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Original Article

Abscisic acid regulates maternal behavior in rats via PPARγ receptors and hypothalamic oxytocin signaling

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Abstract

Background: Abscisic acid (ABA) is present in brain regions associated with parental care and aggression. Maternal care is pivotal in early mammalian development, significantly influencing emotional regulation, stress resilience, and adaptive fitness in offspring. **Aims:** The present study investigates the influence of intra-paraventricular nucleus (PVN) administration of ABA on maternal behavioral responsiveness in lactating female rats. **Methods**: On postpartum day 5, lactating rats (n=6 per group) received intra-PVN injections of ABA (5, 10, or 20 µg/rat) via stereotaxic surgery. Ten min after injection, maternal behaviors, including durations of low and high kyphosis, pup retrieval times, and durations of body and anogenital licking were recorded for 1 h. Additionally, the percentage of oxytocin-immunoreactive cells in brain was quantified. **Results:** ABA administration (20 µg/rat) significantly enhanced most maternal behavior indices ($P<0.05$). These ABA-induced improvements in maternal care were significantly inhibited by GW9662, a peroxisome proliferator-activated receptor γ (PPARγ) antagonist ($P<0.01$). Furthermore, ABA treatment significantly increased number of oxytocin-immunoreactive cells in the paraventricular (Pe) and anterior parvocellular parts (PaAP) of the PVN ($P<0.001$); this increase was reversed to control levels following blockade of PPARγ receptors ($P<0.001$). **Conclusion:** These findings suggest that central ABA administration can enhance maternal care behaviors in lactating rats, potentially through activation of PPARγ signaling and increased expression of oxytocin in the maternal brain. However, further research is required to determine whether ABA has a physiological role under normal conditions.

Key words: Abscisic acid, Maternal care, Oxytocin, Paraventricular nucleus, PPARγ

Introduction

Maternal behavior refers to a series of responses exhibited by females aimed at ensuring the survival, growth, and developmental well-being of their offspring from birth until independence. These behaviors are most prominent immediately postpartum and manifest in species-specific ways (Pires *et al*., 2013; Fleming and Kraemer, 2019). In rodents, maternal care primarily involves pup-licking and grooming, crouched nursing, retrieval of pups, nest construction and maintenance (Capone *et al*., 2005; Bridges, 2015). Specifically, in rats, maternal nursing behavior encompasses lactation, pup cleaning through licking, and protective aggression toward perceived threats to the litter (Bosch and Neumann, 2008; Numan and Stolzenberg, 2009). Licking and grooming behaviors further strengthen mother-pup bonding, stimulate pup circulation, and promote healthy development (Winters *et al*., 2022). Additionally, rat mothers actively engage in nest building and demonstrate retrieval behaviors by relocating pups when encountering potential threats (Khant Aung *et al*., 2022).

The physiology of maternal care in mammals is described by the presence of a variety of neural networks in hypothalamic nuclei, including the paraventricular nucleus (PVN), medial preoptic area (MPOA), anterior hypothalamus (AH), and adjacent structures such as the bed nucleus of the stria terminalis (BNST) (Numan *et al*., 2005; Numan, 2007, 2012). Specifically, PVN neurons play a crucial role in facilitating the maternal responses in rodents, as disruption of the PVN function is shown to significantly alter maternal behavior. For example, damage to PVN increased maternal aggressive behavior on the fifth day after delivery in rats (Giovenardi *et al*., 1998), while electrolytic lesion of the PVN in lactating rats was associated with a decrease in the incidence and duration of attacks on the aggressor male and reduced weight gain in the pups (Consiglio and Lucion, 1996).

Maternal behavior is closely associated with changes in endocrine hormone secretion during pregnancy and parturition. Particularly, changes in the secretion of pregnancy hormones like prolactin and oxytocin are essential stimulators of maternal behaviors and lactogenesis in mammals (Bridges, 2015). Administration of prolactin has been found to enhance maternal care in hypophysectomized female rats (Bridges *et al*., 1985). Also, mice with a mutant oxytocin receptor gene exhibited impaired maternal behavior (Rich *et al*., 2014). Additionally, parturition increases Fos expression in oxytocin neurons within PVN, enhancing olfactory preferences for pup odor (Munetomo *et al*., 2015), suggesting that oxytocin may contribute to the underlying mechanisms that modulate maternal behaviors.

Abscisic acid (ABA) is a signaling molecule which is implicated in the regulation of several physiological functions in plants and animal species (Guri *et al*., 2007; Raghavendra *et al*., 2010; Sah *et al*., 2016), and is synthesized by a wide range of animal cells (Le Page-Degivry *et al*., 1986; Li *et al*., 2011). It is found in high concentrations in the hypothalamus and hippocampus of rats (Qi *et al*., 2015). ABA modulates various physiological functions like immune and inflammatory responses, energy and glucose homeostasis as well as neurological processes (Guri *et al*., 2007; Guri *et al*., 2010; Li *et al*., 2011; Lievens *et al*., 2017). Mechanistically, ABA activities are mainly mediated through activation of peroxisome proliferator-activated receptor γ (PPARγ) (Bassaganya-Riera *et al*., 2010; Kline *et al*., 2010; Bassaganya-Riera *et al*., 2011; Kooshki *et al*., 2021). PPARγ is expressed highly in astrocyte glial cells and also in hypothalamic neurons and modulates various physiological processes including nociception, inflammatory responses, and cell metabolism (Moreno *et al*., 2004; Wang, 2010; Warden *et al*., 2016; Korbecki *et al*., 2019). Intriguingly, during pregnancy, PPARγ activation is associated with reduced placental abnormalities in rodents (Kadam *et al*., 2015).

