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Possible influence of breathing on detection frequency and intensity rating in gas chromatography-olfactometry

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Abstract

Gas chromatography-olfactometry (GCO) is a useful method to screen potent odorants in food aromas. In this technique, the human olfactory sense is used to detect the odor-active compounds eluted from a gas chromatograph. A particularity of GCO, compared to other sensory analyses, is to combine two discontinuous phenomena:the aperiodic and unpredictable elution of odorous compounds from the chromatographic column and the breathing process. We wanted to see whether absence of detection could be attributed to expirations and whether odor intensity rating was influenced by the relative temporal positions of compound elutions and inspiration periods. A tendency for higher odor detection frequency by fast-breathing subjects was observed in the three experiments. It appeared that a subject who was asked to breath faster rated odors more intensively, suggesting a possible individual influence of breathing. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Gas chromatography-olfactometry; Breathing; Odor detection; Intensity

1. Introduction

Gas chromatography-olfactometry (GCO) uses the human nose as a detector for the odorous volatile compounds eluted from a gas chromatograph (GC). As reviewed by Blank (1997), "GC-sniffing" is a common technique used for the screening of potent odorants in food aroma extracts. When combined with molecular identification, it allows the determination of the key compounds responsible for the flavors and off-flavors of food.

Different techniques have been developed to assess the odorant potency of each compound detected by the sniffers. This potency can be estimated by the extract dilution factor (Acree, Barnard & Cunningham, 1984; Ullrich & Grosch, 1987), by the odor detection frequency (Pollien, Ott, Montigon, Baumgartner, Munoz-Box & Chaintreau, 1997) or by a time-intensity evaluation (Etiévant, Callement, Langlois, Issanchou & Coquibus, 1999; Miranda-Lopez, Libbey, Watson & MacDaniel, 1992). However, in each method, most individual results are poorly repeatable and this variability is not simple to understand. Using a dilution technique, Abbott, Etiévant, Issanchou and Langlois (1993) reveal gaps in the coincident responses of four out of six panelists: for a series of dilutions, a sniffer may not detect an odor at a certain retention index but then may detect an odor at this same retention index at higher dilutions. Guichard, Guichard, Langlois, Issanchou and Abbott (1995) report coefficients of variation up to 109% for an intensity measurement with three replicates. Many human parameters may be responsible for the variable response in sensory analysis. In GC-sniffing techniques, particular factors must be considered because the odorants are only presented for a few seconds, contrary to other sensory experiments where the panelist can

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evaluate a product aroma during a longer period. For instance, vigilance, adaptation (Kleykers & Schifferstein, 1995) and breathing (Acree, 1997; Pollien et al., 1997) must be considered in GC-sniffing, in addition to other general parameters such as training and individual sensitivity.

This paper focuses on the influence of breathing on GCO quantitative results, i.e. detection frequency and intensity measurement. Although this factor has been cited by some authors, no experiment to understand and to measure its influence has yet been published. The problem in GC-sniffing is to combine two discontinuous phenomena: the aperiodic and unpredictable elution of odorous compounds from the chromatographic column and the breathing process in which a time of no perception (expiration) alternates with a time of possible perception (inspiration) with a rhythm unique for each subject but not totally stable. The odor intensity evaluation by GCO should not be a problem, as justified by Da Silva, Lundhal and MacDaniel (1994) because a single sniff of 0.4 s on average (shorter than a normal inspiration) is sufficient to detect and evaluate intensity of a single odor (Laing, 1983) as compared to the longer elution duration of a compound from a GC column (for example, it can vary from 3 to 25 s for peaks from a beer aroma extract detected by a flame ionisation detector). However, one must not forget that the normal expiration duration [2.46 s on average, with a normal inspiration of 1.54 s, according to Hermann and Cier (1969)] is large enough to miss a compound elution.

This observation leads to the first question: are the missed responses due to synchronized expirations and compound elutions? In other words, do subjects naturally breathing faster miss fewer odorants than slow breathing subjects? Another particularity of working with a GC, as an olfactometer, is that the elution rate of each volatile compound follows a rough gaussian curve, which means that, in the middle of the elution, the compound concentration is higher than at the beginning or at the end of the elution time. This fact leads to a second question which is: is the variability in intensity response due to the relative position of inspiration compared to the gaussian distribution of concentration delivery with time? These questions are important for several reasons. Firstly, if it is possible to understand why an odorous compound is detected only occasionally, we should keep sporadic data even if the detection frequency is less than 50%, contrary to the reject criteria of Miranda-Lopez et al. (1992). Secondly, if subjects who have faster breathing rates miss fewer compounds, this criterion should be included in the panel selection process. These questions are now studied through three complementary experiments conducted with a GCO apparatus coupled with a "breathing recorder" that was elaborated in our laboratories.

