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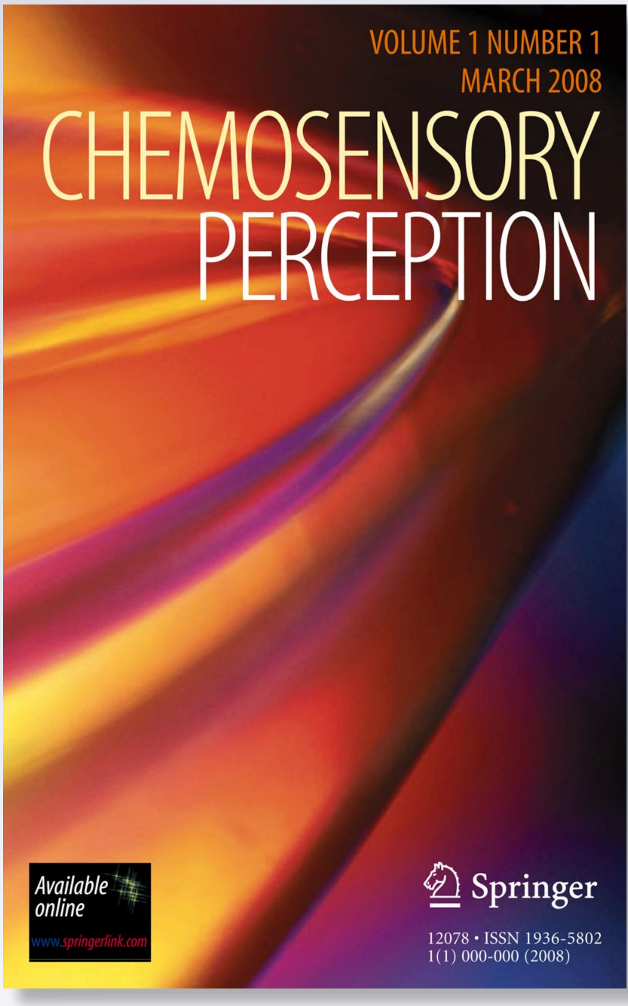

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Does Length of Sampling Affect Quality of Body Odor Samples?

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Abstract A number of recent studies use body odor samples to study how odor affects various human social interactions. However, the methods used vary considerably, and only limited attention has been paid to the validity of the particular approaches adopted. One of the crucial points in body odor sampling is its length. Here we report the results of a study that tested the effects of using two different sampling periods, namely 12 and 24 h, on subsequent odor perception. Our results show significantly higher ratings of pleasantness and attractiveness, and lower ratings of intensity and masculinity, for the 12-h sampling condition. Further analysis of the data distribution suggests higher skew and a potential floor effect in hedonic ratings derived from the 24-h condition. These data imply that sampling length has a significant impact on perceived body odor quality and should thus be considered when designing studies in this area and in interpretation of results across studies.

Keywords Armpit · Attractiveness · Mate choice · Methodology · Olfaction · Smell

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Introduction

Body odor has recently attracted much attention from researchers as it plays a significant role in various social interactions and mate choice, in particular (for recent reviews, see Grammer et al. 2005; Havlicek and Roberts 2009; Havlicek et al. 2010; Lenochova and Havlicek 2008). In spite of this interest, surprisingly little attention has been paid to the validity of methods used in this field. Protocols for collection of body odors usually specify, in various ways, the following: restrictions on body odor donors, medium used for sampling, length of sampling, and length and temperature of sample storage. All of these might affect the outcome of the study. The body odor samples in adult individuals are mostly collected from the armpit (axilla) as its concentration of hair and secretory skin glands create a rich source of distinctive odor, which is thought to have evolved for communication purposes (Comfort 1971).

