Pheromones, Development, and Behavior: Do Human Pheromones Exist?

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1. What are pheromones?

Pheromones are like hormones in that they are bioregulators (i.e., chemicals that play an important role in turning on, off, or maintaining physiological and behavioral processes). Testosterone and progesterone are good example of bioregulator hormonal compounds. Recall that in ringdoves, as testosterone increases in males (as day light becomes longer), courtship behavior increases. Oxytocin is another bioregulator compound with many functions as mentioned earlier. For example, when we watched the piglets nurse in lab, milk letdowns were regulated by oxytocin releases into the blood stream.

![Diagram](image)

**Figure 1.** Interactions among chemical signaling systems and their respective environments. Bioregulators traveling through internal (e.g., blood stream) or external (water, air) environments are called hormones (or neurotransmitters, etc.) and pheromones respectively.

An interesting question is whether all bioregulators must be in the organism?

Can chemical bioregulators be transmitted between organisms?

Yes, bioregulators called **pheromones**, first defined by Karlson and Lüscher (1959) as “substances secreted to the outside of an individual and received by a second individual of the same species in which they release a specific reaction, for example, a definite behavior or developmental process”. — The fundamental question is whether there are chemicals that fit this definition, especially for mammals.

Pheromones were first discovered in insects (e.g., airborne compounds that attract male moths or compounds released by army ant larva that excite workers to forage).
1.1. Releaser

These compounds elicit dramatic behavioral effects and are often referred to as releaser pheromones. E.g., Ant trail-following pheromones.

1.2. Primer

These compounds cause longer-term changes in neuroendocrine or developmental state are usually referred to as primer pheromones. Compounds that alter the developmental process are good examples, such as puberty acceleration pheromones in mice.

2. Pheromones in mice

2.1. The Bruce effect (i.e., pregnancy block and estrous induction). In this phenomenon, the pregnancy of a recent inseminated female mouse is terminated by the exposure to an unfamiliar male. The fetuses fail to implant. The actual male mouse does not need to be there only his urine. It turns out that a testosterone-dependent odor from a strange male is enough to cause the pregnancy block and the induction of estrous several days later.

2.2. The Lee-Boot effect. This phenomenon occurs when female are kept in close proximity to each other and the pheromones they produce cause the estrous cycle of females in the group to become longer and even stop depending on the number of mice and how closely they are packed. The pheromone is released in the urine.

2.3. The Whitten effect. This phenomenon occurs when a group of female mice, whose estrous cycles become very long or stopped, is exposed to the urine of a male mouse. The estrous cycles of these females begin again at about the same time and it blocks the Lee-Boot effect.

2.4. The Vandenbergh effect. This phenomenon occurs when male mouse urine is placed in relatively close proximity to a developing female mouse pup. The mouse pup will have accelerated puberty (i.e., she will start cycling earlier than without the presence of the male pheromone).
2.5. **Puberty delay.** Juvenile female house mice treated with the urine of reproductive females that have been housed together exhibit a delayed onset of puberty.

<table>
<thead>
<tr>
<th>Name</th>
<th>Chemical structure</th>
<th>Origin</th>
<th>Possible chemosignalling function in female mice</th>
<th>Detection threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,5-dimethylpyrazine</td>
<td></td>
<td>Female urine</td>
<td>Puberty delay</td>
<td>$10^{-6}$–$10^{-7}$ M</td>
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<td>2-sec-buty-4,5-dihydrochiazole</td>
<td></td>
<td>Male bladder urine</td>
<td>Oestrus synchronization, Puberty acceleration</td>
<td>$10^{-10}$–$10^{-9}$ M</td>
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<tr>
<td>2,3-dehydro-exo-brevicomin</td>
<td></td>
<td>Male bladder urine</td>
<td>Oestrus synchronization, Puberty acceleration</td>
<td>$10^{-10}$–$10^{-9}$ M</td>
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<td>α- and β-famesenes</td>
<td></td>
<td>Male preputial gland</td>
<td>Puberty acceleration</td>
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<td>2-heptanone</td>
<td></td>
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<td>Oestrus extension</td>
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<td>6-hydroxy-6-methyl-3-heptanone</td>
<td></td>
<td>Male bladder urine</td>
<td>Puberty acceleration</td>
<td>$10^{-8}$–$10^{-7}$ M</td>
</tr>
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**Figure 2.** Structure, origin and function of prospective pheromones in mice.

