Male Axillary Extracts Contain Pheromones that Affect Pulsatile Secretion of Luteinizing Hormone and Mood in Women Recipients

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Abstract

Human underarm secretions, when applied to women recipients, alter the length and timing of the menstrual cycle. These effects are thought to arise from exposure to primer pheromones that are produced in the underarm. Pheromones can affect endocrine (primer) or behavioral (releaser) responses, provide information (signaler), or perhaps even modify emotion or mood (modulator). In this study, we extracted underarm secretions from pads worn by men and placed the extract under the nose of women volunteers while monitoring serum luteinizing hormone (LH) and emotion/mood. Pulses of LH are excellent indicators of the release of gonadotropin releasing hormone (GnRH) from the brain's hypothalamus. In women, GnRH's positive influence on LH affects the length and timing of the menstrual cycle, which, in turn, affects fertility. Here we show that extracts of male axillary secretions have a direct effect upon LH-pulsing and mood of women. In our subjects, the putative male pheromone(s) advanced the onset of the next peak of LH after its application, reduced tension, and increased relaxation. These results demonstrate that male axillary secretions contain one or more constituents that act as primer and modulator pheromones

Introduction

Odors play an important role in mammalian reproductive biology. In non-human mammals, chemical signals, known as pheromones, emitted from one animal can cause a variety of changes in behavior and/or physiology when received by a conspecific (for reviews see 1 and 2). Historically, two broad categories of pheromones were defined, viz., releaser pheromones, which generate immediate, primarily behavioral responses (such as sexual attraction and/or copulation), and primer pheromones, which generate slower physiological/endocrine/neuroendocrine responses, including hormonal changes that alter reproductive function (3). A third category, signaler pheromones was introduced to encompass chemical signals in which information is conveyed but no obvious primer or releaser effect could be established (4). Jacob and McClintock (5) recently introduced the concept of modulator pheromones as an additional group of potential chemical signals. These are chemicals that have the potential to affect the state or mood of the recipient and/or regulate multisensory inputs during exposure. The authors (5) suggest that two steroids may fulfill the criteria as modulators when used at concentrations well above reported endogenous levels. Recently, Chen and Haviland-Jones (6) provided data suggesting that information about human emotional state is contained within axillary secretions and that other people could accurately infer the emotional state of the donor after sniffing the secretions.

In humans, several studies have indicated that interpersonal relations among women, as well as between men and women, may alter reproductive endocrinology, suggesting the presence of primer pheromone activity. These relations include menstrual synchrony among women, first documented in all-women groups (7) and later replicated in a variety of other situations (see1and 2 for reviews).

Russell and colleagues (8) were the first to present evidence that suggested menstrual synchrony could be mediated by axillary secretions. Further studies employing these secretions from women also suggested that extracts of pooled samples, collected from women across the menstrual cycle, could be used to bring a recipient group of women into synchrony with the donors (9). Extracts of male axillary secretions also appear

to affect women with a history of irregular cycle lengths. Lengths of the menstrual cycles of these women showed a significant shift (*vs.* controls) toward the normal cycle-length of 29.5±3 days (10) upon application of male axillary secretion to the upper lip of the recipient women.

Additional support for axillary components from women having primer-pheromone activity is contained within the studies by Stern and McClintock (11) and Shinohara et al. (12). These studies have focused upon female axillary secretions being applied to women recipients. The results of Stern and McClintock (11) suggest that exposing women with normal menstrual cycles to axillary extracts from women in their follicular phase (the days following menses but several days prior to ovulation) shortens the length of the recipient's menstrual-cycle by 1.7 ± 0.9 days. Exposing these same women to axillary extracts collected near the time of ovulation of the donors lengthens the recipient's menstrual-cycle by 1.4 ± 0.5 days. Interestingly, similar effects on the lengths of the estrous-cycles of female rats were noted when the rats were exposed to the follicular and ovulatory odors of other rats (13,14). Studies that have sought to provide a measure of primer pheromone activity by looking for a change in the length of the menstrual cycle have received some criticism for statistical and/or methodological errors (15-18). Other criticisms have been fueled by the intra- and inter-subject variability found in normal, consecutive menstrual cycles (19,20).

Shinohara and coworkers (12) recently demonstrated changes in frequency of LH pulses in women exposed to female axillary secretions. These investigators demonstrated an average 28% decrease in LH pulse frequency in women receiving follicular phase secretions and a 16% increase in LH pulse interval in women receiving ovulatory phase secretions. The authors suggest that the changes in LH pulsing caused by axillary components are modulating the time of ovulation and changing cycle length; these changes may provide an objective measure of human primer pheromone activity.

Exogenous stimuli, such as a conspecific's chemical signal, appear to alter reproductive function by influencing the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator. Although GnRH levels are not easily measured in humans, alterations in GnRH output can be inferred by measuring LH pulsing in blood. Changes in pulse-frequency, -height and -interval occur across a normal menstrual cycle. As women

move from the early follicular phase of the menstrual cycle towards ovulation, LH pulses increase in frequency; however, LH pulse amplitude decreases in the mid-follicular phase and increases at the time of ovulation. LH pulse frequency and amplitude progressively decrease across the luteal phase as the next menses draws near (21).

