All participants were blindly assigned to placebo (N=45) or control (N=45) groups prior to their arrival in the laboratory. There were no significant age and sex differences between the two groups (placebo: 49% males, mean age 25.4±2.5, age range 20-33; control: 49% males, mean age 26.1±3.2, age range 21-37). Of these 90 participants, 57

participants (30 from the control group and 27 from the placebo group) completed two additional creativity tests (see study design). Here too, no age and sex differences were found (placebo: 52% males, mean age 25.8±2.6, age range 22-33; control: 50% males, mean age 26.2±3.1, age range 21-32). This study was approved by our Institutional Review Board, Wolfson Medical Center Helsinki Committee. All patients provided written informed consent.

Procedures: The experiment took place in an olfactory research lab, in which experiments typically ask subjects to rate odors and perform tasks. Participants signed informed consent, came into the experiment room, were presented with an odorant (cinnamaldehyde, Sigma-Aldrich, CAS 104-55-2) in a jar and were asked to smell it, and to rate its pleasantness, familiarity and intensity. Importantly, the placebo group was also told that the odorant is "a unique odor, developed in our lab, which increases creativity and lowers inhibitions". All aspects of the experiment, except the suggestion, were identical between the two groups; the only thing that differed between their experiences was what we told them about the nature of the substance.

Participants rated the odor on a visual analog scale as mildly pleasant (normalized scores to a scale of 1-100: 64.2 ± 22.8), mildly familiar (65.5 ± 25) and relatively intense (73.3 ± 16.4). There were no significant differences between the placebo and control groups in odor ratings (pleasantness: MW U=950, $p = 0.61$; two-tailed $t_{88} = 0.54$, $p = 0.59$; familiarity: MW U=989, $p = 0.85$; two-tailed $t_{88} = 0.15$, $p = 0.89$; intensity: MW U=776, $p = 0.06$; two-tailed $t_{88} = 1.85$, $p = 0.07$, a trend towards the placebo group to perceive the odor as more intense). This result rules out the possibility that differences in group performance were due to differential perception of the odor. There was no significant correlation between odor ratings and performance on the creativity tests. There is evidence that cinnamon odor increases attention and memory[169,170] , although conflicting evidence[171] was also reported. Since both groups were equally exposed to the odor, any presumed effect of the odor itself would affect both groups.

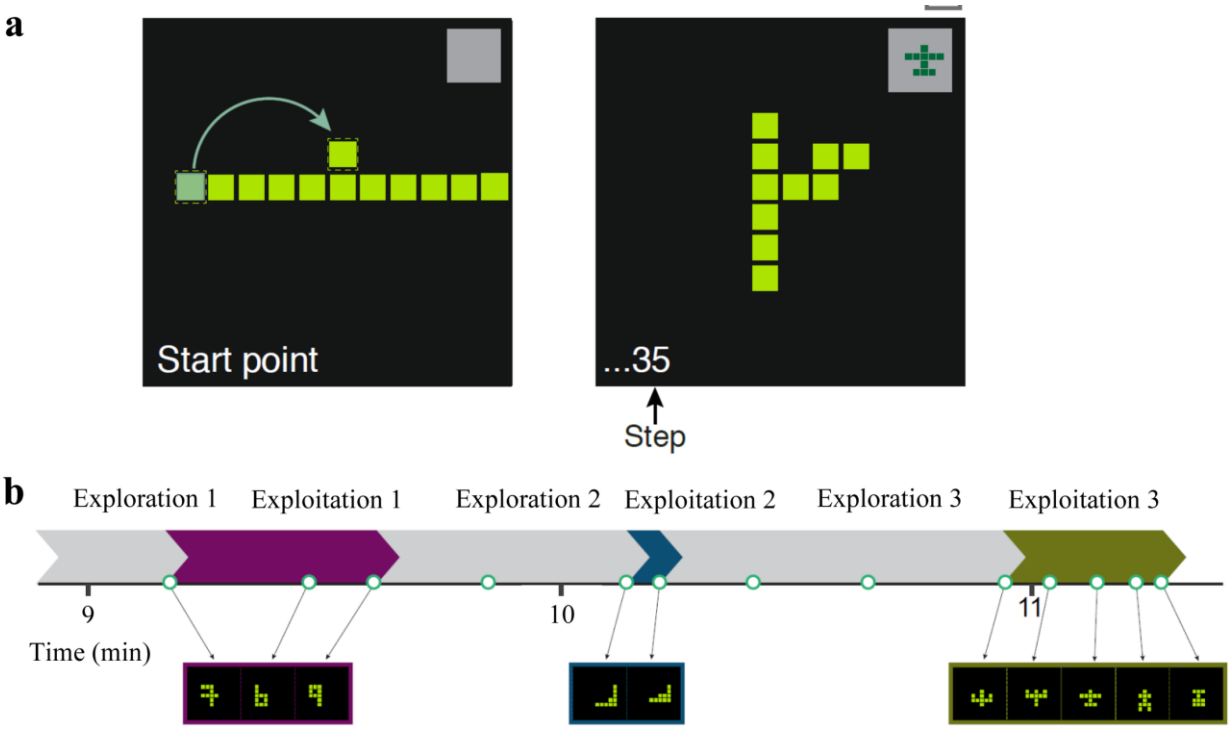
Following the odorant presentation, the participants were introduced to the creative foraging game (CFG), and played it for 10 minutes. Fifty-seven participants (30 from the control group and 27 from the placebo group) continued the experiment as followed: They were asked to smell and rate the odorant again, the placebo group were told this was to maintain the effect of the odor and the control group were told it was to get odor ratings at different time points, and then completed two classical creativity tests - the alternate uses test (AUT) and a subset of Torrance test of creative thinking (TTCT), in a counter-balanced order, each for 10 minutes as well. The total duration of the experiment was approximately 40 minutes.

