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2-Heptanone Produces Sensorial-Emotional Changes, Depending on Length of Exposure

La 2-heptanona produce cambios sensorio-emocionales, dependiendo del tiempo de exposición

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Abstract

2-Heptanone is an alarm pheromone contained in some human fluids, but its role is unknown in chemical communication. In part one of this study, a sample of 24 women provided urine specimens taken around their supposed ovulation days, and a second sample 12 days later. As exclusion criteria, women with anxiety (based on the State-Trait Anxiety Inventory, Spielberger), mood disorders (based on the Clinical Diagnosis of Depression Questionnaire), and premenstrual dysphoric disorder (based on the Daily Symptoms Report) were not included in the study. Gas chromatography/mass spectrometry indicated that urinary 2-heptanone content was increased approximately two-fold during pre-menstruation compared with the days around ovulation. In part two of this study, 141 male and female volunteers, sniffed this ketone and with a simple questionnaire it was determined that the longest tested duration (180 s) of sniffing 2-heptanone lowered the acceptance of sniffing this ketone again, compared with the shorter sniffing durations (5 and 60 s), with no differences between sexes. The increased concentration of 2-heptanone during the day before menstruation may be considered as part of the functional changes preceding menstruation and sniffing this ketone may produce sensorial-emotional changes depending on time of sniffing, the significance of this deserves further study.

Keywords: 2-Heptanone, Chemosignals, Menstrual cycle, Pheromones, Olfactory system

Resumen

La 2-heptanona es una feromona de alarma detectable en algunos fluidos humanos, pero se desconoce su papel en la comunicación química. En la primer parte del estudio, 24 mujeres jóvenes y sanas, proveyeron una primera muestra de orina alrededor del día de ovulación y una segunda 12 días más tarde. Como criterio de exclusión, se descartaron mujeres con ansiedad (Inventario de Ansiedad Estado-Rasgo, Spielberger), trastornos del estado de ánimo (Cuestionario de diagnóstico clínico de depresión) y trastorno disfórico premenstrual (basado en los síntomas diarios de informe). La cromatografía de gases/espectrometría de masas indicó que el contenido urinario de 2-heptanona aumentó aproximadamente al doble antes de la menstruación en comparación con los días cercanos a la ovulación. En la segunda parte del estudio, otros 141 voluntarios (femeninos y masculinos) inhalaron esta cetona y con ello se determinó que con el tiempo más prolongado (180 s) de exposición por olfateo a la 2-heptanona disminuyó la aceptación para olerla nuevamente, en comparación con los tiempos más cortos de olfateo (5 y 60 s). Lo anterior se evidenció mediante las respuestas a un cuestionario simple. No hubo diferencias significativas por género. El aumento de la concentración urinaria de 2-heptanona, durante el día antes de la menstruación, se consideraría como parte de los cambios funcionales premenstruales y el hecho de oler esta cetona produciría cambios sensorial-emocionales dependiendo del tiempo de exposición, cuyo significado requiere mayor estudio.

Palabras clave: 2-heptanona, quimioseñales, ciclo menstrual, feromonas, sistema olfatorio

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Attractiveness during the menstrual cycle depends on estradiol and progesterone levels (Puts et al., 2013). Males perceive (Singh & Bronstad, 2001) and distinguish (Gildersleeve, Haselton, Larson & Pillsworth, 2012) women´s scents in the follicular phase as more pleasant compared with the luteal phase. Women and men usually prefer women´s scents in the fertile-phases to their scents in other phases of the menstrual cycle (Woodward, Thompson & Gangestad, 2015). Men exposed to the scent of an ovulating woman display higher levels of testosterone than when exposed to the scent of a non-ovulating woman, which may be related with the beginning of a romantic courtship (Miller & Maner, 2010).

Compared with the luteal phase, the amygdala is activated during the follicular phase, providing women with greater social sensitivity and seemingly facilitating social interaction (Derntl et al., 2008b). Accuracy in facial emotion recognition, a prerequisite for successful social interaction, also improves (Derntl, Kryspin-Exner, Fernbach, Moser & Habel, 2008a); and the luteal phase relates with a decreased identification of negative emotions, as angry or sad (Guapo et al., 2009). Through hormonal control, mating is facilitated during the follicular phase, and this situation shifts during the luteal phase, involving emotional processing. However, it is unknown whether the existence of additional cues, such as some odoriferous substances, may indicate a period of infertility.

