

A Postpartum Model in Rat: Behavioral and Gene Expression Changes Induced by Ovarian Steroid Deprivation

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Background: Postpartum depression (PPD) affects approximately 10% to 20% of women during the first 4 weeks of the postpartum period and is characterized by labile mood with prominent anxiety and irritability, insomnia, and depressive mood. During the postpartum period, elevated ovarian hormones abruptly decrease to the early follicular phase levels that are postulated to play a major role in triggering PPD. However, the underlying neurobiological mechanisms that contribute to PPD have not been determined.

Methods: In the present study, we examined the effect of ovarian steroids, administered at levels that occur during human pregnancy followed by rapid withdrawal to simulate postpartum conditions, on behavior and gene expression in the rat.

Results: The results of behavioral testing reveal that the hormone-simulated postpartum treatment results in the development of a phenotype relevant to PPD, including vulnerability for helplessness, increased anxiety, and aggression. Real-time quantitative polymerase chain reaction (PCR) demonstrated transient regulation of several genes, including Ca^{2+} /calmodulin-dependent protein kinase II (CAMKII), serotonin transporter (SERT), myocyte enhancer factor 2A (MEF2A), brain-derived neurotrophic factor (BDNF), gamma-aminobutyric acid type A receptor $\alpha 4$ (GABAARA4), mothers against decapentaplegic homolog 4 (SMAD4), and aquaporin 4 (AQP4) that could underlie these behavioral effects.

Conclusions: These studies provide an improved understanding of the effects of withdrawal from high doses of ovarian hormones on behavior and gene expression changes in the brain that could contribute to the pathophysiology of PPD.

Key Words: Aggression, anxiety, behavior, depression, gene profiling, postpartum depression

Postpartum depression (PPD) is a serious medical condition that affects approximately 10% to 20% of mothers during the first 4 weeks after delivery (1). Symptoms of PPD can include labile mood with prominent anxiety and irritability and depressive mood (2,3). Increased aggression and infanticidal thoughts are mainly linked to postpartum psychosis (4,5), which takes the form of mania or severe depression and complicates one in 1000 deliveries (5).

Pregnancy, delivery, and the postpartum period are characterized by robust hormonal change. By the end of third trimester, plasma estrogen and progesterone rise gradually and reach levels approximately 50-fold and 10-fold higher than maximal menstrual cycle levels, respectively. After parturition, these hormones rapidly drop to early follicular phase levels by days 3 to 7 (3). These endocrine events are purported to play a major role in triggering PPD. A pharmacological model of PPD supports this hypothesis; simulating the postpartum state by administering pregnancy levels of gonadal steroids and rapidly withdrawing them significantly increases the incidence of depression in women with a history of PPD more than in nondepressed women. Although statistically not significant, the scores of depression scales are higher in the withdrawal period in nonde-

pressed women, indicating that these endocrine events provoke mood changes in postpartum women (6). However, the underlying neurobiological mechanisms that contribute to PPD have not been determined.

Several studies have been conducted to address this issue using rat models (7–9). One study found an antidepressant-like response in the forced swim test (FST) in the early, but not the late, pregnancy/postpartum period (7). Another study examined the influence of hormone-simulated pseudopregnancy (HSP) to investigate the withdrawal effect of gonadal steroids on the FST (8,9). Ovariectomized rats were administered 17 β -estradiol benzoate (2.5 μg) and progesterone (4 mg) for 16 days, followed by 8 days of a high dose (50 $\mu\text{g}/\text{day}$) 17 β -estradiol benzoate treatment to mimic the rat pregnancy, combined with different time periods of withdrawal. The results demonstrate that rats receiving HSP followed by withdrawal exhibit significantly decreased antidepressant-like responses in the FST. However, this regimen was based on the plasma concentration of estrogen and progesterone that occurs in the course of rat pregnancy, where progesterone peaks during the middle of gestation and falls to estrous cycle levels by the time of delivery (10). Conversely in humans, both estrogen and progesterone increase gradually throughout the pregnancy (11). Progesterone has been shown to alter neurotransmission, indicating a crucial role in the pathophysiology of PPD (3). Thus, novel strategies may be necessary to address the pathophysiology of human PPD.

Based on these considerations, we have developed a novel strategy using endocrine conditions that mimic human pregnancy and the postpartum period. We examine the influence of a high dose of estrogen and progesterone and rapid withdrawal on behavioral alterations in models of depression, anxiety, and aggression, all symptoms of PPD, and gene expression changes. We refer to this as hormone-simulated pseudopregnancy-human (HSP-H) and the withdrawal period as hormone-simulated postpartum period-human (HSPP-H). The results identify a behav-

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ioral phenotype consistent with the symptoms of PPD and novel gene expression changes that could contribute to PPD. Although the use of human endocrine conditions in rodents has limitations, this model will be useful for future studies of this devastating disorder.

Methods and Materials

Animal Treatment

Female Sprague Dawley rats (250 to 280 g, Charles-River Laboratories, Wilmington, Massachusetts) were group housed and maintained on a 12-hour light/dark cycle (7:00 AM–7:00 PM lights on) with free access to food and water. All animal use procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by Yale University Animal Care and Use Committee.