Creativity tasks: I used three creativity tests: the Creative Foraging Game (CGF[168]) in which participants are asked to search for interesting and beautiful shapes in a defined geometric space, the Alternate Uses Test (AUT[172]) which is a verbal test for divergent thinking, and the figural Torrance test (TTCT[173]) which is a visual test for divergent thinking. Scoring of all tests was blind for condition.

Creative foraging game (CFG): The creative foraging game (CFG[168]) is a computer game in which participants create shapes by moving one of ten identical squares at each step, keeping the squares connected by an edge (Figure 15A). The initial condition is ten squares in a horizontal line. Participants are asked to explore the space of possible shapes (a total of 36,446 shapes), and when they come across a shape they find interesting or creative, to select it to a gallery. This is done by pressing a gray square at the top-right side of the screen, which saves the current shape to the gallery (Figure 15A). The gallery has no limit on the number of shapes. After the game, players perform another task - choosing the five most creative shapes from their gallery. This task is not analyzed in the current study. The automated algorithm allows constant recording and analysis of players' moves and reaction times. We use the method described in Hart et al[168] to analyze the CFG. During the game participants alternate between two phases termed *exploration* and *exploitation*[168]. The start and end time points of these phases are automatically defined by a segmentation algorithm. The algorithm uses the time points of choosing gallery shapes as the input. Briefly, exploration phases are defined by increasing time intervals between gallery choices and exploitation phases are defined by decreasing time intervals between gallery choices (see ref [168] for details). Exploration phases can include a single gallery shape. As shown in Ref [168], gallery shapes found in a given exploitation phase have shared perceptual meaning, and are consistently re-discovered by different participants. By clustering the shapes that are re-discovered by different participants, Hart et al. defined shape categories of meaning (SCM). Three examples of SCMs are shapes that resemble airplanes, numerical digits and letters (Figure 15B). Other SCMs include more abstract shapes with visual similarity. In Ref [168], 14 shape categories of meaning were found and were reliably distinguished in a separate discrimination experiment in which people who did not play the game classified shapes from the same SCM versus shapes from another SCM.

CFG Scoring: In creativity tests such as AUT and TTCT, experimenters have access to the solutions provided by the participants verbally or in drawn form, but not to intermediate steps or solutions. I reasoned that such forwarded solutions correspond, in the CFG, to the gallery shapes found in exploitation phases, and thus I scored exploitation gallery shapes rather than all gallery shapes. Fluency was scored as the total number of gallery shapes found by a player in all

Figure 15: Creative Foraging Game (CFG). a: CFG interface. Left: starting point; right: example of a shape. b: Exploration phases are followed by exploitation phases in which participants usually find shapes with shared perceptual meaning. Purple: numbers; green: airplanes. Data for Figure 1b is taken from one of the participants from Ref [168].



exploitation phases. Originality was scored as follows: each shape in the CFG was assigned a probability to be found, based on a database of 100 games by participants who did not take part in the current study[168]. The originality score of a participant is equal to the average of the minus log of the probabilities of all gallery shapes found in that player's exploitation phases. The flexibility score of a player is the number of different SCMs found by that player based on the SCMs found in a combined dataset of the 100 players of Ref [168] plus the 45 players in the group tested (control or placebo), plus the number of exploitation gallery shapes that did not fall into any of the SCMs. The out-of-the-boxness (OB) score for each participant was the fraction of exploitation gallery shapes that lie outside of the standard set of SCMs defined by the 100 players of Ref [168]. To test the robustness of this result, we used a random subset of 75 out of the 100 players of Ref [168] as a database for the SCM, and found similar results regarding higher OB in the placebo group. It was not possible to use less than 75 players because it is no longer possible to detect SCMs. Note that OB differs from originality because OB considers categories of shapes rather than specific shapes. OB differs from flexibility because it concerns the fraction of gallery shapes outside the SCMs, rather than the number of different SCMs found.