In rodents, the emission of olfactory signals is able to modify behavior when perceived by another member of the same species. The active compounds are largely volatile substances that tend to bind to proteins excreted in the urine (Novotny, 2003), providing information about age (Osada et al., 2003), reproductive and sexual status (Achiraman et al., 2011), and stressful situations (Gutiérrez-García et al., 2006). Two ketones released in dominant rats, 2-heptanone and 6-methyl-5-hepten-2-one, generate psychosocial stress when perceived by subordinate rats (Pohorecky et al., 2008). In rodents, 2-heptanone is considered an alarm pheromone. It is present in rodent urine, and its content increases in rats that are subjected to unavoidable stress, while the smell of urine that comes from preputial gland-stressed rats produces anxiety-like behavior in the short term. In the long-term, it can produce despair in otherwise intact rats (Gutiérrez-García, Contreras, Mendoza-López, García-Barradas & Cruz-Sánchez, 2007).

These observations suggest that duration of exposure may influence emotional reactions produced by contact with 2-heptanone, while the role played by 2-heptanone may depend on gonadal functions. Both possibilities require further exploration.

Human skin emits a wide variety of volatile substances as a primary source of body odor (Gallagher et al., 2008). Approximately 1,840 volatile organic compounds have been identified in healthy individuals. Among these, 2-heptanone has been found in feces, urine, breath, and saliva; but not skin (Lacy Costello et al., 2014). Whether the release of this alarm substance is linked to the menstrual cycle and whether the smell of 2-heptanone produces time-dependent emotional sensations in humans, are unknown.

This study consists of two complementary designs: The first, aimed to determine changes in urine 2-heptanone depending on menstrual cycle phases in healthy young volunteers. The second, aimed to explore if inhalation of 2-heptanone by healthy volunteers produces any emotional reactions.

Method

This study was conducted according to the Helsinki Declaration, and was authorized by the Psychology School´s Ethical Committee (Universidad Veracruzana, Mexico). All participants gave written informed consent for sample collection and application of the tests.

Part 1: Measure of 2-heptanone

Participants

Twenty-four healthy women participated in this part of the study. They were 23-33 years old (mean 21.3 ± 0.63 years). An interview was conducted to exclude from the study women who suffered from any general systemic disease, premenstrual dysphoric disorder, or mood disorders, women who were taking hormonal or psychotropic medication, and women who used alcohol or drugs. At the time of invitation, volunteers were asked to complete a brief interview directed to meet criteria for study inclusion, and to determinate the regularity of their past three menstrual periods. Only volunteers with a regular menstrual cycle were included. Other exclusion criteria included pregnancy and their own decision not to enter the study.

All of the participants were instructed to measure their axillary temperature daily immediately after awakening during two consecutive menstruation cycles and keep the corresponding record. A temperature increase of 0,5-1,0ºC in approximately the middle of the menstrual cycle was considered the day of ovulation. From this date onward, the next menstruation was calculated as occurring approximately 14 days later. In the second recorded cycle, women were asked to complete daily the Daily Symptoms Report (DSR, Freeman, De Rubeis & Rickels, 1996). On these days, when volunteers detected an increased axillary temperature, they were instructed to collect a 10 ml sample of their first urine of the day, immediately after awakening. A second urine sample was collected approximately 12 days later. Volunteers agreed to deliver the urine samples to the principal researcher within three hours after collection and where they were immediately stored at -20ºC. On the same day of urine collection, they were asked to attend an interview (8:00 to 10:00 A.M.). In these two sessions, test scores were obtained on the two scales of the State-Trait Anxiety Inventory (STAI-S [for state anxiety] and STAI-T [for trait anxiety] (Spielberg & Díaz-Guerrero, 1975) and for depression on the Clinical Diagnosis Questionnaire (CDQ, Calderón Narvaez, 1992).