We hypothesized that a human male primer pheromone could act to alter menstrual cycle length and timing by altering LH parameters in a fashion analogous to that previously reported for female goats (22) and ewes (23). In these animals, exposure of estrogen-primed females to either the male goat or his hair decreased the LH pulse interval, bringing-on the next pulse after exposure to male stimuli faster than exposure to control conditions (22,23). Data presented herein demonstrate that applications of extracts of male axillary secretions cause a significant decrease in the latency to the next LH pulse, when compared to application of a control substance. In addition to this primer pheromone effect, women also reported experiencing less tension and to be more relaxed during exposure to the male extract, suggesting the presence of a modulator pheromone. These effects may serve as objective measures of primer and modulator pheromone activity that could guide an analytical isolation of the active axillary constituents.

Methods and Materials

Eighteen heterosexual women were enrolled in the protocol, which was approved by the Institutional Review Board at the University of Pennsylvania.

Subjects were given a gynecologic exam and Pap smear; each had a full history taken. Each subject met the following criteria: a) was between 21 and 45 years of age (median and average ages: 27 yr and 28.2 ± 5.7 yr [s.d.], respectively); b) had regular menstrual cycles of 25- to- 35 days duration in the previous year (by self-report) and must not have skipped any cycle ; c) had stable exercise and sleep patterns; e) had not taken any hormonal medication in the 3 months preceding the study; f) had a normal physical exam with no acne, hirsutism, or galactorrhea, and body weight within 15% of normal according to the Metropolitan Life tables; g) had normal thyroid function tests; h) had serum prolactin levels < 15 ng/ml; and i) was heterosexual. All

subjects displayed normal olfactory ability and no general anosmia as evidenced by their ability to perceive dilute solutions of trimethylamine (0.25%) and 3-methyl-2-hexenoic acid (0.1%).

Once admitted to the study, subjects participated for 3 menstrual cycles discussed below as Baseline, Test and Final cycle.

Baseline Cycle. Beginning with the first or second day of menses, subjects recorded their basal body temperature (BBT) on a standard calendar every morning. Morning urine samples were tested for LH daily, from day 10 until the LH surge (using a commercially available kit; OvuQuick One Step, Henry Schein, Melville, NY) to determine the time of ovulation. Approximately seven days following ovulation, serum progesterone levels were measured; a level greater than 4ng/ml was required for subjects to be included in the study.

Test Cycle. BBT, ovulation detection and luteal phase progesterone measures were recorded as in the Baseline Cycle. However, within the first 7 days of the Test Cycle onset (the first day of menses is day 1 of the menstrual cycle), subjects were admitted to the General Clinical Research Center (GCRC) at the Hospital of the University of Pennsylvania for a 12 hr period: 8 am to 8 pm. During this time, subjects received 0.5 ml of control (ethanol) or male extract applied to the nasal region/upper lip every 2 hr using a cotton pad. As described below, both extract and control were fragranced to be indistinguishable. Blood samples (~5 ml) were drawn through a heparin lock every 10 min. The nurses who drew the blood and applied the stimuli as well as the subjects were blind to the nature of the applied materials.

Since we had no a priori knowledge regarding potential lasting effects of male extracts upon LH pulsing parameters, half of the women were assigned to receive the control treatment during the first 6 hr (8 am-2 pm) of their stay in the CRC and the male extract during the final 6 hr (2pm-8pm); the other half received the treatments in reverse order.

Final Cycle. As a check on any changes in menstrual cycle length, patients were followed for an additional cycle by basal body temperature and urine LH measurements as they were during their baseline

cycle. Luteal phase progesterone levels were measured 7-10 days after the urinary LH indicated ovulation had occurred.

Male Extract and Control Solutions. The extract was created by using axillary secretions from 4-6 male donors. Secretions were collected on cotton pads, as previously described (10, 24). Three extracts were employed in the course of this study. Male donors were all healthy and ranged from 22-45 years of age. All refrained from using deodorants/antiperspirants and washed/bathed using only plain soap with little to no fragrance (Ivory Soap) for a total of 4 weeks. One week into this protocol axillae were swabbed and axillary odor was assessed. Axillary swabs were used to generate bacteria cultures and determine the type and number of endogenous cutaneous bacteria. Following this, subjects collected axillary secretions on cotton pads 3x/week. All pads were frozen (-10º C) until extracted. Male donors had no contact with and were not familiar with the female recipients.

We had no knowledge about the structural nature of the active axillary constituents, consequently, we arbitrarily chose to standardize all extracts to 0.5µg/ml of 3-methyl-2-hexenoic acid (3M2H), the major, analytical odorant found in the male axillary extracts (24). This was done in the following manner: half of each extract was concentrated to ~ 10 ml and a 5 µl portion was analyzed by gas chromatography/mass spectrometry (GC/MS), as per previous studies (24), to determine the concentration of 3M2H. After quantifying the concentration of 3M2H, portions of the concentrated extract were added to the more dilute extract such that the concentration of 3M2H in the extract to be applied to subjects was ~0.5µg/µl.