The potential effect of ABA on maternal caregiving responses remains unexplored. Given the high expression of PPARs throughout the PVN in hypothalamus (Yu *et al*., 2012), and their contribution in maternal behaviors (Lendvai *et al*., 2016) and regulation of maternal behavior-related hormones (Heaney *et al*., 2003), we hypothesized that ABA may modulate maternal behaviors via interaction with PPARγ. Thus, here we investigate the possible interaction between ABA and PPARγ within PVN, and whether it is associated with modulation of maternal behaviors, as well as the impact on oxytocin protein expression in the maternal brain.

Materials and Methods

Animals

Adult Wistar rats (230-250 g) were purchased from the Animal Resources Centre, Kerman, Iran. The rats were acclimated in the Shahid Bahonar University animal house for two weeks before starting the experiments. Six rats were kept per cage in controlled conditions (room temperature of 23 ± 1°C, with a regular 12 light/12 dark cycle). There was *ad libitum* access to food and water. To mating, three virgin female rats were kept overnight in a cage with one adult male breeder. Effective mating was determined by presence of a vaginal plug, and the pregnant rats were kept in individual cages. Litter sizes were standardized to eight pups per dam. All the experiments were permitted by the ethics committee of Shahid Bahonar University of Kerman (IR.UK.VETMED.REC.1399.015).

Experimental design

On postpartum day 5, a time point at which maternal care behaviors (e.g., pup retrieval, nest-building, and kyphotic nursing posture) are reliably expressed and associated maternal hormones (oxytocin, prolactin) have reached stable, measurable levels (Bosch and Neumann, 2008; Numan and Stolzenberg, 2009), lactating rats were randomly assigned into six groups (n=6 per group): a control group (no treatment), a sham-operated group (vehicle only, 1 µL DMSO/rat), three ABA-treated groups receiving doses of 5, 10, or 20 µg ABA/rat, and a GW9662 + ABA group which received the selective PPARγ antagonist GW9662 (3 µg/rat), injected 15 min prior to ABA (20 µg/rat). Time and dose were selected according to a previous study (Naderi *et al*., 2017; Mollashahi *et al*., 2018). The drugs were administered intra-PVN three days after surgery as a recovery period. The time course of the experiments is described in Fig. 1.

Maternal behavior testing

The following maternal behaviors were observed and recorded for 1 h after treatment:

Nest construction: defined as the dam carrying nesting material (with mouth, paws, or snout) to the nest site, actively constructing or reorganizing a nest. The total duration (in seconds) spent performing this behavior during the observation period was recorded.

Pup retrieval: defined as the dam orienting toward, approaching, sniffing, gently picking up displaced pups with her incisors, carrying them back, and placing them into the nest. Both the total number of pups retrieved and the total duration (in seconds) spent retrieving pups during the observation period were recorded.

Licking the pups: assessed as the cumulative duration (in seconds) the dam spent licking the pups' bodies and anogenital regions. Individual licking frequency or the number of pups licked simultaneously were not differentiated.

High kyphosis (actively hovering over pups): defined by the dam actively positioning herself over pups and engaging in behaviors such as pup licking, self-grooming, or rearranging nesting materials or facilitating pups' access to her nipples. The total duration (in seconds) spent in this posture was recorded.

Low kyphosis: Maternal nursing behavior was characterized by the dam standing immobile over her litter, with limbs rigidly splayed and a pronounced dorsal arch (kyphosis) of the spine. During periods of low or

absent kyphosis, dams typically refrained from licking pups or engaging in active behaviors. The total duration of this posture (in seconds) was recorded (Daoura *et al*., 2013).

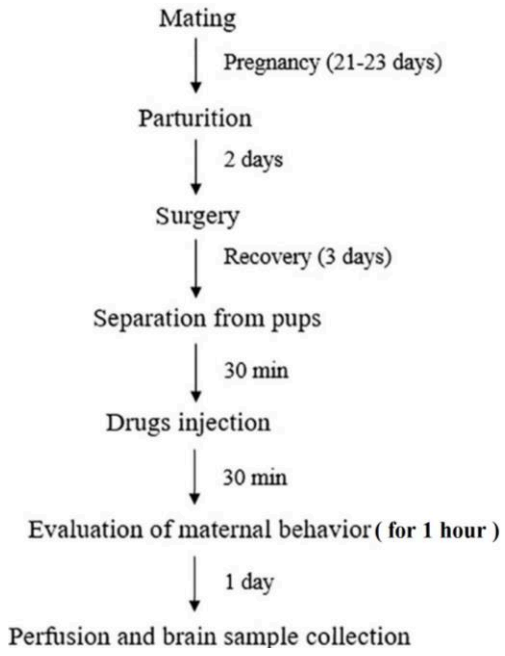

Fig. 1: Experimental timeline and course schedules

All the behavioral assessments were done in the light phase of the postpartum day 5. At least 30 min before beginning the experiments, mothers and their pups in the home cage were moved to the experimental room for habituation. Then, the mothers were conveyed to another cage for injecting the drugs and the pups were dispersed over the floor in the mother's home cage away from the nest area. Thirty min after the last drug injection, the mothers were returned to the home cages with their pups and maternal behavior was assessed for 1 h by the quantity of the time that mothers spent licking the pup's anogenital region and other parts of the body and total times of nest building including retrieval of the nest material. In addition, pup retrieval latencies were evaluated by the latency time to retrieve the first pup and time to retrieve the total litter in the nest. Maternal performances were further evaluated for 1 h by assessment of the time that mothers spent on eating, sleeping, and self-grooming behaviors. The observer was blind to the experimental condition of the animals.