This part of the study was designed to explore changes in the urinary content of 2-heptanone related to the menstrual cycle. The volunteer´s health in this part of the study was confirmed by diverse instruments: Scores on the DSR did not reach the criterion level for premenstrual tension. This instrument has been validated and is currently used in clinical trials. The investigators used a version of the STAI that was validated for the Mexican population (Spielberg & Díaz-Guerrero, 1975). The scores on the STAI did not reach the criterion level for state anxiety. Lastly, the Depressive Syndrome version of the CDQ has also been validated in México (Torres-Castillo, Hernández & Ortega-Soto, 1991) with acceptable sensitivity and specificity (Jurado et al., 1998). The scores on the CDQ did not reach the criterion level for depression. None of the applied scales indicated anxiety, depression, or premenstrual dysphoric disorder.

Head-Space-Gas Chromatography/Mass Spectrometry Analysis

The urine samples were stored at -20°C and then thawed at room temperature before analyzing. The analysis of volatile compounds was performed using a gas chromatograph (Agilent Technologies, 6890N) equipped with a static head-space sampler (Agilent Technologies, 7694E). A 10 ml urine sample was introduced into a 20 ml headspace vial and sealed with a PTFE/silicone rubber Teflon cap. Each vial was equilibrated at 85°C for 45 min in the static headspace sampler. The headspace samples were injected into a DB-5 capillary column (J&W Scientific, 60.0 m x 0.25 mm x 0.25 µm film thickness). The injector temperature was 250°C, with helium (1.0 ml/min) as the carrier gas. The oven temperature was maintained at 40°C for 5 min and was then increased to 210°C at a rate of 30°C/min. Finally, the temperature was increased to 213°C at a rate of 3°C/min for 3 min. 2-Heptanone was identified by mass spectrometry using a mass detector (Agilent Technologies, 5975 inert XL). Mass spectra were obtained by ionization by electronic impact at 70 eV, and 2-heptanone was identified based on its retention indices and by matching its 70 eV mass spectra with those contained in the mass spectra (HP Chemstation-NIST 05 Mass Spectral search program, version 2.0d). In addition, a comparison with a 2-heptanone standard (catalog no. 537683, Sigma-Aldrich) was analyzed under the same conditions.

Part 2: Inhalation of 2-heptanone

Participants

One hundred forty-one young, healthy, undergraduate students participated in this part of the study (47 men and 94 women, 18-36 years old, mean: 20.2 ± 0.21 years). All volunteers were students with good hygienic habits and they used minimal additional cosmetics and perfumes above normal soap. By means of a brief interview, individuals with a history of general systemic disease were excluded. Other exclusion criteria included nose surgery, nasal illness, allergies, flu, and a smoking habit.

Because emotional states vary during the menstrual cycle (Reed, Levin & Evans, 2008), volunteers were divided into three groups and tested as follows: The A group included volunteers with menstruation two weeks prior to the tests (n = 46, 18-27 years old, mean age 19.9 ± 0.32 years), the B group included volunteers who were tested approximately three weeks after their previous menstruation (n = 48 women, 18-36 years old, mean age 20.4 ± 0.45), and the C group included age-matched males (n = 47, 18-21 years old, mean age 20.0 ± 0.29 years).

Sniffing 2-heptanone

In a classroom, at the same hour of day (between 10:00 A.M. and 12:00 P.M.), each participant received a threaded glass tube (16 x 100 ml; no. 0825, Pyrex, México City, México) that contained 0.41 mg/ml 2-heptanone (Sigma Chemical, St. Louis, MO, USA) from a previously prepared stock. The selected concentration (1:1000 ml) corresponded to the olfactory detection range reported for mice (Leinders-Zufall et al., 2000). The distance from the tube to the nose was 5 cm.

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Procedures: Experimental groups

A first group (17 men and 30 women) was asked to gently but continuously sniff the contents of the test tube for 5 s. A second group (15 men and 30 women) sniffed 2-heptanone for 60 s. A third group (15 men and 34 women) did so for 180 s. After sniffing the contents, all of the volunteers were asked to complete a brief, simple questionnaire that was specifically designed to test any sensorial-emotional experience as a consequence of olfactory perception. Each volunteer sniffed the ketone only once.