Armpit odor is a result of metabolic activity of axillary microflora which mostly consists of coryneform bacteria, *Propionibacterium*, *Staphylocous*, *Micrococcacae*, and eukaryotic *Malasezia* (Leyden et al. 1981; Rennie et al. 1991; Taylor et al. 2003; Wilson 2005). Their products include various branched and unbranched fatty acids, particularly 3-hydroxy-3-methylhexanoic acid, sulfur-containing alkanols, cholesterol, 16-androstenes (primarily 5a-androstenone, 5a-androstenol, and androstadienone), and numerous other compounds (Gautschi et al. 2007; James et al. 2004; Natsch et al. 2004, 2006; Zeng et al. 1991, 1996). Penn et al. (2007) using stir-bar sorptive extraction estimated number of axillary compounds over 4,000 and 373 markers showed consistency over several samplings. The substrate for axillary microflora is mostly produced by secretion from abundant apocrine glands; however, this has only slight odoriferous quality, and

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characteristic axillary odor develops only after bacterial activity (Shelley et al. 1953).

These facts have several important consequences for researchers. Studies on human perception of axillary body odor mostly capture odor from odor donors on either cotton pads (e.g., Havlicek et al. 2005, 2006; Roberts et al. 2005; Ferdenzi et al. 2009) or T-shirts (e.g., Wedekind et al. 1995; Wedekind and Furi 1997; Jacob et al. 2002; Roberts et al. 2008). However, these media catch not only the odoriferous chemicals but also axillary microflora, meaning that the samples can undergo further metabolic action, with resulting change in odor quality, even after samples have been collected. For this reason, fresh samples can be used for ratings only for a restricted period. To avoid this limitation, samples may be frozen to limit bacterial activity and other changes in chemical composition of the samples. Two independent studies have shown that this procedure preserves the samples' perceptive quality quite well (Lenochova et al. 2008; Roberts et al. 2008).

Another consequence of bacterial activity on odor samples is that the length of sampling could affect the quality of the collected samples. Sampling length varies highly in previous studies, ranging from 13 min (Chen and Haviland-Jones 2000) up to 7 nights (Hold and Schleidt 1977) (see Table 1 for details). However, studies typically sample odor over either 24 h (e.g., Ferdenzi et al. 2009; Havlicek et al. 2006; Lenochova et al. 2008) or two consecutive nights (e.g., Sergeant et al. 2007; Thornhill and Gangestad 1999; Wedekind et al. 1995). Is there any rationale for this? Our answer is no, as to date there has been no agreement or methodological investigation determining the optimal length of sampling, even though this could have significant impact on study results. To fill this methodological gap, we carried out a simple experiment in which we compared the quality of samples collected from the same individuals over different periods of time (12 and 24 h). We chose the 24-h sampling period because several of our previous studies used this period (e.g., Havlicek et al. 2005, 2006; Lenochova et al. 2008), and we chose the 12-h period because, in the aforementioned studies, there were some indications of an over-representation of the ratings on the lower (for hedonic ratings) or upper end (for intensity) of the rating scales. We expected that a shorter sampling period might avoid these problems.

Material and Methods

Participants

All participants, students at the University of Vienna, were recruited via posters, handouts given out during lectures, or personally by PL. They were not reimbursed financially, but they received a 150 g chocolate bar and a perfume tester in return for their time and potential inconvenience.

Odor Donors

Seven men, aged 23 to 32 (mean, 25.9 years), mean body weight 71.3 (range, 60–87 kg) and body height 180.9 (range, 165–202 cm), participated as body odor donors. None of them smoked, reported any serious disease, or shaved their armpits.

Raters

The odor samples were rated by 25 women aged 19 to 32 (mean, 23.9 years). Eleven of them used hormonal contraception. Cycle length reported by non-users varied between 23 to 31 days. To control for a potential effect of hormonal contraception (HC) use on odor perception (see Roberts et al. 2008), we included this binary variable into the analysis. However, we did not find significant effects of HC on any of the rated variables (e.g., pleasantness) or an interaction with tested conditions (i.e., 12/24 h). We also checked for a confounding effect of tobacco-smoking among raters. Seven raters reported being smokers, but analyses including smoking as a binary variable did not reveal significant effects of smoking on any of the rated variables or interaction with length of sampling. We therefore present results without these two potential confounding variables.

