Importance of early lighting conditions in maternal care by dam as well as anxiety and memory later in life of offspring

Shigeru Toki[1], Shigeru Morinobu[1], Akihiro Imanaka[1], Shigeto Yamamoto[1], Shigeto Yamawaki[1], Ken-ichi Honma[2]

[1]Department of Psychiatry and Neurosciences, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University
[2]Department of Physiology, Hokkaido University Graduate School of Medicine

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Abstract

Rodent studies have revealed that the early rearing environment plays an important role in the development of stress vulnerability, memory and cognition. Although early lighting conditions (ELC) are involved in these neuronal developments through both maternal and offspring behavior, their influence has not been fully elucidated. Thus, by using Sprague-Dawley strain rats, we examined whether ELC affected maternal care by the dam and the subsequent neurodevelopment of the offspring. Prolonged dark phase conditions (PDC; L/D=6:18h) and prolonged light phase conditions (PLC; L/D=18:6h) were administered from postnatal day 2 to postnatal day 14. Throughout this period, maternal care and circadian rhythmicity of dams were investigated. In adolescence and adulthood of the offspring, we measured anxiety-like behavior, social interaction, object recognition memory, activity rhythm and corticosterone response to stress with hippocampal expression of NMDA and glucocorticoid receptor mRNAs. PDC altered maternal care and circadian rhythmicity in the dam compared with normal lighting conditions (NLC) and PLC. PDC markedly increased anxiety-like behavior, decreased social interaction and object recognition memory and inhibited corticosterone feedback in offspring later in life. Furthermore, hippocampal levels of glucocorticoid receptor mRNA and NR2B mRNA in rats subjected to PDC were significantly lower than in animals subjected to NLC. In the adult offspring, the circadian rhythm of locomotor activity was not affected. These findings suggested that ELC affect mother-infant interactions and subsequently, at least partially alter neurobehavioral development of offspring.

Introduction

Rodent studies have shown that early life experiences based on mother-infant interactions have long-lasting influence on neuronal development and subsequently regulate behavioral, cognitive, and neuroendocrinological function in adulthood. For example, early adversity such as maternal separation or low maternal care was demonstrated to be closely involved in enhanced activity of the hypothalamic-pituitary-adrenocortical (HPA) system in response to stress in later life (Ader & Grota, 1970; Rosenfeld et al., 1992; Pihoker et al., 1993; Liu et al., 1997; Biagini et al., 1998; Caldji et al., 1998; Liu et al., 2000a; Kalinichev et al., 2002; Levine, 2005). In addition, a series of studies by Meaney and colleagues showed that maternal care, especially licking/grooming, which forms the basis for tactile stimulation (Jutapakdeegul et al., 2003), is critical for development of emotionality (Liu et al., 1997; Caldji et al., 1998; Caldji et al., 2003; Menard et al., 2004; Zhang et al., 2005) and memory function in pups (Liu et al., 2000b; Bredy et al., 2003a; Bredy et al., 2003b; Bredy et al., 2004). In line with this maternal mediation hypothesis earlier postulated by Levine (Levine, 1967), and Smotherman and Bell (Smotherman & Bell, 1980), Meaney predicted that variations in maternal care can account for the effects of various postnatal manipulations on the phenotype of

Coresspondence: Professor Dr. Shigeto Yamawaki, as above.
E-mail: yamawaki@hiroshima-u.ac.jp
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offspring (Champagne & Meaney, 2001; Cameron et al., 2005).

It has been reported that the peripartum environment involving such factors as poverty, food availability, stress exposure, etc., directly affects characteristics of maternal care and mother-infant interaction in many species (Gray, 1994, 1995; Champagne & Meaney, 2001; Cameron et al., 2005; Pryce et al., 2005). Although it is conceivable that lighting conditions are among the major environmental factors affecting rearing behavior, few studies have examined the influence of lighting conditions on maternal care and neuronal development in mammals. There is a clear circadian rhythm to nursing behavior in many species, including the mouse (Hoshino et al., 2006), rat (Ader & Grota, 1970; Grota & Ader, 1974; Lee & Williams, 1977; Coble et al., 1994) and rabbit (Jilge, 1993, 1995). In rats, maternal care changes over the diurnal cycle, with the amount of time spent nursing being relatively high during the light phase (Ader & Grota, 1970; Grota & Ader, 1974). Recently, it was demonstrated that the Clock mutation in the suprachiasmatic nucleus in the hypothalamus impaired nurturing behavior by destroying the circadian secretion of prolactin in the mouse (Hoshino et al., 2006). Since the biological clock is strongly controlled by light, whether the lighting environment affects nurturing behavior may be an important question with regard to the early neonatal environment. It was suggested that entrainment to a new lighting condition was accompanied by stress (Stephens, 1980; Munck et al., 1984), therefore it is also likely that circadian change-induced stress may affect maternal care by dams. Thus, it could be predicted that altered photoperiodic conditions would change the acrophase in the dam and subsequently influence mother-infant interaction. Also, it could be predicted that offspring exposed to various levels of maternal care might correspondingly exhibit altered development of emotionality and memory functioning.

In this context, to elucidate whether early lighting conditions (ELC) affect maternal care and the circadian rhythm of the dam, we examined whether prolonged dark phase conditions (PDC) as well as prolonged light phase conditions (PLC) during the postpartum period in rats changed active nursing behavior and locomotor activity. Furthermore, we also examined whether alterations in ELC during the neonatal period of rats induced anxiety-like behavior and dysfunction of hippocampus-dependent memory or the HPA system in response to stress in later life to elucidate the importance of ELC in the development of stress vulnerability.

Materials and Methods

Animals

One hundred and four pregnant Sprague-Dawley dams were housed individually in standard polycarbonate cages. Environmental conditions were consistent among the different manipulated groups with the exception of lighting (temperature 23±2oC, humidity 60%). Light intensity at cage level was approximately 100 lux. Rat chow and tap water were available at all times. Pregnant dams were inspected daily in the morning for delivery, and the day of birth was designated as postnatal day 0 (PND 0). Only litters with 11-15 pups were used in this study; furthermore, there were no differences in mean litter size among groups of mothers exposed to various light conditions. Litters were left intact, and on PND 10, pups were gently counted, sexed and weighed. As another parameter of somatic change, we observed the day of eye opening. On PND 22, all litters were weaned and counted again. Male rats under similar lighting conditions and from the same litters were housed in groups of 3 in standard cages with free access to food and water under a 12-h light/dark cycle (lights on 8.00 h-20.00 h).

All procedures involving animals were conducted in accordance with the Guiding Principles on Animal Experimentations in Research Facilities for Laboratory Animal Science, Hiroshima University, and approved by the Hiroshima University Animal Care Committee.

