



# Premenstrual enhancement of snake detection in visual search in healthy women

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It is well known that adult humans detect images of snakes as targets more quickly than images of flowers as targets whether the images are in color or gray-scale. When such visual searches were performed by a total of 60 adult premenopausal healthy women in the present study to examine whether their performance would fluctuate across the phases of the menstrual cycle, snake detection was found to become temporarily enhanced during the luteal phase as compared to early or late follicular phases. This is the first demonstration of the existence of within-individual variation of the activity of the fear module, as a predictable change in cognitive strength, which appears likely to be due to the hormonal changes that occur in the menstrual cycle of healthy women.

Mood, cognition, and social behavior may fluctuate in women across the phases of the menstrual cycle according to changes in the blood concentration levels of a variety of hormones<sup>1–3</sup>. The influence of hormonal changes caused by ovulation has become an intensive focus of research recently. Such changes are associated with predictable changes in cognitive strength in both men and women<sup>4</sup>. According to various measures, women are most attractive when they are most susceptible to becoming pregnant (right before ovulation), and least attractive when they are least susceptible to becoming pregnant (during menstruation)<sup>5,6</sup>. Likewise, men are able to recognize these cues<sup>7</sup>. Around the period when women are most fertile, men as their partners are extra protective and vigilant. In contrast to such changes in cognitive strength, spatial abilities of women are found to deteriorate according to the secretion of female hormones, especially of estradiol<sup>8</sup>.

In contrast to the substantial evidence about such influence of the ovulation phase, however, the amount of available evidence for hormonal effects during other phases of the menstrual cycle is still meager, except regarding a pathological syndrome that is known as the premenstrual syndrome (PMS), whose most common symptoms are anxiety, irritability, and nervous tension<sup>9,10</sup>. PMS occurs up to 14 days prior to menses, continues during the luteal phase, when estradiol and progesterone concentrations are high, and disappears rapidly after the onset of menses. Although only roughly 30% of premenopausal women can be diagnosed with PMS on the basis of a variety of questionnaires and prospective daily ratings<sup>11,12</sup>, it is also true that the overwhelming majority of all of healthy women experience some level of premenstrual symptoms during the luteal phase<sup>13</sup>. Given the robustness and the prevalence of this phenomenon, it is surprising that there have been virtually no reported attempts to reliably assess the influence of the premenstrual hormonal changes in healthy women behaviorally or experimentally. The establishment of a technique for such assessment would obviously be relevant to investigating the relationship between the adverse mood and behavior fluctuations experienced premenstrually by many women besides those suffering from PMS and the hormonal changes underlying those fluctuations. In order to pursue this issue, in the present study, we investigated the within-individual variation of how rapidly healthy women performed snake detection in visual search across the phases of the menstrual cycle.

It is well known that humans are extremely sensitive to biologically threatening stimuli<sup>14</sup> and that this is typically the case for their response to poisonous snakes<sup>15</sup>. Recent investigations have shown that human adults have an attentional bias for the detection of fear-relevant stimuli such as snakes compared to neutral stimuli such as flowers<sup>16–18</sup>. In those studies, typically the researchers presented adults with 3-by-3 matrices of images of fear-relevant stimuli and neutral stimuli. The images were presented either in black and white or in color. When reaction times (RTs) were measured, they were found to be significantly shorter for fear-relevant targets than for neutral targets whether the images were in color or gray-scale. More recent studies have documented that preschool children, 8- to 14-month-old infants, and even non-human primates also detect snakes more quickly



than flowers in gray-scale<sup>19–23</sup>. On the basis of their findings, the authors of those studies argued the possible evolution of a fear module in primates that enables them to experience fear of snakes.

Here, we reasoned that if heightened anxiety was experienced during the luteal phase of most healthy women, it would be reflected in the performance of visual search as the enhanced detection of snake images as targets, which are biologically relevant threatening stimuli. Namely, we hypothesized that snake images as targets would be detected more rapidly during the luteal phase of each woman as compared to other menstrual phases of the same woman.

