

# Estrogen receptor 2 mediates intraspecific aggressive behaviors of the female *Cricetulus barabensis* in the estrous cycle

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The social behavior mechanisms have not been thoroughly reported in the solitary female striped dwarf hamster (*Cricetulus barabensis*). In this study, the handling bag test and neutral arena measurements were used to detect the changes of aggression in the face of rivals of different genders of wild striped dwarf hamsters. We found that female hamsters had the highest aggressive performance in proestrus, followed by estrus, and the lowest in metestrus and the dioestrus, and the increased aggression during the proestrus or estrus period was low-intensity aggression such as intimidation, shock, boxing and counterattack, or even ritualized non-harmful behaviors to drive away opponents. When confronted with male individuals, aggression in females decreased significantly during estrus. The concentration of plasma estradiol was the highest in estrus and the lowest in metestrus and dioestrus. In contrast, estrogen receptor 2 relative expression in the hypothalamus is the lowest in proestrus and highest in metestrus and dioestrus. Besides, both estradiol levels in plasma and estrogen receptor 2 mRNA in the hypothalamus were associated with aggression. These results will broaden our understanding of the molecular mechanism of how breeding phenotype is an essential driver in changing the social behavior of female *Cricetulus barabensis*.

## Keywords

Hypothalamus; Behavioral endocrinology; Estrous cycle; Estrogen receptor 2; Social behavior mechanisms; Rodent

## 1. Introduction

Aggressive behavior is an evolutionarily conserved, heritable trait essential for survival and fitness [1]. One possible factor determining the fitness of these different strategies is population density. A study on the natural population of *Microtus pennsylvanicus* found that female aggression levels increased significantly at relatively low densities [2]. As population density increases, social pressures may limit subordinate age and sex groups' access to concentrated food sites [3]. Populations with high diffusion efficiency tend to exhibit boldness and high aggression [4].

Aggression is an essential social behavior that promotes survival and reproductive fitness across animal systems [5]. Individuals with intense aggression can occupy a higher social class and increase the individual's fitness. However, in various intraspecific-sexual stimulation and endocrine environ-

ments, females will display ferocious aggression, which is also the result of adaptation to their living environment and long-term evolution. In a broader sense, female aggression's function seems to suppress the rivals' reproductive success [6]. While research on the neuroendocrine mechanisms underlying this complex behavior has traditionally focused on the classic neuroendocrine model, circulating gonadal steroids are transported to the brain and act on neural circuits closely related to aggression [5].

Estradiol is the primary circulating estrogen hormone and can regulate many tissue functions in physiology. The estradiol receptor, estrogen receptor 2 (*ESR2*), which was identified in 1996 [7], is proven to be critical in mediating estradiol signaling in the ovary, prostate, lung, cardiovascular and central nervous systems [8]. It is predominantly found with high concentrations in the hippocampus and cerebral cortex in rodents and humans [9]. Studies have reported that *ESR2*-knockout mice were more aggressive than wild-type mice, indicating *ESR2*-mediated actions of gonadal steroids are critical in inhibiting the aggressive behaviors of pubertal and young adult mice [10]. Also, as aggression is an energy-consuming physiological behavior, monitoring mice's metabolic rate may reflect fitness-related behavioral traits (e.g., estrus) in different animal models.

Differences between results from different settings emphasize the role of environmental and social factors in modulating hormones' effects [11]. However, recent studies have suggested that this paradigm is oversimplified [1, 3, 12]. Laboratory research has described detailed descriptions of aggression and defense patterns in the rat, mouse, and hamster. These results showed many similarities and differences between these species [12]. It is still necessary to provide more materials to explore the regulation mechanisms of periodic changes in aggressive female behaviors. Therefore, our study aims to clarify the relationship between intra-species aggressive behaviors and the plasma estradiol concentrations or relative expression of *ESR2* mRNA and offer new experimental data to explain how breeding phenotype changes social behavior.

## 2. Methods

### 2.1 Animals

Live traps caught wild striped dwarf hamsters from the Qufu region in the Shandong Province of China (35° 46' N 117° 0' E). The region belongs to a continental monsoon climate, with four distinct seasons and noticeable temperature changes with the seasons. The primary vegetation is wheat, corn, peanut and other crops. Animal sampling was completed in the spring of 2016.

After capture, hamsters were domesticated in an animal house at the School of Life Science, Qufu Normal University. Polyethylene (PE) plastic feeding boxes were raised in single cages (290 × 188 × 125 mm). Food (standard rat chow, Jinan Pengyue experimental animal breeding Co. LTD) and water were provided *ad libitum*. All animals were housed in a pathogen-free environment and were provided free access to food and water under a 12-hour light/dark cycle in the testing center. After two weeks of acclimatization, 60 adult female hamsters and 20 males (20–30 g) were selected for the study. Specifically, 10 female hamsters were used to determine female hamster's estrous cycle by vaginal smears, and the other 50 female hamsters were used for the aggressive behavior assessment. All procedures used in this study were approved by the Institutional Animal Care and Use Committee of Qufu Normal University. All efforts were made to minimize the number of animals used and animals' suffering in accordance with the ARRIVE guidelines.