Experimental schedule (Figure 1)

For the behavioral evaluation of dams, the amount of maternal care from PND 2 to PND 14 and the circadian rhythm of locomotor activity (PND 4-6, 10-12) were examined. Additionally, a 1-h focal observation of maternal care was undertaken on PND 5. For the behavioral assessment of offspring, the elevated plus maze test, social interaction test, and object recognition test were administered during the dark active phase (21.00 h-1.00 h) on PND 42 (adolescence) and PND 84 (adulthood). Additionally, the circadian rhythm of locomotor activity in offspring was measured on PND 42-44 and PND 84-86. Raters unaware of the lighting conditions conducted data analysis. No more than two animals per experimental group were from any single litter. Particularly for real-time quantitative polymerase chain reaction (RT-PCR), there was only one pup per litter representing 7-9 litters per group. No animal was used in more than one experiment. A total of 413 rats were used in these behavioral analyses and a different set of rats was used for each behavioral test. The test apparatus and arena were wiped down with 10% ethanol between each test session. Each group consisted of non-fasted male rats that had not been acclimatized to the experimental apparatus (15-19 for the elevated plus maze test, 8-9 pairs for the social interaction test, 11-12 for the object recognition test and 10-12 for the locomotor test; each group was comprised of animals from ≥10 different litters). Only male rats were used to eliminate the effects of the estrous cycle in female rats on anxiety and memory functioning. In conjunction with the object recognition test, the hippocampal levels of NMDA receptor subunits were measured by RT-PCR in adult offspring. Furthermore, in adult offspring we measured changes in plasma corticosterone levels by radioimmunoassay and mRNA levels of hippocampal glucocorticoid receptor by RT-PCR in response to a single immobilization stress.

Manipulations of lighting conditions

From PND 2-14, dams and pups were exposed to the following postnatal lighting manipulations: normal lighting conditions (NLC) (n = 35), continuing maintenance of normal lighting conditions; PDC (n = 34), prolonging the dark phase by a 6-h delay of light onset (L/D = 6:18 h; lights on at 14.00 h and off at 20.00 h); and PLC (n = 35), prolonging the light phase cycle by a 6-h delay of light offset (L/D = 18:6 h; lights on at 8.00 h and off at 2.00 h). Dams and pups were then transferred back to normal lighting conditions.

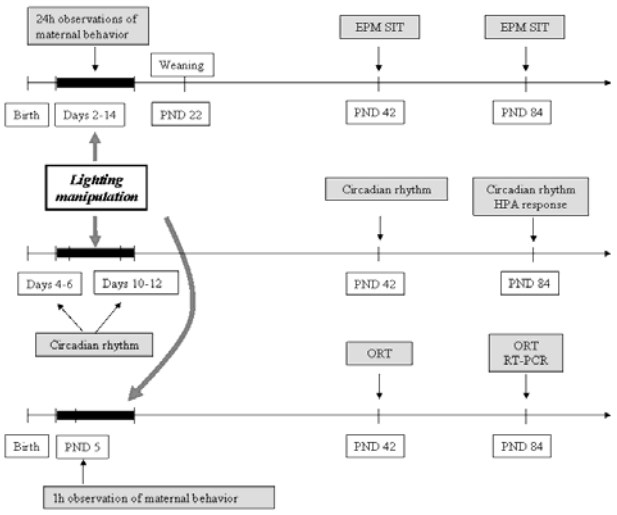

Fig. 1. Schematic representation of the experimental design. Lighting manipulations were administered to dams and pups from PND 2 to PND 14. 1-h and 24-h observations of maternal behavior and measurement of circadian rhythm of locomotor activity were performed in 3 sets of animals. After weaning, elevated plus maze test, social interaction test and object recognition test were administered and locomotor activity was measured on PND 42 and PND 84. Corticosterone response to restraint stress and hippocampal glucocorticoid receptor and NMDA receptor mRNA expressions were investigated on PND84. Each rat was used only once in one experiment, including all behavioral and molecular experiments.

24-h intermittent observation of maternal behavior

We examined maternal behavior using a version of the procedure described by Myers et al (Myers et al., 1989). Throughout PND 2-14, all cages were continuously video-recorded for behavioral scoring. Small infrared cameras (1/cage) with adjustable lenses were mounted on a standard laboratory rack to face the short side of the cages. Maternal behavior was scored blindly at 3-min intervals for 1 h (20 samples/h) every third h, starting at 9.00 h. Thus, for each dam a total of 160 samples per day (8 × 20) were obtained. The following behaviors were scored: (1) mother off pups, (2) mother licking and grooming pups, (3) mother nursing pups in either an arched-back posture, (4) a "blanket" posture in which the mother lays over the pups, (5) a passive posture in which the mother is lying either on her back or side while the pups nurse, or (6) an undetectable state when poor visibility prevented unambiguous identification of the behavior. Overall, the latter accounted for 3.2% of all observations. Maternal observations were converted to a percentage of the times the dam engaged in each of these behaviors over the total number of time points observed. Licking and arched-back nursing were combined into a single category of active nursing according to the work of Meaney's group (Champagne et al., 2003a). Contact was defined as any behavior that involved physical contact or close proximity to pups and almost invariably implied nursing and/or licking/grooming (Champagne et al., 2003a).

1-h focal observation of maternal behavior

Because scores by the above method do not necessarily reflect either the number or length of licking/grooming bouts (LG bouts) but emerge as a function of both frequency and duration, another groups of individual NLC, PDC, and PLC mothers (NLC n = 11; PDC n = 10; PLC n = 11) were observed continuously for 1-h between 15.00 h and 19.00 h on day 5 of lactation. The observer noted the onset and offset of each individual bout of pup licking/grooming, which provided a direct measure of duration.

Dams' circadian rhythm of locomotor activity

Circadian rhythm of locomotor activity was measured by automatic actography in which the level of activity was estimated as the number of interruptions of near infrared rays; the counterinterface was connected to a personal computer. Individual standard cages, in which dams and pups were maintained, were set within the apparatus and monitored. In order to measure the locomotor activity of only dams, the infrared sensors were set at a height of 10 cm from the floor. Movements detected were recorded every 10 min throughout the experiment. Activity counts were summed over 60 min and mean values over the 72 h were calculated. The resulting 12 mean patterns (72 data points each, expressed as percentages of the total mean value of the corresponding subject) were averaged at each time point during the day. Additionally, the mean values for 3 continuous days were depicted in each group.

Elevated plus maze

The plus-maze consisted of 2 open arms (50 × 10 cm) and 2 enclosed arms (50 × 10 × 38 cm) opposite each other at a height of 73 cm above the floor. Lighting on the open arms was 55 lux. At the beginning of each test, the rat was placed in the center facing a closed arm. During the 5-min exposure, the number of entries into each of the arms and the time spent therein were monitored by a video camera. An entry was scored when 2 forepaws passed over the open or closed dividing line. Data were processed to yield the ratio of time spent in the open arms versus total time and the number of entries into each arm of the maze.

Social interaction test

The social interaction test was performed in an open-field arena (48 cm square) dimly lit with white light (65 lux). All sessions were recorded by a video camera located above the apparatus. A male rat was placed simultaneously with an unfamiliar partner rat into the social interaction apparatus. During the 10-min test period, the number and duration of social interactions (sniffing, crawling over, following, etc.) were scored by a rater unaware of lighting conditions to which the animal had been subjected.