2. Materials and methods

2.1. Development of a "breathing recorder" to measure breathing events during GCO sessions

Some medical applications, in oto-rhino-laryngologia and pneumologia, use apparatus that allow the measurement of temporal and volumic parameters of the breathing process (e.g. apparatus used in rhinomanometry or in objective olfactometry). However, none of these could be applied to our problem because it would have interfered with the sniffing of odorants. Although the air temperature sensor was not a cumbersome solution, it is too sensitive to temperature variations in the room. Finally, the piezo-electric relative pressure sensor appeared to be convenient. The breathing recorder consists of a stainless steel tubing (0.5 mm i.d.) placed in the glass cone sniffing port and connected to the pressure sensor. The difference between atmospheric pressure and the pressure in front of a breathing subject's nostril produces resistance changes in the sensor which are converted to an analog signal. In the first prototype (used in experiment 1 only), this signal was displayed on a X–Y plotter. In the definitive model, the analog signal was converted to a digital signal by an analog to digital converter board. This signal was recorded simultanuously with the chromatographic signal given by the flame ionisation detector (FID) and the olfactometric signal (odor detection times and intensities indicated by the sniffers). After processing, all three signals were displayed and saved in computer files. Our system recorded inspiration and expiration times, but not breathing amplitude. Signals were processed by programs written with Matlab 5.2 (Scientific Software, Sèvres, France) in experiment 2 and by the HP ChemStation A 06.01 software for GC (Hewlett Packard, Les Ulis, France) in experiment 3.

2.2. Presentation of the experiments

Three studies with three different panels were conducted. The first two experiments, conducted at ENSIA laboratory in Massy, studied both breathing influence and another important parameter in GC-sniffing: the influence of the air make-up rate and hygrometry in the sniffing port (Hanaoka, Sieffermann & Giampaoli, 2000). In this laboratory, odor intensity was indicated by the subject on a computer screen scale by means of a computer mouse. The third experiment was conducted at TEPRAL in Strasbourg, where the sensory data were obtained by finger-span cross-modality matching. In each experiment, except the first, the subject breathing characteristics are means calculated on 10 to 90 measures of expiration and inspiration duration or number of respirations per min, during time periods of different sessions where no odor was detected. The theoretical

quantity for each compound was determined in the carrier gas after the split between the sniffing port and the FID. The compounds used in the studies were chosen among those used by Etiévant et al. (1999) for their sniffers' training and evaluation. The sniffing port was a glass cone in all experiments.

2.2.1. Experiment 1

The panel consisted of four subjects, trained during two sessions, in order to have a short familiarization with the apparatus. They were two men (SB and AN) and two women (SH and MM) from the ENSIA laboratory, aged 22 to 29, with breathing rates from 7.3 to 22.5 respirations.min^{-1} (Fig. 1). They evaluated, during 12 sessions, the intensity of five compounds mixed in ethanol: 3-methyl-1-butanethiol, 0.16 g. l^{-1}(9 ng eluted in the sniffing port); benzaldehyde, 14.2 g.l^{-1} (852 ng); guaiacol, 4.5 g.l^{-1} (271 ng); 2-phenylethanol, 10.5 g.l^{-1} (628 ng) and vanillin, 5.9 g.l^{-1} (355 ng). One μl of the solution was injected in a split–splitless injector (250°C, split ratio 1:7.3). The five compounds were eluted within 15 min in the order cited above, after separation in an HP-5 capillary column (Hewlett Packard;24 m × 0.32 mm; 0.52 μm thickness). The carrier gas was helium at 1.2 ml min^{-1}, and was equally divided between the flame ionisation detector (FID) and the sniffing port. The oven temperature program of the GC (HP 5890 serie II) was: 4°C. min^{-1} from 120 to 180°C and 20 min at 180°C. Breathing was not recorded during these sessions but during an additional 6 min session where the same subjects evaluated the intensities of four compounds (those presented above, except vanillin). The breathing parameters measured in this additional session were compared to the number of odors detected during the 12 preceding analyses.