Hormone-Simulated Pseudopregnancy-Human and Hormone-Simulated Postpartum Period-Human

Rats underwent ovariectomy (OVX) followed by continuous release pellet implantation (.5 mg of 17 β estradiol [E2] and 50 mg of progesterone [P4] 21-day release or placebo) (Innovative Research of America, Sarasota, Florida) under pentobarbital/atropine anesthesia to mimic serum levels of those during human pregnancy (11). After 21 days treatment, the hormone pellets were completely removed under isoflurane anesthesia. In accordance with previous studies using the rat model of the postpartum period, the 22nd to 28th days were considered as the HSPP-H (7–9). Behavioral tests were conducted in the following experimental groups: placebo treatment (OVX), 21-day treatment of HSP-H, and HSPP-H followed by different periods of hormonal withdrawal (1 day–7 days; HSPP-H/1–7). Separate groups of rats were tested on the forced swim test or the other behavioral tests. Rats that underwent FST were tested only once at each time point. Rats that underwent the open field test (OFT) on withdrawal day 4 were used for further behavioral analysis, i.e., elevated plus maze (EPM) on withdrawal day 5, learned helplessness (LH) or resident intruder test on withdrawal day 7 (Table 1). For gene expression, we used OVX, HSP-H, and HSPP-H/4 rats that were not subjected to the behavioral studies to avoid stress effects. Rats were sacrificed by decapitation, and brains were removed, dissected, and stored at -80°C .

Learned Helplessness

This test was performed with a shuttle box for active and passive avoidance (Med Associates Inc., St. Albans, Vermont)

Table 1. Experimental Timeline

Experimental Group	HSP Treatment		Behavioral Tests
	21 Days	Withdrawal	
OVX	Placebo	Pellet removal on day 21	FST on WD day 0, 1, 4, or 7; or
HSPP-H	E & P	Pellet removal on day 21	OFT on WD day 4, EPM on WD day 5, and LH or RIT on WD day 28
HSP-H	E & P	Sham surgery on day 17	FST on day 21; or OFT on day 21, EPM on day 22, and LH or RIT on day 24

E, estrogen; EPM, elevated plus maze test; FST, forced swim test; HSP-H, hormone-simulated pseudopregnancy-human; HSPP-H, hormone-simulated postpartum period-human; LH, learned helplessness test; OFT, open field test; OVX, ovariectomy; P, progesterone; RIT, resident intruder test; WD, withdrawal day.

during light cycle (1:00 PM–5:00 PM). Rats were subjected to 60 inescapable electric foot shocks (.65 mA; 15-sec duration, at random intervals; mean 30 sec; average 22–38 sec) in the shuttle box. Twenty-four hours later, all rats were placed back in the same shuttle box and tested for shuttle escape learning. The testing paradigm used fixed-ratio (FR) trials, first with 5 FR1 trials (one cross) to terminate the grid shock, followed by 25 FR2 trials (two crosses) to terminate the shock. The entire shuttle box escape test session lasted for 45 min per subject. The mean latencies to terminate the shock for the FR1 and FR2 trials were analyzed separately for all experimental groups (OVX, $n = 16$; HSP-H, $n = 18$; and HSPP-H/7, $n = 17$).

Forced Swim Test

This paradigm was performed as described previously (12–14) during light cycle (1:00 PM–5:00 PM). On day 1, the animals were placed in a Plexiglas cylinder (25 cm diameter \times 65 cm height) with water at a depth of 45 cm (24°C – 25°C) for 15 min. Then, the rats were removed from water, dried, and kept warm for 30 min. On day 2, the rats were placed back into the water for 5 min, and the sessions were videotaped (OVX, $n = 24$; other groups, $n = 6$). A time-sampling technique was used to rate the behavior at the end of each 5 sec period during the 300 sec test session. The behaviors scored in the FST were 1) immobility (rat making only those movements necessary to keep its head above water); 2) swimming (rat staying afloat, pedaling, and making circular movements around the tank); and 3) struggling/climbing (rat making active attempts to escape from the tank).

Open Field Test

Spontaneous locomotor activity was measured in the open field test in a square arena (76.5 \times 76.5 \times 49 cm) using a standard procedure (15) during the dark cycle (9:00 PM–1:00 AM). Animals that underwent OVX, HSP-H, and HSPP-H/4 were used ($n = 16$ /group). The open field was divided into two areas, a peripheral area and a square center (40 \times 40 cm). Rats were allowed to explore for 5 min. The test room was dimly illuminated under red light. The computer software (EthoVision; Noldus, Alexandria, Virginia) calculated the distance moved and the time spent in the center of the open field.

Elevated Plus Maze Test

This test was carried out during dark cycle (9:00 PM–1:00 AM). This model consists of an elevated (50 cm above the floor) plus-shaped maze with two opposite enclosed and two open arms, measuring 50 cm long and 10 cm wide, placed in a red dimly lit room. The testing procedure was described previously (16). Each animal underwent OVX, HSP-H, and HSPP-H/4 ($n = 14$ /group) was placed in the center of the maze facing a closed arm. An entry into an arm was defined as the animal placing all four paws into it. The cumulative time spent in the open/closed arms and in the center and the number of open-arm and closed-arm entries were recorded through a 5-min session. Data were expressed as the time spent in the open arms, the closed arms, and the center (second); the number of entries to the closed arms; and the total number of entries (open + closed arms).

Resident Intruder Test

Aggression toward an unfamiliar intruder was evaluated as described previously (17–19). This test was carried out during the dark cycle (9:00 PM–1:00 AM). A female resident rat that underwent OVX, HSP-H, or HSPP-H/7 treatment ($n = 10$ /group) confronted a naive male intruder rat (240–270 g) for 10 min. A similar paradigm (female resident and smaller male intruder) has

Table 2. Primers Used for Quantitative Real-Time PCR

Gene ID	Left Primer Sequence	Right Primer Sequence
AQP4	AGATCAGCATCGCCAAGTCT	GGGTGTGACCAGGTAGAGGA
BDNF	AAGGCTGCAGGGGCATAGAC	TGAACCGCAGCCAATTCTC
CAMKIIA	TATCGTCCGACTCCATGACA	CTGGCATCAGCCTCACTGTA
CAMKII B	GGCCAGCAAATGCAAAGG	GTTCCCGCAAATCCAAACC
CAMKII D	CCAAAGACAATGCAGTCAGAAGAG	GACCCCGAACGATGAAAGTG
CAMKII G	GCAGGCTTGGTTTGGTTTTG	TCCATAGGGATCTTCTCAAGAC
GABAARA4	TGAAATCCTGAGGTTGAACAATATG	GACAGATTTCTTCCATTCCTGAAG
MEF2A	GCCTCCGAGGGACTAGTG	GCGCTGGTCAA TGAGTAATCAG
SERT	ACTGGGCCAGTACCACCG	TCGGGCAGATCTTCCTCC
SMAD4	GGCATTGGTGTAGACGACCT	CGGTGGAGGTGAATCTCAA

