Olfactory threshold to bourgeonal and sexual desire in young adult males.

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Running head: Olfactory sensitivity and sexual desire

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Abstract Olfactory receptors have been found to be expressed also in the human spermatogenic cells giving rise to the hypothesis that they might play a role in fertility and sexual behavior. For instance, bourgeonal has been demonstrated to be an agonist of sperm cells olfactory receptor, OR1D2. Since OR1D2 is activated by bourgeonal, the aim of the present study was to investigate the purported relationship between olfactory threshold to bourgeonal and sexual desire in a group of normosmic young adult males. We have hypothesized, in fact, that a lower sexual desire could be related to a lower olfactory sensitivity to bourgeonal.

To test the hypothesized influence of olfactory sensitivity to bourgeonal on male sexual desire, we have examined 37 healthy young adult male volunteers (mean age= 24.9±3.6, range 20-36 years) as to their bourgeonal odor threshold and the intensity of sexual desire using the International Index of Erectile Function (IIEF) scale. In addition, samples of DNA were collected and allele and genotype frequency of the OR1D2 single nucleotide polymorphisms (SNPs) were evaluated in order to study the relationship between sexual desire and OR1D2 SNPs expression.

The olfactory median threshold was 13.75. Accordingly, the participants were divided into two groups with a bourgeonal olfactory threshold of, respectively, ≥13.75 and <13.75. Data analyses showed that IIEF1 scores did not differ between the two groups, while the IIEF2 score was higher in the ≥13.75 group (p=0.0753, trend towards significance). Moreover, the analysis of allele and genotype frequency of the OR1D2 SNPs did not find any significant association between allele and genotype frequency and sexual desire (IIED2) in the two groups. Furthermore, SNPs distribution did not differ between the two groups.

Our study found a significant trend between bourgeonal olfactory sensitivity and sexual desire in a group of young adult males. Practical and cognitive implications of olfaction on human sexuality are tentatively discussed.

Introduction

Animal noses can detect an enormous variety of odors at very tiny concentrations. This ability is mediated by olfactory receptors (OR) expressed in the olfactory epithelium (Buck, 2004). OR expression is not restricted to sensory neurons in the nose as ectopic OR transcripts have been found in different tissues such as myocardial, erythroid cells, ganglia of the autonomic nervous system, pyramidal neurons in the cerebral cortex, etc. (Drutel et al., 1995; Feingold et al., 1999; Weber et al., 2002; Otaki et al., 2004) Interestingly, Parmentier et al. (1992) demonstrated the existence of about twenty human olfactory receptors (hORs) in sperm cells, prompting the hypothesis that ORs could be involved in chemosensory signaling pathways; hence in direct sperm chemotaxis. About ten years later, Spehr et al. (2003) identified and cloned the human olfactory receptor OR1D2 (also known as hOR17-4), having a clear involvement in chemotaxis. These authors also showed that the OR1D2 receptor was activated by bourgeonal (a synthetic compound containing an aldehyde group connected to an aromatic ring via a carbon chain odorant). Shortly thereafter, they found that OR1D2 was expressed in the olfactory mucosa having analogous chemical behavior. (Spehr et al, 2004) It was hypothesized that olfaction might play an important role in fertility and human sexual behavior.

It is thought that sexual behavior could be driven by pheromones which have been defined as chemical signals between organisms of the same species that communicate beneficial information from one individual to another. (Hoover, 2011) Pheromones were described for the first time more than 50 years ago by Karlson and Luscher (1959). At any rate pheromones, and their role in humans, are still a controversial issue (Doty, 2010) and, so far, no pheromones have been conclusively identified (Wyatt, 2009). Nevertheless, recent results supported the hypothesis that the chemistry of axillary secretions (which can be considered a human pheromone source) and their effects on conspecifics in humans are analogous to other mammalian pheromone systems. (Wysocki and Preti, 2004) In particular, a compound in women's armpit extract, likely a human pheromone, apparently causes menstrual synchrony in females living in close quarters. (Wyatt, 2009) Thus,

pheromones have finally been proposed to have a direct impact on human sexual desire by influencing hormone balance. (Wyart et al, 2007)