Object recognition test

The object recognition test using 2 different colored bottles as novel objects was performed according to the method of Ennaceur and Delacour with a minor modification (Ennaceur & Delacour, 1988). The male rats were handled twice per day for 1 min for 5 days and, on the subsequent day, habituated to the empty open-field

arena (48 cm square) for 1 h. The training session began 24 h after habituation. During the training session, the 2 identical objects described above were placed into the arena where habituation had taken place, and each rat was allowed to explore freely for 3 min. During the retention test, the rat was placed back into the same apparatus 1 or 24 h after the training, and one of the familiar objects was replaced by a new object. The rat was allowed to explore freely for 3 min. The time spent exploring each object and the total time spent exploring both objects were recorded. To analyze cognitive performance, a discrimination index was calculated as the difference in time exploring the novel and familiar object, expressed as the ratio of the total time spent exploring both objects.

Offspring's circadian rhythm of locomotor activity

On PND 42-44 and PND 84-86, male offsprings' circadian rhythm for locomotor activity was measured and calculated as described above for the dam.

Immobilization stress and plasma corticosterone measurement

On PND 84, to study the HPA response to stress in adult offspring, a single immobilization stress experiment was performed between 21:00 h and 23:00 h with blood sampling from the tail vein at 0, 30, and 120 min after the beginning of immobilization. Immobilization stress was applied as described previously (Morinobu et al., 2003). Animals were immobilized in clear plastic cone bags, sized so that animals were equally immobilized. After centrifugation (500 x g at 4˚C for 30 min), plasma samples were frozen and stored at –70˚C until the day of analysis. The plasma corticosterone level was determined using the rat corticosterone [125I] assay system (Amersham).

Real-time quantitative polymerase chain reaction (RT-PCR)

To collect tissue for the assessment of NR1, NR2A, and NR2B mRNA expression by RT-PCR, rats were decapitated rapidly less than 30 s after removal from the home cage. To collect tissue for the assessment of glucocorticoid receptor (GR), rats were decapitated immediately after immobilization stress for 2h. The entire hippocampus was dissected, frozen on dry ice and stored at -80oC until the time of assay. RT-PCR was conducted as described previously (Suenaga et al., 2004). Total RNA was extracted using the RNAqueousTM Total RNA Isolation kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. After treatment with RNase-free DNase I (Takara, Shiga, Japan), a single-stranded cDNA was synthesized using reverse transcriptase (Toyobo, Osaka, Japan). RT-PCR was performed with an ABI7700 sequence detection system (Applied Biosystems) to quantify relative mRNA levels in samples. RT-PCR was performed to amplify the mRNA of NR1, NR2A, NR2B and GR. The primers and TaqMan hybridization probes were designed using Primer Express software (Applied Biosystems). Table 1 shows the sequences and fluorescent dyes of the PCR primers and TaqMan probes. The TaqMan probe, which was designed to hybridize to the PCR products, was labeled with a fluorescent reporter dye at the 5'-end and a quenching dye at the 3'-end. PCR was carried out with TaqMan Universal PCR Master Mix (Applied Biosystems). All standards and samples were assayed in triplicate. Thermal cycling was initiated with an initial denaturation at 50oC for 2 min and 95oC for 10 min. After this initial step, 40 cycles of PCR were performed. Each PCR cycle consisted of heating at 95 oC for 15 s for melting and at 60oC for 1 min for annealing and extension. The PCR assay for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed using the TaqMan Rodent GAPDH Control Reagents kit (Applied Biosystems). The mRNA levels of NR1, NR2A, NR2B and GR were detected by RT-PCR and the ratio of the concentration of the target molecule to that of GAPDH (target molecule/GAPDH) in unknown samples was calculated.

Table 1: Primers and TaqMan Probes

NR1:
Forward primer 5'-GTTCTTCCGCTCAGGCTTTG-3'
Reverse primer 5'-AGGGAAACGTTCTGCTTCCA-3'
TaqMan probe 5'-FAM-CGGCATGCGCAAGGACAGCC-TAMRA-3'
NR2A:
Forward primer 5'-AGCCCCCTTCGTCATCGTA-3'
Reverse primer 5'-GACAGGGCACCGTGTTCCT-3'
TaqMan probe 5'-FAM-AGGACATAGACCCCCTGACTGAGACCTGTG-TAMRA-3'
NR2B:
Forward primer 5'-CCCCCAAGTTCTGGTTGGT-3'
Reverse primer 5'-TTTTGGGAACGAGCTTTGCT-3'
TaqMan probe 5'-FAM-TTGGCCGTCTTGGCCGTATCAGG-TAMRA-3'
GR:
Forward primer 5'-TTCGAAGGAAAAACTGCCCAG-3'
Reverse primer 5'-CGAGCTTCAAGGTTCATTCCA-3'
TaqMan probe 5'-FAM-TGCCGCTATCGGAAATGTCTTCAGG-TAMRA-3'

Primers and TaqMan probe for each gene. The nucleotide positions are ranging from 2521 to 2586 (66 bp) from the sequence of the NMDA receptor subunit NR1 cDNA (GeneBank No. x63255), from 1254 to 1324 (70 bp) from the sequence of the NMDA receptor subunit NR2A cDNA (GeneBank No. D13211), from 376 to 439 (63 bp) from the sequence of the NMDA receptor subunit NR2B cDNA (GeneBank No. NM_012574), and from 1482 to 1551 (69 bp) from the sequence of the GR cDNA (GeneBank No. Y12264).

Data analysis

SPSS was used for all analyses. Data were analyzed by repeated-measures ANOVA for split-plot designs, following a statistical method by Macri et al. (Macri et al., 2004). For analysis of 24-h maternal behavior, the general model was 13 d × 8 h × 3 treatments. Treatment was a between-litter factor while all other variables were within-litter factors. Additionally, for the analysis of diurnal pattern, one-way ANOVA with Bonferroni test was performed at each time point during day. Data on 24-h maternal behavior were transformed to the arc sine of the square root of the relative frequencies of behavioral scores. For reasons of clarity, all figures are based on non-transformed values.

Differences in 1-h maternal behavior and the locomotor activities of the dam at each time point, body weight and day of eye opening of pups, and the subsequent behavioral and molecular variables of

offspring in adulthood among the 3 lighting conditions were determined by one-way ANOVA with the Bonferroni test.

The general model for analysis of plasma levels of corticosterone was 3 time × 3 treatments. Treatment was a between-litter effect while time points were within-litter factors. When appropriate, the Bonferroni test was performed. Values of $P < 0.05$ were considered significant.

Results

Growth, survival and neurodevelopmental milestones among offspring of NLC, PDC and PLC mothers

The mean body weights of male offspring of NLC, PDC and PLC mothers did not differ at any time-point (Table 2). The eye-opening day was similar among the 3 groups (Table 2). At weaning (PND 22), survival rates among these 3 groups were almost equal (99%). These findings suggest that variations of lighting conditions do not affect gross development.

Table 2: Other reproductive parameters

	NLC	PDC	PLC
Pup body weight (g)			
PND10	14.35±0.67	13.39±0.45	14.26±0.26
PND22	63.10±0.90	61.95±1.77	62.65±1.24
Eye opening day (PND)	13.78±0.10	13.56±0.12	13.69±0.08
1-h focal observation			
Duration of LG bout (sec)	54.50±3.13	18.26±4.13[ab]	76.13±5.43[a]
Numbers of LG bout (sec)	6.45±0.74	2.80±0.59[ab]	7.64±0.64

PND, post natal day.
LG, licking/grooming
[a] P<0.05: compared with NLC
[b] P<0.05: compared with PLC

Mean values ± SEM of pup body weight (n = 17-19 pups, ≥ 12 litters, per group), eye opening day (n = 49-54, ≥ 24 litters, per group) and variables measuring maternal behavior for NLC, PDC and PLC mothers.