Instrument

The olfactory perception questionnaire was based on Distel et al. (1999), with some modifications. It consisted of intermixed positive and negative statements that explored (a) pleasant/unpleasant perception ("*Smelling this substance produces a pleasant/unpleasant feeling*"), (b) relaxation/nervousness ("*Smelling this makes me relaxed/nervous*"), and (c) acceptance/refusal ("*I do/do not desire to smell it again*"). The volunteers were asked to freely select one (but no more than three) of the possible responses that best represented their experience after sniffing 2-heptanone. For each questionnaire, selected statements received a score of 1, and statements that were left blank received a score of 0.

Statistical analysis

General data was analyzed by one-way ANOVA. A paired *t*-test was used to compare the amount of urinary 2-heptanone from females who were included in the first part of the study in two longitudinal samples: days around ovulation vs. the expected day before menstruation. The same statistical test was used to analyze the scores from the CDQ and STAI tests. Values of $p \leq .05$ were considered statistically significant. The data are expressed as mean ± standard error of the mean.

The nonparametric Cochran *Q* test was used for the sensorial emotional questionnaire that was applied after sniffing 2-heptanone. Values of $p \leq .05$ were initially considered statistically significant. As the first step in the analysis, a general profile of selected statements was analyzed (six statements, Bonferroni adjustment, $p \leq .008$). The A, B, and C groups were then compared (Bonferroni adjustment, $p \leq .01$). The effect of duration of sniffing (5, 60, and 180 s) on the number of responders for each statement was then analyzed (Bonferroni adjustment, $p \leq .01$).

Results

Volunteers were classmates, therefore the samples were very similar, except in age, but no statistically significant differences were found ($F_{(2,136)} = 0.387$, $p = 0.680$).

Tests

None of the participants reached the criterion for a diagnosis of premenstrual syndrome, according to DSR scores ($t_{23} = 1348$, $p = 1.91$). The scores on both of the STAI scales indicated mild anxiety, but no significant differences were found between the days around ovulation and premenstrual days (STAI-T: $t_{23} = 1.602$, $p = 0.123$; STAI-S: $t_{23} = -0.196$, $p = 0.846$). No significant differences were found on the CDQ ($t_{23} = 1.750$, $p = 0.093$), in which the scores were within the normal mood range (table 1).

Table 1.
Scores on scales.

Scale	Peri-ovulation	Before menstruation	*p*
DSR	9.6 ± 1.36	11.9 ± 1.97	.191
STAI-T	53.2 ± 1.86	50.6 ± 2.24	.123
STAI-S	50.5 ± 1.81	51.0 ± 2.3	.846
CDQ	30.5 ± 1.53	28.2 ± 1.71	.093

Note: CDQ = Clinical Diagnosis Questionnaire (Calderón Narvaez, 1992), DSR = Daily Symptoms Report (Freeman, Rubeis de & Rickels, 1996), STAI-S & STAI-T = State-Trait Anxiety Inventory (STAI-S [for state anxiety] and STAI-T [for trait anxiety], Spielberg & Díaz-Guerrero, 1975).

Urinary 2-heptanone

The urinary concentration of 2-heptanone was higher in the samples obtained before menstruation compared with the samples obtained around ovulation (t_{23} = 3.643, $p < .001$; Fig. 1).