Sexual desire has been defined as the sum of the forces that lean a person towards and away from sexual behavior. According to Levine (2003) three forces interact to generate sexual desire: sex drive (which is the biological component which has a neuroendocrine physiology), sexual motivation (the psychological component which is influenced by personal mental states, interpersonal states and social contexts) and sexual wish (which is culturally driven). A recent preliminary study by Ottaviano et al (2013) found that olfaction sensitivity and sexual desire were significantly related in normosmic young adult males. The authors hypothesized that young males with higher olfactory sensitivity could be more sensitive to female body odors and experience consequently a stronger sexual desire. Previously, studying a group of hypo-anosmic patients, Gudziol et al. (2009) found that the loss of olfactory function did not have a direct impact on their sexual appetite, but also found that sexual desire was significantly greater prior to the loss of sense of smell. This was in agreement with a previous report by Van Toller (1999), who emphasized that anosmics often stated that their interest in sex has dwindled. Finally, an association between olfaction and sexual desire was also reported by Grammer et al (2005).

As bourgeonal appears to be involved in human sexual behavior (Spehr et al, 2004) and fertility (Ottaviano et al, in press; Sinding et al, 2013), in the present study we investigated the relationship between olfactory sensitivity to bourgeonal, sexual desire and the frequency of 3 single nucleotide polymorphisms (SNPs) of OR1D2 gene in a group of healthy young adult males. Our primary aim was to establish whether there was a correlation between bourgeonal olfactory sensitivity and sexual desire intensity. The 3 SNPs [SNP reference ID number (rs) 769423, 769424 and 11078437] were selected from the 13 SNPs recently evaluated in another study (Ottaviano et al, in press) as being the most promising in understanding unexplained male infertility and low bourgeonal olfactory threshold.

The present study may have practical implication for a better understanding of the role of odors to drive attitudes, and human sexuality on our everyday lives.

Materials and methods

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and has been approved by the University Hospital Ethics Committee (prot. n. 2244/2011). Written informed consent was obtained from all participants before undertaking any study-related procedure.

Participants

Thirty-seven healthy, non-smoker male volunteers ranging in age from 20 to 36 years (mean age 24.9 ± 3.6 years; range 20-36 years), recruited at the Department of Neurosciences of Padova University (Italy) attended the study. All participants received a complete medical check in order to exclude ENT and urological disorders. They all exhibited normal nasal and paranasal sinus function, having scored less than 1 on the Sinonasal Outcome Test (SNOT 22) (Hopkins et al, 2009), as described elsewhere (Ottaviano, Lund et al, in press); and did not suffer from asthma or allergies. None of them had a history of prior sinonasal surgery or head trauma or used drugs that might have affected olfactory function. Also, their genitourinary systems were normal. Finally, none of them were taking β-blockers or salicylic acid therapy (whose circulatory changes might cause an erectile dysfunction affecting, in turn, sexual desire).

Olfactory testing

All participants underwent a quick olfactory screening with the *Nez du Vin* test (McMahon and Scadding, 1986) as done in previous studies (Ottaviano et al, 2012). The test involves identifying six aromas (lemon, mint, strawberry, pine, vanilla, smoke) by giving multiple-choice answers. As all volunteers revealed a normal sense of smell (scores of 5 or 6 out of 6), they were then investigated to ascertain their odor threshold for bourgeonal [3-(4-tert-butylphenyl)-propanal; Santa Cruz biotechnology, Heidelberg, Germany], as described elsewhere (Ottaviano et al, in press). In particular, similarly to the odor threshold for n-butanol (Kobal et al, 1996; Hummel et al, 2007), for

the determination of the odor threshold for bourgeonal, the odour was presented using sticks with a single-staircase, with a "three alternative forced choice" procedure. For this purpose, 29 dilutions were prepared in a geometric series starting from a 4% bourgeonal solution. The dilution ratio was 1:2 and the three sticks were presented in a randomized order. The Bourgeonal (CAS# 18127-01-0) was of the highest available purity and was obtained from SIGMA Aldrich (Milan, Italy). For bourgeonal dilutions, near odorless diethyl phthalate [SIGMA Aldrich (Milan, Italy)] was used as the solvent. For odor presentation, the stick's cap was removed for approximately 3 seconds and the stick's tip was placed approximately 2 cm in front of both nostrils. Three sticks were presented in a randomized order, with two containing the solvent and the third the odorant. Subjects had to identify the odor-containing stick. Threshold was defined as the mean of the last four of seven staircase reversals. The subjects' scores ranged between 1 (the lowest olfactory performance) and 29 (the highest olfactory performance). The median olfactory threshold was 13.75. Accordingly the participants were divided into two groups with bourgeonal olfactory threshold respectively ≥13.75 and <13.75.