Different mothering behaviors among NLC, PDC, and PLC mothers

24-h intermittent observation of maternal behavior

Figure 2A depicts mean daily levels of active nursing from PND 2-14 for NLC, PDC and PLC mothers. Active nursing gradually decreased across days (days, $F_{12,396} = 34.94$, $P < 0.001$). However, ELC significantly affected levels of active nursing (treatment, $F_{2,33} = 13.18$, $P < 0.001$). Both NLC and PLC mothers exhibited significantly higher levels of active nursing than PDC dams (post hoc test, NLC vs PDC $P < 0.001$, PLC vs PDC $P < 0.001$). This difference between PDC and the other 2 conditions emerged on PND 3 and remained stable throughout the remaining period except on PND 10. Analysis of the diurnal pattern (Figure 2C) revealed that active nursing was elevated almost throughout the day in NLC and PLC dams compared to PDC dams (one-way ANOVA with Bonferroni test at each time point, NLC vs PDC $P < 0.05$, at 21.00-22.00, 24.00-1.00 and 6.00-7.00 h, $P < 0.10$, at 9.00-10.00, 12.00-13.00 and 15.00-16.00 h, PLC vs PDC $P < 0.05$, at 15.00-16.00, 21.00-22.00, 24.00-1.00, 3.00-4.00 and 6.00-7.00 h, $P < 0.10$, at 9.00-10.00 and 18.00-19.00 h). Differences in active nursing time were not consistent with contact time with pups (Figure 2B and Figure 2D). Contact time with pups was not affected by neonatal lighting manipulations, indicating that differences in active nursing were not simply due to individual differences in the active interacting time of dams. Analysis of the diurnal pattern (Figure 2D) revealed that, while contact time with pups in PDC mothers was higher at 12.00-13.00 ($P = 0.048$) and also tended to be higher at 15.00-16.00 h ($P = 0.053$), contact in PLC mothers was higher at 18.00-19.00 ($P = 0.003$) and 21.00-22.00h ($P = 0.027$), compared with NLC mothers.

1-h focal observation of maternal behavior

Overall, length of LG bouts ranged from 3 s to over 309.1 s. As shown in Table 2, there were significant group differences in mean duration ($F_{2,29} = 44.0$, $P < 0.001$). While the duration of LG bouts was reduced under PDC relative to NLC and PLC (post hoc test, PDC vs NLC $P < 0.001$, PDC vs PLC $P < 0.001$), duration of bouts under PLC was increased relative to NLC (post hoc test, $P = 0.004$). In addition, PDC mothers licked less frequently than NLC and PLC mothers (post hoc test, PDC vs NLC $P = 0.002$, PDC vs PLC $P < 0.001$).

Dams' circadian rhythm of locomotor activity

Figure 3A shows locomotor activity of NLC, PDC and PLC mothers on PND 4-6. Locomotor activity in PDC mothers was higher at 10.00 h ($p = 0.006$) and lower at 21.00 h ($p = 0.005$). Compared with NLC mothers, in PDC mothers there was a 1.5-2.0 h phase delay at the onset of activity and a 2.5-3.0 h phase delay at the offset of activity. Locomotor activity in PLC mothers was lower at 21.00 h ($p = 0.001$). In PLC mothers, there was a 2.0-2.5 h phase delay at the onset of activity in comparison with NLC mothers. Figure 3B shows locomotor activity of NLC, PDC and PLC mothers on PND 10-12. Locomotor activity in PDC mothers was higher from 9.00 h to 11.00 h ($p < 0.001$, $p = 0.003$ and $p = 0.005$) and lower at 18.00 h and 20.00 h ($p = 0.004$ and $p = 0.001$). Compared with NLC mothers, PDC mothers exhibited a 2.5-3.0 h phase delay at the onset of activity and a 3.0-3.5 h phase delay at the offset of activity. Locomotor activity in PLC mothers was higher at 9.00 h and 11.00 h ($p < 0.001$ and $p = 0.006$) and lower at 20.00 h, 21.00 h and 23.00 h ($p = 0.001$, $p = 0.001$ and $p = 0.003$). In comparison with NLC mothers, PLC mothers exhibited a 3.0-3.5 h phase delay at the onset of activity and 1.0-1.5 h phase delay at the offset of activity.

ANOVA revealed that daily means (counts/min) of activity differed among the 3 groups (NLC: 67.5, PDC: 66.1, PLC: 38.9) on PND 10-12, but not on PND 4-6 (Figure 3C). On PND 10-12, PLC mothers exhibited significantly less movement than NLC and PDC mothers ($p < 0.05$).

In summary, PDC and PLC mothers were not entrained to the altered lighting conditions on PND 4-6, and it was also unclear whether PDC mothers were entirely entrained on PND 10-12. It is possible that entrainment in PLC mothers appeared to take place at the later time only because light inhibited activity. Only in PLC mothers was a large increase in activity at the beginning of the dark