The fundamental problem with the pheromone concept is that all identified chemicals only work in combination with other chemical and only with in specific social and environmental contexts. See review of “*The great pheromone myth*.”
3. Rats: Complex effects of chemical cues

Pheromonal effects are best documented in house mice (*Mus musculus*), however, there are chemical cue effects in Norway rats (*Rattus norvegicus*) as well. In a study I did a few years ago (Schank & Alberts, 2000), I took my cue from Calhoun (1962) on how female rats often isolated their litters from other rats and even blocked the entrance to the nest with grass and other material. Based on this observation, I hypothesized that the effects of chemical cues on the development and reproduction of a dam’s pups may be mediated by her encounters with male urine while away from the nest. To study possible effects of male urine on mothers and the development of her pups, I constructed the habitat illustrated in Fig. 3.

**Figure 3.** Illustration of the habitat layout used in this study.

Urine was collected and frozen from stranger males. When the pups were 14 days of age, male urine was placed twice a day (morning and late afternoon) in the a small stainless steel box that only the mother could sniff. A control group received only distilled water.

There were a number of physiological effects detected.

**Mother effect:** The period between parturition and the second lactational estrus was 2 days longer in mothers that received male urine than in those that received only distilled water and there was no difference in the length of time to the next estrus (Fig 4).

**Figure 4.** (a) Effect of male urine on the interval between parturition and the second lactational estrus and next estrus in the dams (*indicates a significant effect, P < 0.02). (b) Effect, via the dam, of male urine on cycle length in female pups (* indicates a significant effect, P < 0.002).
Effects on Offspring:

1. **Day of opening.** Vaginal opening of male urine (MU) pups was more than 1 day later than distilled water (DW) pups (mean MU: 36.14 days vs. DW 34.8 days).

2. **Day of first estrus.** The first estrus of MU pups was 1 day later than DW females (mean MU 36.8 days vs. DW 35.8).

3. **First Three Estrous Cycles.** The first estrous cycle of MU offspring was shorter than for DW offspring (see Fig. 4).

4. **Litter Size.** MU females (now adults) had larger litters than DW females (mean MU: 11.8 pups vs. DW 9.43 pups).

4. The vomeronasal organ (VNO)

It turns out that there is a special organ in most (but not all mammals) that detect pheromones and indirectly affect the hypothalamus.

![Diagram of the vomeronasal organ](image)

**Figure 5.** The rodent vomeronasal organ. (A) Sagittal view showing the location of the tubular vomeronasal organ in relation to the main olfactory epithelium. (B) Diagram of a coronal section of the vomeronasal organ, which is enclosed in a cartilaginous capsule. The sensory epithelium containing the vomeronasal sensory neurons is located medially. Changes in blood flow to the laterally located blood vessel change the volume of the lumen and pump stimuli in and out.
Figure 6. Neural pathways for vomeronasal processing. The V1Rs are a class of G-protein-coupled receptors cells that are expressed by vomeronasal sensory neurons in the apical zone of the sensory epithelium. These V1R sensory neurons (shown in blue) project to the anterior sub-region of the accessory olfactory bulb (AOB). The V2Rs are also G-protein-coupled receptors, but their structure differs markedly from the V1Rs. The V2Rs have a large extracellular amino terminus and form a receptor complex with members of the M10 family of MHC class Ib molecules and β2- microglobulin (β2m). The V2R-expressing sensory neurons (shown in red) are located in the basal zone of the sensory epithelium and project to the posterior sub-region of the AOB. The information from the V1Rs and V2Rs, which is processed separately in the AOB, converges in completely overlapping projections to the bed nucleus of the stria terminalis (BNST), bed nucleus of the accessory olfactory tract (BAOT), medial amygdala (MeA) and the posteromedial cortical nucleus (PMCo) of the amygdala. Though not shown here, there are then projections from the amygdala to the hypothalamus.

Olfactory receptor neurons project connection to the main olfactory bulb (MOB), whose mitral/tufted cells project into the olfactory cortex. However, vomeronasal receptor neurons connect to the accessory olfactory bulb (AOB), whose mitral cells connect to hypothalamic areas via the medial amygdala (see Fig. 6). This is exactly what we would expect if pheromones act like hormones.
4.1. Do humans have a VNO?

This has been a topic of considerable controversy. For a long time, it was unclear whether humans even had a VNO structure, but now we can say with near certainty that humans have a VNO structure. If this VNO structure works, then it would be possible for there to be pheromones that have at least some of the effects identified for mice and rats.