Odor Sampling Procedure

The donors were sent all experimental instructions via E-mail several days before the experiment, and if needed, ambiguities were discussed individually. They were also given a pack of experimental materials (a white cotton T-shirt, a bar of non-perfumed soap, two cotton pads, surgical tape, and two zip-lock plastic bags). Sterilized cotton pads packed in aluminium foil with an oblong tape attached to the pad (tape, 11×15 cm; pad, 10.5×6 cm) were used as the sampling media. The donors were asked to follow a schedule of dietary and behavioral restrictions on the day prior to the sampling and during the sampling day. They were instructed to refrain from (1) using perfumes, deodorants, antiperspirants, aftershaves, and shower gels; (2) eating meals containing garlic, onion, chilli, pepper, vinegar, blue cheese, cabbage, radish, fermented milk products, and marinated fish, (3) drinking alcoholic beverages or using other drugs, and (4) smoking. Additionally, they were asked to avoid strenuous physical activities, sexual intercourse, or sleeping in the same bed with their partner or pet during this time.

On the evening before sampling, the donors used a non-perfumed soap (Sara Lee Household and Body Care,

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Table 1 Summary of studies using axillary samples as stimuli

Authors	Sampling length	Medium	Experimental task	Odor donors
Chen and Haviland-Jones 2000	13 min	Gauze pads	Recognition	14 F/11 M
Albrecht et al. 2011	20 min	Cellulose pads	Emotion/hedonic ratings	13 M
Chen et al. 2006	20 min	Rayon-polyester pads	Reaction time/hedonic ratings	3 F/4 M
Mujica-Parodi et al. 2009	20 min	Cotton pads	Discrimination/hedonic ratings	52 F/92 M
Zernecke et al. 2010	20 min	Cotton pads	Detection/localization	20 M
Zhou and Chen 2009	20 min	Rayon-polyester pads	Emotion perception	8 M
Platek et al. 2001	30 min	Gauze pads	Recognition/hedonic ratings	18 M/32 F
Adolph et al. 2010	60 min	Cotton pads	Hedonic ratings	6 M
Prehn-Kristensen et al. 2009; Pause et al. 2009	60 min	Cotton pads	Hedonic ratings	21 F/28 M
Ackerl et al. 2002	70 min	Cellulose pads	Recognition/hedonic ratings	42 F
Haegler et al. 2010	75 min	Cotton pads	Risk game	21 M
Prehn et al. 2006	90 min	Cotton pads	Hedonic ratings	12 M
Rantala et al. 2006	5 h	T-shirts	Hedonic ratings	19 M
Doty et al. 1978	8 h	Gauze pads	Hedonic ratings	5 F/5 M
Cernoch and Porter 1985	8 h	Gauze pads	Head orientation	118 F/60M[a]
Roberts et al. 2005	8 h	Cotton pads	Recognition	32 F
Roberts et al. 2011	8–10 h	Cotton pads	Hedonic ratings	20 M
Ferdenzi et al. 2009	24 h	Cotton pads	Hedonic ratings	38 M
Havlicek et al. 2005	24 h	Cotton pads	Hedonic ratings	48 M
Havlicek et al. 2006	24 h	Cotton pads	Hedonic ratings	19 F
Havlicek and Lenochova 2006	24 h	Cotton pads	Hedonic ratings	17 M
Lenochova et al. 2008	24 h	Cotton pads	Hedonic ratings	39 M
Kohoutova et al. forthcoming	24 h	Cotton pads	Hedonic ratings	45 M
Lenochova et al. forthcoming	24/12 h	Cotton pads	Hedonic ratings	7 M/10 M/12M[b]
Russell 1976	24 h	T-shirts	Recognition	13 F/16 M
Gangestad and Thornhill 1998	2 nights	T-shirts	Hedonic ratings	42 M
Jacob et al. 2002	2 nights	T-shirts	Hedonic ratings	6 M
Kuukasjarvi et al. 2004	2 nights	T-shirts	Hedonic ratings	82 M
Roberts et al. 2008	2 nights	T-shirts	Hedonic ratings	97 M
Sergeant et al. 2007	2 nights	T-shirts	Hedonic ratings	20 M
Thornhill and Gangestad 1999	2 nights	T-shirts	Hedonic ratings	82 F/80 M
Thornhill et al. 2003	2 nights	T-shirts	Hedonic ratings	100 F/97 M
Wedekind and Furi 1997	2 nights	T-shirts	Hedonic ratings	4 M/2 F
Wedekind et al. 1995	2 nights	T-shirts	Hedonic ratings	44 M
Wedekind et al. 2006	2 nights	T-shirts	Hedonic ratings	38 M
Weisfeld et al. 2003	2/3 nights	T-shirts	Recognition/hedonic ratings	55 F/55M[c]
Ferdenzi et al. 2010	3 nights	T-shirts	Recognition/hedonic ratings	14 F/20 M
Chen and Haviland-Jones 1999	3 days	Gauze pads	Hedonic ratings	10 F/10 M; 5 G/5B
Dubas et al. 2009	3 nights	T-shirts	Recognition/hedonic ratings	99 G+B
Martins et al. 2005	3 days	Cotton pads	Preference test/hedonic ratings	12 F/12 M
Olsson et al. 2006	3 nights	Cotton pads	Recognition/hedonic ratings	26 F/11 M
Porter et al. 1985	3 nights	T-shirts	Recognition	15 F/15B+G; 12 F/12 M
Porter et al. 1986	3 nights	T-shirts	Recognition	40 F+M
Porter and Moore 1981	3 nights	T-shirts	Recognition	16 G/8B; 13 G/7B
Rikowski and Grammer 1999	3 nights	T-shirts	Hedonic ratings	19 F/16 M
Singh and Bronstad 2001	3 nights	T-shirts	Hedonic ratings	19 F
Mallet and Schaal 1998	4 nights	T-shirts	Recognition/hedonic ratings	9 G/9B
Hold and Schleidt 1977	7 nights	T-shirts	Recognition/hedonic ratings	24 F/24 M