The extract was then scented with a deodorant fragrance supplied by the Mennen division of Colgate-Palmolive to a level of 0.005%. A similar volume of ethanol was used to dilute an aliquot of fragrance to 0.005% for use as an odor-matched control.

Mood Ratings: At 1 and 4 hours into each 6-hour exposure session, subjects were asked to provide ratings, on a 7-point, Likert-type, categorical scale, of the following: energetic, sensuous, tense, tired, calm, sexy, anxious, fatigue, relaxed, and active (see ref 25 for a full discussion of this type of scaling method). Subjects were allowed to self-interpret these adjectives. Horizontal scales, each on a separate sheet of paper,

were composed from 1, labeled “I am not at all …” to 7, which was labeled “I am extremely …” Adjectives were scored by circling a numeral, one at a time. After completing each scale, the forms were collected by the nurse so that subjects could not refer to previous ratings.

Lutenizing Hormone Levels and Pulse Detection. Each blood sample from our subjects was analyzed for LH using a standard immunoradiometric assay (IRMA; 26) by the Chemistry Core Lab of the GCRC of the Hospital of the University of Pennsylvania. **After collection, blood samples were allowed to stand on ice for 30 minutes and then centrifuged. Serum was separated and frozen at -20°C until analysis. The minimum** detectable level of LH was 0.1 mIU/ml of blood. Analyses of **serum** samples from groups of 3 to 6 subjects were run at different times during the course of this study. A coefficient of variation (CV) for the assay was calculated for each “batch” of samples by analysis of a pooled serum sample doped with standards. The CV ranged from 2.8 to 12.3% during the course of this study.

The location, amplitude and frequency of the LH pulses in the data were determined using the pulse detection software “ Cluster Analysis” of Urban et al. (27). With this program, significant pulses were detected using 2x1 cluster sizes (2 test samples for a nadir and 1 for a peak) and t values of 2x2 to minimize false positives and negatives in the pulse detection. In addition to these parameters, we employed 0.75, 1.0, and 1.5 mIU/ml as minimum peak sizes for a LH pulse. All analyses yielded similar results. The results using 1.5 mIU/ml as a minimum peak size for a pulse are presented here.

Data Analysis. The mean LH levels, LH pulse amplitude, LH pulse frequency and latency to next LH pulse in the control and extract periods of the follicular phase were compared using repeated measures analysis of variance for each variable, independently. Sequence of applications formed the independent groups factor; all others were repeated measures. Latency to the next LH pulse after an application also was analyzed with the nonparametric Wilcoxon Signed Ranks Test. Possible diurnal effects were evaluated with paired-samples t-tests while ignoring type and order of stimulus application.

Categorical mood ratings were analyzed with the nonparametric Sign Test or the Wilcoxon Signed Ranks Test. Prior to analyses of mood ratings, the two ratings in each phase (Male Extract and Control) were

compared with the Sign Test to determine whether there were any significant differences between ratings within each experimental condition: There were none so the two ratings in each phase of the experiment were averaged for subsequent analysis.

Results

Eighteen ovulating, heterosexual women completed the protocol. The ages of the women ranged from 25-45 years.

Post-hoc interviews with all subjects demonstrated that none correctly guessed the true nature of the stimuli that were being delivered to their nasal region. All subjects perceived "alcohol" and/or "fragrance." None guessed that sweat/axillary-secretion extracts had been applied when it was revealed to them at the end of the interview. No changes in menstrual cycle length attributable to our 6-hour application of extract were noted.

Figure 1 presents examples of the pulse patterns seen in our female recipients. Relative to the average latency to the next pulse during control applications (59 ± 5 min), male axillary extracts shortened the time to the next LH pulse by an average of 20% (to 47 ± 5 min; see Figure 2). Since our study was a repeated measures design, the error bars in Figure **2** can be misleading. Hence, average latency difference scores were calculated by subtracting the average latency to the next pulse during control applications from the average latency to the next pulse during application of male extract. These difference scores are seen in Figure 3.

The reduction in time between exposure to the extract and the onset of the next LH pulse was most effective following the first two applications (see Figure **4**) and suggests that there may be a refractory period following repeated exposures to male stimuli. Relative to the control condition, male extract did not affect the average number of LH pulses, the average LH pulse amplitude, or the basal level of LH (see Table 1).

We also noted significantly greater average LH pulse heights in the second 6-hour interval (afternoon to early evening [2 pm-8 pm]; 4.51 mIU/ml) than in the first interval (morning; 3.85 mIU/ml; $t_{(17)} = 5.18$, $p < 0.001$) and significantly higher basal LH levels in the afternoon (3.10 mIU/ml) than in the morning (2.80 mIU/ml; $t_{(17)} = 3.44$, $p < 0.005$), both of which appear to agree with published findings that LH levels in the

afternoon are elevated, relative to the morning (28). The average number of pulses in the morning (4.3) was not different from the number in the afternoon (4.2). We also examined the possibility that a diurnal influence might be responsible for the decreased latency to the next LH pulse that was observed subsequent to application of male extract. We ignored the type of stimulus application and compared the latencies to the next LH pulse after an application in the first 6 hours (8 am-2 pm) to the latencies noted during the next 6 hours (2 pm-8 pm). The results of the analysis of variance indicated that there was no significant difference between the morning (latency = 55 ± 5 min) and afternoon (latency = 51 ± 4 min) sessions ($F_{(1,17)} = 1.75$, $p > 0.20$).