2.2.2. Experiment 2

Six women working at ENSIA who did not participate in the first experiment formed the second panel. They were 23 to 42 years old and had breathing rates from 11.8 to 30.8 respirations. min $^{-1}$ (Fig. 2). After a longer familiarization with the GCO task with training sessions of gradual difficulty, subjects had to rate intensities of 10 compounds presented 30 times each. In order to avoid memory effects, each compound was contained in five solutions out of six, as described in Hanaoka et al. in press. Each solution contained seven to nine compounds diluted in dichloromethane. The compounds were: 3-methyl-1-butanethiol, 0.1 g.l^{-1} (14 ng) eluted in the sniffing port), 2-hexanone, 15 g.l^{-1} (1901 ng), octanal, 4.9 g.l^{-1} (628 ng), nonanal, 1 g.l^{-1} (127 ng), furfural, 14.7 g.l^{-} (869 ng), citronellal, 4.7 g.l^{-1} (596 ng), benzaldehyde, 9.9 g.l^{-1} (1256 ng), 1-octanol, 9.8 g.l^{-1} (1241 ng), 2-phenylethyl acetate, 4.9 g.l^{-1} (621 ng) and guaiacol, 0.5 g.l^{-1} (65 ng). One μl of each solution was injected in a split–splitless injector (250°C, split ratio 1:2.9). The 10 compounds were eluted within 20 min in the order cited above, after separation in a DB-WAX capillary column (J.W. Scientific Inc.; 30 m × 0.32 mm; 0.5 μm thickness). The carrier gas was helium at 3.8 ml min^{-1}, and was equally divided between the FID and the sniffing port. The oven temperature program of the GC (HP 5890 serie II) was: 6 °C. min^{-1} from 70 to 140°C, and then 30°C. min^{-1} from 140 to 200°C, and 7 min at 200°C. Breathing was recorded during each analysis.

2.2.3. Experiment 3

In this study, a single trained sniffer breathed with two different rates: 9.2 and 22.2 respirations C.min^{-1} (Fig. 3) and evaluated six ethyl butyrate concentrations in 12 replicates for each breathing rate. Thus, in this experiment, the differential sensitivity of one subject to different compounds or of different subjects to one compound was avoided. The six concentrations in ethanol were chosen in order to present theoretical sensory intensities with a geometrical progression, using Stevens' coefficient, $n = 0.35$ (Etiévant et al., 1999). The concentrations for the six solutions were: solution C1, 13.3 g.l^{-1} (1112 ng eluted in the sniffing port), solution C2, 7.9 g.l^{-1} (656 ng), solution C3, 4.1 g.l^{-1} (342 ng), solution C4, 1.8 g.l^{-1} (147 ng), solution C5, 0.5 g.l^{-1} (44 ng), solution C6, 0.07 g.l^{-1} (6 ng). One μl of each solution was injected in a Programed Temperature Vaporizer

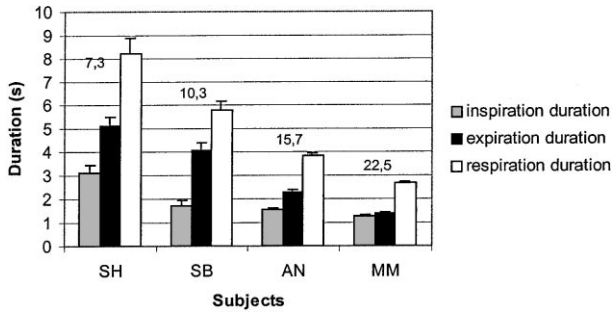

Fig. 1. Breathing features of experiment 1 panel. Bars: confidence interval around mean at level 95%. Number: breathing rate (number of respirations/min).

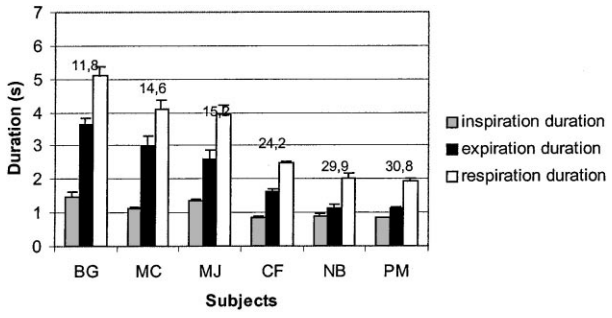

Fig. 2. Breathing features of experiment 2 panel. Bars: confidence interval around mean at level 95%. Number: breathing rate (number of respirations/min).