**Figure 7.** illustrates the location of the human VNO, however, there are some problems.

**Figure 7.** Schematic diagram showing the approximate location of the human vomeronasal organ (VNO) at the base of the nasal septum. Abbreviations: MOB, main olfactory bulb; OE, olfactory epithelium. The accessory olfactory bulb (AOB) has not been demonstrated in humans.

**Problems with the Human VNO.**

1. **Accessory Olfactory Bulb.** An anatomically distinct structure from the main olfactory bulb in rodents and other mammals with VNOs does not appear to exist in humans. (uh-oh)

2. **Pseudogenes.** Vomeronasal pheromone detection begins by the binding of pheromones to pheromone receptors located on the cell membrane of sensory neurons of the VNO. This triggers a signal transduction pathway that ultimately leads to the activation of the hypothalamus.
However, Zheng and Webb (2003) show that TRP2 ion channel and V1R pheromone receptors, two components of the vomeronasal pheromone signal transduction pathway, have been impaired since shortly before the separation of hominoids and Old World monkeys $\approx$ 23 million years ago. Moreover, random inactivation of pheromone receptor genes is an ongoing process even in present-day humans. (Pseudogenes are sequences of genomic DNA with such similarity to normal genes that they are regarded as non-functional copies or close relatives of genes.)

3. **Vomeronasal System Development.** Although the VNO is neurally connected early in fetal development, it subsequently degenerates, with no connections to the olfactory bulb (Fig. 8)

Figure 8. Schematic drawings of three phases of the presumptive vomeronasal system in human fetuses, in comparison with the olfactory system. A: The first phase. The vomeronasal organ (VNO) is connected with the vomeronasal bulb (VNB) via the vomeronasal nerves (VNNs). The olfactory mucosa (OM) is located in the dorsal part of the nasal cavity. The olfactory nerves (ON) originating from the OM terminate in the olfactory bulb (OB). FL, frontal lobe; V, ventricle. B: The second phase. In parallel with the degeneration of the VNB, the size of the VNN becomes smaller; the degeneration of the VNN takes place. As the vomeronasal epithelium of the VNO becomes thinner, the volume of the vomeronasal lumen increases. In contrast, the size of the OM and OB continues to increase. C: The third phase. The VNO and the brain are disconnected, although the VNO continues to grow. The development of the OM and ON is still in progress.

5. **Menstrual Synchrony (McClintock Effect)**

The VNO is the main pheromone detection system in mammals, which have a functional VNO. It is thought by some that the main olfactory system can detect some pheromones, but this is controversial.

If humans release, detect, and respond to pheromones, it almost certainly has to be through the main olfactory system. But, for there to be human pheromones, there must be something they cause in human physiology and/or behavior.

Many authors have argued that the most likely place to find human pheromones is as a cause or mechanism for menstrual synchrony among women.
5.1. The best case for human pheromones

![Graph showing menstrual cycle synchrony over time.]

**Figure 9.** McClintock’s (1971) results for groups with 5 to 10 women (mostly 10 women).

The very first study was conducted 37 years ago and published in 1971 by Martha McClintock. In that study, she analyzed the menstrual cycles of 135 women from October to April. She reported a small, but apparently statistically significant increase in synchrony as measured by the absolute mean difference among cycle onsets. However, since publication, serious problems have been discovered (e.g., see Wilson, 1992).

5.2. Does menstrual synchrony exist?

![Graphs showing menstrual cycle patterns over time for different groups.]

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5.1. The best case for human pheromones

![Graph showing menstrual cycle synchrony over time.]

**Figure 9.** McClintock’s (1971) results for groups with 5 to 10 women (mostly 10 women).

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5.2. Does menstrual synchrony exist?
Figure 10. Cycle onsets for 9 of 29 groups Chinese women where sets of nearest onset dates (indicated by open circles) deviated from a uniform distribution around a circle. Each group is designated as Grade-Room number. Note that cycle variability causes convergence and divergence of onsets.

Many methodological problems have been pointed out, but the central problem is cycle variability. Synchrony cannot occur if women don’t all run on exactly the same frequency. They don’t, so synchrony turns out to be impossible (Fig. 10).

6. Do menstrual-cycle altering pheromones exist?

Several studies over the last 20 years have been published reporting the existence of pheromones that can alter the length of menstrual cycles in women (lengthen
them, shorten them, or make them variable). However, if menstrual synchrony does not exist, then this would logically imply that pheromones that could cause it should not exist.