Immunohistochemistry

To explore the potential mechanism underlying the effects of ABA, the percentage of oxytocin-positive cells in the hypothalamic regions paraventricular (Pe) and anterior parvocellular (PaAP) was determined using immunohistochemistry. The next day after behavioral assessment, perfusion and brain sample collection were performed. Set serial sections showing the location of the Pe and PaAP were prepared from all groups (control, ABA, and ABA+GW) (n=4). The sections were washed six times in 0.05 M TBS (50 mM Tris, 150 mM NaCl, pH 7.6) to eliminate cryoprotectant, then heated for 10 min at 90°C in 0.01 M Tris (pH 10) to recover oxytocin antigenic site. After heat-induced epitope retrieval, the samples were rinsed with Tris-buffered saline (TBS, 10 mM) (0.025% triton-X100) twice for 5 min each. After blocking with 1% BSA and 5% normal goat serum in TBS-T for 2 h, the slides were incubated with the primary antibodies (Santa Cruz Biotechnology, USA), at 4°C overnight. Then, the samples were washed in TBS-T twice for 5 min and then incubated with biotinylated secondary antibody (Santa Cruz Biotechnology, USA) with TBS in the darkness for 60 min. Slides were stained with 3, 30-diaminobenzidine (DAB) and were washed with 0.3% H_2O_2 (Sigma-Aldrich) in TBS-T for 10 min for endogenous peroxidase blocking before incubating with HRP-conjugated goat anti-rabbit antibodies (Santa Cruz Biotechnology, USA) for 60 min. The slides were rinsed with TBS-T, 3 times for 2 min each, and incubated with DAB solution. After DAB staining, the slides were rinsed in distilled water, dehydrated in 70%, 95%, and 100% ethanol, respectively, 2 times for 3 min each, washed in xylene 2 times for 5 min each and then mounted with cytoseal (Thermo Scientific, Waltham, MA, USA). To quantify oxytocin expression, histological sections of the Pe and PaAP parts of the PVN were examined under light microscope. Oxytocin-positive immunoreactive cells were counted under ×40 magnification. The obtained values represent the percentage of oxytocin-positive cells relative to the total number of cells in the target area. Data were analyzed using one-way ANOVA.

Surgery

On the second day after parturition, each lactating rat was deeply anesthetized by the infusion of ketamine (50 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) mixture, and fixed on a stereotaxic device (stoelting, USA). The 22-gauge guide cannula was bilaterally inserted in the PVN at the coordinates of 7.8 mm posterior to bregma, ± 0.6 mm lateral to the midline, and 7.9 mm ventral to the skull surface. Upon surgery, each mother was returned to the home cage with her pups. Each rat was checked for any alteration in maternal behaviors induced by surgery and the animals that show abnormal maternal behavior toward pups were excluded from the experiment. Moreover, following the experiments, methylene blue was injected through the guide cannula to approve the correct location of the cannula. If the cannula was not fixed in the correct place, the data set from that rat was excluded. In total, there were four mis-cannulated rats. Figures 2A and B is a representative of brain coronal section showing the injection site.

Drugs

(±)-cis, trans-ABA, and GW9662, a PPARγ receptor antagonist, (both Sigma Aldrich, USA) were dissolved in absolute dimethyl sulfoxide (DMSO) and then diluted with saline solution. The total concentration of DMSO was less than 0.1%.