Women were less tense (exact, 2-tailed, significance = 0.012) and more relaxed (exact, 2-tailed, significance = 0.022) during exposure to the male extract, relative to the control stimulus (Figure 5). No other effects on mood were noted. Mood ratings also were reanalyzed to determine whether there was a diurnal effect: There was none. The effects on moods appear to be specific to the male extract: A woman nurse was in close contact with the subject throughout the experiment yet the mood effect was noted only during application of the male extract.

Discussion

Our data are the first evidence that male axillary extract causes neuroendocrine and mood alterations in women. Male extract brings on a subsequent LH pulse sooner than it would occur under endogenous rhythms, suggesting that one or more extract components influence the GnRH pulse generator. In non-human mammals, studies have demonstrated that conspecific chemosignals may cause a rapid increase in LH. Although the neuroendocrine response in women was not like that of female rodents (29), it did rival that seen in goats. In ovariectomized female goats, which had been primed with estradiol to suppress the GnRH pulse generator, exposure to the chemical signals present on the hair of male goats decreased the intervals between LH pulses by about 10%, relative to control exposures, during 4 hours post exposure (30). In contrast to these effects in female goats, under normal cycling conditions in women, we observed a decrease in latency of 20%: an effect that was two-fold greater than noted in animals that had been hormonally manipulated to maximize an effect.

This study presents a potential mechanism by which male axillary secretions may alter menstrual cycle length and timing. The change in LH pulsing is predominantly seen during the first 2 applications of extract and suggests that the male stimulus may only have a transient effect upon the GnRH pulse generator. In addition to the noted endocrine change, which may be accurately described as a primer pheromone effect, the mood data, demonstrating a lessening of tension and increased feeling of relaxation, suggest the presence of a modulator pheromone. Whether these two effects are mediated by the same or different compounds, or sets of compounds, is unknown. No other behavioral effects were noted as a result of extract application.

All subjects perceived that “alcohol” or “fragrance” was being applied to them during their stay in the GCRC; none guessed the true nature of the stimuli or the delivery sequence. This suggests that conscious awareness of body odor is not necessary to elicit the primer and modulator pheromonal responses we observed.

LH pulse maxima (heights) during extract application were significantly greater relative to control applications in the afternoon and early evening. Further, LH pulse maxima during afternoon control applications were greater than both morning control and morning extract maxima. These results suggest a diurnal shift in LH pulse maxima. LH has been reported to increase to maximal levels during the11 am to 5 pm time-frame (28); this time encompasses 3 hr in our morning/afternoon session (8 am-2 pm) and 3 hr in our afternoon/early evening session (2 pm-8 pm). Alternatively, male extract may have a “lingering effect,” which is reflected as higher levels of LH during afternoon applications of the control stimulus. Male extract may "uncouple" the controlling effect that endogenous opiates have upon the GnRH pulse generator. In this regard, its action appears similar to a small, acute dose of an opiate antagonist, such as naloxone. In the follicular phase of the human menstrual cycle (31), as well as in oviods (32), chronic administration of opiate antagonists raises tonic LH levels. As reported above, we may have observed this in subjects receiving male extract; however, we cannot say with certainty that a diurnal effect is not responsible for the elevated LH. Future studies should attempt to resolve this question by employing the same 12-hour protocol reported herein and exposing subjects to the control stimulus every 2 hours for the entire 12 hours.

Other studies that have examined the effects of male and female axillary secretions upon the menstrual cycle have relied upon changes in cycle length as a measure of activity. The results reported here present a potential neuroendocrine measure of extract activity and a mechanism by which axillary secretion components may change cycle length. A periodic acceleration of LH pulses might be expected to occur during intimate heterosexual encounters as part of a stable heterosexual relationship. This pattern may ultimately result in a greater frequency of pulses and entrain the pulse generator as well as stimulate ovarian follicles with a subsequent increase in tonic estrogen levels; this increase has been demonstrated in domestic ruminants (33, 34), and is suggested in data from humans, where plasma estrogen levels in women having regular, weekly heterosexual intercourse are higher than in women having infrequent or no heterosexual intercourse (35).

The recent study by Shinohara and co-workers (12) describes a reduction in LH pulse interval in women exposed to the follicular phase secretions of women donors and an increase in the LH pulse interval in women exposed to ovulatory phase secretions. These results suggest that female axillary secretions affect other women; however the effects are different from those of male axillary secretions used in our study. Aside from the first pulse after application, we did not observe a significant increase or decrease in pulse intervals across the time we monitored LH. There are, however, significant methodological differences between the studies. The application schedule and protocol of Shinohara et al. differed from ours. These investigators applied their stimuli every 30 minutes; we applied every 2 hr. In addition, they did not use each subject as her own control as we did. In their study, women were randomly assigned to receive isopropanol (control), follicular phase secretions, or ovulatory phase secretions. The latter two were presented on axillary pads moistened with isopropanol swiped on the upper lip; we used an ethanol extract of axillary pads. Despite these differences, these results support and strengthen the impact of our data: 1) axillary secretions from women affect the LH pulses of other women; and 2) the changes seen in LH pulses are of the same magnitude as those seen by us. Shinohara et al. reported that follicular phase secretions decrease LH pulse interval by 28% and ovulatory phase secretions increase pulse intervals by 16%; we noted a decrease in the latency to the next LH peak by 20%. Shinohara and coworkers did not examine this parameter. Based upon earlier studies by the same authors (36,