Sexual desire assessment

Participants' sexual desire was assessed using part of the International Index of Erectile Function (IIEF) scale, which is a brief, multidimensional, self-administered, proven method to measure several dimensions of male sexual functioning. (Rosen et al, 1997) For the purposes of the present study, participants only answered the following questions: "How often have you felt sexual desire?" (IIEF1) and "How would you rate your level of sexual desire?" (IIEF2). The possible answers for IIEF1 were: 1 (almost never or never), 2 (occasionally), 3 (sometimes), 4 (often), 5 (almost always or always). For IIEF2 they were: 1 (very low), 2 (low), 3 (moderate), 4 (high), and 5 (very high).

DNA sample extraction

Genomic DNA was obtained by brushing the oral mucosa. DNA samples were then quantified by measuring the adsorbance at 260 nm by means of nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies, DE, USA).

OR1D2 SNPs analysis

The OR1D2 suitable primers of the 3 SNPs (rs769423, rs769424, rs11078437) were used (see, table 1). The Polimerase Chain Reaction (PCR) was performed following standard protocol (Ottaviano et al, in press). The PCR products were analyzed on 1% agarose gels and, before sequencing, performed in a core facility, they were purified using ExoSAP (GE Healthcare, Milan, Italy) to remove primers and PCR reagents.

Data analysis

Wilcoxon rank sum test was used to compare IIEF1 and IIEF2 scores' distribution between the higher olfactory threshold group (≥13.75) and the lower olfactory threshold group (<13.75). The correlation between IIEF1 and IIEF2 scores was analyzed with non-parametric Spearman correlation test. The association between SNPs and sexual desire was analysed in each of the olfactory threshold group with the Fisher's exact test. The same test was used to evaluate the SNPs differences between the two groups.

A p-value <0.05 was set for the statistical significance. Values in the range of $0.10>p \geq 0.05$ were considered as indicating a statistical trend.

The SAS 9.2 (SAS Institute Inc., Cary, NC, USA) for Windows was used for all analyses.

Results

IIEF 1 and IIEF 2 scores were shown to be correlated (r=0.4334, p=0.0074). IIEF1 scores did not differ between young males with olfactory threshold under vs over or equal to the median value of 13.75 (p= 0.5763). (Table 2) IIEF2 scores showed a statistical trend towards a significant difference

between the two groups (p=0.0753), being IIEF2 higher in the group with olfactory threshold ≥13.75. (Table 2)

Analyzing the 3 SNPs of OR1D2, we were not able to find any statistically significant association between allele and genotype frequency and sexual desire (IIED2) in either of the two groups (rs769423: p=1.0000 for both groups with bourgeonal olfactory threshold <13.75 and ≥13.75; rs769424: p=1.000 and p=0.2353 respectively for the group with bourgeonal olfactory threshold <13.75 and for the other with olfactory threshold ≥13.75; rs11078437: p=1.000 and p=0.2778 respectively for the group with bourgeonal olfactory threshold <13.75 and for the other with olfactory threshold ≥13.75). Finally, no SNPs distribution differences were found between the two groups (rs769423: p=0.3398; rs769424: p=0.6599; rs11078437: p=0.6039). (Table 3)

Discussion

Sexual desire has three components: sex drive, sexual motivation, and sexual wish. (Levine, 1987). The first one is a biological variable that has an anatomical and neuroendocrine physiology. Sex drive and sexual function could be influenced also by nasal function, as underlined by recent studies. (Gunhan, 2011; Benninger and Benninger, 2009) Another study by Gudziol et al. (2009), who studied a group of non-congenital hypo-anosmic patients, reported that sexual desire had been significantly greater prior to the loss of sense of smell. Moreover, Van Toller (1999) emphasized the fact that anosmics often stated that their interest in sex was dwindled. Very recently, a study from Ottaviano et al. (2013) showed an association between olfactory threshold and sexual behavior in young adult males.