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Table 1 (continued)

Authors	Sampling length	Medium	Experimental task	Odor donors
Lundstrom and Jones-Gotman 2009	7 nights	T-shirts	Identification	20 F/40 M
Schleidt 1980	7 nights	T-shirts	Recognition/hedonic ratings	25 F/25 M
Schleidt et al. 1981	7 nights	T-shirts	Recognition/hedonic ratings	71 F/71 M

It shows authors of the studies, sampling length, sample collection medium, experimental task (i.e., what were odor raters requested to do), odor donors' sex, and sample size. Note that studies on infants are not included

F indicates women, *M* indicates men, *G* indicates prepubertal girls, *B* indicates prepubertal boys

[a] It is not clear from the methods whether different samples were used across individual studies described in the paper

[b] 12-h condition applies to study 3 with 12 M donors

[c] Number of child odor donors is not clear from the methods

Stockholm, Sweden) and put on a new white 100% cotton T-shirt, previously washed twice without washing powder as the first layer of their clothes. They were asked to wear this T-shirt for the night and the following day to avoid odor contamination from other clothes and environment, while following an experimental protocol and restrictions used in previous studies (Havlicek and Lenochova 2006; Kohoutova et al. forthcoming; Lenochova et al. 2008).

The next day, at noon, the donors washed both their armpits with the non-perfumed soap. Subsequently, they fixed a cotton pad into one armpit and put on the T-shirt again. The side of armpit was randomly assigned to avoid possible side differences (Ferdenzi et al. 2009). Later, at midnight, the donors washed the second axilla with the non-perfumed soap and applied the second cotton pad, while keeping the first pad on. This procedure and schedule approximately balanced the length of the active and sleeping phase in both types of samples. Consequently, we received the 12- and 24-h samples from each donor at noon on the following day. The pads were stored in zip-lock plastic bags and labelled to distinguish the samples worn in the left and right armpits. The donors' conformity with the instructions was checked by a questionnaire. No serious violations occurred: One donor reported having a small amount of onion, and another one had a little pepper on the day before sampling.