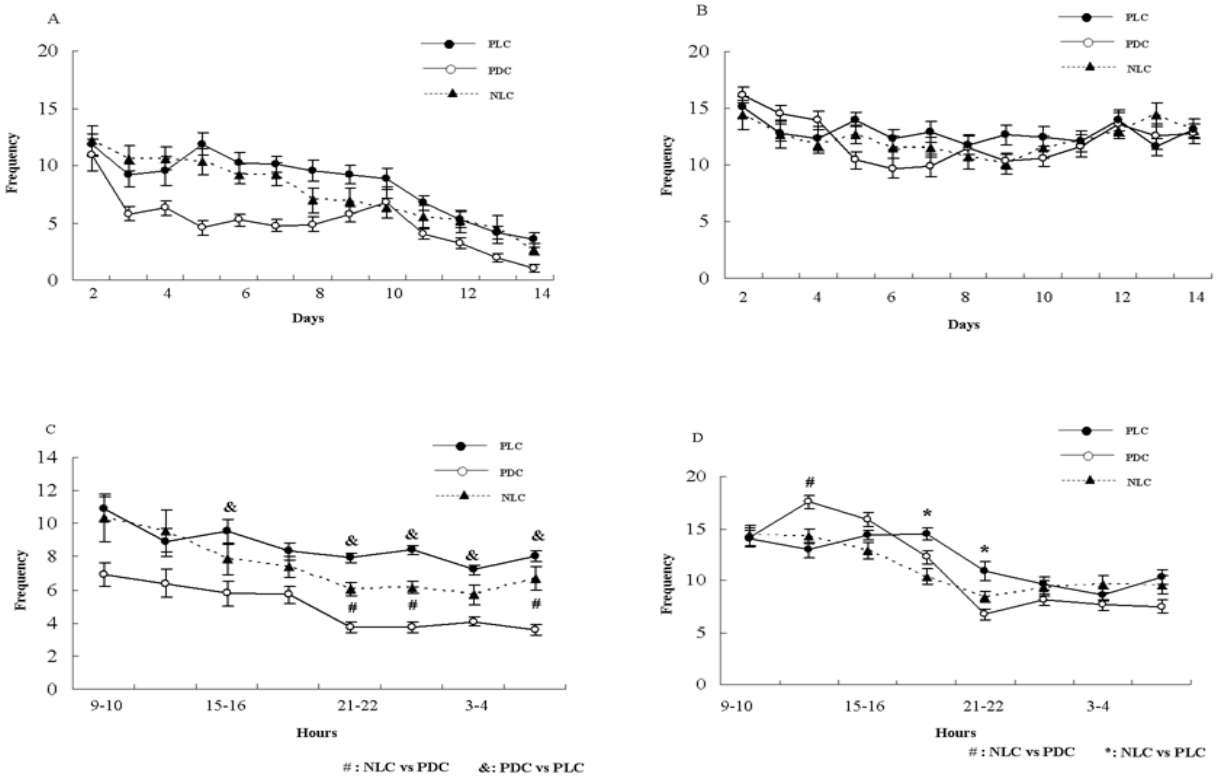

Figure 2. Effects of ELC on maternal behavior. (A, B) Daily frequency (mean/h ± SEM) of (A) active nursing and (B) contact time with pups by dams subjected daily to normal lighting condition (NLC; n = 12), prolonged dark phase cycle (PDC; n = 12) or prolonged light phase cycle (PLC; n = 12). Daily scores are based on 8 daily 1-h sampling sessions. (C, D) Diurnal patterns of (C) active nursing and (D) contact time scores (mean ± SEM) by NLC (n = 12), PDC (n = 12) and PLC (n = 12) dams. Active nursing was elevated almost throughout the day in NLC and PLC dams compared to PDC dams (NLC vs PDC $P < 0.05$, at 21-22, 24-1 and 6-7 h, $P < 0.10$, at 9-10, 12-13 and 15-16 h, PLC vs PDC $P < 0.05$, at 15-16, 21-22, 24-1, 3-4 and 6-7 h, $P < 0.10$, at 9-10 and 18-19 h). While contact time with pups in PDC mothers was higher at 12-13 ($P < 0.05$) and tended to be higher at 15-16 h ($P < 0.10$), contact time in PLC mothers was higher at 18-19 and 21-22 h ($P < 0.05$), compared with NLC mothers. Scores from each of 8 daily 1-h sampling sessions were averaged across PND 2-14. #$P < 0.05$; NLC vs PDC, &$P < 0.05$; PDC vs PLC, *$P < 0.05$; NLC vs PLC.

period observed, indicating strong pressure to begin activity, consistent with results of previous studies (Boon et al., 1997; Benstaali et al., 2001).

Influence of ELC on circadian rhythm of locomotor activity in adolescent and adult offspring

Figure 4A shows locomotor activity of NLC, PDC and PLC offspring in adolescence. Compared with NLC offspring, locomotor activity in PDC offspring was higher at 10:00 h and 13:00 h ($p = 0.016$ and $p = 0.04$). Locomotor activity in PLC offspring was lower at 9:00 h ($p = 0.001$) and higher at 10:00 h and 11:00 h ($p = 0.003$ and $p = 0.009$). Figure 4B shows locomotor activity of NLC, PDC and PLC offspring in adulthood. Compared with NLC offspring, PDC offspring exhibited no significant difference in locomotor activity. Locomotor activity in PLC offspring was lower at 9:00 h ($p = 0.004$) and higher at 19:00 h ($p = 0.001$). There were no significant differences among the 3 groups during the dark active phase both in the adolescent and adult animals.

ANOVA revealed that daily means (counts/min) of activity did not differ among the 3 groups at any time point.

Anxiety phenotypes in adolescent and adult, NLC, PDC and PLC offspring

Elevated-plus maze test in adolescent and adult offspring

ANOVA revealed that the ratio of time spent in open arms (vs. total time) in the elevated-plus maze was significantly affected by ELC in both adolescent ($F2,45 = 18.38$, $P < 0.001$) and adult ($F2,51 = 12.47$, $P < 0.001$) animals (Figure 5A). Analysis of the number of open-arm entries revealed a significant effect of ELC in adolescent ($F2,45 = 13.13$, $P < 0.001$) and adult animals ($F2,51 = 16.09$, $P < 0.001$) (Figure 5B). Percentage of time spent in open arms and the number of open-arm entries were reduced in PDC compared with NLC and PLC at both ages. With PLC, the number of open-arm entries was increased compared with NLC and PDC at both ages. Groups did not differ in the number of closed arm entries in both