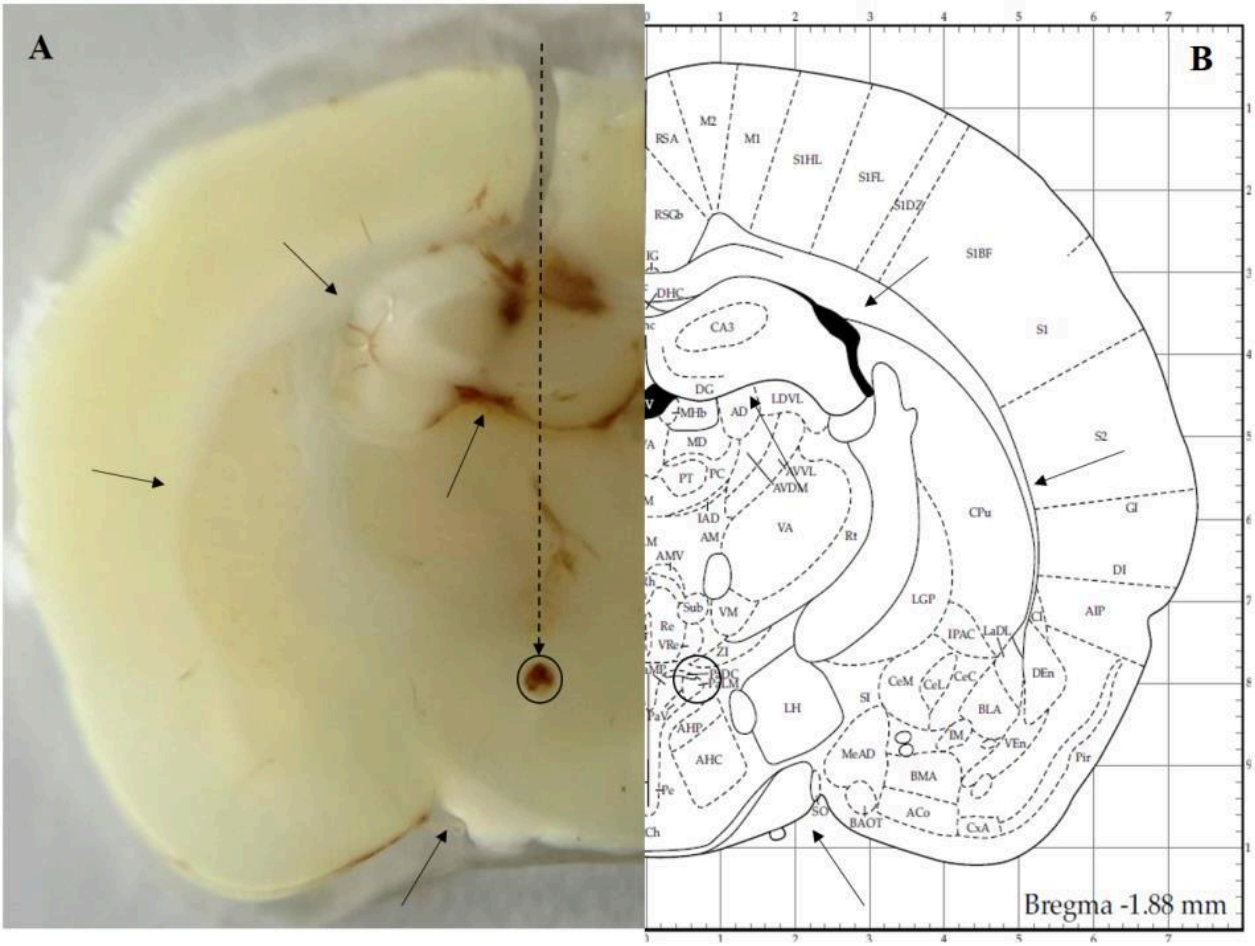

Fig. 2: Stereotaxic surgical procedure for guide cannula implantation in the paraventricular nucleus (PVN). (**A**) A representative photograph exhibiting appropriate cannula implantation into the PVN region, and (**B**) A typical section of basolateral amygdala (BLA) taken from the Paxinos and Watson rat brain atlas

Microinjections

The drugs were administered into the PVN at a rate of (1 µL/min) through a 27-gauge internal cannula coupled to a 1 µL Hamilton syringe by a polyethylene tube. The injection needle was set 1 mm beyond the reference cannula. The needle was left in the site for 1 min after drug administration to prevent backflow.

Statistical analysis

The data are expressed as mean±SEM. The Kolmogorov-Simonov test was used to explore the data normality. In the maternal behavior indexes, the data did not show a normal distribution. Therefore, the data were analyzed by the non-parametric Kruskal-Wallis test followed by the Mann-Whitney U-test of multiple comparisons. The immunohistochemistry data display alteration in level of oxytocin protein showed normal distribution and evaluated by one-way ANOVA. Post hoc tests were performed using Tukey's test. A P-value of less than 0.05 was considered as the level of significance.

Results

Assessment of maternal behaviors

The groups displayed significant difference in low H [=10.193, P=0.037, df=4] and high [H=13.805, P=0.008, df=4] kyphosis behavior (Figs. 3A-F). Time of low and high kyphosis behavior were increased in ABA treated groups (10 and 20 µg/rat) in comparison with control group (P<0.05, for both comparisons). The time taken to retrieve all the pups was decreased in the group of rats that received ABA at the dose of 20 µg as compared to control (P<0.05; Fig. 3C). Moreover, time of nest-building behavior was significantly different among groups [H=11.704, P=0.020, df=4]. As shown in Fig. 3D, ABA treatment at 5 and 20 µg/rat was able to increase the nest-building time as compared to other groups (P<0.01). Similarly, the time that dams spent licking the pup's body was significantly increased in the ABA (20 µg/rat) group as compared to control group (P<0.05; Fig. 3F). However, intra-PVN administration of ABA only at the dose of 10 µg/rat could increase the time of pups' anogenital regions licking by dams as compared to control group (P<0.01; Fig. 3E).

Importantly, the increasing effect of ABA (20 µg/rat) on kyphosis behavior, pups anogenital regions liking time as well as nest-building time were prevented by selective PPARγ antagonist GW9662 (3 µg/rat; Figs. 4A-4D). In addition, pretreatment with GW9662 suppressed

ABA (20 µg/rat) effect on the time to retrieve the pups (Fig. 4E).

Table 1 shows the total time that lactating dams spent on eating, self-grooming, and sleeping during the experiment. The groups showed significant differences in eating [Chi-square=20.149, P=0.001, df=5] and self-grooming [Chi-square=19.019, P=0.002, df=5] behavior. However, the treatments did not alter the length of the time that lactating dams spent sleeping [Chi-square=1.724, P=0.886, df=5]. In the ABA (20 µg/rat) group, the dams' eating time was decreased in comparison with the control rats (P<0.05). Additionally,