This logical implication suggests that we should look closer at the few studies reporting human female pheromones that alter menstrual cycle lengths.

6.1. Early studies

![Figure 1](image1.png)

**Figure 1.** Analysis of the data for Russell et al. (1980; a) and Preti et al. (1986; b) using the Kupier-Stephens (Stephens 1965) circular statistic for testing non-uniform distributions on circle. For Russell et al. (1980) a 28-day clock was assumed and the black dots, “•”, represent cycle onset for each recipient (a). For Preti et al. (1986), a 30-day clock was assumed and again black dots represent cycle onsets (b). In both cases, the recipient onsets were neither synchronized nor clustered in time.

**Russell et al. (1980)** were the first to report pheromonal influence on the menstrual cycle. Five experimental subjects received axillary secretions from a single donor three times a week for a period spanning 5 menstrual cycle onsets (Russell et al. 1980). The donor had 28-day cycles throughout the study and was one of the researchers (Wilson 1992). Russell et al. (1980) applied a repeated-measures analysis of variance to differences between the donor and secretion recipients and reported a statistically significant decrease in cycle onset differences indicating that a process of synchronization had occurred.

However, the statistical analysis they applied was inappropriate for rhythmic data. What is required is a circular statistic that can tell us whether the women were
synchronized on a clock that is, for example, a 28-day clock. When we test for synchrony, we find that the women were not synchronized (Fig. 11a).

**Preti et al. (1986)** conducted an experiment design similar to that used by Russell et al. (1980). Ten women were in the experimental condition, nine were in the control condition, and there were four donors. Pads with axillary secretions from three cycles were collected for a total of 12 cycles and extracts from five of these cycles were combined to conform to days 2, 5, 8, 11, 14, 17, 20, 23, 26, and 29 of a 29-day menstrual cycle (Preti et al. 1986). Thy reported a statistically significant increase in synchrony to donors due to modulation of recipient cycle length (Preti et al. 1986). Wilson (1987) pointed out a plethora of errors in this study. Most importantly, the presentation of secretions was not in 29-day cycles but, random cycles. In any case, the women did not synchronize when the appropriate statistics are used (Fig 11b).

6.2. **Stern & McClintock (1992)**

6.2.1. **Experimental Design**

In the most famous study was conducted by Stern and McClintock (1998). They hypothesized that pheromones released during the follicular phase shorten the cycles of recipients while pheromones released during the ovulatory phase lengthen the cycles of recipients. As with the previous two studies, axillary secretions were collected from nine donors who wore pads and did not use deodorant soaps or perfumes. To avoid as much as possible consciously detectable odors, putative pheromones were extracted with alcohol (Stern and McClintock 1998).

![Figure 12. Stern and McClintock’s crossover experimental design. Cycle 1 is their baseline control cycle and cycles 2 through 5 are treatment cycles (grey represents follicular odor and black represents ovulatory odor). In the SM transformation, cycle 1 is subtracted from cycles 2 and 3. Cycle 3 is both a treatment and control condition and is subtracted from cycles 4 and 5.](image-url)
6.2.2. Results

Figure 13. 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).

What they found was that apparently, ovulatory secretions lengthened cycles a little and shortened them about the same amount (Fig. 13). But, was their method of transforming the data a mistake (see Fig 12)?
6.2.3. What does this data transformation do?

Figure 14. Two simulated data sets of random cycle lengths drawn from a truncated normal distribution (Schank 2000, 2001a) with mean and standard deviation from Stern (1992) and Stern and McClintock (1998). Graphs a and b are the data sets plotted untransformed. Neither are statistically significant $F(1, 19) = 0.564, p = .462$ (a) and $F(1, 19) = 1.721, p = .205$ (b). After the SM-data manipulation from a to c and b to d (see Fig. 12), the data now have a radically different pattern and are statistically significant, $F(1, 19) = 4.729, p = .043$ (c) and $F(1, 19) = 4.89, p = .04$ (d).

There is a fundamental flaw in data transformation used to analyze these data. By subtracting treatment cycle 3 (see Fig. 12) from cycles 4 and 5, the data were manipulated in a way that was highly biased towards finding results similar to those reported in Stern and McClintock (1998) as can be shown by constructing artificial data sets of randomly related cycles and then applying their data transformation. Specifically, the Stern-McClintock method (SM) illustrated in Fig. 12 will tend to increase the spread in positive and negative directions (Fig. 14).
6.3. Breastfeeding pheromones?