AQP4, aquaporin 4; BDNF, brain-derived neurotrophic factor; CAMKII, Ca²⁺/calmodulin-dependent protein kinase II; GABAARA4, gamma-aminobutyric acid type A receptor α 4; MEF2A, myocyte enhancer factor 2A; PCR, polymerase chain reaction; SERT, serotonin transporter; SMAD4, mothers against decapentaplegic homolog 4.

been shown to result in significant aggressive behavior in a previous report (19). The behavior of residents during confrontation was videotaped and latency to the first attack was monitored. Frequency and duration of behavioral acts were manually scored for each animal. The following behaviors were distinguished: 1) lateral threat; 2) offensive upright; 3) keep down; 4) clinch; 5) chase; 6) investigating opponent; 7) anogenital sniffing; 8) social grooming/crawl over; 9) mounting; 10) ambulation; 11) rearing; 12) grooming; 13) inactivity; 14) freeze; 15) submissive posture; and 16) keep off. Offensive behavior includes lateral threat, offensive upright, keep down, clinch, and chase. Social explore includes investigating opponent and anogenital sniffing. Social interaction includes social grooming/crawl over and mounting. Nonsocial explore includes ambulation and rearing. The duration of the different behavioral categories was cumulated and expressed as a percentage of the 10-min confrontation.

Real-Time Quantitative Reverse Transcript Polymerase Chain Reaction

To identify the candidate genes that are regulated by HSP-H and HSPP-H, DNA microarray analysis was performed as described previously (20) (Supplement 1). Differential expression of candidate genes obtained from DNA microarray analysis was confirmed by real-time quantitative reverse transcript polymerase chain reaction (RT-PCR). Total RNA (3 μ g) from hippocampus, a region that is implicated in the pathophysiology of mood disorders (21), was reverse transcribed using SuperScript II first-strand synthesis system for RT-PCR (Invitrogen, Carlsbad, California). Primers used for RT-PCR are designed using Primer express software (Applied Biosystems, Foster City, California) and listed in Table 2. Real-time quantitative reverse transcript polymerase chain reaction was performed in the ABI PRISM 7900HT fast real-time polymerase chain reaction (PCR) system (Applied Biosystems) using SYBR green detection (QuantiTect SYBR green PCR kit, Qiagen, Valencia, California). The following temperature profile was used: 40 cycles of 15 sec at 95°C followed by 1 min at 60°C. Cycle thresholds were automatically determined within the log-linear phase of the reaction using Sequence Detection Systems (SDS) plate utility software, version 2.2 (Applied Biosystems). The comparative cycle threshold method (Δ Ct method) was used to calculate relative expression levels. Briefly, to calculate Δ Ct, cycle number at threshold crossing values (Ct) for an internal control housekeeping gene (cyclophilin) was subtracted from Ct for a gene of interest. Each expression value relative to cyclophilin was determined as $2^{-\Delta$ Ct and normalized by OVX ($n = 6$ /group).

Statistical Analysis

The data obtained in the experiments were analyzed by a one-way analysis of variance (ANOVA), followed by Fisher's protected least squares difference (PLSD) or Scheffe's test for the post hoc comparison of the mean. All statistical data in the text

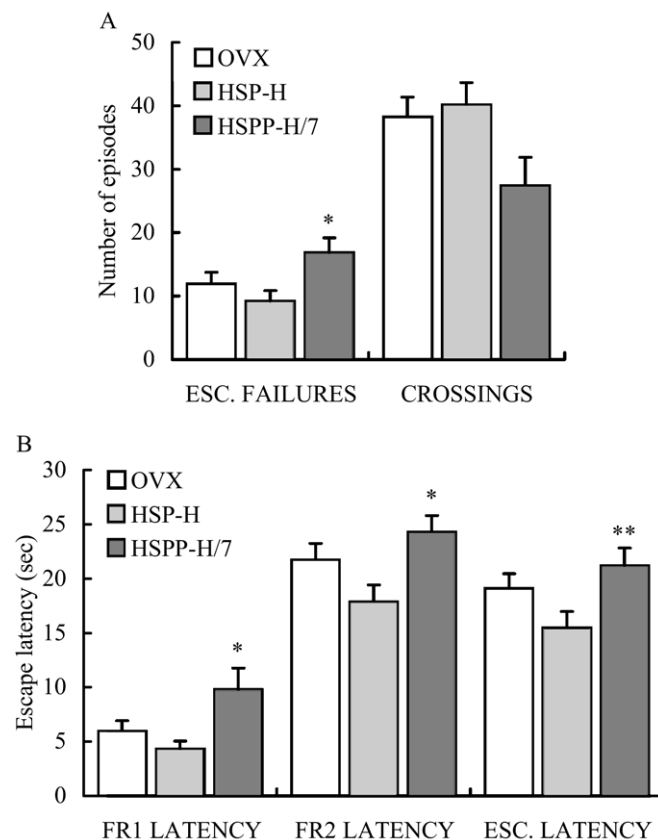


Figure 1. Influence of HSP-H and HSPP-H on the learned helplessness test. Escape failures, number of crossings, and escape latency (FR1, FR2, and total mean value) were determined. The results are the mean \pm SEM (OVX $n = 16$, HSP-H $n = 18$, HSPP-H/7 $n = 17$). ANOVA and Scheffe: * $p < .05$, difference in comparison with HSP-H, ** $p < .01$, difference in comparison with HSP-H. Left top to right top, $F(2,48) = 4.181, p < .05$; $F(2,48) = 3.437, p < .05$. Left bottom to right bottom, $F(2,48) = 4.663, p < .05$; $F(2,48) = 4.690, p < .05$; $F(2,48) = 5.323, p < .01$. ANOVA, analysis of variance; FR1, fixed-ratio one cross; FR2, fixed-ratio two crosses; HSP-H, hormone-simulated pseudopregnancy-human; HSPP-H, hormone-simulated postpartum period-human; OVX, ovariectomy.

are expressed as mean ± SEM; differences were considered statistically significant only if $p < .05$.