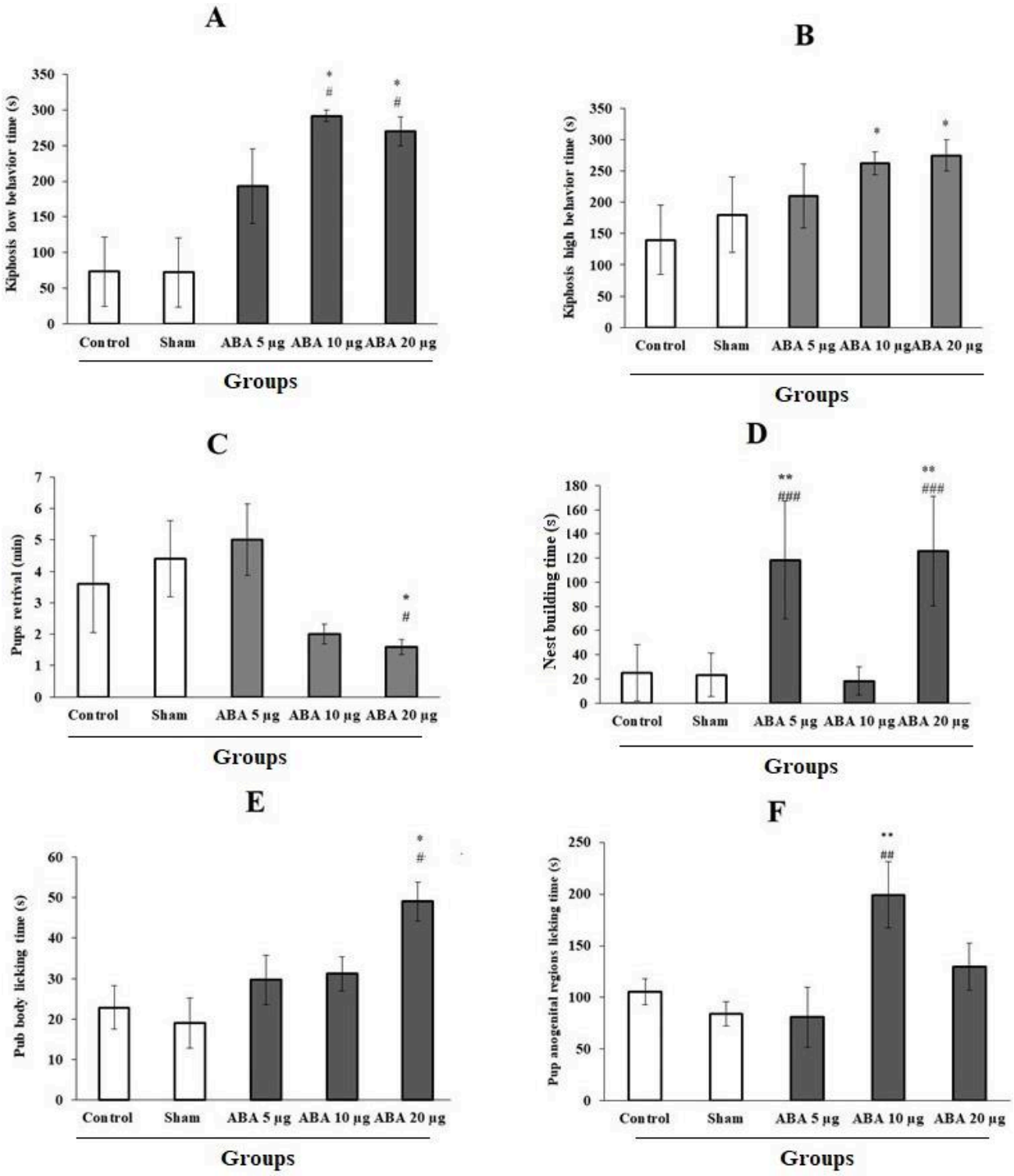

Fig. 3: The effects of intra-PVN administration of abscisic acid (ABA) (5, 10, and 20 µg/rat) on: (**A**) Maternal behavior indices including low kyphosis behavior, (**B**) High kyphosis behavior, (**C**) Pups' retrievals time, (**D**) Nest building time, (**E**) The time of licking body, and (**F**) Anogenital regions in lactating female rats (n=6). Values are expressed as means±SEM. Six rats per group were used. ** P<0.01, * P<0.05 vs control group; ### P<0.001, ## P<0.01, # P<0.05 vs sham group