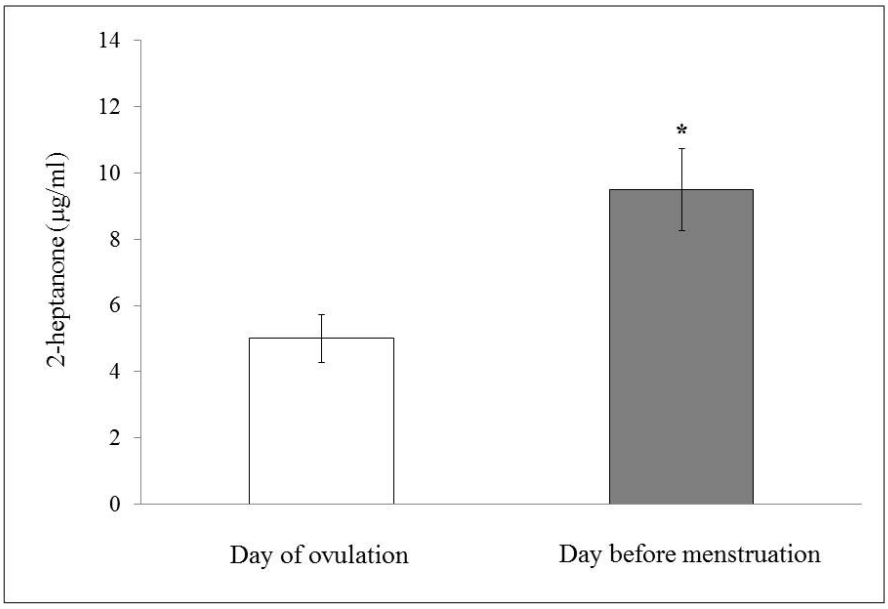

Figure 1. The urinary content of 2-heptanone was higher in women before menstruation compared with days around ovulation. *$p < .001$ (paired *t*-test).

Sniffing 2-heptanone

Sensorial-emotional responses

The general profile of selected statements, independent of group but considering the duration of sniffing, indicated that the majority of the responses after sniffing 2-heptanone for 5 s occurred for statements that explored feelings of *pleasure* (60.4%) and *acceptance* (72.9%; Cochran *Q* test = 75.806,

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df = 5, $p < .0001$). Similar distributions of responses were observed after sniffing the ketone for 60 s (*pleasant* = 55.5%, *acceptance* = 57.7%; Cochran *Q* test = 47.741, *df* = 5, $p < .0001$); and after sniffing the ketone for 180 s, but with a reduction of responders (*pleasant* = 39.5%, *acceptance* = 43.7%; Cochran *Q* test = 19.951, *df* = 5, $p < 0.001$). Therefore, with the longest duration tested, a reduction of pleasure and acceptance was observed among responders.

Non-significant differences were found in the number of responders for the following statements among the A, B, and C groups: *pleasant* (Cochran *Q* test = 2.000, *df* = 2, $p = 0.367$), *unpleasant* (Cochran *Q* test = 1.000, *df* = 2, $p = 0.606$), *relaxed* (Cochran *Q* test = 0.060, *df* = 2, $p = 0.970$), *nervous* (Cochran *Q* test = 2.700, *df* = 2, $p = 0.259$), *acceptance* (Cochran *Q* test = 0.787, *df* = 2, $p = 0.674$), and *refusal* (Cochran *Q* test = 3.125, *df* = 2, $p = 0.209$).

An analysis of duration of sniffing, independent of group, indicated non-significant differences in *pleasant* (Cochran *Q* test = 3.257, *df* = 2, $p = 0.196$), *unpleasant* (Cochran *Q* test = 0.461, *df* = 2, $p = 0.793$), *relaxed* (Cochran *Q* test = 0.001, *df* = 2, $p = 1.000$), *nervous* (Cochran *Q* test = 0.375, *df* = 2, $p = 0.829$), and *refusal* (Cochran *Q* test = 4.800, *df* = 2, $p = 0.090$). The only statement with significant differences based on duration of sniffing was acceptance (5 s: 75.5%; 60 s: 57.7%; 180 s: 42.2%; Cochran *Q* test = 10.242, *df* = 2, $p < .005$).

Discussion

The present study sought to determine variations in menstrual cycle-related urinary content of 2-heptanone and sensorial-emotional reactions to sniffing 2-heptanone in young, healthy volunteers in different groups. The urinary content of 2-heptanone was higher before menstruation compared with peri-ovulation days. Therefore, premenstrual increase in urinary release of 2-heptanone observed in this study appears to involve a functional process. Independent of gender, differences in sensation depended on the duration of sniffing: longer inhalation time was associated with less acceptance of sniffing 2-heptanone again.