Results

Behavior Analysis

Learned Helplessness Test. Animals exposed to repeated inescapable foot shock (IES) exhibit “helpless” behavior characterized by an inability to respond in a subsequent escapable situation. This escape deficit is reversed by chronic antidepressant treatments (22,23). There were significant effects of treatment on escape failure and escape latency of FR1, FR2, and the mean [$F(2,48) = 4.181, p = .0212$; $F(2,48) = 4.663, p = .0141$; $F(2,48) = 4.690, p = .0138$; $F(2,48) = 5.323, p = .0082$]. The HSP-H/7 animals displayed a significantly increased escape failure and latency to escape after exposure to IES compared with OVX. There were no significant behavioral changes between the OVX and HSP-H groups (Figure 1, Table 3). The results demonstrate that the hormonal deprivation subsequent to HSP-H treatment increases helplessness in the LH.

Forced Swim Test. In this paradigm, the time spent in immobility is typically decreased by antidepressant administration (12). The results revealed significant differences between the groups in mean time spent in immobility and struggling/climbing [$F(4,43) = 5.410, p = .0013$; $F(4,43) = 4.746, p = .0029$]. Immobility was significantly decreased in the HSP-H/4 and HSP-H/7 groups and struggling/climbing was significantly increased in the HSP-H/7 group compared with OVX. There was no significant interaction between treatment group and swimming [$F(4,43) = 2.317, p = .0724$] (Figure 2, Table 3).

Open Field Test. The open field test, as well as EPM, were conducted to test the possibility that the decreased immobility observed in HSP-H on the FST was due to increased spontaneous locomotor activity or increased anxiety. Time spent in the center of a novel open field and the distance moved are indications of anxiety and locomotor activity, respectively (15). Four days’ withdrawal was examined, as decreased immobility in the FST was observed at this time point. Hormone-simulated pseudopregnancy-human treatment and HSP-H did not significantly influence time in the center field or the distance moved

Table 3. Behavioral Profile of HSP-H and HSP-H Animals

Behavioral Tests	HSP-H	HSP-H
Learned Helplessness		
Escape failures	–	++/helpless
Escape latency	–	++
Forced Swim Test		
Immobility	ne	+++
Swimming	ne	+
Struggling/climbing	ne	++
Open Field Test		
Time in center	ne	ne
Total distance	ne	ne
Elevated Plus Maze		
Open arm	–	+++
Total arm	--	ne
Resident Intruder Test		
Social behaviors	ne	ne
Aggression: time	+++++	+++
latency	+++	+++

HSP-H, hormone-simulated pseudopregnancy-human; HSP-H, hormone-simulated postpartum period-human; ne, no effect; +, indicated behavior induced; –, opposite effect observed.

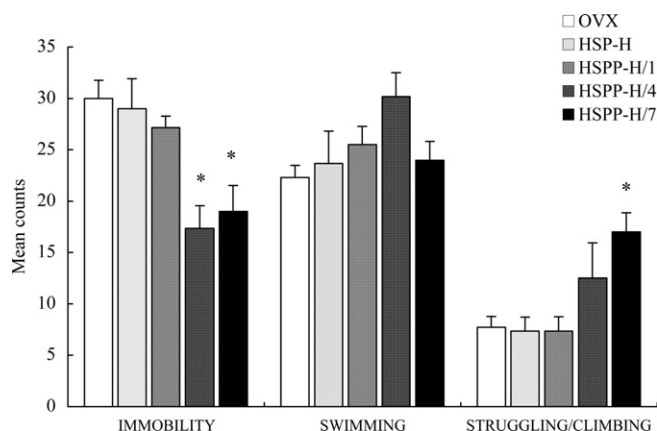


Figure 2. Influence of HSP-H and HSP-H on the FST. Each behavior (immobility, swimming, struggling/climbing) was scored during the first 5 min of day 2 trials. The results are the mean ± SEM (OVX $n = 24$; HSP-H, HSP-H/1, HSP-H/4, and HSP-H/7 $n = 6$ each). ANOVA and Scheffe: * $p < .05$, difference in comparison with OVX. Left to right, $F(4,43) = 5.410, p < .005$; $F(4,43) = 2.317$, not significant (ns); $F(4,43) = 4.746, p < .005$. ANOVA, analysis of variance; FST, forced swim test; HSP-H, hormone-simulated pseudopregnancy-human; HSP-H, hormone-simulated postpartum period-human; ns, not significant; OVX, ovariectomy.

[$F(2,45) = .566, p = .5717$; $F(2,45) = 1.176, p = .3177$], although there was a tendency for a decrease in center time in HSP-H treated groups (Figure 3, Table 3).