Alternate uses test (AUT): I followed the protocol of Ref [174]. Participants were given a list of five common items (shoe, pin, sheet, nail and button) and asked to list as many alternate uses as possible for each object within 10 minutes, while trying to think of original uses (the most common everyday use was indicated in parenthesis). Only responses that did not reiterate the given common uses were counted and included. Suggested uses which were meaningless were discarded. Scoring included fluency, originality and flexibility.

AUT scoring: I followed the procedure of Ref [174]. Fluency was scored by the number of alternate uses found for each object averaged over the five objects. Originality was defined as statistically infrequent responses of all responses provided per object. Specifically, for each object, a list of all obtained uses was collected from all participants. Two raters grouped similar uses into groups, with inter-rated agreement of 89.5% (Kappa coefficient 0.79). For example button as an earring/ as jewelry were grouped together, as were shoe to throw at someone/ as a weapon/ to hit someone. An infrequency score was assigned to each group as follows: answers which were listed by 5% or more of the participants were given a score of 0; answers provided by 2-5% were scored 1, and answers less frequent than 2% were scored 2. The total originality score of a participant is the mean over the infrequency score of all responses. Flexibility was scored by the number of different categories (groups) of the solutions of each object. The total flexibility score is the mean over the number of categories for each object of that participant.

Torrance test of creative thinking (TTCT), figural part, circles subset: I followed the protocol of the TTCT manual[175]. Participants were given a printed page with 35 identical circles with 1.27 cm (0.5 inch) radii in a 5x7 array. They were asked to draw as many different drawings/ideas as possible within 10 minutes, while trying to make each drawing/idea original and creative. Each drawing must include at least one circle and must be given a title. As before, scoring included originality, flexibility and fluency. Fluency was scored by the number of drawings generated from the circles. Originality and flexibility were both scored based on the TTCT scoring guide[175], which specifies originality scores (0, 1, 2 or 3) for about 150 potential drawings, in about 60 different categories. According to the guidelines, a drawing which is not specified in the manual gets 3 points for originality. As before, the total originality score of a participant is the mean over the originality score of all his/her drawings. When two or more circles are combined to create a single drawing, they are given a bonus originality score, which is added, according to the guidelines, to the total originality score. Flexibility was scored by the number of different categories of the drawings of each participant, using the manual. A drawing which was not mentioned in the manual was given a category according to those specified in the manual, or provided with a novel one, if

none would fit. The total flexibility score is the mean over the number of categories of that participant.

Analysis: To compare the control and placebo groups, I used the Mann-Whitney (MW) non-parametric test, which is appropriate because the data is not necessarily normally distributed. I note here also results for the historically more widely used two-tailed t-test: In all cases where I compared groups of total size N=90, the t-test gave results almost identical to the MW test: when MW was significant $p<0.05$ so was the t-test, and when MW>0.05 so was the t-test. In cases where N=57 (comparison with the AUT and TTCT tests), T-test results sometimes showed a trend p=~0.1 when the MW test showed significance or trend for significance $p<0.06$. These cases are AUT originality: MW p = 0.048, T-test p = 0.11; AUT fluency MW p = 0.056, T-test p = 0.12; AUT flexibility MW p = 0.06, T-test p = 0.11. All statistically significant results reported here employed the Benjamini-Hochberg multiple-hypothesis correction using FDR=0.15.

RESULTS:

The comparison between the placebo and control group is shown in Table 5. I found no significant effect of placebo on the TTCT test, and hereafter I focus on the CFG and AUT tests.

Table 5. Performance of the placebo and control groups in the three creativity tests.

Test	Measure	Placebo median ±MAD	Control median ±MAD	p-value MW	Effect size
Creative	Originality	7.0±0.5	6.7±0.4	**0.036***	0.46
Foraging	Fluency	14±6.6	16±8.6	0.73	--
Game (CFG)	Flexibility	10±7.2	11±6.5	0.96	--
	Out-of-the-boxness	0.47±0.17	0.35±0.15	**0.008****	0.6
	Exploitation phases	5±2	5±2.2	0.7	--
Alternate	Originality	3.6±1.3	2.6±1.5	**0.048***	0.43
Uses Test	Fluency	4.2±0.9	3.2±1.1	0.056†	0.43
(AUT)	Flexibility	3.8±0.8	3±1.1	0.06†	0.44
Torrance Test	Originality	28±13	28±9	0.75	--
of Creative	Fluency	10±5	8.5±4	0.34	--