### 2.2 Determination of the estrous cycle

First, the stage of the estrous cycle can be roughly judged by vulva observation. 10 adult female hamsters were continuously for 16 days. Vaginal smears were used to determine the exact estrous cycle according to the type of cells and the percentage of keratinocytes in the vaginal smear. The time points of the behavioral and physiological tests were determined. Under laboratory domestication conditions, female hamsters have a stable estrous cycle, generally about 4 days. Each estrous cycle goes through four stages. Therefore, the following points are selected for the follow-up behavioral and physiological experiments: Day1 (20:30~21:30, proestrus), Day2 (18:30~20:00, estrus), Day3 (13:00~14:30, metestrus), Day4 (16:00~17:30, dioestrus).

### 2.3 Quantification of aggressive intraspecific behavior

The aggressive behavior of hamsters is determined by handling bag test and neutral arena measurements. The handling bag test is used as an index to measure rodents' docility/ferocity [13]. In this study, the other 50 hamsters were used to assess female hamsters' aggressive behaviors at each estrous stage. After the hamster was transferred to a mesh bag, the length of the animal biting and struggling for 1 min was counted as a measure of its ferocity [13, 14].

Intraspecific aggression of female hamster was observed in 15-minute paired encounters in a neutral arena. A transparent plexiglass observation box was set up in the experiment, with a size of 40 cm × 40 cm × 40 cm. The obser-

vation box was divided into two same parts. The bottom of the box is paved with sawdust as cushion material. A red incandescent lamp and the video monitoring system are suspended 1.5 m above the arena for behavioral observation. In this test, same-sex or different-sex aggressive encounters of female hamsters were staged, recorded and analyzed. Female hamsters in different estrous cycles were placed on one side of the neutral arena. A weight difference of less than 5% female or male hamster based on the weight matching principle was placed on the other side of the neutral arena [15]. After 2–3 minutes of adaptation, the partition was removed, and the encounter behavior was recorded in the night vision mode for 15 minutes. The experiments in female vs female and female vs male groups were carried out alternately. The same hamsters were used discontinuously for the target female hamsters. For the target female hamster, the same opponent hamster is used discontinuously. Numbers and durations of threat, plunge, boxing, hit back, bites, wrestle, as well, as chases were quantified using our suite for monitoring aggressive behaviors.

Meanwhile, numbers and durations of escape, flee, good defensive and side defensive were named defensive behavior [16, 17]. Social behaviors include the numbers and durations of investigation, approach, intimacy, and grooming. Video analysis adopts focus sampling. JWatcher 1.0 software is used to input detailed behavioral indicators.

### 2.4 Resting metabolic rate (RMR)

RMR of the females was qualified as the rate of oxygen consumption, using an open-flow respirometry system (Q-Box RP2LP, Qubit, Canada). Each animal was placed individually in a 650 mL Plexiglass cylindrical metabolic chamber. The chamber temperature was controlled at 29 °C, which lies within the thermal neutral zone for *C. barabensis* [18]. Body mass was recorded before and after each measurement. Air was pumped at a 490–650 mL/min rate through a cylindrical sealed Perspex chamber with rubber stoppers, immersed in a water bath ( $\pm 0.5$  °C). Gases leaving the chamber were dried (silica gel) and directed through CO<sub>2</sub> and the O<sub>2</sub> analyzer at a 145–150 mL/min flow rate. The data were averaged and collected every 10 s (within 0.5 h), and the lowest rate of oxygen consumption over 5 min was taken to estimate RMR. Because animals were probably not post-absorptive during the measurement, we classified metabolic measurements as RMR rather than the basal metabolic rate (BMR). Thus, like many recent metabolic studies on small, wild-caught endotherms [19, 20], our RMR measures include a non-quantified metabolic contribution from the heat increment of feeding.

### 2.5 Blood sampling, estradiol quantification and tissue collection

After the behavioral and physiological experiments, 15 hamsters in each estrous cycle were killed by CO<sub>2</sub> asphyxiation. Blood samples were processed, and plasma estradiol concentrations were quantified using a commercially available enzyme immunoassay system (Multiskan MS 352). Hy-

pothalamus were removed and frozen in liquid nitrogen, and then stored at  $-80^{\circ}\text{C}$  for the subsequent fluorescence quantitative PCR experiments.