Elevated Plus Maze Test. This test relies on the inherent conflict between exploration and avoidance of a novel area. The time spent on the open arms and the number of entries into the open arms are inversely correlated with anxiety. Previous studies demonstrate that anxiolytic agents increase and that anxiogenic drugs decrease the time and entries in the open arms (16). The amount of time spent in the open arms of the OVX females is similar to that in previous reports (24,25). Analysis of variance revealed a significant effect of treatment on time spent in open arms and number of total entries [$F(2,27) = 15.119, p < .0001$; $F(2,27) = 16.569, p < .0001$]. Hormone-simulated postpartum period-hu-

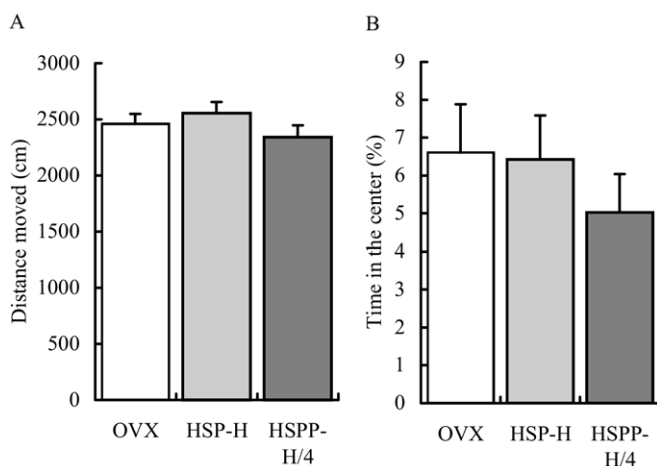


Figure 3. Influence of HSP-H and HSP-H on the open field test. Distance moved and the time spent in the center that indicate locomotor activity and anxiety, respectively, were calculated. The results are the mean ± SEM ($n = 16$ /group). Left to right, $F(2,45) = .566, ns$; $F(2,45) = 1.176, ns$. HSP-H, hormone-simulated pseudopregnancy-human; HSP-H, hormone-simulated postpartum period-human; ns, not significant.

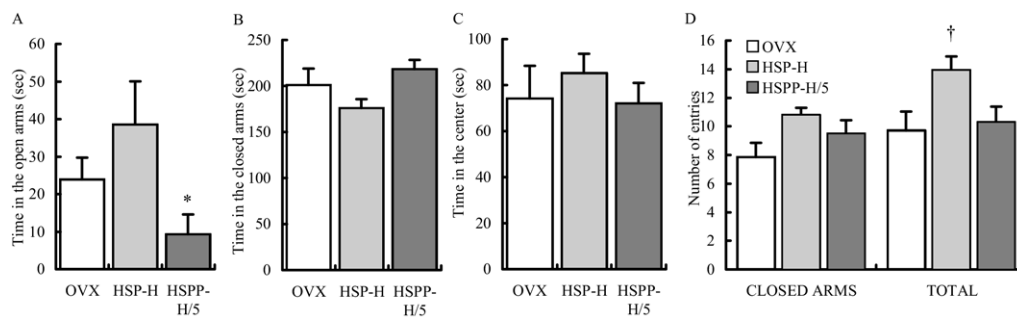


Figure 4. Influence of HSP-H and HSPP-H on the elevated plus maze test. This test was conducted to evaluate the influence of HSP-H and HSPP-H treatments on anxiety. The results are the mean ± SEM (*n* = 14/group). ANOVA and Scheffe: * *p* < .05, difference in comparison with HSP-H. † *p* < .05, difference in comparison with OVX. Left top to right top, *F*(2,39) = 3.297, *p* < .05; *F*(2,39) = 2.744, ns; *F*(2,39) = .425, ns. Left bottom to right bottom, *F*(2,39) = 3.020, ns; *F*(2,39) = 4.073, *p* < .05. ANOVA, analysis of variance; HSP-H, hormone-simulated pseudopregnancy-human; HSPP-H, hormone-simulated postpartum period-human; ns, not significant; OVX, ovariectomy.

man/5 animals spent significantly less time in the open arms than HSP-H animals, whereas HSP-H rats showed a significant increase in the number of entries compared with OVX (Figure 4, Table 3).

Resident Intruder Test. This test was performed to evaluate whether maternal aggression is induced by HSP-H or HSPP-H. Intrusion of an unfamiliar rat into the home cage provokes a typical offensive and aggressive behavioral pattern of the resident rat (18). The results revealed a significant effect of treatments on offensive behavior and attack latency [*F*(2,27) = 15.119, *p* < .0001; *F*(2,27) = 16.569, *p* < .0001]. Hormone-simulated pseudopregnancy-human animals showed a significantly increased time in offensive behaviors toward the intruder compared with OVX and HSPP-H/7 animals. Both HSP-H and HSPP-H/7 showed a significant reduction in the attack latency to the intruder. There was no significant influence on other social and nonsocial behaviors (Figure 5, Table 3). These results indicate that the HSP-H treatment induces aggression in female rats that persists for at least 7 days after withdrawal.

Gene Expression: RT-PCR

Differential expression of candidate genes obtained from microarray results (see Supplement 1 for details) was confirmed using real-time quantitative RT-PCR. The results revealed that Ca²⁺/calmodulin-dependent protein kinase IIδ (CAMKIID) is significantly upregulated by HSP-H and returns to OVX levels after 4 days withdrawal. Ca²⁺/calmodulin-dependent protein kinase IIα (CAMKIIA) expression was significantly decreased in the HSPP-H/4 group compared with OVX and HSP-H. Other CAMKII subtypes, including CAMKIIβ (CAMKIIB) and CAMKIIγ

(CAMKIIG), were not regulated. The expression of the serotonin transporter (SERT) was significantly increased by HSP-H and returned to OVX levels after 4 days withdrawal. Myocyte enhancer factor 2A (MEF2A) and brain-derived neurotrophic factor (BDNF) expressions were significantly attenuated after hormonal deprivation but were not altered by the HSP-H per se. Gamma-aminobutyric acid type A receptor α4 (GABAARA4) and aquaporin 4 (AQP4) expressions were significantly downregulated by HSP-H and returned to OVX levels after 4 days withdrawal. Expression of mothers against decapentaplegic homolog 4 (SMAD4) was significantly decreased in the HSP-H group compared with OVX (Figure 6, Table 4). Other genes that have been implicated in mood disorders, including the glucocorticoid receptor (26), vascular endothelial growth factor (VEGF) (27), glycogen synthase kinase 3β (GSK3B), Akt/protein kinase B (28), and interleukin-18 (29), were not altered in the present model (not shown).