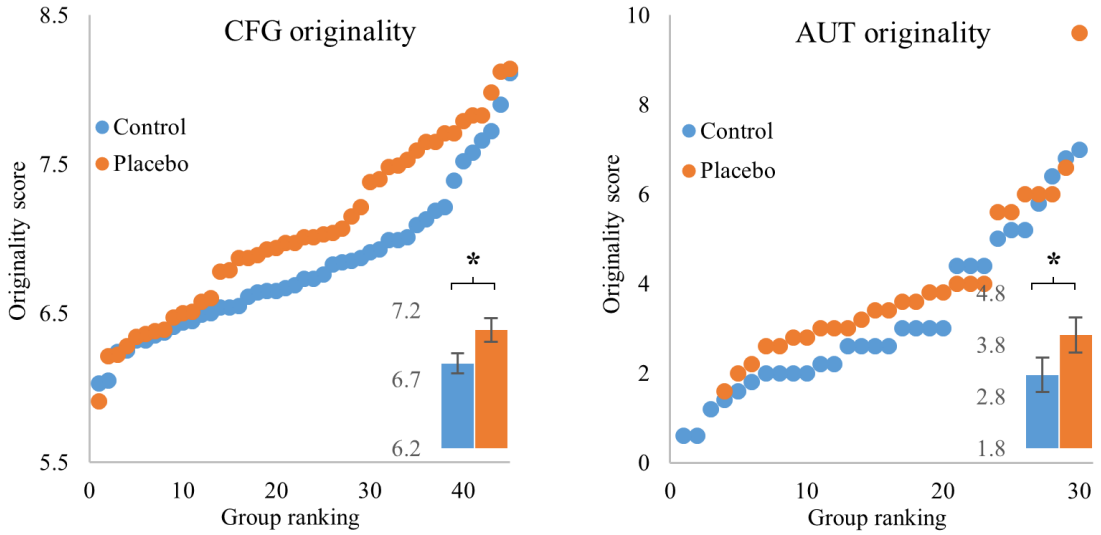
Thinking (TTCT)	Flexibility	7±3	7±3	0.78	--

** p value < 0.01; * p value < 0.05; † trend, p value <= 0.06

The placebo group showed higher originality

I first consider originality – the extent to which a player finds solutions not found by other participants (Methods). Participants in the placebo group showed significantly higher originality than the control group, both in the CFG (MW U=753, p = 0.036; Figure 16, left panel), and in the AUT (MW U=278, p = 0.048, Figure 16, right panel). The effect size was medium (CFG: Cohen's D=0.46; AUT: Cohen's D=0.43).

Figure 16: The placebo group showed significantly higher originality in both the CFG (left) and AUT (right). Main plot in each panel is a scatterplot of originality scores of each group, ranked from lowest to highest score. X axis is the ranking and Y axis is originality score. Insets are bar graphs of mean originality in each group, error bars are standard error of the mean. *p value < 0.05.

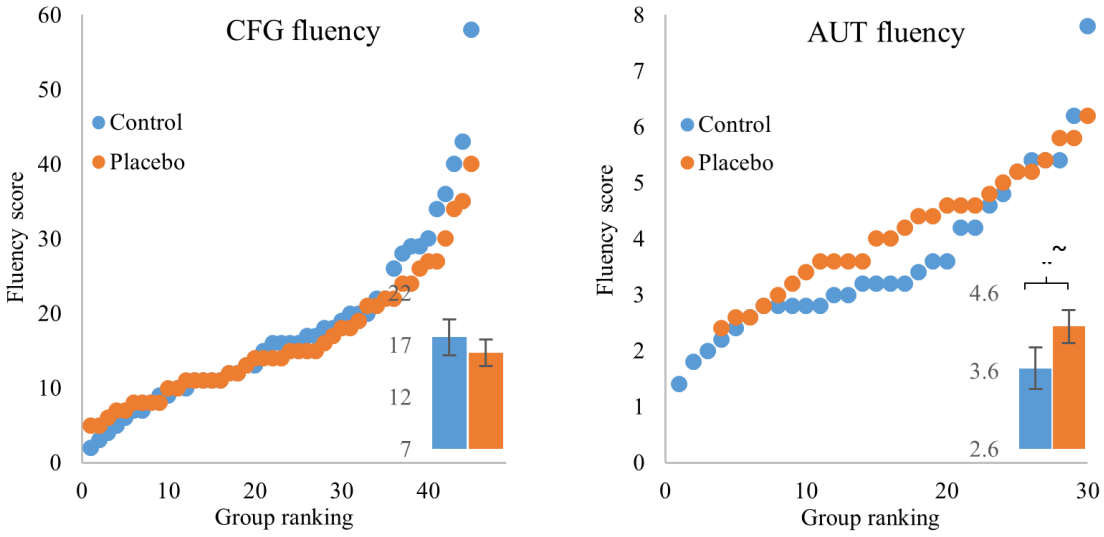


Fluency and flexibility did not significantly differ between the groups in the CFG, and were marginally significant in the AUT, in favor of the placebo group

I next compared fluency, defined as the overall number of solutions. In the CFG, I defined fluency as the number of shapes selected to the gallery during exploitation phases, finding no significant difference between control and placebo (MW U=970, p = 0.73). In the AUT test, fluency showed

a trend for being higher in the placebo group than in the control group (MW U=286, p = 0.056, Cohen's D=0.43) (Figure 17).