## 2.6 Total RNA extraction and real-time fluorescent quantitative PCR

Total RNA was isolated from the hypothalamus according to Trizol Reagent Kit (Takara) instructions. The purity was assessed by the OD260/OD280 value using ultraviolet spectrophotometer (Germany Eppendorf). The integrity was detected by agarose gel electrophoresis. RT-PCR amplification was performed in triplicate using an SYBR Green PCR Reagent system, with  $\beta$ -actin as an internal control. The primer sequences are listed below: *ESR2*: F: GAGCATCCCTCTTTGAACT, R: CTCTCCTTTAGCAACCCATT (5'-3');  $\beta$ -actin: F: GAGACCTTCAACACCCAGC, R: ATGT-CACGCACGATTTCCTCC (5'-3'). The fabrication of the standard curves, the product specificity and the validation of amplification efficiency are described previously [21].

## 2.7 Statistical analysis

SPSS 20.0 (SPSS Inc., Chicago, USA) and GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA) were used for data processing and analysis. Shapiro-Wilk and Levene, respectively, were used to test data normality and homogeneity of variance. One-way analyses of variances (ANOVAs) were used to examine the estrous cycle's effects on aggression variables, RMR, circulating estradiol and *ESR2* relative expression. Tukey's HSD posthoc analyses were conducted to analyze the interaction of photoperiod and week. Pearson correlations were used to quantitatively assess relationships between aggression and estradiol concentrations or *ESR2* relative expression. Data are expressed as means  $\pm$  standard error of the mean (means  $\pm$  SEM).  $P < 0.05$  indicate a statistical difference.

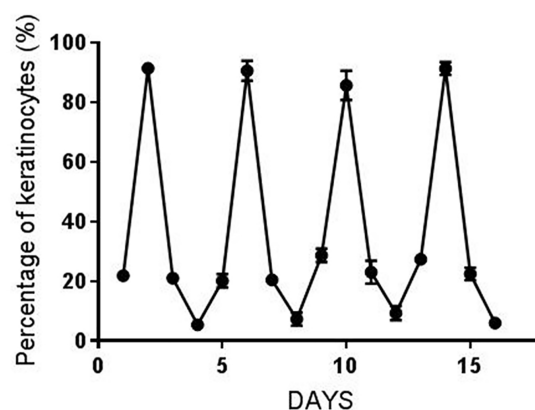
# 3 Results

## 3.1 The estrous cycle of hamster

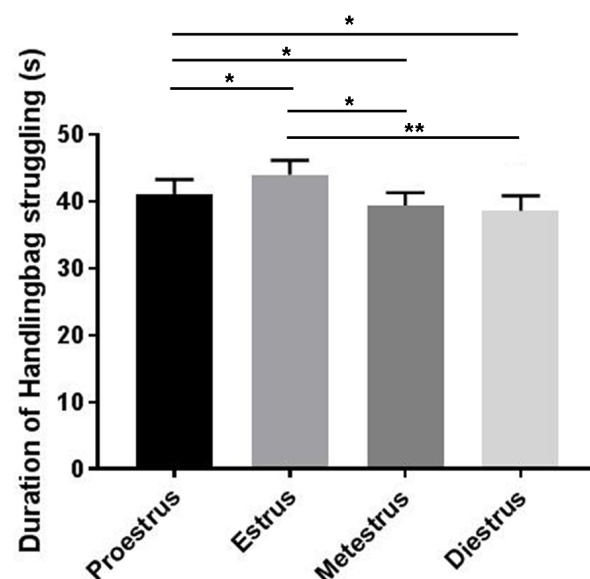
The percentage of keratinocytes in the vaginal smear shows regular changes. The time of proestrus and estrus was relatively short, while the metestrus period was the longest (Fig. 1). The duration of the whole estrous cycle may fluctuate irregularly. This fluctuation has a certain compensation; if the previous estrous cycle is prolonged, the next estrous cycle will be shortened. This change is mostly caused by the extension or shortening of the metestrus.

## 3.2 Duration of handling bag struggling

In different stages of the estrous cycle, the female hamsters showed extreme differences in the duration of biting and struggling within 1 min ( $F_{(3,156)} = 23.433$ ,  $P < 0.001$ ). Among them, the duration of biting and struggling in estrus was significantly higher than that in proestrus. The duration in proestrus was significantly higher than that in metestrus and dioestrus (Fig. 2).



**Fig. 1. The vaginal smear shows changes in the estrous cycle of *Cricetulus barabensis*.** Tukey's HSD posthoc analyses indicated that the percentage of keratinocytes have significant differences between the days of 1d, 5d, 9d and 13d ( $F_{(3,36)} = 4.471$ ,  $P = 0.009$ ), and they were all in the proestrus range. However, there was no significant difference between the days of 2d, 6d, 10d and 14d ( $F_{(3,36)} = 0.707$ ,  $P = 0.554$ ), also the days of 3d, 7d, 11d and 15d ( $F_{(3,36)} = 0.221$ ,  $P = 0.881$ ), and the days of 4d, 8d, 12d and 16d ( $F_{(3,36)} = 0.812$ ,  $P = 0.596$ ). Data are expressed as means  $\pm$  SEM. Significance was determined by ANOVA, followed by Tukey's HSD posthoc analyses.