Discussion

Behavioral Studies

In the LH behavioral despair model, the HSPP-H animals showed significantly increased escape failures and latency to escape, interpreted as a measure of helplessness or depressive-like behavior. Conversely, in the FST, the HSPP-H animals displayed a significant decrease in immobility, which is typically interpreted as a measure of antidepressant activity (12,30). Although the opposing effects observed in LH and FST tests appear contradictory, there are conflicting reports on FST behav-

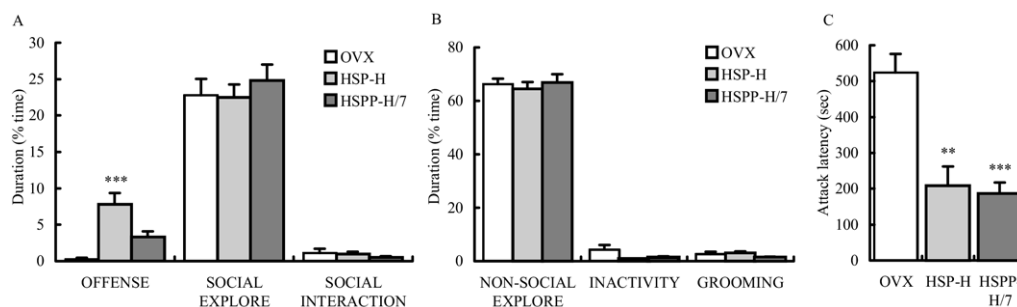


Figure 5. Influence of HSP-H and HSPP-H on the resident intruder test. The results are the mean ± SEM (*n* = 10/group). ANOVA and Scheffe: **p* < .01, ***p* < .001, ****p* < .0001, difference in comparison with OVX. † *p* < .05, difference in comparison with HSP-H. Left top to right top, *F*(2,27) = 15.119, *p* < .0001; *F*(2,27) = .385, ns; *F*(2,27) = .746, ns. Left bottom to right bottom, *F*(2,27) = .250, ns; *F*(2,27) = 3.449, *p* < .05; *F*(2,27) = 2.291, ns; *F*(2,27) = 16.569, *p* < .0001. ANOVA, analysis of variance; HSP-H, hormone-simulated pseudopregnancy-human; HSPP-H, hormone-simulated postpartum period-human; ns, not significant; OVX, ovariectomy.