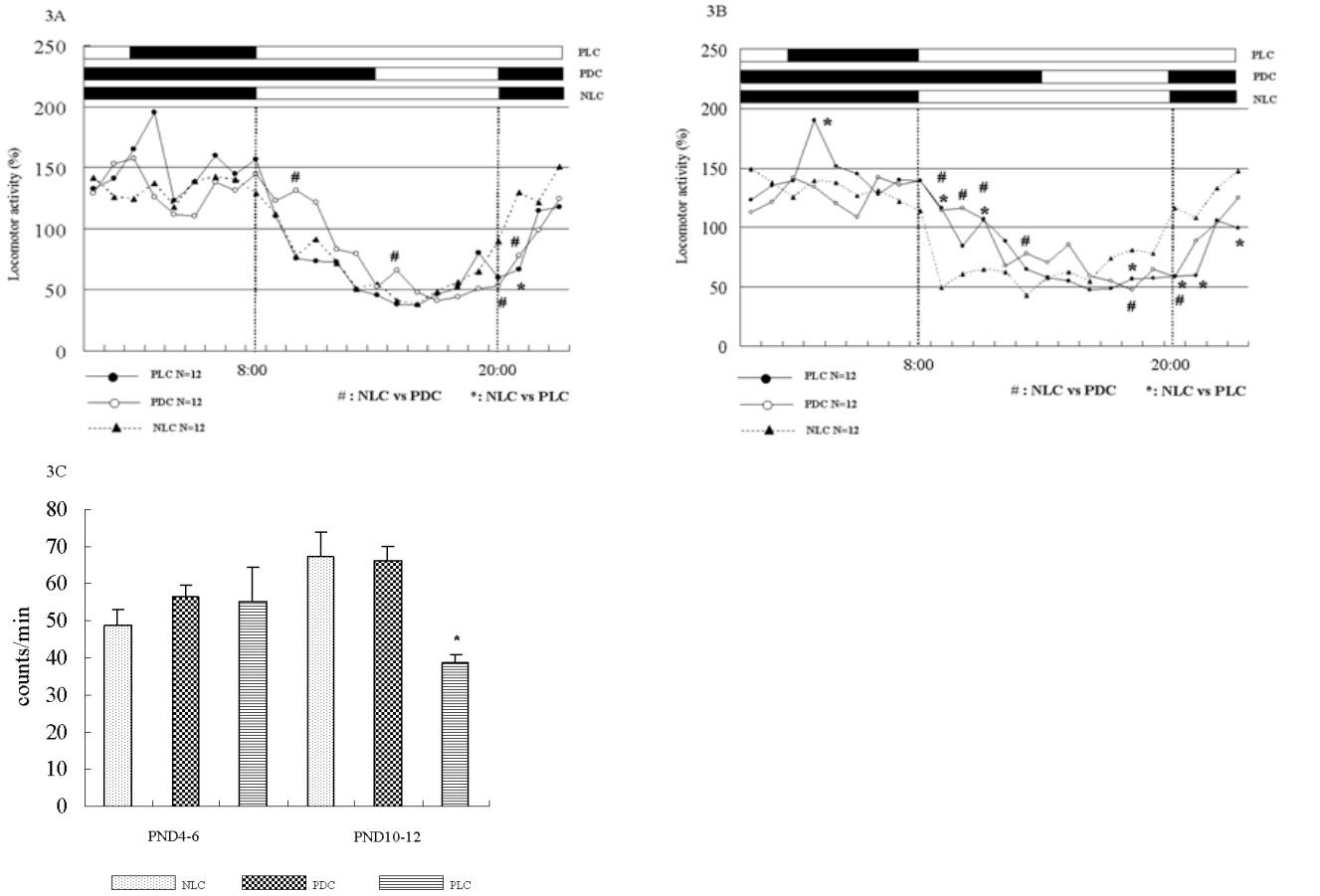

Fig. 3. Comparison of circadian rhythm of locomotor activity among NLC, PDC and PLC dams. Motor activity as measured by an actimeter of NLC (closed triangle, n = 12), PDC (open diamonds, n = 12), and PLC (closed diamonds, n = 12) mothers over 72 h. (A) 72 1-h percentages of the total mean value of the corresponding group on PND 4-6 were averaged. While locomotor activity in PDC mothers was higher at 10.00 h and lower at 21.00 h ($p < 0.05$), that in PLC mothers was lower at 21.00 h ($p < 0.05$). (B) 72 1-h percentages of the total mean value on PND 10-12 were averaged. Locomotor activity in PDC mothers was higher from 9.00 h to 11.00 h ($p < 0.05$) and lower at 18.00 h and 20.00 h ($p < 0.05$). (C) Mean values (counts/min) of each group over a 72-h period on PND 4-6 and PND 10-12. On PND 10-12, PLC mothers exhibited significantly less movement than NLC and PDC mothers ($p < 0.05$). Results are means ± SEM. *#$p < 0.05$; compared with NLC mothers.

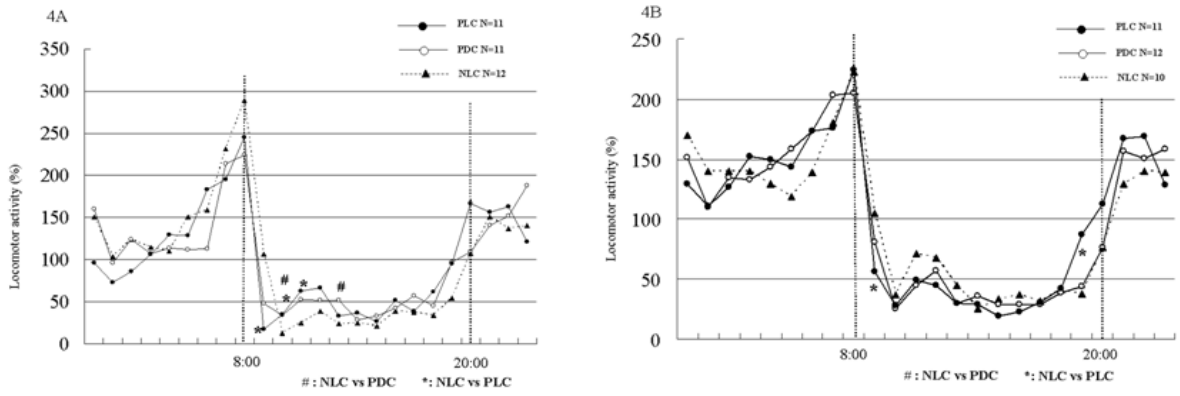

Fig. 4. Circadian rhythms of locomotor activity in NLC, PDC and PLC offspring. Motor activity as measured by an actimeter of NLC (closed triangle), PDC (open diamonds), and PLC (closed diamonds) offspring over 72 h. Vertical bars represent the change in lighting condition (L:D = 12:12, lights on 8.00 h-20.00 h). (A) 72 1-h percentages of the total mean value of the corresponding group on PND 42-44 were averaged. Compared with NLC offspring, locomotor activity in PDC offspring was higher at 10:00 h and 13:00 h ($p < 0.05$). Locomotor activity in PLC offspring was lower at 9:00 h and higher at 10:00 h and 11:00 h ($p < 0.05$). (B) 72 1-h percentages of the total mean value of the corresponding group on PND 84-86 were averaged. Locomotor activity in PLC offspring was lower at 9:00 h and higher at 19:00 h ($p < 0.05$). *$p < 0.05$; compared with NLC offspring.