**Fig. 2. Duration of handling bag struggling in the estrous cycle of *Cricetulus barabensis*.** The duration of biting and struggling in estrus was significantly higher than that in proestrus, and the duration in proestrus was significantly higher than that in metestrus and dioestrus. Data are expressed as means  $\pm$  SEM. \*:  $P < 0.05$ , and \*\*:  $P < 0.01$ .  $P < 0.05$  indicates a significant difference. Significance was determined by ANOVA, followed by Tukey's HSD posthoc analyses.

## 3.3 Behavior changes in a neutral arena

In the neutral arena, the attack time and numbers of female *C. barabensis* on female and male hamsters showed significant difference in estrous cycle ( $F_{(3,196)} = 8.2$ ,  $P < 0.001$ ;

$F_{(3,196)} = 5.236, P = 0.002$ ;  $F_{(3,196)} = 6.491, P < 0.001$ ;  $F_{(3,196)} = 5.021, P = 0.002$ ). The attack time was highest at proestrus when placing a female with a female or a male, while the attack time was higher at estrus than metestrus and diestrus when placing a female with another female (Fig. 3A). Interestingly, there was no significant difference in attack time among estrus, metestrus, and diestrus when placing females with males (Fig. 3A). The attack number was highest at proestrus when placing a female with a female or a male. The attack number was lower at estrus versus proestrus, but estrus was higher than metestrus and diestrus when placing a female with another female. Simultaneously, there were no significant differences in attacking numbers at estrus, metestrus and diestrus when placing a female with another male (Fig. 3B).

Moreover, the defense time and numbers of female *C. barabensis* against females and males were shown significant differences in the estrous cycle ( $F_{(3,196)} = 2.721, P = 0.046$ ;  $F_{(3,196)} = 4.206, P = 0.07$ ;  $F_{(3,196)} = 4.178, P = 0.07$ ). The defense time was lowest at proestrus when placing a female with a female, while it was highest at estrus when placing a female with a male (Fig. 3C). Similarly, the defense number was highest at estrus when placing a female with a male. However, there was no significant difference in defense number at four estrous cycle stages when placing females with females (Fig. 3D).

Also, the social behavior duration time and numbers of female *C. barabensis* with male showed significant differences in the estrous cycle ( $F_{(3,196)} = 5.538, P = 0.01$ ;  $F_{(3,196)} = 4.706, P = 0.03$ ). The social behavior duration time and number were highest at estrus when placing a female with a male. Simultaneously, there were no significant differences in the social behavior duration time and number at the other three stages of the estrous cycle when placing a female with a male (Fig. 3E-F). The social behavior duration time and number were shown no differences at four estrous cycle stages when placing a female with a female (Fig. 3E-F).

### 3.4 RMR

RMR of female hamsters in different stages of the estrous cycle showed significant differences ( $F_{(3,196)} = 12.897, P < 0.01$ ). Among them, RMR in estrus was significantly higher than that in the other three stages, and in metestrus was significantly lower than that in the other three stages. However, there was no significant difference between the stages of proestrus and dioestrus (Fig. 4).

### 3.5 The levels of estradiol in plasma and ESR2 in hypothalamus among estrous cycle

The estradiol levels in the plasma of female hamsters showed significant differences in different estrous cycle stages, and the significance was determined by ANOVA, followed by Tukey's HSD posthoc analyses. Among them, the content in estrus is the highest ( $P < 0.05$ ), and the proestrus was also significantly higher than the metestrus and the dioestrus ( $P < 0.05$ ). Estradiol content was lowest in the

metestrus and the dioestrus, and there was no significant difference between the two stages (Fig. 5). However, there were significant differences in the relative expression of *ESR2* in the hypothalamus of female hamsters at each stage of the estrous cycle. Among them, the relative expression in the metestrus is the highest, and the expression in the proestrus is significantly lower than that in the other three periods.

### 3.6 Relationships between aggression and plasma estradiol or relative expression of *ESR2*

Pearson correlations were performed to assess relationships between aggression and estradiol concentrations or *ESR2* relative expression. It showed that the levels of aggression were near related to plasma estradiol levels and the *ESR2* mRNA in the hypothalamus in female *C. barabensis*. Moreover, the plasma estradiol level displayed positive associations with the handling bag struggling time, attack time vs female, and RMR. In contrast, relative expression of *ESR2* mRNA in hypothalamus females displayed negative associations with handling bag struggling time, attack time vs female, attack time vs male and attack number vs male (Table 1).