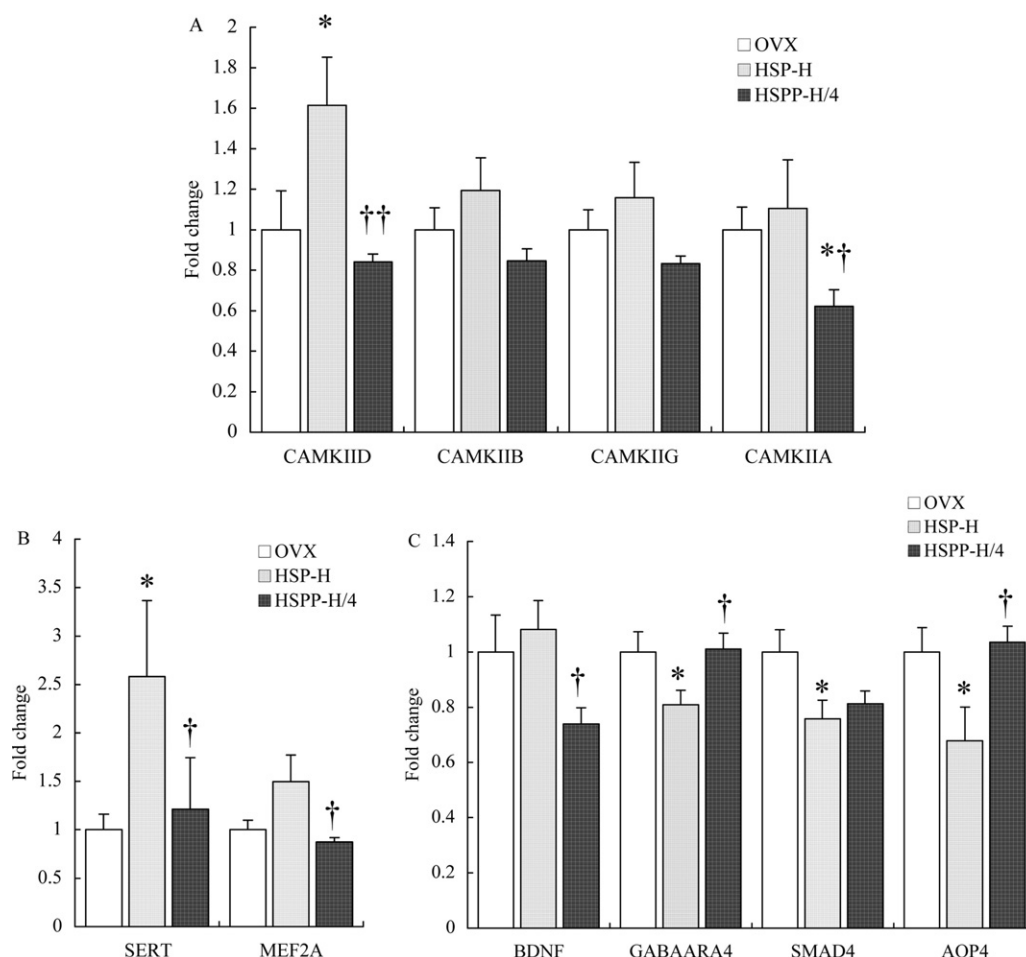


Figure 6. Real-time PCR of selected genes identified by microarray to be regulated by HSP-H and HSPP-H. Fold change was calculated as $2^{-\Delta Ct}$ and normalized by OVX as described in Methods and Materials. The results are the mean \pm SEM ($n = 6/\text{group}$). ANOVA and Fisher's PLSD: * $p < .05$, † $p < .05$, †† $p < .01$, difference in comparison with HSP-H. **(A)** CAMKII: Left to right, $F(2,15) = 5.239, p < .05$; $F(2,15) = 2.207, ns$; $F(2,15) = 1.874, ns$; $F(2,15) = 4.648, p < .05$. **(B)** Upregulated genes: Left to right, $F(2,15) = 3.398, p < .05$; $F(2,15) = 3.732, p < .05$. **(C)** Downregulated genes: Left to right, $F(2,15) = 3.889, p < .05$; $F(2,15) = 4.059, p < .05$; $F(2,15) = 3.695, p < .05$; $F(2,15) = 4.424, p < .05$. ANOVA, analysis of variance; CAMKII, Ca^{2+} /calmodulin-dependent protein kinase II; HSP-H, hormone-simulated postpartum period-human; HSPP-H, hormone-simulated postpartum period-human; ns, not significant; OVX, ovariectomy; PCR, polymerase chain reaction; PLSD, protected least squares difference.

ior in postpartum rat models. One study reported decreased immobility in early pregnancy and no effect in postpartum (7), while other studies have reported that an HSPP-H regimen that mimics rat pregnancy (i.e., progesterone [4 mg/day] terminated in day 14), increased immobility (8,9). The differences in the progesterone treatment between the latter and the present study (continuous release pellet, 50 mg/21 days) could underlie the behavioral differences observed.

To examine the possibility that the decreased immobility observed in HSPP-H on the FST was influenced by altered spontaneous locomotor activity or anxiety, we examined behaviors in the OFT and EPM. The results of the OFT demonstrate that the HSP-H, with or without withdrawal, has no significant effect on locomotor activity. Hormone-simulated postpartum period-human showed a small trend for decreased time spent in the center, indicative of increased anxiety. This was confirmed by the EPM, which demonstrates a highly significant increase in anxiety, indicating that hormone withdrawal also leads to increased anxiety. The reason for the discrepancy between the OFT and EPM is not clear but could be related to the greater level of anxiety that is encountered in the open arm of the EPM relative

to that in the open field. It is also possible that the elevated anxiety observed in the HSPP-H withdrawal group could contribute to the altered behavior in the FST. This is supported by previous studies demonstrating that agents that increase anxiety, including pentylentetrazol, a corticotropin-releasing factor receptor type 1 (CRF1) agonist, or a diazepam inverse agonist, all decrease immobility time in the FST, similar to the effects observed in the present study (31–33). In addition, exposure to stress produces a state where subsequent antidepressant treatment increases, instead of decreases, immobility (34). These studies indicate that enhanced anxiety could increase struggling and climbing in the FST and thereby account for the observed decrease in immobility. In summary, the data are consistent with the hypothesis that hormone withdrawal increases behavioral despair in the LH paradigm and that this is accompanied by increased anxiety in situations that induce a relatively high state of anxiety, such as the open arm in the EPM and water/swimming in the FST.

The elevated anxiety observed in the EPM is in line with the clinical features of PPD; women with PPD are more likely to present with anxious features compared with patients with

Table 4. Genes Regulated by HSP-H and HSPP-H Treatments

Protein	Gene ID	Fold Change (ANOVA and Fisher PLSD <i>p</i> Value)		
		HSP-H/OVX	HSPP-H/OVX	HSPP-H/HSP-H
Ca ²⁺ /Calmodulin-dependent Protein Kinase II α	CAMKIIA	+1.11 (ns)	–1.61 (.0242)	–1.78 (.0147)
Ca ²⁺ /Calmodulin-dependent Protein Kinase II β	CAMKIIB	+1.19 (ns)	–1.18 (ns)	–1.41 (ns)
Ca ²⁺ /Calmodulin-dependent Protein Kinase II δ	CAMKIID	+1.61 (.0280)	–1.19 (ns)	–1.92 (.0078)
Ca ²⁺ /Calmodulin-dependent Protein Kinase II γ	CAMKIIG	+1.16 (ns)	–1.20 (ns)	–1.39 (ns)
γ -Amino-Butyric Acid Type A Receptor α 4	GABAARA4	–1.24 (.0318)	+1.01 (ns)	+1.25 (.0219)
Serotonin Transporter	SERT	+2.58 (.0425)	+1.21 (ns)	–2.13 (.0261)
Aquaporin 4	AQP4	–1.48 (.0282)	+1.04 (ns)	+1.52 (.0164)
Myocyte Enhancer Factor 2A	MEF2A	+1.49 (ns)	–1.15 (ns)	–1.72 (.0206)
SMAD, Mothers Against Decapentaplegic Homolog 4	SMAD4	–1.32 (.0205)	–1.23 (ns)	+1.07 (ns)
Brain-Derived Neurotrophic Factor	BDNF	+1.08 (ns)	–1.36 (ns)	–1.47 (.0170)

ANOVA, analysis of variance; AQP4, aquaporin 4; BDNF, brain-derived neurotrophic factor; CAMKII, Ca²⁺/calmodulin-dependent protein kinase II; GABAARA4, gamma-aminobutyric acid type A receptor α 4; HSP-H, hormone-simulated pseudopregnancy-human; HSPP-H, hormone-simulated postpartum period-human; MEF2A, myocyte enhancer factor 2A; ns, not significant; PLSD, protected least squares difference; SERT, serotonin transporter; SMAD4, mothers against decapentaplegic homolog 4.

nonpostpartum depression (35). This finding is also supported by animal studies demonstrating that pseudopregnant rats that undergo estrogen and progesterone withdrawal display an enhanced level of anxiety (36). Progesterone elicits an anxiolytic effect through its metabolite allopregnanolone, which interacts with the gamma-aminobutyric acid type A receptor (GABAAR) in a benzodiazepine-like manner (37–39). Therefore, it is possible that progesterone deprivation leads to a rebound increase in anxiety.