Figure 17: The placebo group showed a trend for higher fluency in the AUT (right) and no such difference in the CFG (left). Main plot in each panel is a scatterplot of fluency scores of each group, ranked from lowest to highest score. X axis is the ranking and Y axis is fluency score. Insets show the bar graphs of mean fluency in each group, error bars represent standard error of the mean. ~ p value < 0.06.



I also compared flexibility between the placebo and control groups, defined as the number of categories of suggested solutions. I find that flexibility in the CFG was not statistically different between the placebo and control groups (MW U=1005, p = 0.96). In the AUT, the placebo group showed a trend towards higher flexibility (MW U=287, p = 0.06).

The placebo group showed greater out-of-the-boxness in the CFG

When comparing flexibility of the two groups in the CFG, we noticed that the placebo group found many shapes that did not fit any category previously discovered by a database of 100 games[168]. We devised a score for this effect, the extent to which players found categories of shapes that were non-standard, naming it "out-of-the-boxness", OB. I asked, for each participant in our study, what fraction of the gallery shapes lies outside of the standard set of shape categories (SCM, see Methods). Note that OB differs from originality because it considers categories of shapes rather than specific shapes. I find that the OB of the placebo group was significantly higher than the control with medium-to-large effect size (MW U=683, p = 0.008; D=0.6). Figure 18 shows that this effect seems to be spread among all players.

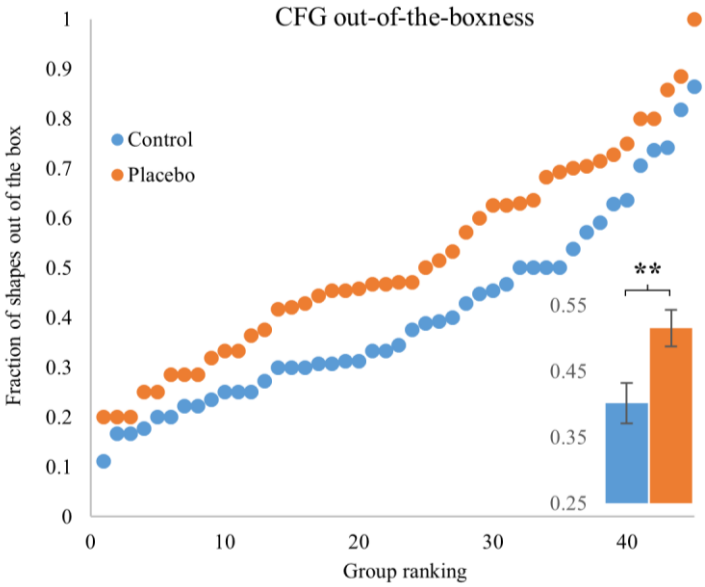


Figure 18: The placebo group displayed higher "out-of-the-boxness" (OB) than the control group in the CFG. Main plot is a scatterplot of OB scores of each group, ranked from lowest to highest score. X axis is the ranking and Y axis is the fraction of shapes outside standard categories. Insets show the bar graphs of mean OB in each group, error bars represent standard error of the mean. ** p value < 0.01.

DISCUSSION

In this study, I demonstrate that placebo can enhance the originality aspect of creativity. I used an odorant together with a verbal suggestion that it enhances creativity and reduces inhibitions, and evaluated creativity using three tests, the classical AUT and TTCT tests and the CFG, an automated creativity test in a well-defined search space. The placebo group showed increased originality in the AUT and CFG, but not the TTCT, by finding solutions rarely found by other players. The placebo group also showed higher out-of-the-boxness in the CFG by finding solutions not included in categories found by a database of 100 players from a previous study. The size of the significant effects was medium to strong, and effects were distributed among most members of the placebo group. Placebo did not seem to strongly affect fluency, the number of solutions found, which suggests that subjects were not simply less selective, but rather genuinely more original.

What are the psychological mechanisms that allow placebo to increase the originality aspect of creativity? There are at least two possibilities. The first mechanism is based on extensive research by Amabile and Deci and Ryan[150,158,159,176-178], which suggests that creativity is modulated by motivation. Extrinsic motivators were shown to be mostly detrimental to creativity, whereas intrinsic motivation is conductive to and strongly associated with creative abilities[150,157,158,176,179-181]. A key factor in intrinsic motivation, according to self-determination theory[159,160] , is the belief in one's competence. For example subjects who practiced encouraging statements (related to self-confidence, releasing anxieties etc.) and omitted self-incapacitating statements showed improved

creativity scores[182]. This is in line with the verbal suggestion in our study that the odorant increases creativity, which may have made subjects feel more competent. Additional components of intrinsic motivation, such as social relatedness, may also have been increased by experimenter effects in the present study, by the experimenter's perceived interest in the effects of the odorant.