## Results

When the collected data were analyzed by a 2 (type of the target image, TARGET) x 2 (phase of the menstrual cycle of the participant, PHASE) ANOVA (analysis of variance), both of the main effects were statistically significant in one of three participant groups (referred to as Group A below) (Fig. 1,  $F(1,19) = 398.16$ ,  $P < 0.0001$ ,  $\eta^2_p = 0.95$  for TARGET,  $F(1, 19) = 63.89$ ,  $P < 0.0001$ ,  $\eta^2_p = 0.77$  for PHASE). There was also a significant interaction between TARGET and PHASE ( $F(1, 19) = 44.36$ ,  $P < 0.0001$ ,  $\eta^2_p = 0.70$ ). Post-hoc analyses, using the least significant difference method (Scheffe's tests), revealed that the participants responded to the snake image as the target more rapidly during the luteal phase than during the early follicular phase (mean RT  $\pm$  SD = 1149  $\pm$  87 ms, and 1345  $\pm$  97 ms, during the luteal phase and during the early follicular phase, respectively,  $P < 0.0001$ ). However, RTs when the participants responded to the flower image as the target did not differ whether they were tested during the luteal phase or during the early follicular phase (mean RT  $\pm$  SD = 1603  $\pm$  86 ms, and 1634  $\pm$  91 ms, during the luteal phase and during the early follicular phase, respectively,  $P = 0.39$ ).

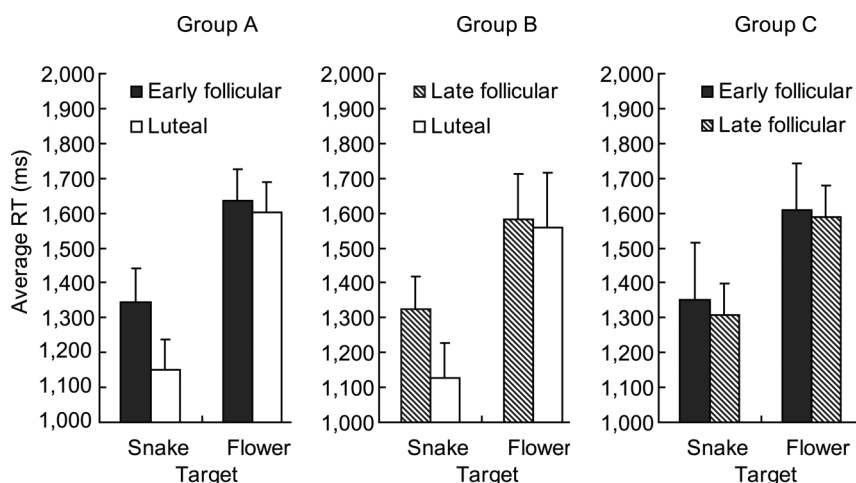
When the data collected from one of the two other groups of participants (Group B) were analyzed, both of the main effects were also statistically significant ( $F(1, 19) = 289.04$ ,  $P < 0.0001$ ,  $\eta^2_p = 0.94$  for TARGET,  $F(1, 19) = 54.39$ ,  $P < 0.0001$ ,  $\eta^2_p = 0.74$  for PHASE). Again, there was a significant interaction between TARGET and PHASE ( $F(1, 19) = 24.10$ ,  $P < 0.0001$ ,  $\eta^2_p = 0.56$ ). Post-hoc analyses revealed that the participants responded to the snake image as the target more rapidly during the luteal phase than during the late follicular phase (mean RT  $\pm$  SD = 1128  $\pm$  100 ms, and 1326  $\pm$  91 ms, during the luteal phase and during the late follicular phase, respectively,  $P < 0.0001$ ). However RTs recorded when the participants responded to the flower image as the target did not differ whether they were tested during the luteal phase or during the late follicular phase (mean RT  $\pm$  SD = 1560  $\pm$  ms, 157, and 1583  $\pm$  128 ms,

during the luteal phase and during the late follicular phase, respectively,  $P = 0.99$ ).

Concerning the data collected from the third group (Group C), the main effect of TARGET was statistically significant ( $F(1, 19) = 231.88$ ,  $P < 0.0001$ ,  $\eta^2_p = 0.92$ ). However, the main effect of PHASE was not significant ( $F(1, 19) = 1.25$ ,  $P = 0.23$ ,  $\eta^2_p = 0.06$ ). There was not a significant interaction between TARGET and PHASE, either ( $F(1, 19) = 2.36$ ,  $P = 0.14$ ,  $\eta^2_p = 0.11$ ), indicating that RTs to snake or flower images as the target, respectively, were not significantly different whether the participants were tested during the early follicular phase or the late follicular phase. Overall mean RT  $\pm$  SD when the target was a snake image was = 1330  $\pm$  131 ms, while that when the target was a flower image was = 1598  $\pm$  114 ms.