37), they propose androstenol (5α-androst-16-en-3α-ol) as the axillary constituent that is responsible for the change in LH. One of these earlier studies reported that women who exhibited menstrual synchrony had lower olfactory thresholds to androstenol (36). In another study (37), women exposed to 2.5 mM androstenol in isopropanol (applied above the upper lip hourly for 4 hours) experienced an increase in their LH inter-pulse interval vs. controls. The level of androstenol applied by these investigators to their subjects (300 ul of the 2.5 mM solution; personal communication to G.P.) far exceeds endogenous levels, reported to be 4-10 ng in male axillae (38, 39). Furthermore, at the level used, the odor of androstenol would have been obvious to the recipients; no control odor was used to exclude the possibility that the observed results were spurious due to a novel odor.

Studies by Jacob and McClintock (5) utilized putative “human pheromones” reported to “stimulate the (human) vomeronasal organ” (40, 41; however, see 42 for a review of experimental evidence for and against the existence of a functional, human VNO). These compounds are steroids, but only one, viz., Δ4,16-androstadienone, has been reported in the axillae, and then in picomolar quantities (38), which are far lower than the levels used by Jacob and McClintock (~ 9 nanomoles per recipient; ref. 5); the other compound, estra-1,3,5(10)tetraen-3-yl acetate, is a synthetic compound, never reported in the axillae. These investigators (5) used such high levels (~1000x greater than endogenous concentration) because they wished to increase the compound’s concentration near the pits of the “vomeronasal organ.” Previous studies from our laboratory have not revealed the presence of this steroid in axillary extracts produced by the extraction techniques we employed in the current study (39). Consequently, although Jacob and McClintock report modulator pheromone activity for androstadienone when used at non-physiological concentrations, we do not speculate on it or any other compound’s role in our study.

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Figure Legends

Figure 1. Shows LH pulse patterns in four representative subjects. The times of control (C) and extract (E) applications are indicated as are the LH peak tops (*). **As described above, all LH pulses (peak tops) have been objectively determined by the "Cluster Analysis" software (27).**

Figure 2. Average latency to the next LH peak subsequent to the application of male axillary extracts (Extract; 47 ± 5 min), applied three times, spaced by 2 hours each, or subsequent to the application of the control solutions (Control; 59 ± 5 min), also spaced by 2 hours each. In an analysis of variance, the main effect of stimulus type on latency to the next pulse was significant ($F_{(1,16)} = 28.34$, $p < 0.001$).

Figure 3. Average latency difference (in minutes) generated by subtracting the average latency to the next LH peak subsequent to the application of the control stimulus from the average latency to the next LH peak subsequent to the application of male axillary extracts. Sixteen of the 18 women had an average latency to the next LH pulse that was shorter in the extract condition than in the control condition; 1 woman had latencies that were equivalent in both conditions (Wilcoxon Signed Ranks Test = 3.54; 2-tailed $p < 0.001$).

Figure 4. Minutes to the next LH peak after each application of male axillary extracts (Male Extract), spaced by 2 hours each, or after application of control solutions (Control), also spaced by 2 hours each. In an analysis of variance, the main effect of application number on latency to the next pulse was significant ($F_{(2,32)} = 4.59$, $p < 0.02$). Order of presentation was not a significant factor ($F_{(1,16)} = 0.61$, $p > 0.44$) and did not enter into any significant interaction with other factors ($p > 0.60$ for all F values).

Figure 5. Mood ratings were analyzed with nonparametric statistics, hence, we present the median ratings for tension and relaxation during the control and male extract phases. Responses could range from 1 ("I am not at all tense/relaxed") to 7 ("I am extremely tense/relaxed"). Scales of this type are routinely used to collect such data (43, 44). Complete data for tension were available for 17 women; 16 had ratings of tension while exposed to the male extract that were equal to or lower than their ratings of tension while exposed to the control stimulus, which is unexpected in a random distribution (Wilcoxon Signed Ranks Test = 2.61; 2-tailed $p = 0.009$). These same women also accounted for the increased ratings of relaxation, although data were available

for all 18 women. The other mood ratings were not significantly different between control and male extract conditions.

Table 1. Number of LH pulses and blood levels of LH during exposure to male axillary extract or control solutions and a statistical evaluation of any difference between them.

Parameter	**Male Extract**	**Control**	***t*-value**	**p-value**
Average Number of LH Pulses	4.3	4.2	0.21	> 0.80
Average LH Pulse Amplitude (mIU/ml)*	4.3	4.1	0.94	>0.30
Basal LH Level Over 6 Hours (mIU/ml; includes pulses)*	2.9	3.0	0.70	>0.40

*Parameter was affected by the order of stimulus delivery; a diurnal effect was noted where morning values were lower than afternoon values.