A second possible psychological mechanism of placebo, as suggested by Weger et al., is to weaken inhibitory mechanisms that normally impair performance[149]. Creativity was found to increase in several studies that tested conditions with reduced inhibitions, such as alcohol consumption[161-163]. Wieth and Zacks showed that creative problem solving was improved when participants were tested during non-optimal times of day, and suggested that this is due to reduced inhibitory control[164]. Moreover, studies which used non-invasive brain stimulation by means of transcranial direct current stimulation (tDCS) found enhanced creativity, and attributed it to reduced inhibitions and diminished cognitive control[174,183]. This effect was suggested to be in line with paradoxical functional facilitation theory, which attributes improved performance of damaged nervous system to release from inhibition[184]. Informal notions in improvisation theatre suggest that the inner critic is a source of inhibition that limits creativity[185]. The verbal suggestion made in our study that the odorant increases creativity and reduces inhibitions may thus work through a reduced-inhibition mechanism and/or by increasing belief in one's competence. Future work can test which of these mechanisms is at play.

It is interesting to note that in terms of the creative product, the CFG and TTCT are both figural tests, whereas the AUT is verbal. Yet, here the placebo effect was beneficial for the CFG and AUT, but not TTCT. Whereas the reason to this difference is not clear to me, I noticed that the two manual tests, AUT and TCTT, did not show significant correlation with each other across participants in fluency, flexibility or originality (all spearman coefficients $R < 0.16$, all $p > 0.24$). A similar lack of correlation between different manual tests for divergent thinking was reported in previous studies[186-190]. This suggests that each test might measure different aspects of creativity. Encouragingly, I find that the AUT and CFG are significantly correlated (composite creativity score correlation $R=0.27$, $p = 0.04$[168]), which may help explain the effect. In addition, enhanced creativity in AUT but not TTCT was also found in a study on the effect of transcranial direct current stimulation (tDCS) on creativity[174]. The authors attributed the effect to release from inhibition. Interestingly, similar to my results, this study also found enhancement in the originality aspect of creativity, but not in fluency or flexibility. This may further imply that the placebo subjects in the present study were similarly less inhibited.

Limitations of the present study include a between-subjects design which cannot measure the ability of placebo to increase creativity within an individual. A benefit of studying a placebo effect on healthy individuals is that unlike the clinic, it is possible to employ both conditions on the same person to control for intra-subject variability. In this study I decided not to use this option because performing the CFG twice may create a learning curve or a habituation effect I wanted to avoid. In addition, although the order of the AUT and TTCT was counterbalanced, the CFG was always completed first. This ordering effect might have contributed to the significant findings on the CFG, given that participants completed it first and could have been more alert at the beginning of the experiment. The approaching significant findings on the AUT could be due to insufficient power. Future work can use the finding that AUT and CGF both pick up on the effects of placebo, and employ CFG and AUT in a counterbalanced way before and after placebo in order to address this issue. The study is limited to a single culture and context, and future work can explore placebo on creativity in other cultures and contexts.

Further research can explore the mechanisms by which placebo can enhance cognitive and creative abilities. Such exploration can include, in addition to tests to elucidate psychological mechanisms, also physiological and neurological measures of systematic and autonomous changes[191,192]. Placebo for enhancing cognitive abilities may thus be a research field with beneficial potential.

Concluding discussion:

In my PhD, I used various behavioral, hormonal, physiological and neural tools in order to provide better understanding of links between olfaction and human behavior in two disease-related conditions (under the guidance of Prof. Noam Sobel), with an emphasis on social olfactory communication, and one health-related phenomenon (under the guidance of Prof. Uri Alon). As I mentioned in the introduction, the link between the latter project and the two former ones is not very tight, yet I feel that the three of them provided me with meaningful tools to continue and explore human behavior in non-invasive methods, to use creative behavioral paradigms and questions to shed light on human phenomena from a neurobiological point of view.

Olfactory social communication has allowed me to find neurobiological mechanisms in two unrelated conditions, autism spectrum disorder (ASD) and recurrent pregnancy loss (RPL). In my first and central project I used a single non-invasive measure of nasal breathing, the sniff response, to infer on olfactory processing in ASD and its underlying neural mechanisms, and provide a potential biomarker for autism. In a second project, I used a more extensive investigation to test a novel hypothesis, studying RPL in the brain, and more specifically in the nose. I joined together behavioral, physiological and neurological evidence to suggest potential olfactory underpinnings of RPL, as is implicated in a well-established mammalian phenomenon (the Bruce effect).

Finally, I applied my experience in the olfactory world together with profound interest in the placebo effect to generate an independent project, taking the placebo effect outside clinical settings and into daily situations, showing that a simple suggestion can lead to enhanced cognitive abilities, as was demonstrated using creativity tests.

To conclude, my PhD journey has led to new discoveries, and has equipped me with necessary tools and methods to continue and explore human behavior in different conditions, and link behavioral phenomena to physiological evidence, providing neurobiological understanding of complex human behaviors.

APPENDIX A: Does human milk contains social chemosignals to facilitate parental behavior in adults?