## 4. Discussion

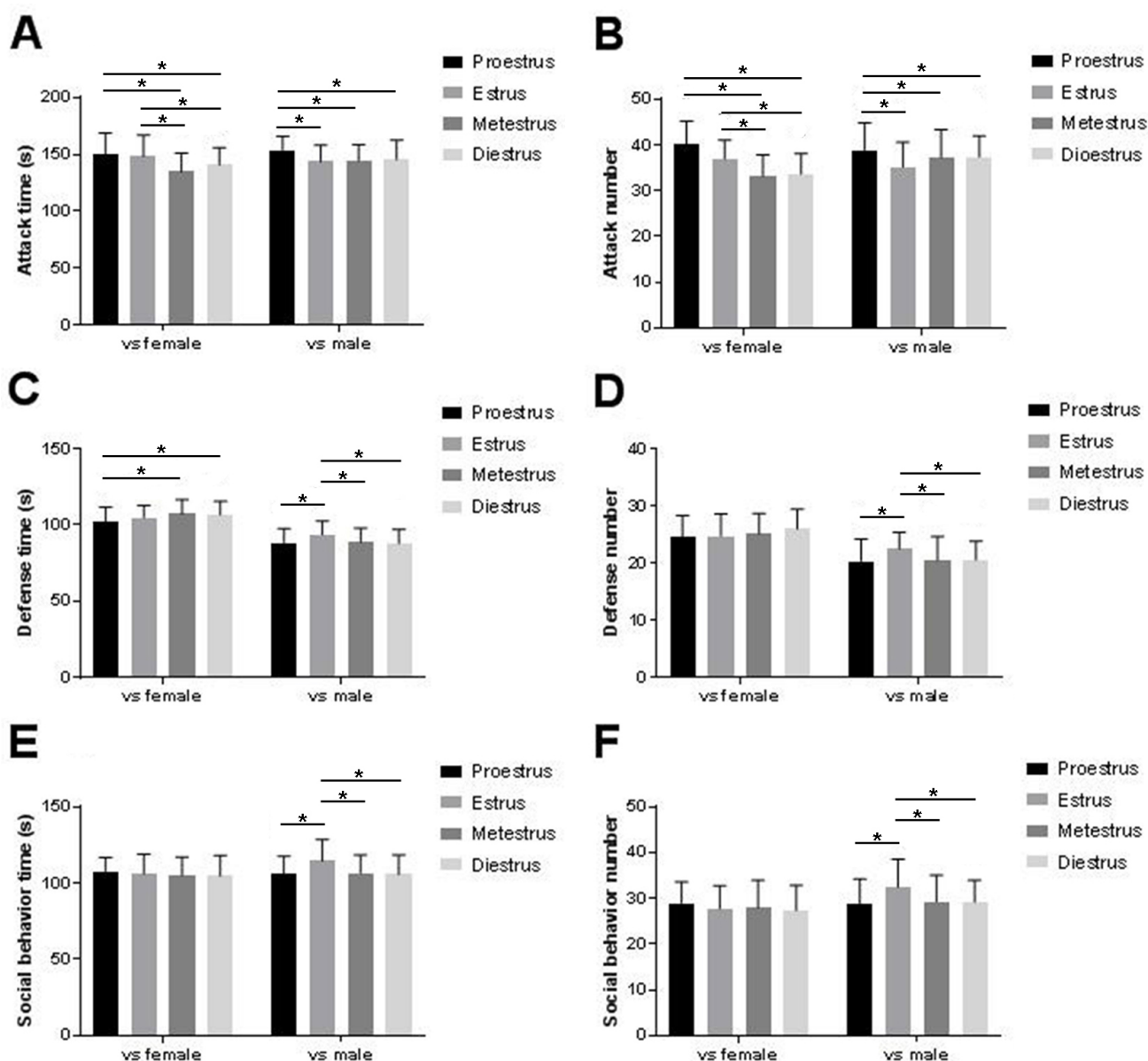
### 4.1 Aggressive behaviors and RMR changed with the estrous cycle

Hamsters have been proven to have sex differences in response to social behavior [22]. Both male and female hamsters will spontaneously display aggressive behavior, and they exhibit similar HPA-axis responsivity [23]. When not in behavioral estrus, female hamsters will readily attack a male or female conspecific. Female hamsters are described as more aggressive than males. Aggressiveness in female encounters depends on the stable 4-day sexual cycle stage, with a decrease on the day of receptivity, i.e., estrus [24]. Striped dwarf hamster (*Cricetulus barabensis*), also known as Chinese hamster, is a solitary species [25]. Females are highly aggressive and dominant over males. Their presence seemed to inhibit aggression between males. During their first pregnancy, young females became aggressive towards any resident adult male of littermate and forced them out of the nest so far shared, where they subsequently gave birth to their litter [26]. Thus, we used female hamsters as the animal model to investigate the mechanism of aggression.