(PTV) used in a fixed temperature mode (200 °C, split ratio 1:5). Separation was performed in a DB-WAX capillary column (J.W. Scientific Inc.; 30 m × 0.32 mm; 0.5 μm thickness). The carrier gas was helium at 2.7 ml. min^{-1}, and was equally divided between the FID and the sniffing port. The oven temperature of the GC (HP 6890) was fixed at 60°C. All six solutions were injected in series always beginning with C1, followed by the other solutions in random injections every 1.5 min. The subject alternated a series with slow breathing and a series with fast breathing. As he could not breath fast during the whole analysis, he breathed more rapidly 20 s before each odor presentation until 20 s after (he had indication of times to begin and to stop).

3. Results and discussion

3.1. Influence of breathing parameters on odor detection

In each experiment where breathing was recorded, we have searched whether the missed detections corresponded to a case where the subject was breathing out. During the 6 min analysis of experiment 1, one of the four subjects missed an odor. It appeared that it was an odor usually detected by this sniffer, and that the compound was almost totally eluted during an expiration. This event encouraged us to study this phenomenon with more analyses and more subjects. In experiment 2, a precise breathing observation was conducted on 1/6 of the measures, which corresponded to 270 measures, i.e. nine compounds evaluated five times by six subjects (data about furfural were dropped because they were rare and unconfirmed with odor description). In these data, it appeared that 19 elutions of compounds were not detected by sniffers. Among these absences of detection (“null values”), only five took place when more than 85% of the FID peak area corresponded to an expiration period. In the same way, only five null values among 16 corresponded to the same case in experiment 3. Therefore, we cannot completely explain the null values by the expiration process. So, we have investigated the same problem indirectly by studying the odor detection frequency. The breathing process can be modelled by a binary system in which inspiration represents a possible odor detection and expiration an impossible detection. Supposedly, if the inspiration occurrence is more frequent, the probability of detection is also higher. That is why we compared the panelists' breathing rate to the number of odors detected. In experiment 1, the two sniffers who breathed the fastest missed fewer odors than the two other sniffers (Fig. 4). The detections were calculated without including 2-phenylethanol because two subjects never detected it (their sensitivity towards this compound was too low). A statistical analysis of experiment 2 data shows that the breathing rates of the six panelists are also negatively correlated with the number of missed odor peaks (Fig. 5, $R^2 = 0.58$ and $P = 0.08$). The third experiment was partly planned to confirm the relationship between breathing rate and odor detection with a single subject adopting different respiration rythms. It appeared that ethyl butyrate, the stimulant used here, was more often detected when the sniffer was asked to accelerate his breathing (Fig. 6), thus confirming the phenomenon observed in experiments 1 and 2, i.e. a faster breathing rate is correlated to a lower number of missed odorants. However, the subject attention was probably increased during the fast breathing periods in the third experiment. This could also explain a higher detection

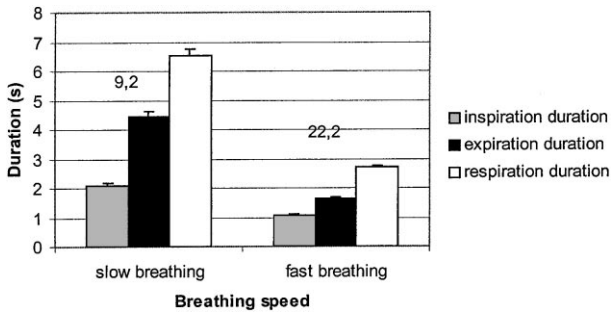

Fig. 3. Breathing features of experiment 3 panel. Bars: confidence interval around mean at level 95%. Number: breathing rate (number of respirations/min).