This project was carried out in collaboration with M.Sc student Reut Weissgross.

In order to reach for the mother's mammary glands and feed off them, offspring rely on sensory input in the form of pheromones emitted from the mother's milk, skin, nipples and the glands surrounding them. In fact, a single molecule was identified and isolated from rabbits' milk which can by itself guide the newborn rabbits to their mother's milk and produce suckling and grasping[70].

Human mothers and infants also display olfactory-based communication, called chemosignaling. It was shown that newborns prefer the smell of their own mother's milk rather than a foreign human milk, and prefer both over formula replacement milk[193]. Human milk was also shown to have a calming effect on newborns[194] and to induce frontal lobe activation in them[195]. Additional evidence showed that body odor of breastfeeding women - taken from their axillary and mammary glands together - induced changes in the timing of ovulation and menstrual cycle of nulliparous women who smelled these mixtures[196], and even increases their sexual motivation[196]. Altogether, these findings imply a social chemosignal conveyed in human breast milk and the glands surrounding it.

In this study, we wanted to check the hypothesis that the human breast milk by itself contains a social chemosignal, which will affect adults – both males and females. We hypothesized that human milk will affect human adults' emotional arousal towards other individuals (infants and adults), especially in the context of parenting, and will also affect their emotions and behavior. This project was performed in full collaboration with Reut Weissgross, a Masters student in our lab, who ran all subjects and took equal part in designing the experiment.

EXPERIMENTAL DESGIN:

Participants: a total of 84 nulliparous subjects completed the experiment: 64 in the main experiment, 32 men and 32 women, wherein subjects were exposed to either human breast milk or nulliparous woman's body odor, in a counter-balanced manner. Additional 20 men participated in two control experiments, wherein we tested each of the main experiment's compounds against a clean pad (without body odor). All subjects provided an informed consent.

Procedures: We used a within-subjects design, counter-balanced for order and double-blind for compound identity. Subjects signed informed consent, then were exposed to either breast milk (2

ml on a clean pad) or control (2 ml cornflower on pad worn by a nulliparous woman to control for general feminine body odor effects). Importantly, both breast milk and control body odor were freshly collected in the morning of each experimental day. After 10 consecutive sniffs and ratings of the odorant, subjects rated their emotional arousal following pictures of either infants, adults or parental situations. Ratings were for 30 images, which were composed of 10 pictures of babies (gender could not be determined), 10 pictures of parental situations: 5 mothers and 5 fathers, and 10 pictures of adults: 5 men and 5 women. Subjects were asked to rate how emotionally engaged they feel towards the image displayed. Next, subjects had a 30-minute break and following which they performed the other condition of the experiment (control/ milk). Saliva samples were collected before and after each part of the experiment (4 samples per participant), but due to negative behavioral results – were not further analyzed.

RESULTS:

Initial experiment: 30 subjects, 16 men and 14 women.

Here we found a significant and strong effect suggesting that after exposure to the odor of human breast milk versus control body odor, subjects displayed higher emotional engagement (t_{29}=2.49, p=0.019). When exploring the different categories of the pictures, we unexpectedly found that the effect was mainly driven by the adults images that contained no babies in them (t_{29}=2.29, p=0.029), followed by the parental images (parents and babies, t_{29}=1.66, p=0.11) and a non-significant effect in the babies images (t_{29}=0.56, p=0.58). We then asked whether men and women displayed a different pattern of response: We found that the main effect over all images was carried by men (t_{15}=2.245, p=0.04), who exhibited higher emotional engagement after sniffing breast milk versus control odor, and not by women (t_{13}=1.33, p=0.21). When breaking the effect according to the different image categories, interestingly, men were leading the effect in the adults images (t_{15}=2.59, p=0.021), and women were more responsive to parental images (t_{13}=2.15, p=0.05).

Extended experiment: additional 34 subjects, 16 men and 18 women; total of 32 men and 32 women.

We then decided to increase sample size to 30 subjects in each gender to further validate the results and this intriguing dissociative response. Unfortunately, the results did not replicate our initial results. Namely, both when looking across all images and when looking at different image categories, no significant differences were found between smelling breast milk body odor and

control body odor (all p values > 0.4). These results remained true when collapsing all 64 subjects, regardless of gender (all $p > 0.16$).

Control experiments: 20 men subjects, 10 in each experiment.

In parallel to the extended experiment, we wanted to account for the possibility that we were actually measuring a response to the control body odor, meaning that the response we found in the initial experiment in men was not higher emotional engagement following exposure to the odor of milk, but rather lowered emotional engagement following exposure to women's body odor. For this aim, we recruited additional 20 men to go through a similar experiment, only now comparing each condition: milk / control odor (BO of a nulliparous woman) to a clean pad.