In this study, through the tests in handling bags and the observations of behavior changes in a neutral arena, we found that the aggression of female *C. barabensis* was remarkably different in the estrous cycle. Several studies have investigated aggression in cycling female hamsters. Some conflicting reports have revealed that during the stages of dioestrus, proestrus or estrus, the levels of aggression may be higher [22, 27]. A previous study found that female hamsters' intra-sexual aggression reached the highest levels on dioestrus days 1 and 2 and gradually decreased during proestrus and estrus [28]. However, our study found the higher levels of aggression occurred during proestrus.

The struggling time of female hamsters in the handling bag experiment showed a similar trend to female opponent





**Fig. 3. Behavior changes in the estrous cycle of *Cricetulus barabensis*.** In the neutral arena, the durations of attack time and numbers of female *C. barabensis* on female and male hamsters showed a significant difference in the estrous cycle (A, B). (C, D) showed the differences in defense time and the numbers of female *C. barabensis* against females and males in the estrous cycle. Moreover, the social behavior time and numbers of female *C. barabensis* against a male in the estrous cycle were shown (E, F). Data are expressed as means  $\pm$  SEM. \*:  $P < 0.05$ , and \*\*:  $P < 0.01$ .  $P < 0.05$  indicates a significant difference. Significance was determined by ANOVA, followed by Tukey's HSD posthoc analyses.

hamsters' aggressive behavior in the neutral arena experiment. As a result, female hamsters exhibited similar aggressive behavior patterns in the face of capture pressure and same-sex competitors. Handling bag test was first used to detect small rodents' personalities under the pressure of capture. Personality characteristics are a series of comprehensive behavioral expressions for animals to be adapted to the habitat environment, reflecting the differences in behavioral expressions of different individuals under specific environmental conditions [29]. The expression of animal behaviors is closely related to how individuals live, and individu-

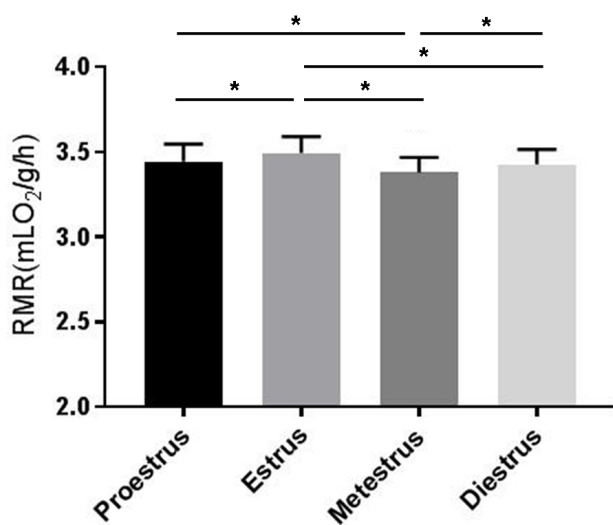
als can adjust their behavioral strategies according to their fitness. Therefore, personality characteristics have environmental constraints [30]. Many studies have shown that the same species' personality is significantly different due to different environmental conditions [31, 32]. Our results confirmed that female *C. barabensis* had different estrous personality, which indicated that hamsters could take different behavioral strategies to adapt to the environmental conditions to have the maximum fitness.

The occurrence of aggressive behaviors is generally restricted by competitive mate, food, population density and

**Table 1. Relationships between aggression, RMR, plasma estradiol and relative expression of *ESR2* mRNA in hypothalamus of female *Cricetulus barabensis*.**

Item	Plasma estradiol		Relative expression of <i>ESR2</i>	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Handling bag struggling time	0.647	<b>0.000</b>	-0.299	<b>0.039</b>
Attack time vs female	0.468	<b>0.002</b>	-0.409	<b>0.004</b>
Attack time vs male	-0.099	0.541	-0.302	<b>0.037</b>
Attack number vs female	-0.222	0.168	-0.091	0.538
Attack number vs male	0.156	0.335	-0.342	<b>0.017</b>
RMR	0.317	<b>0.046</b>	-0.234	0.109

Bold values indicated statistical significance using Pearson correlations ( $P < 0.05$ ).



**Fig. 4. Resting metabolic rates in the estrous cycle of *Cricetulus barabensis*.** RMR in estrus was significantly higher than that in the other three stages and was significantly lower in metestrus than that in the other three stages. However, there was no significant difference between proestrus and dioestrus. Data are expressed as means  $\pm$  SEM. \*:  $P < 0.05$ , and \*\*:  $P < 0.01$ .  $P < 0.05$  indicates a significant difference. Significance was determined by ANOVA, followed by Tukey's HSD posthoc analyses.