Jacob, et al. (2004) hypothesized that axillary and nipple secretions from breastfeeding mothers would increase the menstrual-cycle length variability of women receiving these compounds: “Specially, this novel study hypothesized that pheromones from breastfeeding (non-ovulating) women would increase the variability of ovarian cycles, particularly by lengthening them, and also by shortening them, as the effects of pheromones depend on the state of the ovary at the time of pheromone exposure (Schank and McClintock 1992).” They site Schank and McClintock (1992) as justification for this variability hypothesis, the prediction of continuous exposure to the same pheromones has no effect at all on cycle length with no specific implications for breastfeeding women.

![Figure 15](image)

**Figure. 15.** Cycle length-distributions from Jacob et al. (2004). For cycle 1 (a and b, the first test cycle), SD = 4.29 for the control group (a) and SD = 7.29 for the experimental group (b). Levine’s test using the median yielded a trend ($F(1,47) = 3.44, p = .07$) towards more variability in the experimental than the control conditions. For cycle 2, (c and d), SD = 3.77 for the control group (g) and SD = 3.79 for the experimental group (h). Levine’s test revealed no difference ($F(1,35) = 0.29, p = .59$) in variability between the experimental and the control conditions. Thus, the data do not support the conclusion that breastfeeding women secrete compounds that increase cycle variability.

They did not test for variability in their data (this is bizarre), but when we do, we find no effect at all (Fig. 15)
6.4. Sociosexual Behavior

McCoy and Pitino (2002) reported that a putative menstrual-cycle altering pheromone (Athena Pheromone 10:13) increased the sociosexual behavior of young women (McCoy and Pitino 2002). The chemical nature of Athena pheromones 10:13 has not been revealed and will not be revealed until the putative pheromone is patented (McCoy and Pitino 2002). Women were assigned to either of the two conditions by selecting between one of several identical boxes (on a tray) containing vials with either Athena 10:13 or SD40 alcohol resulting in 23 women in the pheromone condition and 20 women in the placebo condition. Seven sociosexual variables were measured: sexual intercourse (SI), sleeping next to a romantic partner (SNRP), petting/affection/kissing (PAK), informal dates (ID), formal dates (FD), and male approaches (MA). The data were averaged separately for each variable for the two baseline weeks and the six treatment weeks.

McCoy and Pitino (2002:367) reported that “A significantly greater proportion of pheromone users compared with placebo users increased over baseline in frequency of sexual intercourse, sleeping next to a partner, formal dates and petting/affection/kissing....” However, contingency table tests only test the non-independence of data, they cannot determine whether the users increased over baseline. Since the complete data set was published, these claims could be reanalyzed using more appropriate statistical methods (see Fig. 16 below).

When an appropriate statistical analysis is applied, the only statistically significant changes are in the placebo condition. However, the placebo condition is not supposed to change! If it does, this is an indication that something methodologically went wrong with the study. Most importantly, the pheromone did not increase any of the sociosexual behaviors.

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Conclusion: Although pheromones and pheromone reception via the VNO occurs in a wide variety of mammals and can affect development and behavior (e.g., the house mouse and Norway rat), there do not appear to be human pheromones.
Figure 16. Change from baseline (± SE) for variables PKA (a), SNRP (b), SI (c), FD (d), MA, (e). None of the changes from baseline were statistically significant (one-tailed) within the pheromone condition using paired t-tests: PKA ($t = 1.42, df = 18, p = 0.086$), SNRP ($t = 1.24, df = 18, p = 0.115$), SI ($t = 1.43, df = 18, p = 0.085$), FD ($t = 1.58, df = 18, p = 0.066$), and MA ($t = -1.58, df = 18, p = 0.066$). However, for the placebo condition, the variables PKA ($t = 2.20, df = 16, p = 0.017$), SNRP ($t = 2.11, df = 16, p = 0.025$), SI ($t = 2.09, df = 16, p = 0.026$) were statistically significant, FD was close ($t = 1.74, df = 16, p = 0.051$), but MA ($t = 1.33, df = 16, p = 0.101$) was not statistically significant. An “*” indicates statistical significance at $\alpha = .05$ level within treatment conditions. If a Bonferroni correction were applied, then none of these tests would be significant at the $\alpha = 0.0083$ level.
Further Readings:


