Regulation of ovulation by human pheromones

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Pheromones are airborne chemical signals that are released by an individual into the environment and which affect the physiology or behaviour of other members of the same species[1]. The idea that humans produce pheromones has excited the imagination of scientists and the public, leading to widespread claims for their existence, which, however, has remained unproven. Here we investigate whether humans produce compounds that regulate a specific neuroendocrine mechanism in other people without being consciously detected as odours (thereby fulfilling the classic definition of a pheromone). We found that odourless compounds from the armpits of women in the late follicular phase of their menstrual cycles accelerated the preovulatory surge of luteinizing hormone of recipient women and shortened their menstrual cycles. Axillary (underarm) compounds from the same donors which were collected later in the menstrual cycle (at ovulation) had the opposite effect: they delayed the luteinizing-hormone surge of the recipients and lengthened their menstrual cycles. By showing in a fully controlled experiment that the timing of ovulation can be manipulated, this study provides definitive evidence of human pheromones.

The existence of human pheromones was first suggested by the demonstration that women living together can develop synchronized menstrual cycles under specific conditions[2–5]. In rats, a similar process of ovarian synchrony occurs and is mediated by the exchange of two different pheromones[6–7]. One, produced before ovulation, shortens the ovarian cycle; the second, produced at ovulation, lengthens the cycle. These two opposing pheromones were predicted by a coupled-oscillator model of ovarian synchrony and shown by computer simulation to be sufficient for producing not only synchrony, but also the other observed effects of ovarian asynchrony and cycle stabilization[7,8]. By applying this model to humans, we demonstrate the existence of human pheromones and identify a potential pheromonal mechanism for menstrual synchrony, as well as for other forms of social regulation of ovulation.

We found that the recipients had shorter cycles when receiving axillary compounds produced by donors in the follicular phase of the menstrual cycle (-1.7 ± 0.9 days) and longer cycles when receiving ovulatory compounds ($+1.4 \pm 0.5$ days), which represent significantly different opposite effects (Fig. 1). The response was manifest within the first cycle, rather than requiring three cycles of exposure as suggested previously[2,7], and the sequence of compound presentation had no effect. The two types of axillary compounds had effects that were significantly different from each other and from the baseline cycle. The carrier had no effect on cycle lengths of the control recipients. In five of the cycles, women had mid-cycle nasal congestion, which could have prevented their exposure to pheromones; including these cycles in the analysis made the results slightly less robust (follicular compounds: -1.4 ± 0.9 days; ovulatory compounds: $+1.4 \pm 0.5$ days; ANOVA: follicular versus ovulatory compounds $F(1,18) = 4.32$, $P \leq 0.05$; cycle 1 versus

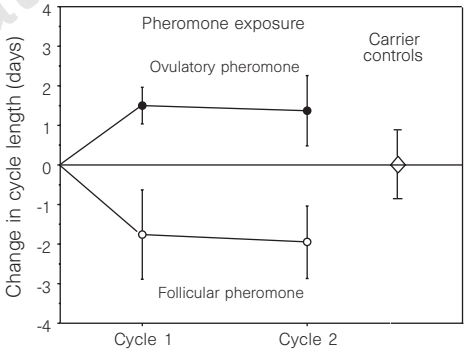

Figure 1 Effect of axillary compounds, donated by women during the follicular or ovulatory phases of their menstrual cycle, on the menstrual cycle length of recipients. This was measured as a change in length from the recipient's baseline cycle with a repeated measures analysis of variance: within-subject factors were follicular versus ovulatory compounds ($F(1,18) = 5.81, P \leq 0.03$) and cycle 1 versus cycle 2 of exposure (not significant, NS); the between-subjects factor was: order of presentation (NS); all interactions between factors were not significant). Cycles were shorter than baseline during exposure to follicular compounds ($t = 1.78, P \leq 0.05$, 37 cycles) but longer during exposure to ovulatory compounds ($t = 2.7$, $P \leq 0.01$, 38 cycles). Cycles during exposure to the carrier were not different from baseline ($t = 0.05$, $P \leq 0.96$, 27 cycles).

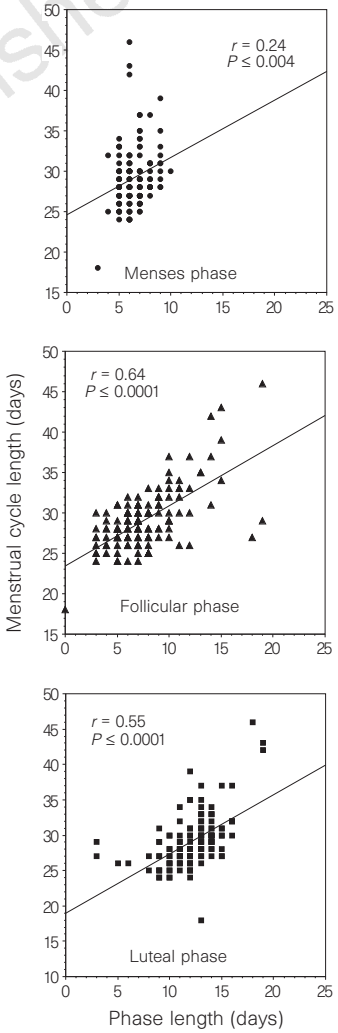

Figure 2 Each of the three phases of the menstrual cycle are variable in length (*x*-axis) and correlate with overall menstrual cycle length (Pearson's *r*), establishing each as a potential mediator of the effects of axillary compounds. Menses phase (●, day 1 to the end of menses); follicular phase (▲, day after menses to the day before the preovulatory LH surge); luteal phase (■, three days after the LH surge to day before menses, verified to be functional by ovulatory levels of progesterone glucuronide (PG) and rise in basal body temperature. The ovulatory phase is a fixed 3 day interval (day of LH surge onset plus 2 subsequent days). Note that the luteal phase of these normal subjects is significantly more variable than the 12–16-day range described in standard medical texts.

cycle 2 of exposure (not significant, NS); order of presentation (NS); all interactions between factors were not significant).

The finding that axillary compounds changed cycle length indicates that the compounds contain pheromones. The existence of two opposing effects, especially one that accelerates ovulation, makes it unlikely that a simple disruption of ovulation by a chemical produced the observed changes[9]. It also suggests two functionally different ovarian-dependent pheromones in humans, as in rats, with opposing effects. The existence of a phase-advance pheromone and a phase-delay pheromone supports the coupled-oscillator model of menstrual synchrony[7,8]. Women reported that they detected only alcohol (the control odorant, and carrier of the compounds), indicating that these changes were due to pheromones that were not consciously detected.

These results are consistent with another central prediction of the coupled-oscillator model: that there are individual differences in sensitivity to pheromones and therefore in the strength of the response[8]. Although a significant proportion of women in this experiment responded to the pheromones with changes in cycle length in the expected directions (68% of women responded to follicular pheromones, 68% to ovulatory pheromones), some women did not. In addition, the range of response magnitude was considerably more than the variation in cycle length typical for this age group[10]: cycles were shortened from 1 to 14 days and lengthened from 1 to 12 days.