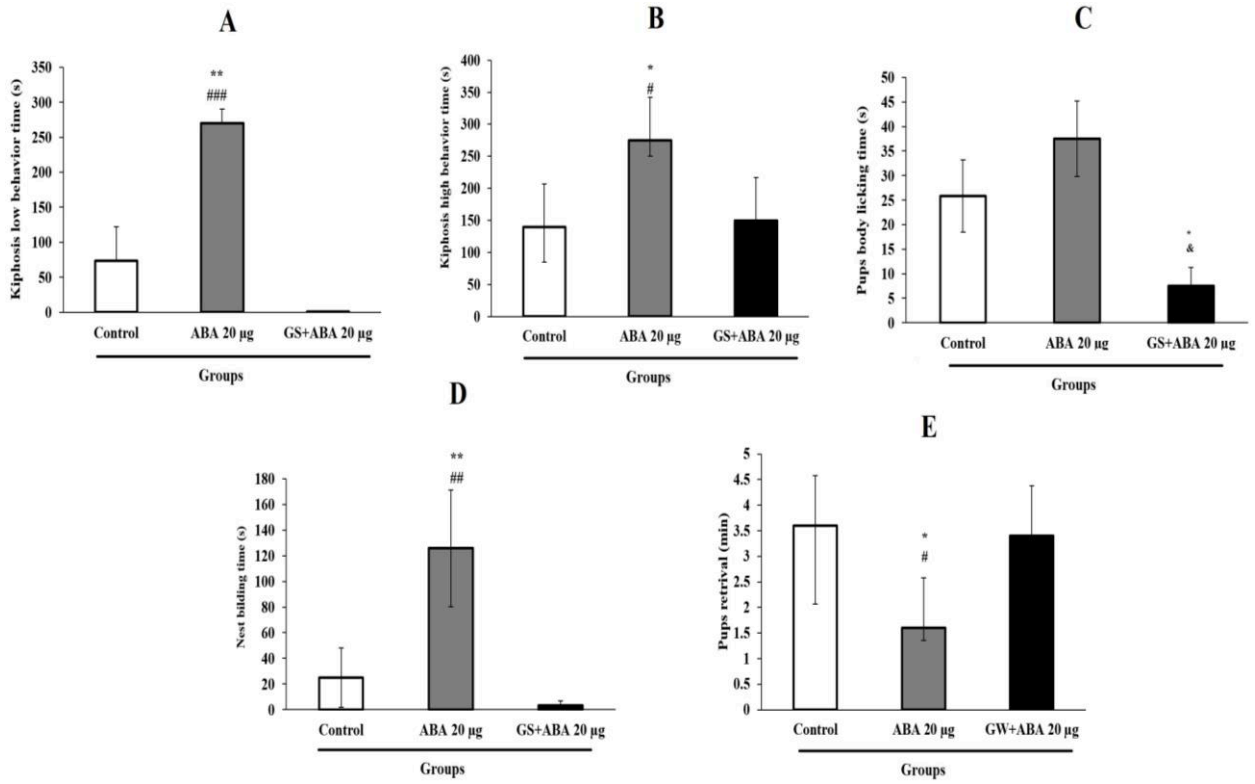

Fig. 4: The effects of intra-PVN administration of abscisic acid (ABA) (20 µg/rat) and ABA (20 µg/rat) plus PPARγ antagonist GW9662 (3 µg/rat) on maternal behavior indices including: (**A**) Low kyphosis behavior, (**B**) High kyphosis behavior, (**C**) The time of licking body regions, (**D**) Nest building time, and (**E**) Pups retrievals time in lactating female rats (n=6). Values are presented as means±SEM. ** P<0.01, * P<0.05 vs control group; ### P<0.001, ## P<0.01, #P<0.05 vs GW+ABA group

Table 1: Comparison of eating, self-grooming, and sleeping behaviors of lactating rats across experimental groups. Observation time was 1 h

Groups	Eating (s)	Self-grooming (s)	Sleeping (s)
Control	156±45.707	32±8.621	300±134.164
Sham	148.3333±36.860	28.5±6.323	225±92.222
ABA (5 µg)	90±28.635	10±6.324**	280±110.272
ABA (10 µg)	52±18.912	19±6.879*	345±93.229
ABA (20 µg)	17±8.717*#&	18±7.483*&	196.66±74.147
GW+ABA (20 µg)	250.3333±16.950	52±6.531	250±83.666

The values are expressed as means±SEM. Six rats pre group were used. * P<0.05, ** P<0.01 vs control group; # P<0.05 vs sham group, & P<0.05 vs GW+ABA group

ABA at all doses was able to attenuate mother self-grooming behavior (P<0.01 and P<0.05), whilst, ABA-induced (20 µg/rat) reduction in eating and self-grooming behavior in lactating rats was inhibited by GW9662 (3 µg/rat).

Immunohistochemistry

Using a representative illustration of rat brain section, the hypothalamic areas Pe and PaAP were defined (Figs. 5A and 5B).

Figures 5C-H indicate oxytocin-immunoreactive cells in the brain sections for control, ABA (20 µg/rat) and ABA plus GW9662 (20 and 3 µg/rat, respectively) treated groups. Quantification of immunohistochemistry sections showed that in the ABA group, the percentage of oxytocin-immunoreactive cells in the Pe and PaAP were significantly increased as compared to control group (Fig. 5C). Whilst, ABA efficiency to increase the expression of oxytocin in the intended areas was prevented by intra-PVN infusion of GW9662 (3 µg/rat) (P<0.001) (Figs. 5I and J).

Discussion

This study identified a novel role for ABA, revealing that local administration of ABA into the PVN of the hypothalamus promotes maternal care in lactating rats. Moreover, ABA increased count of oxytocin immunoreactive cells specifically in the hypothalamic nuclei (Pe and PaAP), which was inhibited by GW9662, a PPARγ antagonist, indicating that PPARγ signaling might be involved in ABA's effects on maternal behavior.