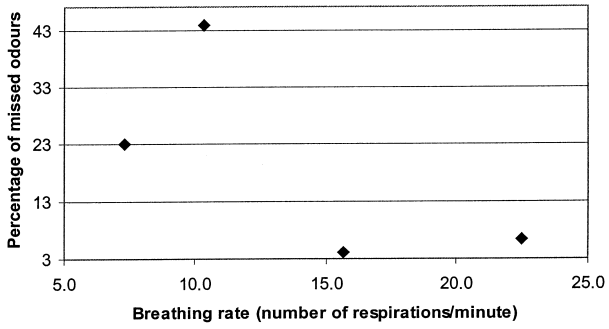

Fig. 4. Panel 1 breathing rates and absence of odor detections.

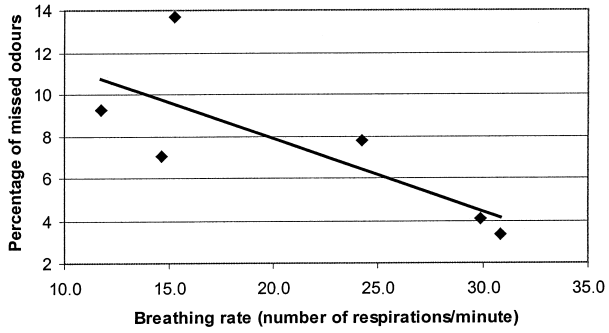

Fig. 5. Panel 2 breathing rates and absence of odor detections. Straight line: linear regression line ($R^2 = 0.58$; $P = 0.08$).

frequency of the odor. Evidently, breathing rate can partly explain the gap in odor detection in these three studies, the remaining gap being explained by the different sensitivities or attention of the panelists.

Some compounds in experiments 1 and 2 were always detected. The compounds that were sometimes not detected had the lowest average intensities (means calculated without the null values) but did not have the shortest elution durations. Fig. 7a–c compare the detection frequencies, the intensities and the FID peak widths (elution durations) in experiment 2. In GC, the peak width is correlated with the concentration. This simply means that the frequency of detection depends on the olfactory detection threshold of the compound. The panelist's breathing rate may then influence the frequency of detection for compounds near their olfactory detection threshold.

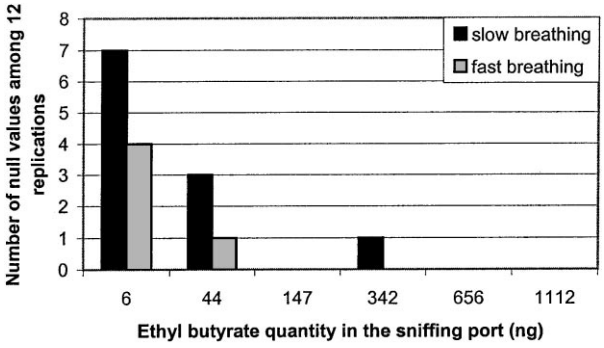

Fig. 6. Number of missed ethyl butyrate peaks during experiment 3.

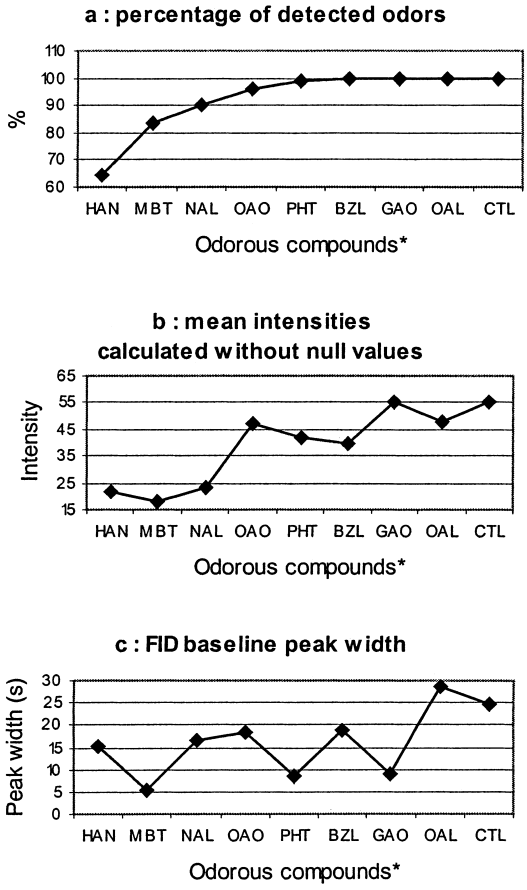

Fig. 7. Comparison of the percentage of odors detected (a), mean intensities (b) and FID peak widths (c) in experiment 2. HAN: 2-hexanone, MBT: 3-methyl-1-butanethiol, NAL: nonanal, OAO: 1-octanol, PHT: 2-phenylethyl acetate, BZL: benzaldehyde, GAO: guaiacol, OAL: octanal, CTL: citronellal.