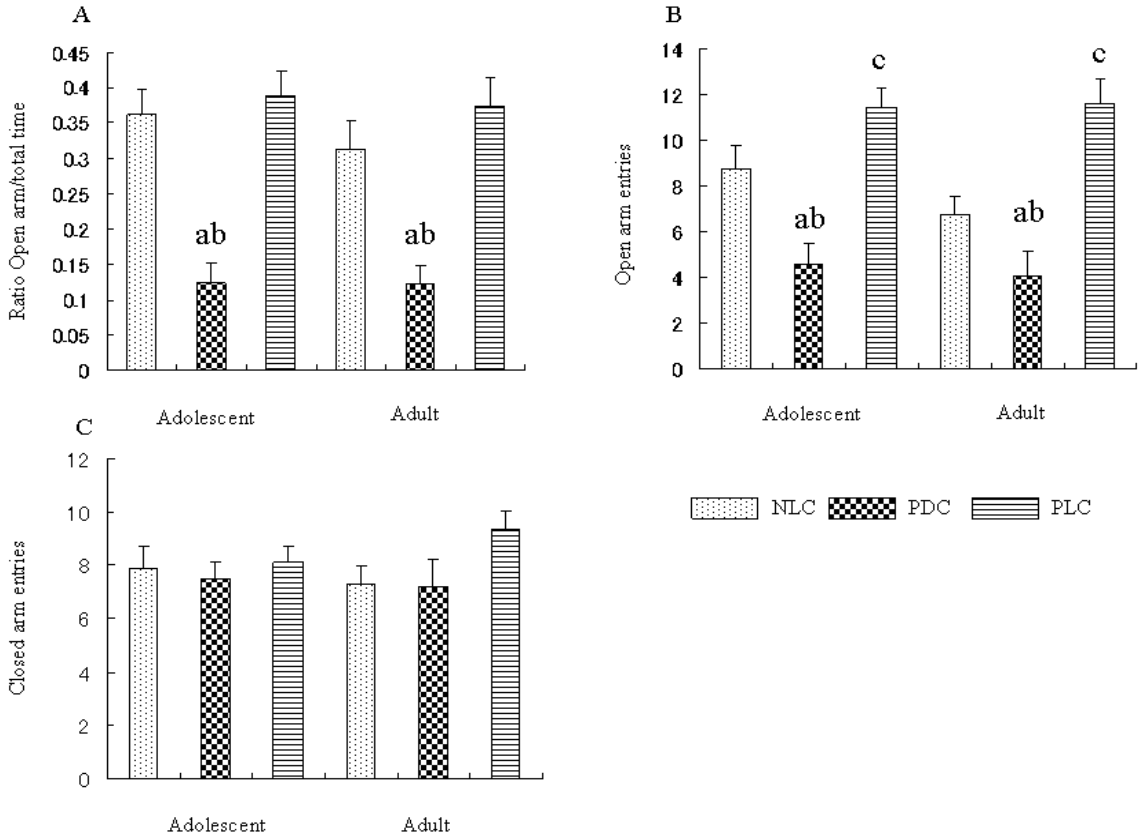

Fig. 5. Prolonged dark phase cycle during neonatal period produces a high-anxiety phenotype in adolescence and adulthood. Results of elevated plus maze test on PND 42 and PND 84 in male offspring. (A) Ratio of time spent in open arms over open arms + closed arms. (B) Number of open arm entries. (C) Number of closed arm entries. Percentage of time spent in open arms and the number of open-arm entries were reduced in PDC compared with NLC and PLC at both ages. With PLC, the number of open-arm entries was increased compared with NLC and PDC at both ages. Results are means±SEM. [a] $P < 0.05$; NLC vs PDC, [b] $P < 0.05$; PDC vs PLC, [c] $P < 0.05$; NLC vs PLC.

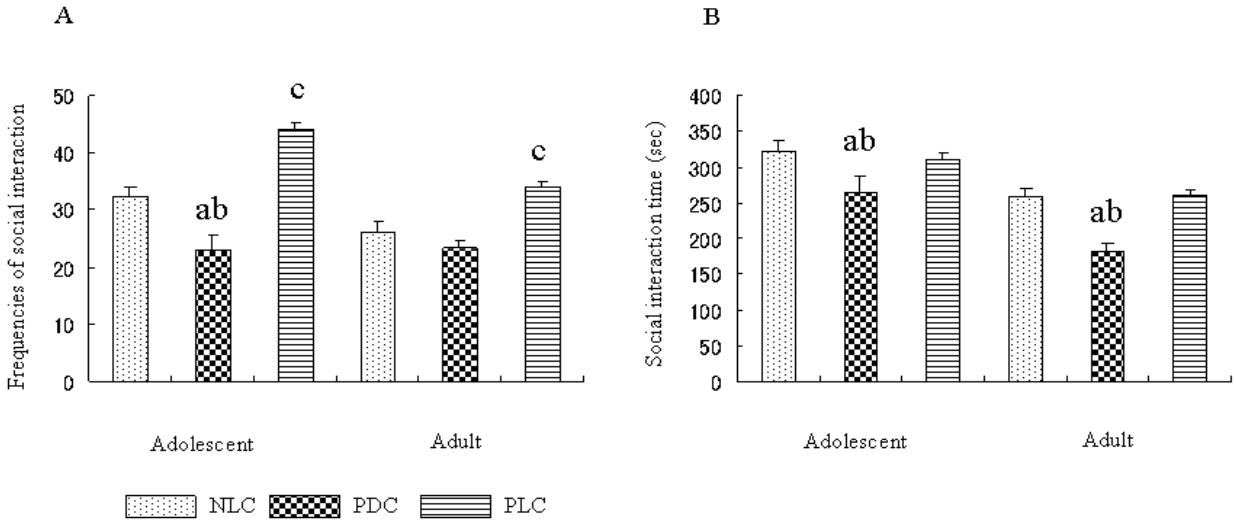

Fig. 6. Prolonged dark phase cycle also reduced social interaction in adolescence and adulthood. Results of social interaction test on PND 42 and PND 84 in male offspring. (A) Frequency of social interaction. PDC decreased the frequency of social interaction, in comparison with NLC and PLC in adolescence. PLC increased the frequency of social interaction compared with NLC both in adolescence and adulthood. (B) Total time spent for social interaction. PDC decreased the time spent in social interaction both in adolescent and adult animals, compared with NLC and PLC. Results are means±SEM. [a] $P < 0.05$; NLC vs PDC, [b] $P < 0.05$; PDC vs PLC, [c] $P < 0.05$; NLC vs PLC.

adolescent and adult animals (Figure 5C).

Social interaction test in adolescent and adult offspring

ANOVA revealed that the frequency of social interaction was significantly affected by ELC in both adolescent ($F_{2,51} = 33.14$, $P < 0.001$) and adult ($F_{2,45} = 13.92$, $P < 0.001$) animals (Figure 6A). PDC decreased the frequency of social interaction compared with NLC and PLC only in adolescence. PLC increased the frequency of social interaction compared with NLC both in adolescence and adulthood. Analysis of time spent in social interaction revealed a significant effect of ELC in adolescent ($F_{2,51} = 3.27$, $P = 0.046$) and adult animals ($F_{2,45} = 21.86$, $P < 0.001$) (Figure 6B). PDC decreased the time spent in social interaction both in adolescent and adult animals, compared with NLC and PLC.

Object recognition memory and the hippocampal NMDA receptor subunit expression in adult NLC, PDC and PLC offspring

Table 3 shows the total time in exploring 2 identical objects in the training trial for the 1-h and 24-h retention test. ANOVA for total exploration time revealed no differences among groups in both adolescence and adulthood. One-sample t test used to examine whether the discrimination index was zero (chance level) showed that all groups spent comparable time exploring each of the 2 identical objects in the training trial.

In the 1-h retention test, one-sample t test revealed that the discrimination index of NLC offspring was significantly different from zero both in adolescence ($t10 = 6.03$, $P < 0.001$) and adulthood ($t11 = 6.90$, $P < 0.001$) (Figure 7A), indicating that rats at both ages readily discriminated the novel object from the familiar object during the 1-h retention test. Moreover, ELC significantly affected the discrimination index both in adolescence ($F_{2,31} = 7.85$, $P = 0.0017$) and adulthood ($F_{2,32} = 16.13$, $P < 0.001$). As shown in Figure 7A, PDC decreased recognition memory both in adolescent and adult animals, compared with NLC and PLC. In contrast, during the 24-h retention trial, no preference for the novel object in the NLC, PDC, and PLC rats was exhibited in both adolescence and adulthood (Figure 7B). As shown in Table 3, ELC did not influence the total amount of time exploring the 2 objects after the 24-h retention interval.

ANOVA revealed that ELC affected hippocampal mRNA levels of NR2B ($F_{2,21} = 5.06$, $P = 0.016$) (Figure 8C), but not NR1 (Figure 8A) and NR2A (Figure 8B). Levels of NR2B were lower in PDC compared with NLC offspring. Downregulation of NR2B is consistent with the finding of impaired object recognition memory in PDC offspring.

Corticosterone response and hippocampal GR mRNA levels in adult NLC, PDC and PLC offspring.