Figure 1

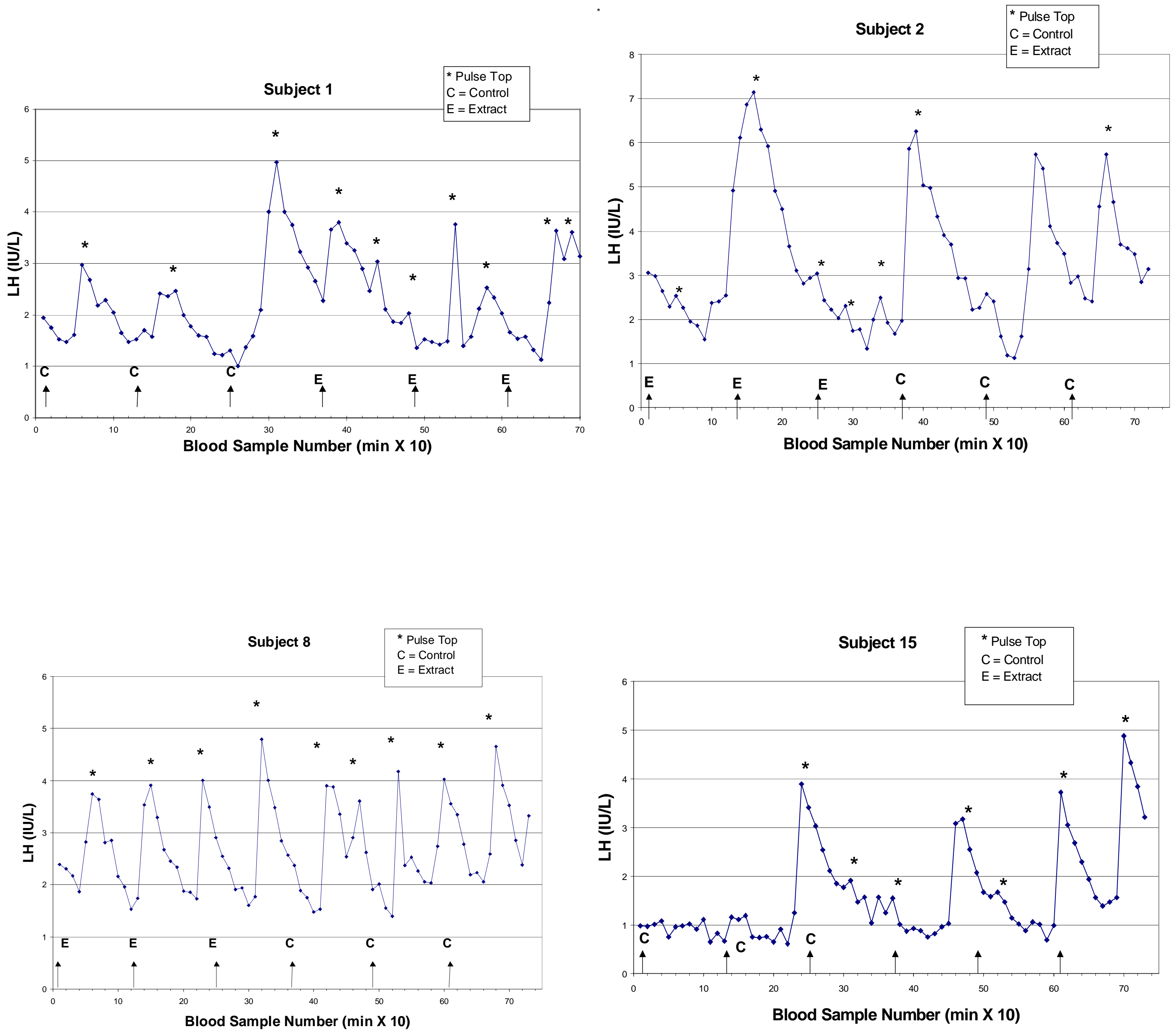

Figure 2.