The resident intruder test revealed increased aggression toward the intruder in the HSP-H and the HSPP-H animals compared with the OVX animals. Previous studies are in agreement with this finding, reporting that in rodents the dam shows an enhanced aggressive behavior toward an intruder after parturition (40–43), although reduced anxiety has been reported (40,44). There is clinical evidence that the symptoms of PPD may include anxiety accompanied by aggression, sometimes resulting in obsessions of child harm (5). Taken together, the increased anxiety and aggression, as well as depressive-like behavior, observed in the current PPD model are more relevant to the clinical manifestations of PPD than those observed in models that more closely approximate the rodent hormone levels and changes.

Gene Expression Analysis

Ca²⁺/calmodulin-dependent protein kinase II is a mediator of diverse physiological responses induced by Ca²⁺-linked signaling that exists in four known isoforms, CAMKIIA, CAMKIIB, CAMKIID, and CAMKIIG (45). Ca²⁺/calmodulin-dependent protein kinase IIA and CAMKIIB are neuron specific, whereas CAMKIID and CAMKIIG are ubiquitous (46). Ca²⁺/calmodulin-dependent protein kinase IIA, the most enriched form, plays a role in synaptic plasticity and memory (47–50) and neurotransmission (51). Postmortem brain studies have suggested the involvement of CAMKIIs in the pathophysiology of psychiatric conditions including bipolar disorder (52), Alzheimer disease (53), schizophrenia, and depression (54). Furthermore, chronic antidepressant treatment increases CAMKII activity (55,56). Our results revealed transient CAMKIID upregulation and decreased CAMKIIA expression during the course of HSP followed by withdrawal, suggestive of possible involvement of these kinases in the pathophysiology of PPD.

Serotonin transporter regulates extracellular serotonin levels (57) and is a primary molecular target of antidepressants (58).

Serotonin transporter gene expression is regulated by cyclic adenosine monophosphate (cAMP)-dependent (59), as well as cyclic guanosine monophosphate (cGMP)-dependent, pathways (60); S100 β , which is astrocyte-specific Ca²⁺-binding protein (61); and steroid hormones including estrogen (59,62). The adaptive downregulation of this protein is postulated to play a major role in the clinical response of antidepressants (63). In our animal model of PPD, we observed a transient increase and a rapid decrease to basal levels of SERT expression in response to HSP-H followed by withdrawal. The large fluctuation of SERT expression that occurs during the perinatal period could contribute to the development and expression of mood disorders.

Myocyte enhancer factor 2A is a transcription factor that plays a critical role in cell differentiation during development of skeletal muscle, as well as the central nervous system (CNS) (64). Although there are no reports of a relationship between MEF2A and mood disorders, our results suggest that decreased MEF2A gene expression after gonadal steroid deprivation could also contribute to the behavioral changes observed.

Brain-derived neurotrophic factor is a small basic protein that supports the survival of neurons (65). Several lines of evidence suggest the involvement of BDNF in the neurobiological basis of depression and antidepressant response. Stress decreases and antidepressant treatment increases the expression of BDNF (66,67), and BDNF infusions produce an antidepressant response in rodent behavioral models (14,23,68). The present results demonstrate that BDNF expression is decreased during the HSPP-H period, an effect that could contribute to the depressive-like behavior observed in the learned helplessness paradigm and possibly the other behavioral responses.

The gamma-aminobutyric acid (GABA) gated chloride channel A (GABAAR) is a pentamer composed of various combinations of α (1–6), β (1–3), γ (1–3), δ , and ϵ subunits that mediate inhibitory neurotransmission in the CNS (69). Withdrawal from the progesterone derivative allopregnanolone increases both the messenger RNA (mRNA) and protein of GABAAR α 4 (GABAARA4), a subunit that is relatively insensitive to benzodiazepines (36,70) and has been reported to be involved in the pathophysiology of PPD and premenstrual syndrome (3). The results of the present study are consistent with these findings and demonstrate that GABAARA4 expression is increased in HSPP-H animals compared with HSP-H.

Aquaporin 4 is the predominant water channel in the brain and might be related to the formation of blood-brain barrier (71).

Increased AQP4 expression has been reported in postmortem brain of bipolar disorder subjects (72). Our results reveal a transient decrease of AQP4 expression by HSP-H followed by rapid recovery.

Mothers against decapentaplegic homolog 4 (SMAD4) is an essential intracellular component of transforming growth factor beta (TGF- β) signaling (73). Enhancement of TGF- β signaling by activin, a member of TGF- β superfamily, promotes an antidepressant-like effect (74). In the present study, SMAD4 expression is decreased by HSP-H, suggesting that the behavioral changes in the postpartum model could be precipitated by attenuated TGF- β signaling.

In summary, we have established a novel animal model of PPD that mimics human pregnancy and the postpartum period. Although there are limitations of our study, including the difference of gestation length between human and rat and the levels of estrogen and progesterone, the results demonstrate a behavioral phenotype that is relevant to PPD symptoms, including increased anxiety, increased aggression, and vulnerability for learned helplessness. Analysis of gene expression reveals transient regulation of several genes encoding regulatory proteins that might be susceptible genes for PPD, a hypothesis to be tested in future studies. We believe these studies provide an improved understanding of the effects of ovarian hormones and withdrawal on behavioral and gene expression changes in the brain that underlie the pathophysiology of PPD.

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