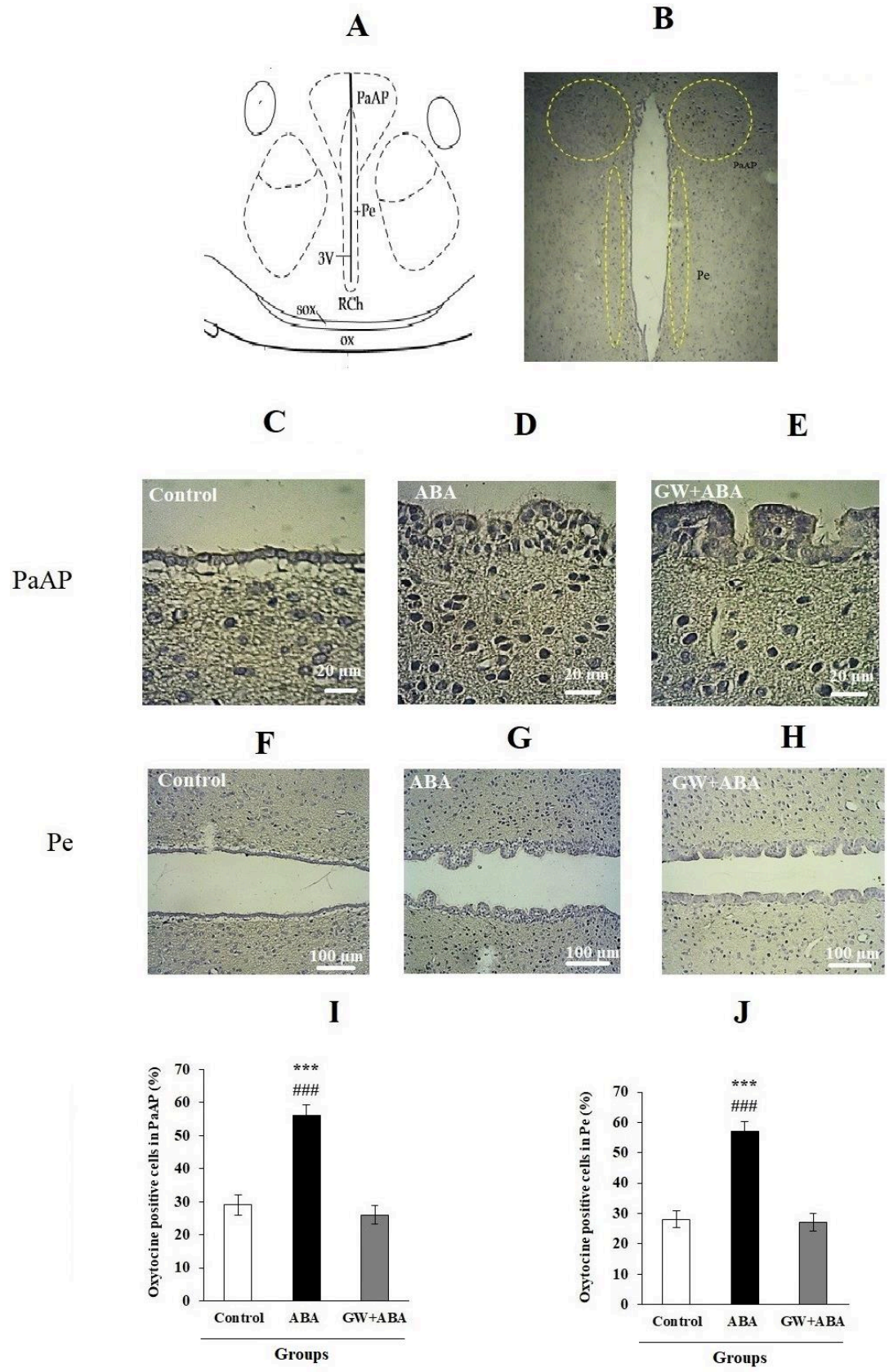

Fig. 5: (**A**) Representative drawing of brain section taken from the Paxinos and Watson rat brain atlas, (**B**) A representative histological section illustrating the location of para ventricular (Pe), and anterior parvocellular (PaAP) part of the PVN with ×4 magnification, (**C-H**) Histological sections (20 µm) of oxytocin immunoreactive cells in the control group and the groups of rats that were infused with abscisic acid (ABA; 20 µg/rat) and ABA+GW9662 (3 µg/rat), and (**I** and **J**) The quantification sections (100 µm) of oxytocin-positive cells in the Pe and PaAP between experimental groups (n=4). Data are expressed as mean± SEM. *** P<0.001 vs control group, ### P<0.001 vs ABA+GW group

Previous studies have shown a rapid ABA uptake in brain regions critical for learning and memory, such as the hippocampus and hypothalamus, suggesting its neuromodulatory role (Kline *et al*., 2010; Korbecki *et al*., 2019). Moreover, central administration of ABA enhances cognitive functions and alleviates pain in rodent models, indicating its broad impact on brain function (Naderi *et al*., 2017; Korbecki *et al*., 2019). The results of this study expand the current knowledge by highlighting ABA's specific involvement in maternal behavior, in line with the well-established role of the PVN in maternal care regulation and its connectivity with other hypothalamic regions (Lee *et al*., 2013; Bridges, 2015; Georgescu *et al*., 2021). Maternal behaviors, including nursing, grooming, nest building, and offspring protection, depend heavily on PVN functionality and its interactions with medial preoptic area, arcuate nucleus, bed nucleus of the stria terminalis (BNST), and the suprachiasmatic nucleus (Lee *et al*., 2013; Bridges, 2015; Georgescu *et al*., 2021). Additionally, the PVN contributes to BNST-mediated regulation of corticotrophin-releasing factor and oxytocin in rats, further elucidating potential pathways involved in maternal behaviors (Dabrowska *et al*., 2011). Our finding that ABA infusion into the PVN enhances maternal care suggests a modulatory mechanism involving interactions among these interconnected nuclei.

We observed variability in ABA's effects regarding dose-dependency, with certain maternal behaviors showed clear dose-dependent responses, while others did not. This variability may result from differences in receptor density, receptor distribution, or downstream signaling pathways within the neural networks responsible for distinct behaviors (Madadzadeh *et al*., 2021). Among behaviors studied, both low (ABA 5 µg/rat) and high doses (ABA 20 µg/rat) significantly increased nest-building time. In contrast, an intermediate dose (ABA 10 µg/rat) specifically enhanced anogenital region licking. Moreover, low and high kyphosis behaviors were enhanced by ABA at 10 and 20 µg/rat doses, reflecting variable sensitivity to ABA concentrations. Previous research on ABA's effects in cognitive, analgesic, and anti-inflammatory models has shown both dose-dependent and dose-independent outcomes (Mollashahi *et al*., 2018; Khorasani *et al*., 2019; Madadzadeh *et al*., 2021).

These discrepancies might be due to nonlinear receptor-ligand interactions and varied receptor sensitivities across brain regions. Further studies focusing on receptor-level dynamics and intracellular signaling cascades at different ABA doses could clarify these observations.