Odor Rating Procedure

The rating session started within an hour after collection of the samples (1 PM) and continued until 8 PM. It is possible that bacterial action may continue to influence odor during rating sessions and that this effect could be different for the 12- and 24-h samples. In other words, the amount of bacteria collected during 24-h period could be higher compared with 12-h and in turn may affect the rate of change in the perception of the samples. To check for this possibility, we compared (using two sample *t* tests) mean ratings performed in between 1 and 4 PM (18 raters) and 4 and 8 PM (seven raters). We found no significant differences (all $P>0.22$) between early (1–4 PM) and later (4–8 PM) ratings in any of the rated variables (e.g., pleasantness), either in the 12- or the 24-h conditions. All 14 fresh samples (seven from the left and seven from the right armpits) were enclosed in clean 200-ml lidded plastic sniffing bottles and labelled by a letter code. The ratings took place in a relatively large and quiet ventilated lecture room. The samples were randomly split into two sets. After sniffing half of the samples, the raters were recommended to have, at least, a 10-min break to avoid possible odor habituation. During the break, they were asked to complete an additional questionnaire. Order of sets and order of stimuli within a set was randomized for each rater.

The stimuli were assessed on seven-point scales for their (1) pleasantness, (2) attractiveness, (3) masculinity, and (4) intensity. Both ends of each scale were anchored by verbal descriptions (e.g., very unpleasant and very pleasant). The ratings were written down immediately after sniffing each stimulus, but the time spent sniffing was not restricted. In the event that the raters found any of the samples too weak, they were instructed to select "I cannot smell the sample" instead of using the scales. Fourteen raters judged at least one sample (range, 1–6 samples) as too weak, and nine samples were rated as too weak at least once (range, 1–7). In total, 31 ratings were considered to weak to rate accurately (in five of these, samples in the 12- and 24-h conditions were marked as too weak), resulting in exclusion of 26 rating pairs (14.9%). Thus, the main analysis is based on 149 rating pairs out of 175 (i.e., seven pairs of samples collected from the same individual and assessed by 25 raters).

Statistical Analysis

To assess the effect of length of sampling on perceived body odor quality, we computed the mean odor ratings for

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each rater, for both of the tested conditions. The mean values were subsequently compared using paired *t* tests. To analyze data distribution, we used a Kolmogorov–Smirnov test to check normality distribution. The statistical package Statistica 7.1 was used for all testing.

Results

First, we found significantly higher ratings of attractiveness ($t_{(24)}$=4.6; P<0.001), pleasantness ($t_{(24)}$=4.8; P<0.001) and lower ratings of masculinity ($t_{(24)}$=4.3; P=0.002) and intensity ($t_{(24)}$=6.3; P<0.001) in the 12-h samples (Fig. 1).

Kolmogorov–Smirnov test on distribution fitting with mean values for each rater as unit of the analysis did not show significant deviation from normality in either variable. In contrast, all variables showed highly significant deviation from normality when individual ratings were analyzed. Visual inspection of the histograms with individual ratings (Figs. 2 and 3) and skewness values (Table 2) clearly suggests more positive skewness of the hedonic ratings collected under 24-h conditions (i.e., ratings of pleasantness, attractiveness, concentrated on the lower side of the scales) and more negative skewness of the masculinity and intensity ratings.

To test whether the number of samples for which odor was too weak to be perceived varies across the conditions, we used a Chi-square test. Participants indicated they could not detect the odor in 23 ratings in the 12-h condition and in eight ratings in the 24-h condition. This difference is highly significant (χ^2=7.8; P=0.005).

Correlational analysis of the individual data showed a highly significant positive correlation between attractiveness and pleasantness, and a negative correlation with masculinity and intensity, in both conditions (Table 3).

Discussion

In our study, we found higher scores of perceived pleasantness and attractiveness in samples collected for 12 compared with 24-h samples. On the other hand, ratings of intensity and masculinity were higher for the longer sampling period. More importantly, we found that data distribution is more skewed (i.e., ratings of pleasantness and attractiveness concentrated at the lower end of the scales, and ratings of masculinity and intensity concentrated at the higher end of the scale) in the 24-h condition for all variables tested. As in the majority of previous studies, we also found hedonic ratings (i.e., pleasantness and attractiveness) being negatively related to ratings of intensity (Doty et al. 1978; Havlicek et al. 2006).