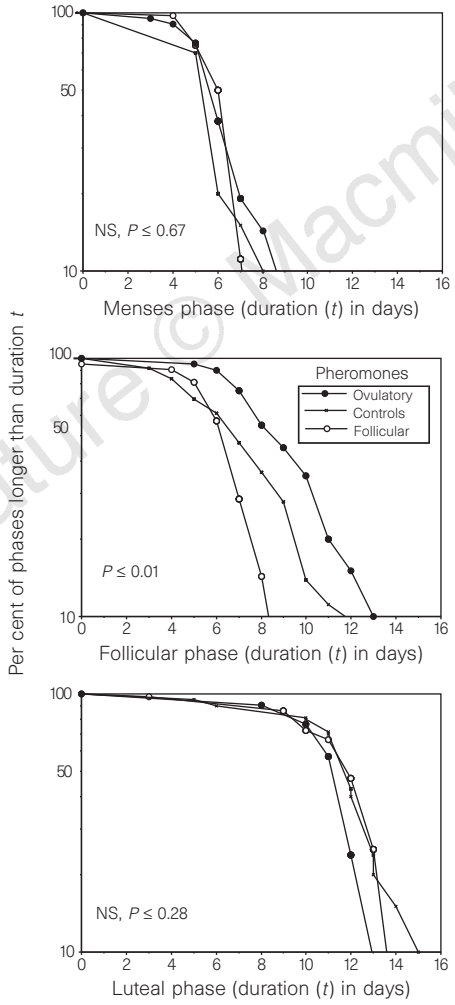

Figure 3 Effect of follicular and ovulatory pheromones on the length of each of the three phases that could mediate the observed change in menstrual-cycle length. Log-survivor analysis of the percentage of phases that are longer than a given length (time (*t*) in days; Mantel-Cox test). The same conclusions were reached with repeated measures analysis of variance on each of these three phases.

There are three phases of the menstrual cycle that vary and might mediate the effect of pheromones on cycle length; each is controlled by different neuroendocrine mechanisms (menses, follicular and luteal phases). To determine the specific mechanism of pheromone action, we measured the luteinizing hormone (LH) and progesterone glucuronide content from urine samples to pinpoint the time of the preovulatory LH surge and verify the occurrence of ovulation. Previous hypotheses have focused on the menses or luteal phases[2–7,11,12], although most medical texts report that the normal luteal phase is relatively fixed in length and it is the follicular phase that varies. In our sample, each of these three phases, including the luteal phase, varied significantly in length (indicated by the range of *x*-axis values in Fig. 2) and correlated sufficiently with cycle length for any of the three hypotheses to be correct.

Nonetheless, we traced all the changes caused by the pheromones presented in our study to the follicular phase (Fig. 3). For the menses and luteal phases, the distribution during the pheromone and control conditions were the same (indicated by overlapping log-survivor curves). Only the follicular phase was regulated, shortened by follicular compounds and lengthened by ovulatory compounds, suggesting that these ovarian-dependent pheromones have opposite effects on the recipient's ovulation by differentially altering the rate of follicular maturation or hormonal threshold for triggering the LH surge.

This experiment confirms the coupled oscillator model of menstrual synchrony and refocuses attention on the ovarian-dependent pheromones that regulate ovulation, producing either synchrony, asynchrony or cycle stabilization within a social group, namely two distinct pheromones, produced at different times of the cycle, which phase-advance or phase-delay the preovulatory LH surge. From this initial test of human ovarian-dependent pheromones, we do not know whether the phenomenon is fragile—that is, limited to modulation of ovulation timing in healthy young women—or robust, and so capable of modulating ovulation in a diverse population for either contraception or treatment of infertility. Moreover, we need to determine whether humans naturally receive compounds that have similar effects in the context of everyday life.

There may be other consequences of ovarian-dependent pheromones in women, in addition to the alteration of the timing of ovulation. Our work in rats and with computer simulations demonstrates that these same ovarian-dependent pheromones have qualitatively different effects depending on the initial conditions under which pheromonal and social interactions begin, as well as on the point in the reproductive lifespan when they occur[7,8,13–15]. Further work in this area may well reveal that, as in rats, social interactions mediated by ovarian-dependent pheromones affect age of puberty, interbirth intervals, age at menopause, and level of chronic oestrogen exposure throughout a women's lifetime.

These data demonstrate that humans have the potential to communicate pheromonally. In other species there are many other types of pheromones, not dependent on ovarian function, which enable individuals to regulate diverse aspects of their internal neuroendocrine states on the basis of information about another's internal state or environment. For example, pheromones influence mating preference in hamsters[16], dominance relationships among male elephants in musth[17], timing of weaning in rats[18] and how rat pups learn to distinguish edible foods from poisons[19], how hamsters recognize individual members of their social group[20], and the level of stress experienced by a mouse in a new environment on the basis of the emotional state of the previous occupant[21]. Well controlled studies of humans are now needed to determine whether there are other types of pheromones, with effects that are as far-reaching in humans as they are in other species. □

Methods

Subjects and procedures. The experiment involved 29 women aged 20–35, who were students or staff at a university, used barrier contraception and had

histories of regular and spontaneous ovulation. They were the first women who met our subject criteria among those responding to our request for volunteers and none dropped out once the experiment had begun. We collected compounds from the axillae of 9 donor women in hormonally distinct phases of the menstrual cycle and applied them daily just under the noses of 20 recipients. All participants were unaware of the experiment's hypothesis and the source of the compounds. The study was presented as focused primarily on the development of non-invasive methods for detecting ovulation, and secondarily on sensitivity to the odour of small amounts of 'natural essences' (consent was obtained for a list of 30 compounds).