## Discussion

In accord with previous reports<sup>16–18</sup>, the present results showed that the female participants detected snakes as the targets more quickly than flowers as the targets, confirming the proposal of the snake detection theory that an evolved bias for the detection of evolutionarily relevant threatening stimuli exists in humans<sup>19–21</sup>. Moreover, the present results revealed the fact that such biased detection became relatively faster during the luteal phase in the menstrual cycle of healthy women, indicating the possibility that a fear module underlying this biased visual search behavior<sup>24,25</sup> was temporarily more activated during this phase, and presumably would continue to be so during the following phase of early pregnancy if they were pregnant<sup>26,27</sup>. This behavioral change should be quite adaptive because it could contribute to women's ability to increase their vigilance towards biologically relevant threatening stimuli around themselves during this period of possible pregnancy. This reasoning is also confirmed by field observations<sup>28,29</sup> of free-ranging nonhuman primates, which showed that adult females during the comparable endocrinological phase were likely to be more isolated from other group members and to interact with them less often. Interestingly, the authors of both of these field studies mentioned such behavioral change as a rudimentary form of PMS, though quantitative measurements of similar changes in humans are almost impossible practically. If these interpretations are correct, the present findings would provide an important step to link previous neuroimaging studies<sup>30–32</sup> to clinical work with women with PMS manifested as emotional and cognitive impairments. In all, this is the first demonstration of the existence of within-individual variation of the activity of the fear module in women, as a predictable change in cognitive strength that appears likely to be due to the hormonal changes that occur in the menstrual cycle, particularly due to increased progesterone and estradiol levels.



**Figure 1 | Experiments.** Mean reaction time (RT) of the participants of the three groups to detect snake or flower targets. Error bars represent SDs.



As for progesterone, however, a recent study<sup>33</sup> found that its administration produced mild sedative-like effects in both men and women, a finding that appears to be opposite to the results of the present study. This apparent contradiction might be related to the fact that two of the molecules metabolized from progesterone, allopregnenolone and pregnenolone, are capable of crossing the blood-brain barrier and affecting neural function<sup>34</sup>, and of increasing the activity of the neurotransmitter GABA, which has effects throughout the cerebral cortex<sup>35</sup>. Research with animals demonstrated increased anxiety upon administration of progesterone, suggesting that progesterone affects parts of the brain related to anxiety and mood<sup>36,37</sup>. Those effects taken together with the current neurological findings would be expected to be mediated by the amygdala and related to the negative mood symptoms in humans that are observed during increased allopregnanolone levels. Indeed, this issue was investigated more recently by a neuroimaging study using functional magnetic resonance imaging<sup>38</sup>. When the recruited women were tested as to whether a single progesterone administration modulated the amygdala response to angry and fearful faces as threatening stimuli, the administration was found to increase the plasma concentrations of progesterone and allopregnanolone to levels that are reached during the luteal phase and early pregnancy. The imaging results revealed that progesterone selectively increased amygdala reactivity<sup>39</sup>. Subsequent functional connectivity analyses also indicated progesterone modulation of functional coupling of the amygdala with distant brain regions.

These findings appear to be quite consistent with the results of the present study, because as the visual part of the fear module as a behavioral and neural system that enables automatic visual detection as a way for snakes to capture attention, the amygdala is regarded to be important for learning what is threatening and for responding appropriately, and to play a major role in helping mammals, including humans, survey and evaluate the environment for danger signals<sup>24–27</sup>. Therefore, increased reactivity of this neural complex located in the temporal lobe is predicted to be the neural correlate to the enhancement of snake detection observed during the luteal phase.

As for estradiol, a recent brain imaging study<sup>31</sup> reported reduced amygdala activity during the late follicular phase, which is caused by the increased level of the hormone in the blood. This fact would imply the possibility of a combined effect of high progesterone and high estradiol. Moreover, in addition to sex hormones, cortisol levels of the blood also increase during the luteal phase<sup>1</sup>. This could somehow contribute to causing women's enhanced attentional bias towards threatening stimuli. Which possibility is more plausible as an interpretation for the present results is difficult to determine, at the moment, from this experiment, in which no blood samples were collected to verify cycle phase or to correlate hormone levels to behavioral effects. Clearly, this is a key issue to be further investigated.