The differences across the two sampling conditions clearly indicate that the length of sampling is a significant factor in studies of body odor perception and that it should be kept constant, at least within individual studies. Across studies, the length of sampling to date has been highly disparate (see Table 1). These studies vary not only in the phenomenon they explore, ranging from kin recognition to MHC preferences but also in the experimental protocol. Some of them employ a recognition paradigm (e.g., of the

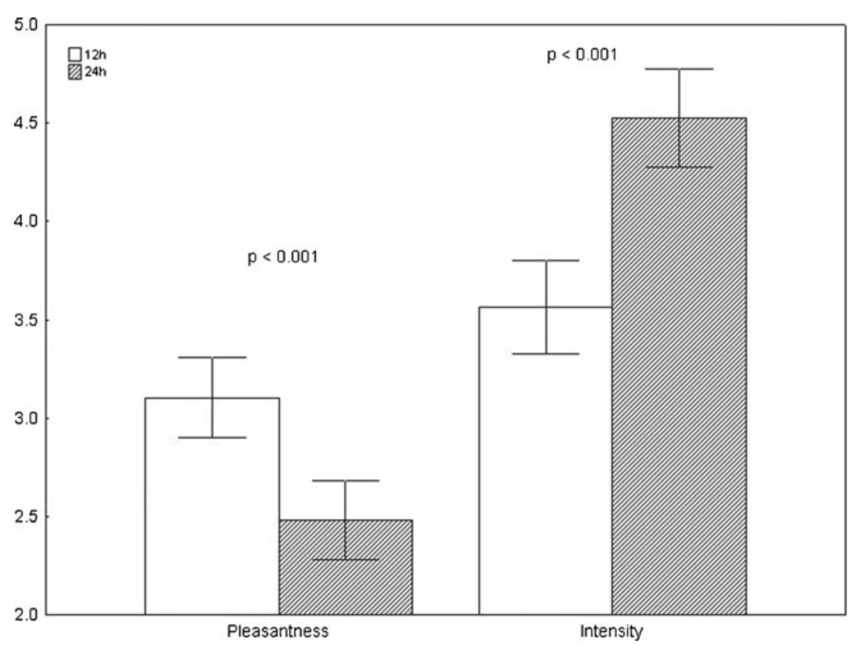

Fig. 1 Mean ratings (±SE) of pleasantness and intensity in 12- (*empty bars*) and 24-h samplings (*striped bars*)

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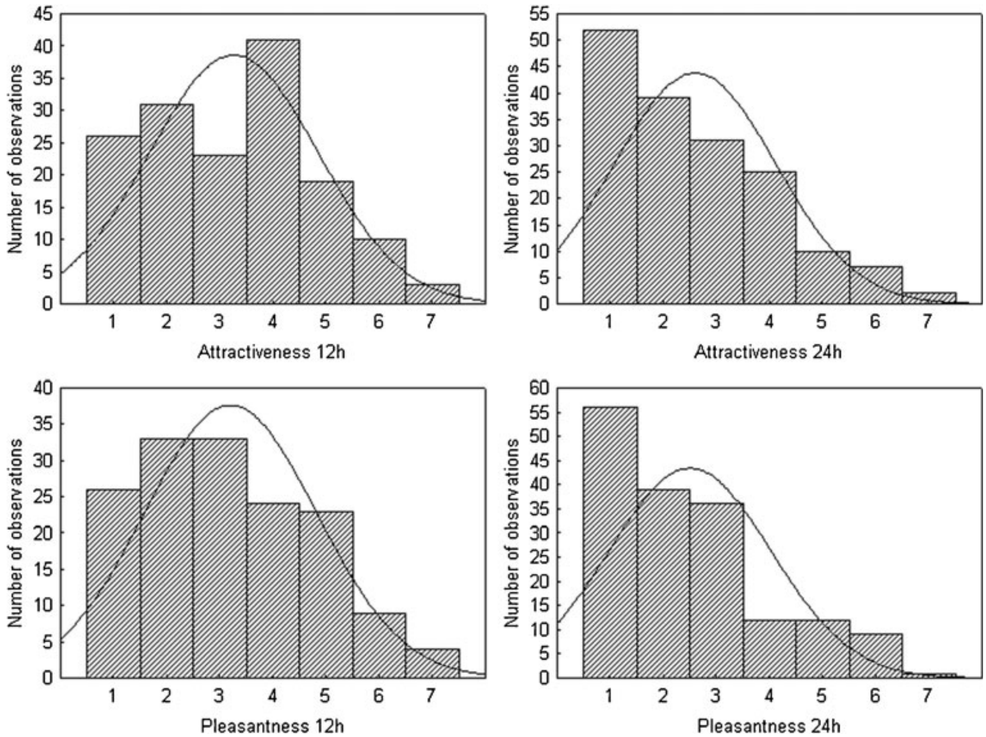