3.2. Influence of breathing parameters on odor intensity measurement

The second possible influence of breathing in GCO responses is on the intensity perception and rating. In a chromatogram, the peak area is proportional to the concentration of the corresponding compound. But if we superimpose on the same time scale a chromatogram and a breathing profile, we can see that the correspondance of FID peaks and inspiration times is very random (Fig. 8). Assuming that the elution time of flavor-active compounds is the same at the FID and at the sniffing outlets, partial FID peak areas were calculated between the beginning and end of inspirations (Fig. 8). We tested whether these partial areas could more accurately predict the intensity noted by a subject, supposing that the "efficient" odorant gas volume is the one eluted during an inspiration (or a sniff). Partial FID areas were therefore measured for 270 FID peaks in experiment 2, thanks to the manual integrating procedure of HP ChemStation. We gave particular attention to the greatest partial area which was thought to correspond to the maximum perceived intensity during elution. Intensity ratings were systematically centered for each subject in order to avoid the bias due to a different use of the scale among the panelists. These centered intensities, or their log value (as referred to Stevens' law),

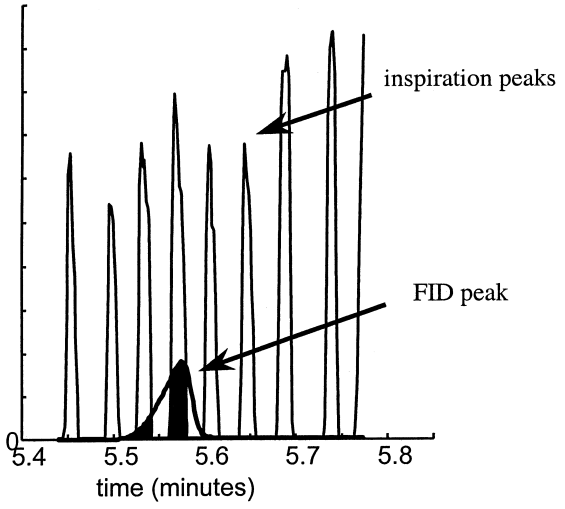

Fig. 8. Flame ionisation detector (FID) and breathing signals superimposition. Darkened areas: partial FID peak areas.

were then compared to different partial peak areas (or area log values) calculated for each compound:maximum partial area, sum of the peak partial areas, mean partial area per peak. Similar regressions were also investigated with raw (uncentered) values, subject by subject, for each compound. In all cases, no correlation could be obtained. One reason could be that the intensity variations were not sufficient (as each compound was always injected at the same concentration) or that subjects' sensitivities were too different to compare their results. In order to avoid this complexity, the intensity of a single compound was evaluated by a unique subject at six different concentrations in experiment 3. The analysis of these data by a General Linear Model Procedure (SAS 6.12 analysis of variance, SAS Institute Inc., Cary, NC, USA) showed, on the intensity ratings, a positive significant effect of concentration ($P=0.0001$), of breathing rate ($P=0.001$), and an interaction between concentration and breathing rate ($P=0.04$), but no replication effect ($P=0.13$). Fig. 9 illustrates these effects: intensity means were higher when the subject breathed faster for solutions C2 (1112 ng), C3 (342 ng), C4 (147 ng) and C5 (44 ng), and these differences are significant at the 5% level with the Student test for concentrations C2, C4 and C5. Intensities are not different with C1 because the subject knew that it was the maximum intensity reference always injected first. As for C6, the lowest concentration, the number of non-detections was certainly too high to obtain significant results in intensity, even if the number of null values was higher with the slow breathing rate (see Fig. 6). During the fast breathing periods, the partial FID areas were smaller since inspirations were shorter. Whereas this should have led to lower intensity ratings, the contrary was observed. Other interesting results were obtained from experiment 3. First, the Newman–Keuls test following the global analysis of variance revealed that the sniffer brought the six concentrations together into 4 groups when breathing slowly, and into 5 groups when breathing quickly, which means that he better discriminated intensities when breathing quickly. Secondly, intensity rating was more repeatable when breathing rapidly, as revealed by the variation coeffi-