Intriguingly, ABA shares structural similarities with retinoic acid (RA), a carotenoid derivative. This provides a mechanistic hypothesis for ABA's observed effects (Zhou *et al*., 2016). Retinoic acid regulates hormones crucial for maintaining pregnancy and lactation, including prolactin, progesterone, and oxytocin (Vershinin, 1999). Consistent with reports that RA increases oxytocin expression in the rat uterus, our findings support the hypothesis that ABA may modulate maternal behaviors via pregnancy-related hormone pathways due to structural and functional similarities with RA (Larcher *et al*., 1995). In this regard, RA and prolactin synergistically increase the expression of specific casein in the mammary alveolar (MAC-T) cells, *in vitro* (Lee *et al*., 2013). *In vivo*, RA stimulates steroidogenesis and progesterone expression via cAMP/PKA pathways (Manna *et al*., 2014; Suwa *et al*., 2016). Moreover, intraperitoneal and *in vitro* administration of RA increased the gene expression of oxytocin in the rat uterus (Larcher *et al*., 1995). Together, these findings suggest that ABA may modulate maternal behaviors through similar mechanism of action of RA.

In our study, ABA administration significantly elevated count of oxytocin immunoreactive cells within critical hypothalamic nuclei responsible for maternal behaviors. Maternal behavior comprises maternal care and maternal aggression (Bosch, 2013). Oxytocin is essential for lactation and maternal care (Bosch, 2013; Yoshihara *et al*., 2018), but does not significantly alter maternal aggression (Bosch and Neumann, 2012), suggesting distinct neurochemical pathways underlie these different behavioral aspects (Pires *et al*., 2013; Froemke and Young, 2021).

Oxytocin's role in maternal care likely involves interactions with hormones such as prolactin, a key regulator of oxytocin neuronal function that influences lactation and maternal behavior (Kokay *et al*., 2006). Prolactin receptors (PRLRs) are expressed on oxytocin neurons in rats, suggesting direct hormonal interactions (Sirzen-Zelenskaya *et al*., 2011; Georgescu *et al*., 2021). Interestingly, intracerebroventricular (i.c.v.) administration of prolactin inhibits oxytocin neuronal activity in virgin and pregnant rats, yet this inhibition is lost during lactation (Augustine *et al*., 2017; Georgescu *et al*., 2021). Prolactin also induces membrane hyperpolarization in paraventricular oxytocinergic neurons (Sirzen-Zelenskaya *et al*., 2011), and increases plasma levels of oxytocin and vasopressin by modulating nitric oxide synthase activity within the supraoptic nucleus and PVN (Vega *et al*., 2010). Together, these findings reveal complex prolactin-oxytocin interactions, (Larsen and Grattan, 2012; Georgescu *et al*., 2021) suggesting this signaling axis might be involved in modulating maternal behaviors by ABA.

Stress modulation could further contribute to ABA's observed effects. Stress and anxiety significantly impair maternal behaviors, as shown in lactating rodents exposed to stress (Gammie and Stevenson, 2006; Sheleg *et al*., 2017). Moreover, Central oxytocin administration reduces anxiety and stress hormone release via ERK1/2 signaling (Windle *et al*., 1997; Windle *et al*., 2004; Blume *et al*., 2008). Thus, increased oxytocin immunoreactive cells induced by ABA could indirectly enhance maternal behaviors by reducing anxiety and emotional distress in lactating rats. Future research should investigate whether ABA directly modulates

stress-related pathways within the PVN.

ABA's impact on maternal behavior may also be related to its reported neuroprotective effects. For example, ABA administration improved cognitive outcomes in Alzheimer's disease models and protected against cognitive impairment (Khorasani *et al*., 2019). Therefore, ABA might promote maternal behavior through similar neuroprotective mechanisms, potentially enhancing hypothalamic neuronal function, a hypothesis that warrants further investigation.

Importantly, we found that administration of the PPARγ antagonist GW9662 suppressed ABA-enhanced maternal behavior in lactating rats, confirming the involvement of PPARγ signaling in ABA-induced maternal behaviors. Previous studies have established that ABA interacts with various PPAR isoforms to facilitate learning, memory, and inflammatory responses (Bassaganya-Riera *et al*., 2011; Mollashahi *et al*., 2018; Khorasani *et al*., 2019); however, our identification of PPARγ's role in maternal care is a novel finding. The role of PPARγ in pregnancy and maternal health is already well-recognized, particularly in maintaining pregnancy and ensuring proper placental development. For example, activation of PPARγ reduces placental abnormalities, whereas its absence increases abortion rates (Lee *et al*., 1995; Kadam *et al*., 2015). Moreover, PPARγ activation mitigates cognitive and behavioral impairments in offspring following maternal immune activation or sleep deprivation by promoting hippocampal neurogenesis (Zhao *et al*., 2019; Han *et al*., 2020). While these findings show the significant association of PPARγ with pregnancy, maternal health, and labor, its precise role in maternal caregiving behaviors is not fully clear. Our study findings might help in filling this gap by highlighting the involvement of PPARγ signaling specifically in maternal behavior. However, this study utilized only one experimental approach involving the PPARγ antagonist GW9662. Therefore, future research should explore endogenous PPARγ activity and the effects of PPARγ agonists to more precisely explore the intracellular pathways possibly involved in maternal behavior modulation by this receptor.

This study demonstrates a novel role for ABA in promoting maternal care through PPARγ activation and increased immunoreactive cells in the hypothalamus. These findings provide valuable insights into maternal behavior's neuroendocrine regulation and highlight ABA's therapeutic potential in maternal health disorders. Future research should focus on detailed ABA-PPARγ signaling pathways and the neuroprotective role of ABA in maternal neural circuits. Such studies could lead to innovative therapeutic strategies for disorders involving impaired maternal behaviors, improving maternal and offspring outcomes.

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Conflict of interest

The authors claim that there are no conflicts of interest.

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