Fig. 2 Histograms of individual ratings of attractiveness (*above*) and pleasantness (*below*) in 12- (*left*) and 24-h (*right*) sampling conditions

kin or emotion) while others rely on hedonic ratings (e.g., attractiveness). There does not seem to be any rationale behind this highly variable pattern apart from the fact that many scholars simply repeated the procedure employed by the Wedekind et al. (1995) highly influential study.

Our analysis of data distribution found an approximately normal distribution in all variables when mean values of individual raters were used but significant deviation from normality when we used individual ratings. This should not be surprising because the variability is strongly reduced by

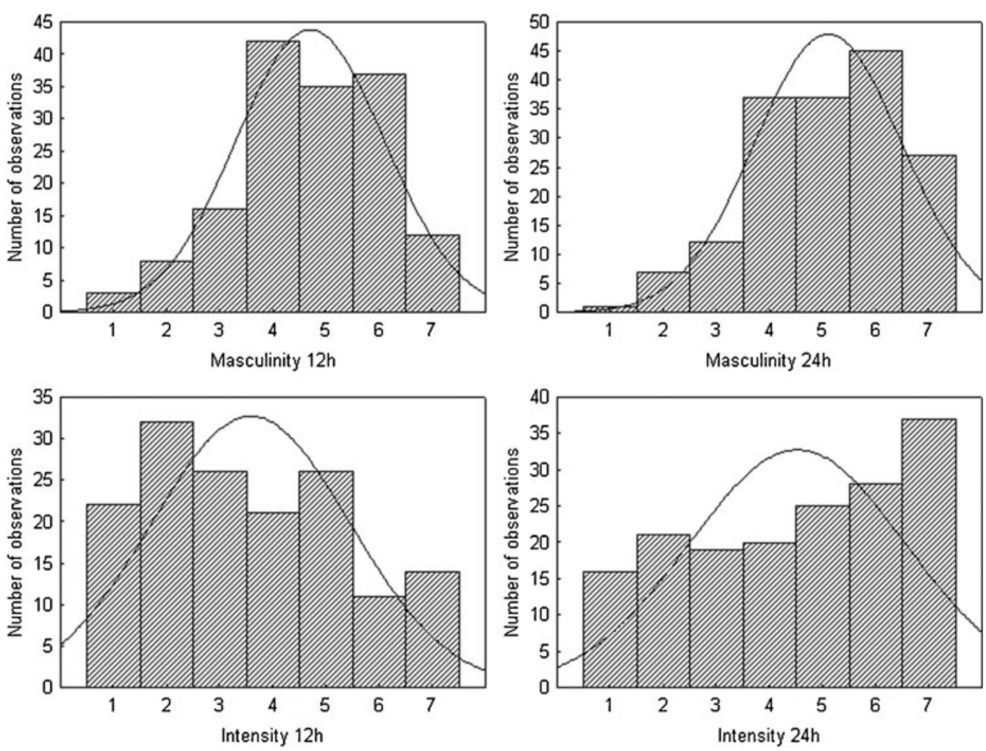

Fig. 3 Histograms of individual ratings of masculinity (*above*) and intensity (*below*) in 12-h (*left*) and 24-h (*right*) sampling conditions

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Table 2 Descriptive statistics including values of skewness of individual variables in 12- and 24-h conditions