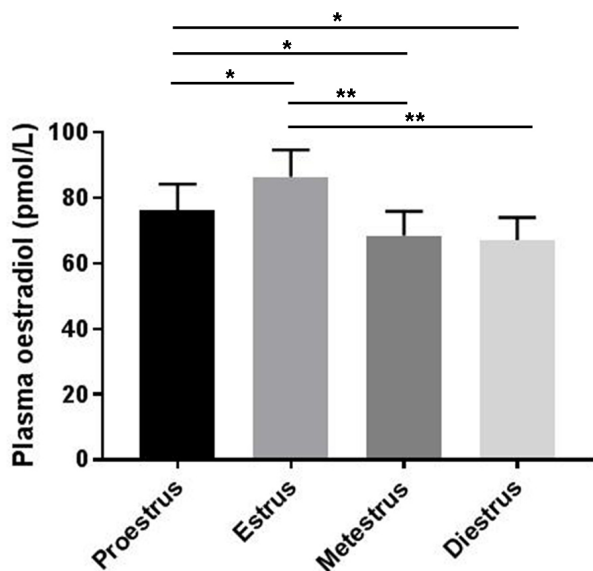
domain behavior. As solitary hamster, female individuals change into fierce and aggressive in proestrus, which is conducive to defeat potential same-sex competitors and overcome external unfavorable environmental conditions, thus to improve their fitness and win more mating opportunities and pass on their genes to their offspring [33]. In the metestrus and dioestrus periods, the need for mating became less urgent. The essential awareness of intraspecies and territorial fights with other individuals was maintained, which resulted in the lowest attack frequency throughout the estrus cycle. In estrus, female hamsters were more aggressive when their opponents were female and less aggressive when their opponents were male. Simultaneously, female hamsters showed the highest defensive and social behaviors when faced with males during estrus, further suggesting that the strategy was an adaptation for mating. It was reported RMR correlates with a range of fitness-related behavioral traits [34], e.g., per-

sonality [35]. Female *C. barabensis* had the highest RMR during estrus, which suggested that estrus was also an energy-consuming physiological process.

In most cases, the enhanced aggression during the proestrus or estrus period is low-intensity, such as intimidation, shock, boxing and counterattack, or even ritualized non-harmful behaviors to drive away opponents. This situation indicates that in the estrous stage, the primary task is mating and reproduction, and the focus of energy consumption and behavior pattern is more inclined to reproductive activities [36]. It is undoubtedly not in line with a life-history strategy to engage in a fierce fight with other individuals. On the other hand, the significant decrease of aggressive behaviors in metestrus is due to estrus' end and a large amount of female hamsters' energy consumption through breeding activities [37]. Therefore the energy is not enough to support too much vigorous sports such as biting, wrestling and pursuing.

#### 4.2 Estradiol level and relative expression of *ESR2* mRNA changed with the estrous cycle

The estrous cycle is the recurring physiological changes induced by reproductive hormones in most mammalian terrestrial females. Based on the classic neuroendocrine model, sexual and aggressive behavior is mainly dependent on estradiol. Therefore, the estradiol level may be positively correlated with the breeding aggression of females. The estradiol receptor *ESR2* can also mediate estradiol signaling in the ovary, prostate, lung, cardiovascular, and central nervous systems. In this study, we found that the estradiol level in plasma and the expression level of *ESR2* mRNA in the hypothalamus of female *C. barabensis* were consistently changed with the estrous cycle, implying the aggression behavior during the estrous cycle may be regulated by the estradiol/*ESR2* in the female *C. barabensis*. Studies have been revealed that estrogens initiate signaling cascades via *ESR2* in the hippocampus [38], thus enhancing synaptic plasticity and performance on hippocampal-dependent cognitive behaviors and physiological relevance of these effects in animals [39]. In the brain, estrogen can pass through the blood-brain barrier and bind to its receptor, such as *ESR2*. Then *ESR2* translocates to the nucleus and binds to the estrogen response element (ERT) to regulate gene expression (Fig. 6). The estradiol/*ESR2* can also