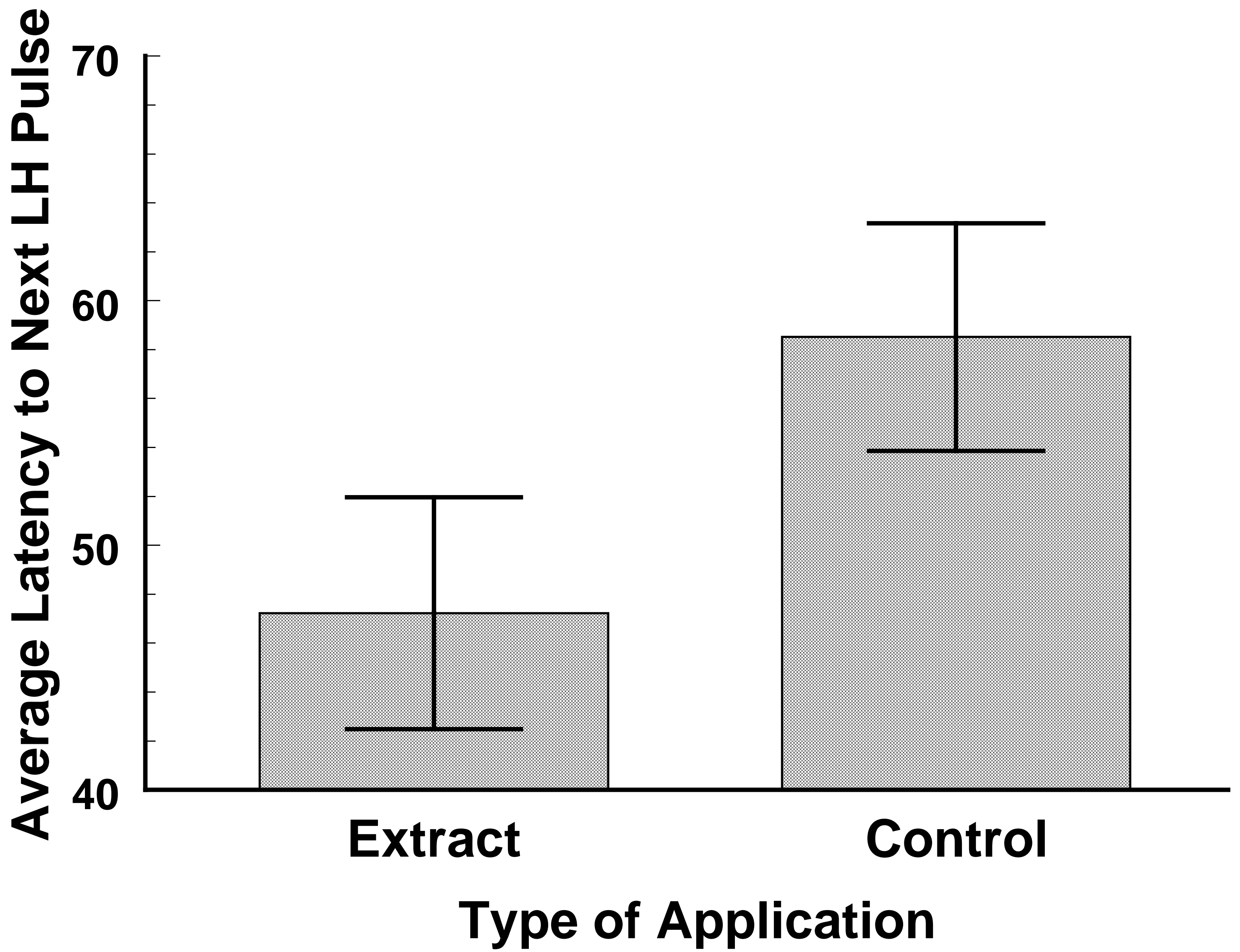

Figure 3.