In the two control experiments: milk odor versus clean pad and body odor versus clean pad, no significant results were observed (milk: all $p > 0.4$, body odor: all $p > 0.37$). This was complementary to our failure to replicate initial results in the extended experiment, and allowed us to conclude this investigation with negative results. Namely, at least in the paradigm we used, the odor of human breast milk (nor the odor of women's BO) did not change levels of emotional engagement in adult subjects exposed to it.

APPENDIX B: Can olfactory processing inform on level of consciousness in disorders of consciousness patients?

This experiment is being carried out in collaboration with Dr. Anat Arzi, Cambridge UK and Dr. Yaron Sacher from the Lowenstein institute

The sniff response is a non-verbal, non-task-dependent measure of olfactory processing. It is completely passive and in order to measure it, breathing is the only action required from the subject. Our lab has already found the sniff response to be an indicative measure of olfactory processing even when subjects are asleep[197] or distracted[26]. Moreover, we demonstrated that the sniff response can inform on impaired brain anatomy[43] or even neurodevelopmental conditions, as autism spectrum disorders[26]. Here, we set out to ask whether the sniff response could be used in order to determine level in consciousness in patients with disorders of consciousness (DOC) who range from no clear signs of conscious awareness (vegetative state/unresponsive wakefulness syndrome) to fluctuating but reproducible signs of awareness (minimal consciousness state). We also ask whether the sniff response as measured in day one of arriving to the rehabilitation intensive care unit - could predict patient's state when released. In other words, we hypothesize that the sniff response can provide meaningful information regarding consciousness levels of rehabilitated patients.

EXPERIMENTAL DESIGN:

Legal guardians (all were families) of all participants signed informed consent to procedures approved by Lowenstein Rehabilitation Center Helsinki Committee. Exclusion criteria is impaired olfactory brain anatomy or impaired nasal breathing. So far we have collected and analyzed data from 20 patients so far, in 49 different sessions, and continue collecting data.

We approach subjects whose families have consented to participate in the experiment in the intensive care unit at the Lowenstein Rehabilitation Unit. While explaining each step to the subject, regardless of their presumed conscious state, we fit them with a nasal cannula (1103, Teleflex medical) which is connected to a spirometer (Spirometer FE141 ADInstruments) and amplifier (Power-Lab Monitoring System, ADInstruments), and provides constant measurement of their respiration. We manually provide them with five odors to smell, in a randomized manner: two pleasant odors (pleasant phenyl-ethyl alcohol, PEA, CAS 60-12-8, Sigma-Aldrich; Herbal Essence, Senseale, Ramat Gan, Israel) two unpleasant odors (butyric acid, CAS 107-92-6, Sigma-Aldrich; Rotten Fish, Senseale, Ramat Gan, Israel) and one blank odor. All odors are presented in

a jar and placed on an odorless pad. Prior to odor presentation, the experimenter prepares the subject by counting backwards from 3 to 1 and then presenting the odorant, for about 5 seconds. An interval of 30 seconds or at least 6 sniffs is maintained between odor presentations. Experiment typically lasts 20 minutes, during which odors are repeated ten times. Following odor presentations, the experimenter conducts the coma-near-coma (CNC) scale to assess the patients' consciousness level. This is the scale that is currently in use at Lowenstein.

PRELIMINARY RESULTS

Analyzing 20 patients in 49 different sessions, so far we several findings: First, similar to healthy individuals, DOC patients too display a sniff response, Namely, taking significantly smaller sniffs for unpleasant odors in comparison to clean air (Wilcoxon sign rank test, $p<0.05$), which implies olfactory processing in these patients. Second, correlating DOC patients sniffing patterns with level of consciousness as measured by the CNC scale, we find a significant correlation. Specifically, as level of consciousness was higher, sniffing patterns were more variable in the first sniff after pleasant odor onset (Spearman r =0.55, p = 0.02) and intriguingly also after clean air onset (Spearman r =0.63, p = 0.007, Figure 19).

We currently continue to collect data in an effort to follow-up on patients from their arrival to the unit until they leave, to obtain higher resolution sampling on sniff dynamics.

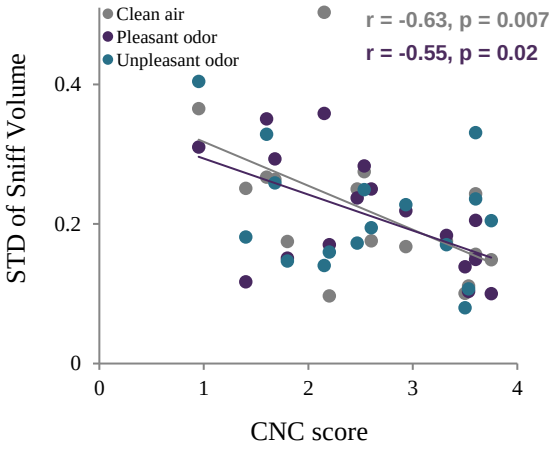


Figure 19. Sniffing is correlated with consciousness level. As conscious level was higher, sniffing patterns were more variable in the first sniff after pleasant odor onset

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