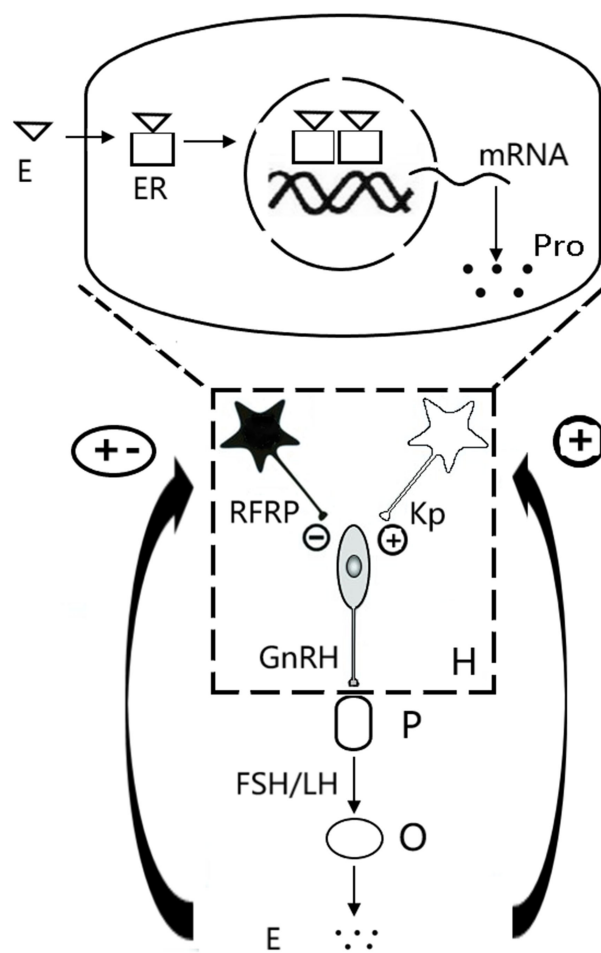


**Fig. 5. Plasma estradiol concentrations in the estrous cycle of *Cricetus barabensis*.** The estrus period's plasma estradiol level was the highest, followed by the proestrus stage, and was lowest in the metestrus and the dioestrus stages. Data are expressed as means  $\pm$  SEM. \*:  $P < 0.05$ , and \*\*:  $P < 0.01$ .  $P < 0.05$  indicates a significant difference. Significance was determined by ANOVA, followed by Tukey's HSD posthoc analyses.

regulate glucose transport, mitochondrial function, and aerobic glycolysis to generate ATP [9]. Therefore, in this study, the aggressive behaviors of the female *Cricetus barabensis* in the estrous cycle may attribute to their expression levels of estradiol/*ESR2* to mediate the hippocampal-dependent cognitive and physiological behaviors as well as brain and body metabolism.

However, recent studies have suggested that this paradigm is oversimplified. Some animals display elevated aggression levels during the non-breeding season, despite gonadal regression and reduced circulating androgens levels. Experimental elevation of gonadal steroids (i.e., estradiol) does not reduce females' aggression, showing that aggression is not suppressed by high hormone levels [40, 41]. Further, aggression is not reduced when estradiol is virtually depleted via ovariectomies, providing vital support that female aggression regulation may be independent of gonadal steroids [41]. According to some studies, low hormone levels could be accommodated by extra-gonadal hormonal precursors. For example, Adrenal dehydroepiandrosterone (DHEA) likely serves as an essential precursor for neural androgen synthesis during the non-breeding season [5]. On the other hand, increased site-specific *ER $\alpha$*  may function as a compensatory mechanism to allow increased responsiveness to estradiol in regulating aggression in place of high circulating concentrations of hormones [42]. In other words, the dynamic changes in estrogen receptors are one of the compensatory mechanisms regulating aggression during reproductive quiescence.

We found that the plasma estradiol levels of female *C. barabensis* were positively correlated to handling bag strug-



**Fig. 6. Mechanism of estrogen and receptor in the estrous cycle.** Estrogens affect reproduction and behavioral regulation by binding to receptors of the hypothalamic in the estrous cycle. E, estrogen; ER, estrogen receptor; H, hypothalamus; Kp, kisspeptin; O, ovary; P, the pituitary body; Pro, protein; RFRP, RFamide-related peptide.

gling time & attack time to female opponents. Simultaneously, the relative expression of *ESR2* in the hypothalamus was negatively related to handling bag struggling time & attack time to both female and male opponents. As a result, the highest aggression of female hamsters in proestrus could be attributed to elevated estradiol in plasma and the lowest *ESR2* in the hypothalamus. In contrast, the decrease of estrus aggression might associate with the higher estradiol in plasma and *ESR2* in the hypothalamus. Besides, in metestrus & dioestrus, the lowest estradiol and the highest *ESR2* co-occurred with the lowest aggression.

In summary, we explored the aggression, personality, RMR and molecular regulation mechanism in the estrous cycle of female striped dwarf hamsters. We firstly demonstrated that aggression tracked phenotype over the estrous cycle. Moreover, we found that both the estradiol level in plasma and the expression level of *ESR2* mRNA in the hypothalamus were associated with the aggression of female *C. barabensis* during the estrous cycle, which indicates an estradiol/*ESR2*-

dependent mechanism for regulation of aggressive behavior throughout the estrus cycle. These results are intriguing, which will broaden our understanding of how breeding phenotype is an essential driver of associated changes in social behavior mechanisms.

## Author contributions

JHX, HLX and LXX conceived and designed research; JHX, HLX and CF performed experiments; JHX and ZW analyzed data, interpreted results of experiments, prepared figures; JHX and ZW drafted manuscript; HLX and MW, provided experimental guidance and suggestions for revision; JHX and LXX edited manuscript and approved final version of manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

All procedures used in this study were approved by the Institutional Animal Care and Use Committee of Qufu Normal University.

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## Conflict of interest

No conflicts of interest, financial or otherwise, are declared by the authors.

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