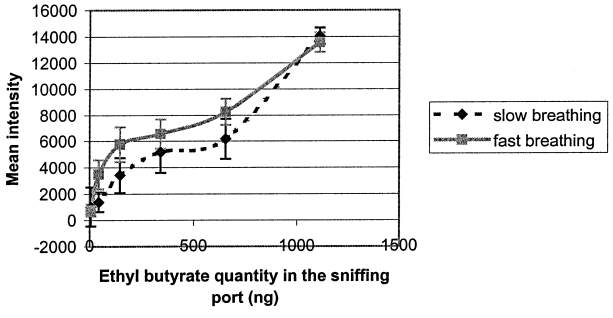

Fig. 9. Intensity rating during fast or slow breathing in experiment 3. Bars:confidence interval around mean at level 95%.

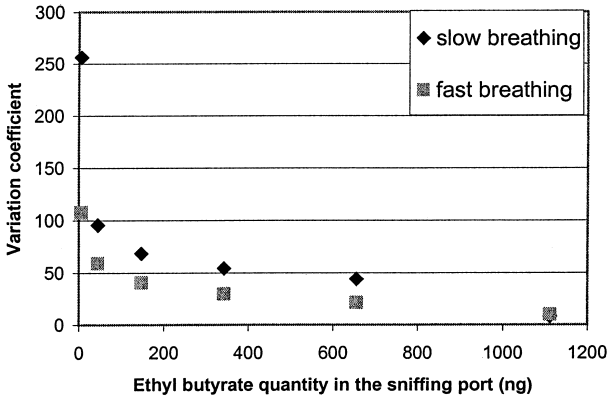

Fig. 10. Variation coefficients (S.D. $\times 100$/mean) of intensity rating for slow or fast breathing in experiment 3.

cients (S.D. $\times$ 100/mean) calculated for each concentration in both cases (Fig. 10). Experiment 3 shows, therefore, that a trained sniffer perceived higher intensities and evaluated different intensities of the same stimulus more precisely (smaller variation coefficients and better discrimination) with a fast breathing rate in GCO. However, the attention factor certainly influenced these results positively because the wait for the odor during fast breathing periods may have increased the subject concentration.

4. Conclusion

The questions raised in the introduction gave partial answers that should be confirmed and completed by further experiments. We first showed that a null value (no detection) was rarely explained by an expiration, assuming that the maximum value for each gaussian distribution of concentrations appeared simultaneously at both outlets (FID and sniffing port) in our experiments. However, the three experiments indicate that subjects breathing more rapidly detected odors more often. No relationship was found between partial FID peak areas and intensities. Two reasons for this could be proposed: our initial hypothesis on the elution pattern at the sniffing and FID outlets may be false, and other human parameters must be taken into account to understand intensity rating in GCO. The gaussian peak at the sniffing port is probably drastically altered between the capillary outlet and the nostril because the conic shape of the sniffing port certainly changes the gas flow characteristics and an additional air flow was used to enhance molecule transportation into the nostrils. However, the third experiment showed that it was possible to enhance the perceived intensity of an odor, to have a better discrimination of different intensities and to rate intensity with more repeatability, by changing a subject's behaviour. The question is therefore:which change in the subject's behaviour of experiment 3 most

influenced the GCO results, breathing rate or attention? If individual sensitivity and vigilance are carefully controlled, it would be interesting to complete studies on the influence of breathing by considering other parameters such as the ratio of expiration and respiration durations, and the occurrence of sniffing episodes (more frequent breathing) during analyses. We actually observed with a panel, not presented in this paper, that the expiration/respiration ratio for some subjects can be far from the literature mean value of 8/13 (Hermann & Cier, 1969). It would be interesting to compare results from subjects with different sniffing frequencies or compare the sniffing frequencies of one subject between the first analyses and the last ones to see whether the breathing behaviour may change with training. A change in the respiratory air flow rate during sniffing episodes could also explain that our FID partial areas alone did not explain the intensity fluctuations. Further research to build a breathing recorder that allows respiratory air flow rate measurement during GCO analysis could be useful.

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