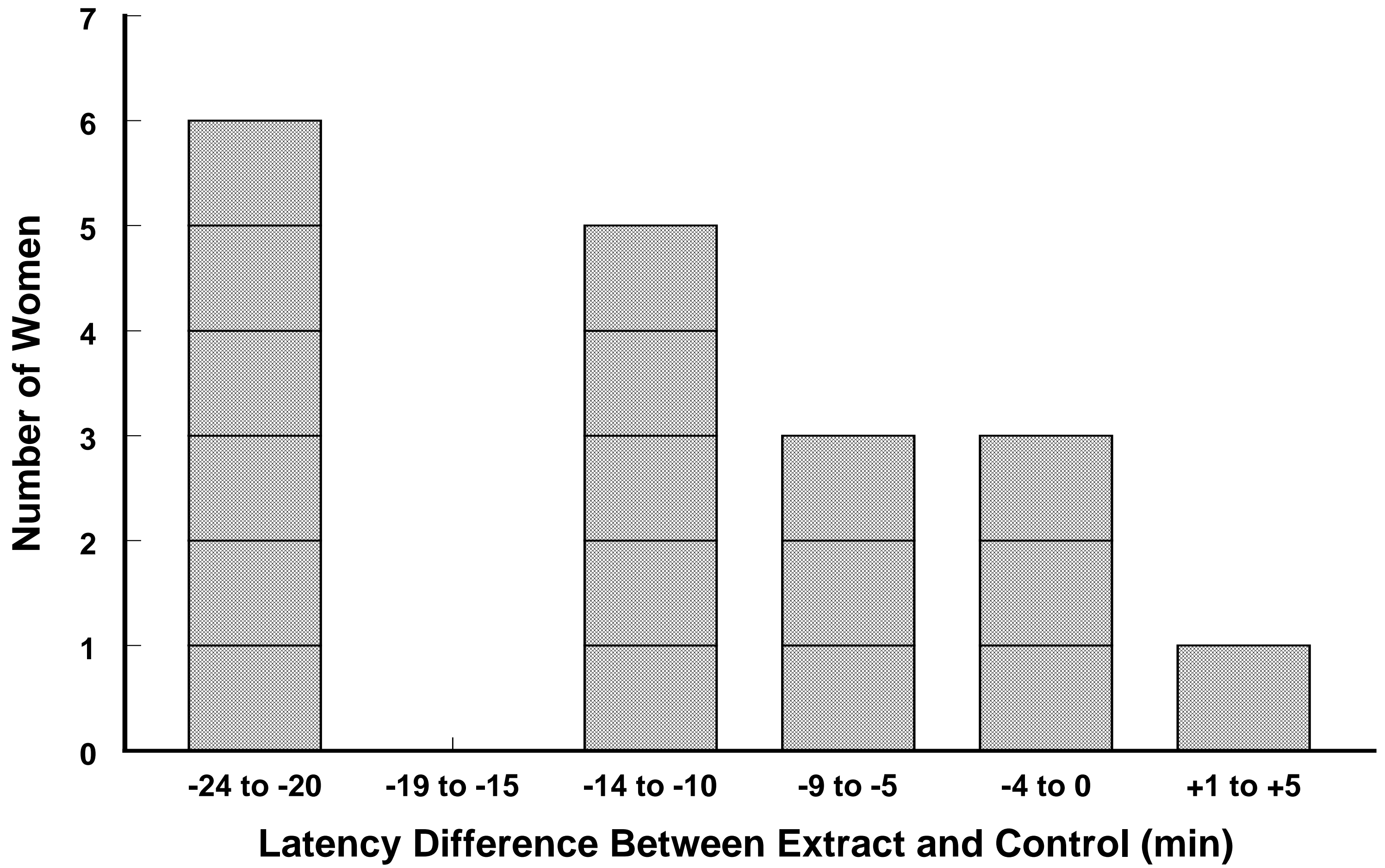

Figure 4.

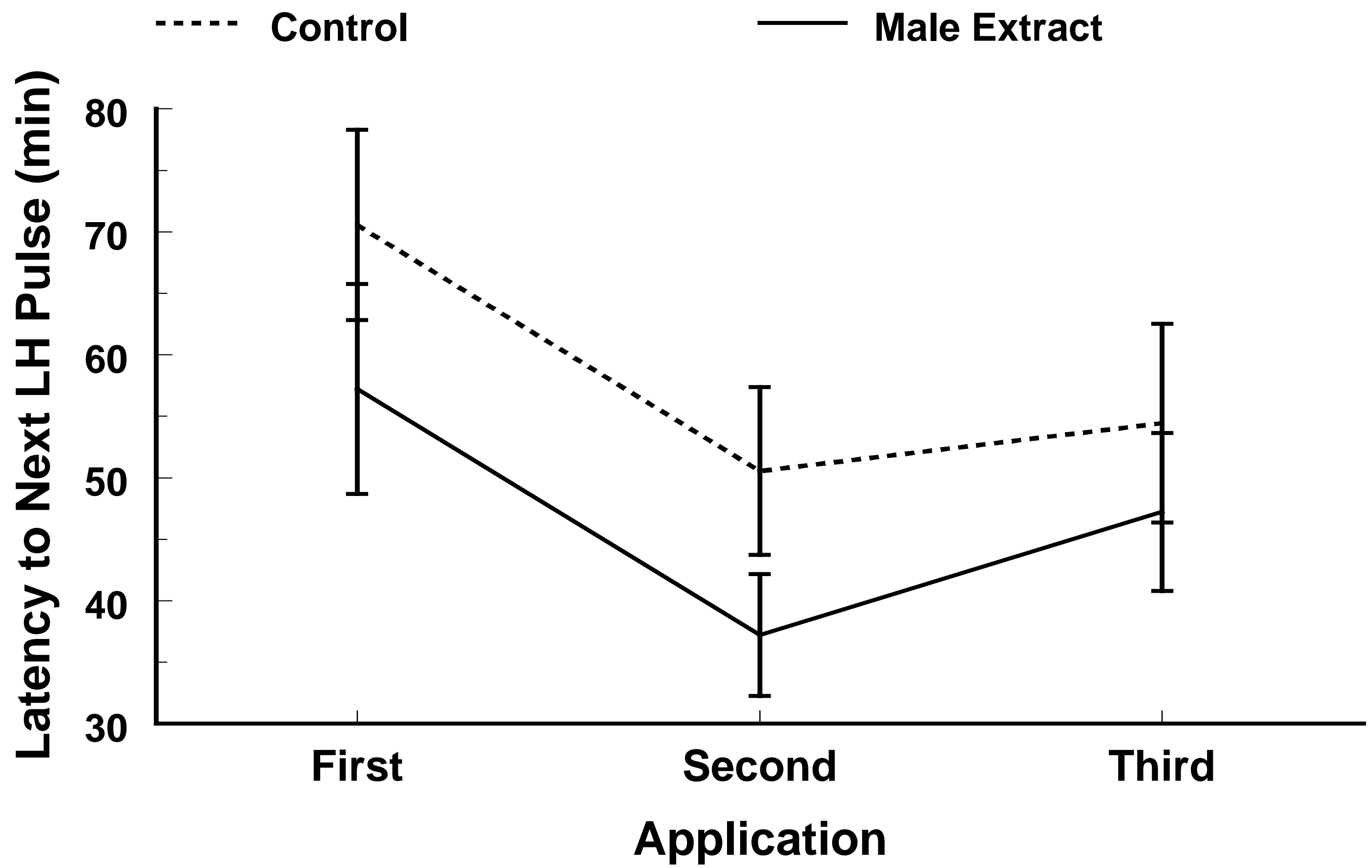

Figure 5.