Variable	*N*	Mean	Min	Max	SD	Skewness	SE Skewness
Pleasantness 12 h	25	3.103	1.000	5.286	1.019	0.073	0.464
Pleasantness 24 h	25	2.478	1.143	5.286	1.006	1.089	0.464
Attractiveness 12 h	25	3.168	1.000	5.143	1.030	0.006	0.464
Attractiveness 24 h	25	2.562	1.000	4.429	0.926	0.373	0.464
Masculinity 12 h	25	4.623	3.000	6.200	0.831	−0.127	0.464
Masculinity 24 h	25	5.087	3.571	6.286	0.780	−0.165	0.464
Intensity 12 h	25	3.566	1.286	6.600	1.185	0.437	0.464
Intensity 24 h	25	4.527	1.143	6.800	1.247	−0.789	0.464

computing a composite score. Furthermore, our seven-point scale is only a rough approximation to a variable which in reality is expected to be normally distributed. We think that simple visual inspection of the data distribution has much higher informative value in this respect. It suggests higher skewness of the hedonic ratings in the 24-h condition, with low values being over-represented. This may thus result in a floor effect, that is, a situation where a majority of participants return the lowest possible values (here, very unpleasant/unattractive), and the data do not reflect true underlying variability. This can in turn obscure the probability of finding the effect tested by a particular study and thus increases the chance of type II error. The lower hedonic ratings in the 24-h condition can be attributed to the activity of bacterial microflora which converts fresh and odorless apocrine secretion into odorous breakdown products. It is currently not clear whether perceptible differences are due to the amount of chemicals produced (generally higher concentrations are perceived as less pleasant) or by subsequent changes in chemical composition. This can be a consequence of further bacterial activity on some of the chemical compounds or of spontaneous oxidative processes, or both. Also, we did not extract cotton oils from the sterilized cotton pads before sampling. It is thus possible that contamination of odor samples by bacterial breakdown product of the cotton oils has occurred. However, in both conditions, we found no significant differences between ratings performed early and later in the session. This suggests that the differences in the two conditions might be due to changes in chemical composition of the axillary compounds occurring and prevailing during the collection period. The stability of the ratings after the samples are obtained from the body is supported by the study of Singh and Bronstad (2001) who let their raters re-evaluate the odor samples (collected over the menstrual cycle) which were left for a week at room temperature, producing similar results as during the original rating session.

On the other hand, the use of the 12-h sampling period may result in a higher number of samples which are too weak to be perceived. Indeed, samples from the shorter sampling period accounted for the majority of below-threshold ratings (23 out of 31 non-detectable samples). This accounts for 13.1% and 4.6% of all samples in the 12- and 24-h sampling periods, respectively. It should be noted, however, that our results are based on adult male axillary odors collected on cotton pads; collection of odor samples from women or prepubertal children who on average have weaker axillary odors (e.g., Chen and Haviland-Jones 1999) could require a different time period. Similarly, odors collected under some specific conditions (e.g., anxiety (Albrecht et al. 2011) or extensive physical activity (Prehn et al. 2006)) could be sampled over a shorter time as they are typically more intense. Furthermore, cotton pads attached directly to the armpit would in general require a shorter collection time as compared with the T-shirt paradigm where contact with the armpit is relatively loose.

Table 3 Correlation coefficients between rated variables in 12 and 24 h sampling conditions

Sampling conditions	12 h			
	Pleasantness	Attractiveness	Masculinity	Intensity
24 h				
Pleasantness		0.91 (152)	−0.40 (152)	−0.55 (151)
Attractiveness	0.84 (165)		−0.35 (153)	−0.54 (152)
Masculinity	−0.38 (165)	−0.28 (166)		0.44 (152)
Intensity	−0.63 (165)	−0.55 (166)	0.47 (166)	

Values in brackets show number of ratings. All correlations are significant at $P<0.001$. Individual ratings served as unit of the analysis

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This, in summary, suggests that one may not expect one ideal length of sampling useful across a broad range of odor studies. However, the optimal sampling length under different paradigms should be carefully considered (e.g., by performing a pilot study).

Conclusions

The results of our study suggest that the length of sampling affects hedonic ratings of odor samples. Specifically, samples collected over 12 h were rated as more pleasant and attractive than those collected over 24 h and were judged as less masculine and intense. Further inspection of the data distribution indicates over-representation of low values in pleasantness ratings of 24-h samples. Our results demonstrate that the precise methodology employed in future odor studies should take account of a trade-off between the shape of the distribution of odor ratings and the proportion of samples that are too weak to be detected. In our view, the significant advantage of avoiding floor/ceiling effects in the dataset overrides the disadvantage of a relatively small number of samples falling below some raters' detection thresholds.

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