Axillary compounds. As in other species, human pheromones might be produced by apocrine glands (active only during reproductive maturity), eccrine glands (which produce sweat that contains compounds found also in saliva and urine), exfoliated epithelial cells or bacterial action[22–24]. We collected compounds from axillae because they contain all four of these potential sources and because the two previous, albeit highly criticized, attempts to study this issue used axillary compounds[3,4,25–28]. The 9 donors bathed without perfumed products every day and then wore 4×4 cotton pads in their axillae for at least eight hours. Each pad was cut into four sections for distribution to different recipients, treated with 4 drops of 70% isopropyl alcohol[25] and then frozen immediately at −80 °C in a glass vial.

Menstrual cycle assessment. Donors provided urine samples every evening, which we assayed for LH to detect the onset of the LH surge that triggers ovulation[29]. This singular hormonal event unambiguously demarcates the follicular from the ovulatory phases of the cycle. The LH surge was used together with data on vaginal secretions, menses, basal body temperature, and a rise in progesterone glucuronide in the postovulatory luteal phase, to classify each pad as containing compounds produced during the follicular phase (2 to 4 days before the onset of the LH surge) or the ovulatory phase (the day of the LH surge onset and the 2 subsequent days). To ensure a similar stimulus for all recipients regardless of individual differences among donors, all 9 donors contributed equally to the follicular and ovulatory compounds received by each subject.

As it is not yet known when during the menstrual cycle women are physiologically most sensitive to putative pheromones, applying compounds every day ensured covering a potentially sensitive period. However, our computer simulation experiments indicated that in rats this pheromonal-sensitive period occurs mid-cycle, around the time of ovulation[8] (a period when women are particularly sensitive to some olfactory stimuli[30]). Any condition preventing exposure to the compounds, such as nasal congestion anytime during the mid-cycle period from three days before to two days after the preovulatory LH, could weaken the effect. We analysed the data taking this into account.

Experimental design. All recipients were studied for one baseline cycle without exposure to axillary compounds. Then, in a crossover experimental design during the next four consecutive cycles, axillary compounds were applied daily by wiping a thawed pad above the recipients upper lip. Half of the recipients ($n = 10$) received follicular compounds daily for two menstrual cycles and were then switched to exposure to ovulatory compounds for the next two cycles. The other 10 recipients received the same compounds in the reverse order. After applying the compounds, recipients were free to go about their normal activities but were asked not to wash their faces for the next six hours. All but two subjects, who missed only the last cycle of their second treatment, completed all five cycles of the experiment.

A between-subjects control group was provided by women (the donors) who collected all ovarian-cycle measures, but received only the carrier above their upper lip each day: 70% isopropyl alcohol. In addition, because the two-day change in menstrual cycle length (expected from the initial study[2]) is substantially less than individual variation in cycle length typical for this age group[10], we created within-subjects controls by measuring the effect on the menstrual cycle in terms of a change in length from each individual subject's cycle preceding each condition. (For experimental subjects this was the cycle that preceded exposure to each type of compound; for control subjects this was the cycle that preceded exposure to the carrier, 70% alcohol).

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Motor role of human inferior parietal lobe revealed in unilateral neglect patients

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The exact role of the parietal lobe in spatial cognition is controversial. One influential hypothesis proposes that it subserves spatial perception[1], whereas other accounts suggest that its primary role is to direct spatial movement[2,3]. For humans, it has been suggested that these functions may be divided between inferior and superior parietal lobes, respectively[2,4]. In apparent support of