The metabolism of 2-heptanone is well known in some microorganisms, namely fungi (Cakmakci et al., 2013; Gehrig & Knight, 1958; Pasanen, Korpi, Kalliokoski & Pasanen, 1997); it is formed from octanoic acid (Larroche, 1996; Larroche, Besson & Gros, 1994). This 8-C fatty acid, following a β-oxidation process, produces β-ketoacids. Once b-ketoacids are decarboxylated through the loss of one carbon, alkan-2 one compounds are formed (Schaft van der, Burg ter, Bosch van den & Cohen, 1992). Octanoic acid and 2-heptanone, among other compounds, are naturally contained in several nutritional sources (Atasoy, Hayaloglu, Kirmaci, Levent & Türkoğlu, 2013; Delgado, González-Crespo, Cava & Ramírez, 2011; Santos, Villarino, Zosa & Dayrit, 2011). Octanoic acid enjoys preferential absorption in the gastrointestinal tract, and its transportation into mitochondria for oxidation occurs independently of the carnitine transport system (Jong-Yeon, Hickner, Dohm & Houmard, 2002; Papamandjaris, MacDougall & Jones, 1998). It is consequently susceptible to metabolic transformation in the human body (Carnielli et al., 1994), suggesting the possibility that 2-heptanone may be formed through fatty acid oxidation. The property of elongation and desaturation of fatty acids also suggests that this ketone may be produced in other organisms. 2-Heptanone is detectable in other mammals (Pohorecky et al., 2008; Wood, 2003), as well as humans (Lacy Costello et al., 2014), though this finding needs to be confirmed.

Cortisol at physiological concentrations is a potent stimulus of lipolysis in human adipose tissue (Djurhuus et al., 2002). The release of cortisol is very sensitive to stress. Independent of gender, subjects who are most sensitive to stress also exhibit the highest cortisol response (Childs, Dlugos & De Wit, 2010). Estradiol plasma levels upon awakening peak in the peri-ovulatory phase, whereas progesterone peaks in the early to mid-luteal phase, but cortisol does not change during the menstrual cycle (Ahn et al., 2011; Gröschl, Rauh, Schmid & Dörr, 2001; Kudielka & Kirschbaum, 2003) or shows slight increases during the mid-luteal phase (Andreano, Arjomandi & Cahill, 2008). In healthy women who are subjected to psychosocial stress, the levels of salivary cortisol are positively associated with post-task subjective stress in the luteal phase, whereas the converse association is observed in the follicular phase (Duchesne & Pruessner, 2013). Therefore, cortisol levels in response to environmental stimuli are higher during the follicular phase than during the luteal phase. Cortisol levels increase in anxious women who are subjected to a stressful situation, particularly during their follicular phase (Hlavacova, Wawruch, Tisonova & Jezova, 2008), which may increase the likelihood of lipolysis, which in turn may explain the increased amount of urine 2-heptanone before menstruation in our study.

In this study, we did not measure the concentration of 2-heptanone that was actually sniffed. However, we maintained the same concentration of 2-heptanone for all of the groups and changed sniffing durations in the different groups of volunteers. In mice, the threshold concentration for perceiving 2-heptanone is approximately 10^{-11}-10^{-10} M (Leinders-Zufall et al., 2000). A similar concentration produces changes in extracellular recordings of the basal amygdala in rats (Contreras, Gutiérrez-García, Molina-Jiménez & Mendoza-López, 2012).

Although 2-heptanone is considered an alarm pheromone (Gutiérrez-García et al., 2007), we did not find any changes in nervous/relaxed states, which may indicate that sniffing 2-heptanone did not produce any signs of anxiety in humans rather than more subtle sensations. Anxiety may be considered an adaptive response (Gutiérrez-García & Contreras, 2013). In rodents that are subjected to unavoidable stress, the urinary delivery of 2-heptanone is reduced by a benzodiazepine (Gutiérrez-García et al., 2006), indicating that 2-heptanone delivery has two complementary meanings: (a) as a chemical signal that indicates the presence of danger and (b) the subject that emits the ketone into its environment experiences some degree of adaptive anxiety, and the receptor individual supposedly reacts according to the duration of exposure.