Bourgeonal has been found to be the OR1D2 most potent agonist in human sperm cells. Moreover, bourgeonal has been shown to be involved in sperm chemotaxis and to have a similar behavior both in human testis and in olfactory epithelium (where it is expressed as well). Olsson and Laska (2010), using a three-alternative forced-choice test procedure, determined the olfactory detection thresholds for bourgeonal in 500 subjects (250 males and 250 females) and found that males detected the odorant at lower concentrations than females. The authors concluded that the human male superiority in olfactory bourgeonal sensitivity could be due to differences in its behavioral relevance for males and females. Given this, in the present study we evaluated the olfactory sensitivity to bourgeonal in a group of healthy young volunteers and tried to analyze any relation between sexual desire and olfactory sensitivity to this compound. A group of healthy volunteers homogeneous from rhinological viewpoint was studied using standardized olfactory threshold measurements. Our results seem to be in line with literature data as the group with better olfactory sensitivity to bourgeonal (threshold ≥13.75) showed a statistical trend towards a stronger sexual desire. It could be that the only marginal significance could be explained by the low number of considered volunteers.

Sexual desire was evaluated by using IIEF1 and IIEF2 which are the only 2 of the 15 questions that make up the International Index of Erectile Function scale to be definitely related to sexual desire. Although IIEF 1 and IIEF 2 were strongly correlated (p=0.0074), in the present study we found an association between bourgeonal olfactory threshold and the IIEF2 score (p=0.0753), but not with IIEF1 score (p= 0.5763). This confirms what we found in a previous study (Ottaviano et al, 2013), i.e. that IIEF2, which is a rate of the sexual desire, is probably more related to sex drive than IIEF1.

Vosshall (2004) investigated the hypothesis that olfaction influences human sexual behavior and even the choice of partner and suggested that a potential female partner produces a particular body scent capable of attracting a male partner by stimulating the latter's olfactory system. Young adult males with higher olfactory threshold for bourgeonal could be more sensitive to female body odors and then could experience a stronger sexual desire than those with a lower olfactory threshold for bourgeonal.

Although we would have expected to find a stronger association between bourgeonal olfactory sensitivity and sexual desire, we believe that our results could be considered in line with

other literature data (Gudziol et al, 2009; Van Toller, 1999; Grammer et al, 2005; Ottaviano et al, 2013) and consider the moderate significance a consequence of the small number of the subjects in our study. Our study supports the hypothesis, already proposed by Olsson and Laska (2010), that sexual selection may act upon the expression of ectopic olfactory receptors such as OR1D2. Although these authors posited that the olfactory receptor SNPs frequency distribution could be different in subjects with different olfactory sensitivity, we were not able to find any relationship between bourgeonal olfactory thresholds, sexual desire and the frequency of the 3 analyzed SNPs. This could be equally due to: 1) the fact that the 3 studied SNPs have been selected because statistically significant (or with a trend towards significance) out of 13 SNPs previously considered in a project that had a different aim (the study of the relation between OR1D2 SNP individual genome, unexplained male infertility and olfactory threshold to n-butanol); 2) the low number of subjects studied.

The results of the present study, in addition to those of a recently published one (Ottaviano et al, in press) lead us to hypothesize that the natural analogous of bourgeonal could behave as human pheromone, influencing sexual behaviour, partner choice and, finally, fertility. In line with this hypothesis, it could be speculated that a potential female partner would produce a specific cocktail of chemo-attractants for her eggs and a similar body odour scent. Such a chemical display would first attract a male partner through the olfactory system and subsequently ensure productive sexual intercourse by attracting his sperm. (Vosshall, 2004)

Conclusions

The present study, evaluating for the first time the relationship between bourgeonal olfactory sensitivity and sexual desire in a group of young adult males, tried to focus on the practical and cognitive implications of olfaction on human sexuality. Although studies on larger numbers of subjects are mandatory in order to confirm our outcomes, the present results could open new perspectives in infertility care, as well as in the development of new contraceptive methods.

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Table legends

Table 1: sequence of the PCR primers used for the OR1D2 SNPs detection
Table 2: sexual desire and bourgeonal olfactory threshold
Table 3: SNPs distribution in the two groups.