## Methods

This investigation was conducted according to the principles expressed in the Declaration of Helsinki. All experimental protocols are consistent with the Guide for Experimentation with Humans and were approved by the Institutional Ethics Committee of the Primate Research Institute, Kyoto University.

**Participants.** The participants were sixty naturally cycling 29- to 30-year-old single women. They were included in the present study after signing informed consent forms. They were healthy, as determined by routine physical and laboratory examinations, and had no current psychiatric disorder, as indicated by a structured interview<sup>40</sup>. They were all right-handed, were free of medication, did not use hormonal contraceptives, and reported no history of psychiatric or somatic disease potentially affecting the brain. They were not pregnant and reported no history of pregnancy. They reported that the onset of menstruation had occurred regularly with intervals of 27–29 days over at least the previous 4-month-period.

Each of the 60 participants underwent the visual search experiment (whose detailed protocols are described below) twice during the study. The first and the second testing were separated from one another by a 2- to 3-month-period for each participant. They were randomly assigned to one of three groups. In one of the three groups

(Group A), the 20 participants underwent the experiment during the early follicular phase (day 5) and during the luteal phase (day 25) of different menstrual cycles. The 20 women in one of the other two groups (Group B) underwent the experiment during the late follicular phase (day 13) and during the luteal phase (day 25) of different menstrual cycles. The remaining 20 (Group C) underwent the experiment during the early follicular phase (day 5) and during the late follicular phase (day 13) of different menstrual cycles. Concerning the menstrual cycles of the participants when the experiment was conducted, their actual cycle length was identified retrospectively, and was confirmed to be between 27 and 29 days. The order of the two different phases in which the first and second testings were to be conducted was determined randomly for a given participant in each of the three groups.

**Materials.** For each experiment, we selected 24 photographs in gray-scale for each stimulus category. In a given trial, 9 of these photographs were displayed in a 3-by-3 matrix. Each matrix contained 1 target image from one category and 8 distracter images from the category: a flower matrix would contain a snake target, and a snake matrix would contain a flower target. This yielded two combinations: a snake among flowers, and a flower among snakes. An RDT151TU (MITSUBISHI) touch-screen monitor was used to present each image matrix on a 38.1 cm (15-in.) screen. Each of the 24 images in the target category served as the target once. Each of the 24 pictures in the distracter category appeared 8 times on average; the different distracters were presented approximately the same number of times across trials. The stimulus order was created by randomly arranging the matrices.

**Procedure and analyses.** In each experiment, the participant was seated in front of the touch-screen monitor (approximately 40 cm from the base of the screen) and was told to place her hands on a set of handprints. This ensured that her hands were in the same place at the start of each trial, making it possible to collect reliable latency data. An experimenter was seated alongside to monitor and instruct her throughout the procedure.

First, a set of 9 practice trials was given to instruct her how to use the touch screen. In the first 3 trials, a display of 1 target (a puppet from an animated cartoon well known to her) and 8 distracter (another puppet, also well known) images was presented. She was asked to touch the target among distracters as quickly as possible, and then return her hands to the handprints. In the next 6 trials, the display consisted of 1 target (a snake or a flower) and 8 distracter (the other) images, and she was asked to touch only the target image. All images used in the practice trials were chosen randomly from the original sets of 24.

When the participant had learned the procedure, a series of test trials followed. The task comprised 48 trials in total ordered in 2 blocks of 24 trials. In each trial, a different image matrix containing 1 target (snake or flower) and 8 distracters (the other) was presented. Between trials, an image of a stuffed animal or a popular character appeared on the screen to keep her attention on the screen. A trial was initiated when the experimenter judged that the participant was looking at the image, causing the next matrix to appear in order to ensure that her full attention was on the screen before each matrix appeared. When the first block was over, another block began. If the first block target was snakes, the next target was flowers, or vice versa. Each participant was randomly assigned to one of 2 block orders.

In each trial, the RT of the participant was automatically recorded from the onset of the matrix to when she touched one of the pictures on the screen. The results described in the text were solely based upon analyses of the RT data collected in this manner (RTs of incorrect responses as well as extreme RT scores—defined as values more than 2 standard deviations above or below the mean relative to each participant's mean RT—were excluded from the analyses).

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## Author contributions

NM conceived of the study, and participated in its design and coordination and drafted the manuscript. MS and NM conducted the experiments, and participated in the data analysis and interpretation. Both authors read and approved the final manuscript.

## Additional information

**Competing financial interests:** The authors declare no competing financial interests.

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