Chemosensory anxiety signals may be mediated by the olfactory system and activate many areas of the human brain (Prehn-Kristensen et al., 2009). In rats, exposed to an alarm pheromone, Fos expression increases in the anterior-division lateral and medial areas of the bed nucleus of the *stria terminalis*, paraventricular nucleus, dorsomedial hypothalamic nucleus, anterodorsal medial, lateral and basolateral amygdaloid nucleus, ventrolateral periaqueductal gray matter, laterodorsal tegmental nucleus, and *locus coeruleus* (Kiyokawa, Kikusui, Takeuchi & Mori, 2005). In mice, the olfactory system receptors have been identified (Xu et al., 2005). In other mammals, similar receptor systems may exist, with information reaching deep temporal lobe structures. The amygdala has been shown to be activated in volunteers who sniffed human sweat samples from donors who were subjected to emotional stress (Mujica-Parodi et al., 2009). The amygdala complex and hippocampus constitute an anatomical substrate of emotional memory (Paz & Pare, 2013; Phelps, 2002), and connections between the olfactory system and amygdala complex have been reported (Gutiérrez-Castellanos, Martínez-Marcos, Martínez-García & Lanuza,

2010). Moreover, the actions of 2-heptanone on this circuit and on the vomeronasal organ have been reported (Contreras et al., 2012; Contreras, Gutiérrez-García & Molina-Jiménez, 2013; Molina-Jiménez, Gutiérrez-García & Contreras, 2013). The lowered acceptance by volunteers who sniffed 2-heptanone for a relatively long time (180 s) seemingly involves the temporal lobe amygdala complex and its olfactory connections, thus providing an emotional experience.

Sweat from anxious men may induce anxiety in females who sniff it (Albrecht et al., 2010), particularly in socially anxious females (Pause, Adolph, Prehn-Kristensen & Ferstl, 2009; Prehn, Ohrt, Sojka, Ferstl & Pause, 2006) or when the donors are stressed female volunteers (Ackerl, Atzmueller & Grammer, 2002). However, no differences by sex were observed in the perception of odors when the scent stimulus came from females (but not so from males) who experienced a "happy situation". When the stimulus came from individuals who experienced a "scary situation", both sexes identified the stimulus as coming from men (but not from women) (Chen & Haviland-Jones, 2000). Female sweat samples from anxious individuals also decreased the positive priming of face perception (Pause, Ohrt, Prehn & Ferstl, 2004).

Notably, in these studies the source of the scents did not include 2-heptanone. Nonetheless, the observations suggest some sex differences in the identification of scents. However, we did not observe any such differences. Our interpretation of the present results is based on the lowering of acceptance to smell 2-heptanone again with the longest sniffing duration. We did not explore specific sex differences in acceptance. Although women are more sensitive to male odors (Singh & Bronstad, 2001), their olfactory threshold is highest during the active menstrual phase compared with other phases of the menstrual cycle (Navarrete-Palacios, Hudson, Reyes-Guerrero & Guevara-Guzmán, 2003), which may help explain the similarities found in responses between sexes when they inhaled 2-heptanone.

Our study has two limitations. First, the menstrual cycle was controlled based solely on changes in body temperature. We did not perform any other precise measures (e.g., analysis of vaginal mucus or hormonal plasma levels). Therefore, ovulation was only inferred. However, in this part of the study, we employed a longitudinal design, in which the same volunteers underwent two stages of the study, including urine collection. Second, we did not include a structured questionnaire to explore sensory-emotional responses in the volunteers who sniffed 2-heptanone. We simply asked the volunteers to mark previously elaborated phrases. However, they were free to select between affirmative and negative responses and to leave some answers blank. Importantly, the results were obtained from different groups of volunteers who were asked to sniff the ketone for different lengths of time.

In conclusion, healthy young women exhibited an increase in urinary 2-heptanone content before menstruation, which may be considered a part of functional changes preceding menstruation, and a relatively long period of time sniffing 2-heptanone decreased feelings of acceptance in healthy volunteers.

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