The increased plasma corticosterone response to stress in PDC offspring was significantly sustained compared with NLC and tended to be sustained compared with PLC offspring (Figure 9). Statistical analysis revealed a significant lighting manipulation × time interaction ($F_{2,72} = 2.95$, $P = 0.026$). ELC had no effect on basal ($F_{2,36} = 0.76$, NS) and peak ($F_{2,36} = 2.05$, NS) levels of corticosterone, but significantly ($F_{2,36} = 4.52$, $P = 0.018$) affected levels at 120 min after starting stress. Post hoc analysis revealed

Table 3: Total exploration time in object recognition test

	ELC	Training	Retention
1h retention interval			
Adolescent	NLC	29.5±1.4	25.1±2.7
	PDC	26.4±1.5	17.4±2.0
	PLC	26.5±0.9	23.9±0.8
Adult	NLC	24.7±0.9	20.9±3.2
	PDC	25.2±1.8	16.8±1.8
	PLC	24.7±1.0	25.9±3.5
24h retention interval			
Adolescent	NLC	26.3±1.5	24.3±1.7
	PDC	24.0±1.7	22.9±1.8
	PLC	26.5±1.0	25.3±1.4
Adult	NLC	23.8±2.0	22.2±2.9
	PDC	23.7±1.2	21.8±2.2
	PLC	23.9±1.3	19.0±1.5

Total time spent exploring the 2 objects (2 identical objects for the training trial, and a familiar and a novel object for the test trial), expressed as means ± SEM in seconds. Statistical analysis is described in Results (n = 11-12 per group).

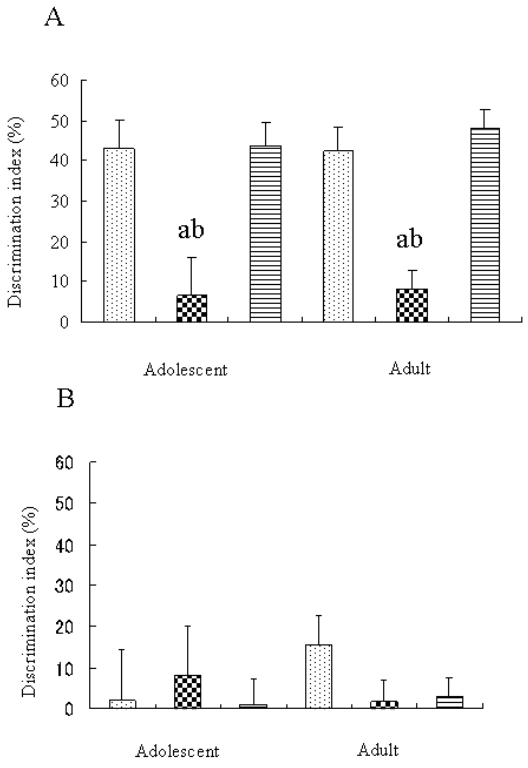

Fig. 7. Prolonged dark phase cycle impaired object recognition memory in adolescence and adulthood. (A) Discrimination index of 1-h retention trial. PDC decreased recognition memory both in adolescent and adult animals, compared with NLC and PLC. (B) Discrimination index of 24-h retention trial. Results are means ± SEM. [a] $P < 0.05$; NLC vs PDC, [b] $P < 0.05$; PDC vs PLC.

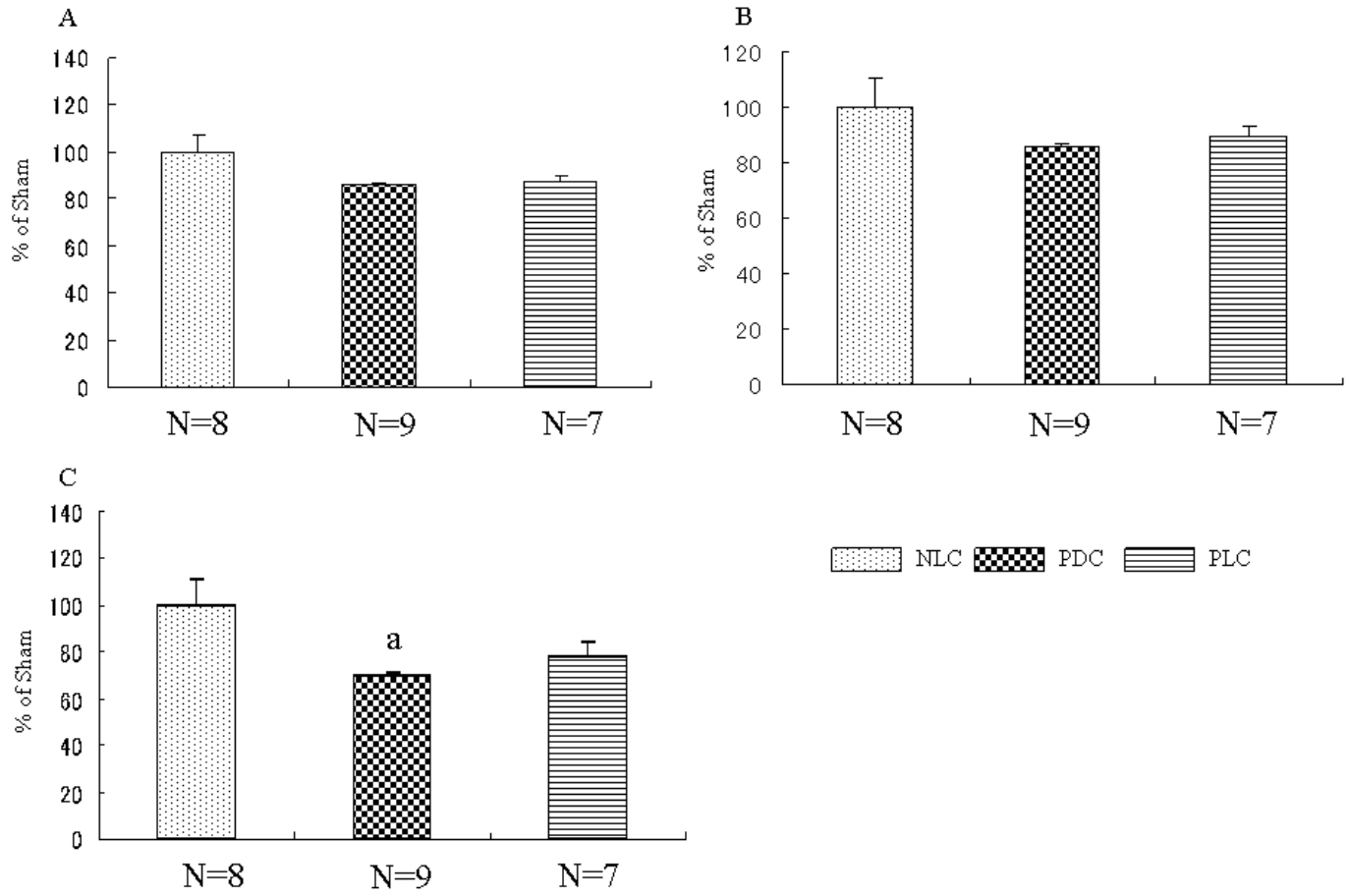

Fig. 8. Influence of ELC on NR1, NR2A and NR2B mRNA expression in the hippocampus as determined by RT-PCR. (A) Influence of ELC on NR1 mRNA levels. (B) Influence of ELC on NR2A levels. (C) Influence of ELC on NR2B levels. Levels of NR2B were lower in PDC compared with NLC offspring. [a] $P < 0.05$; NLC vs PDC.

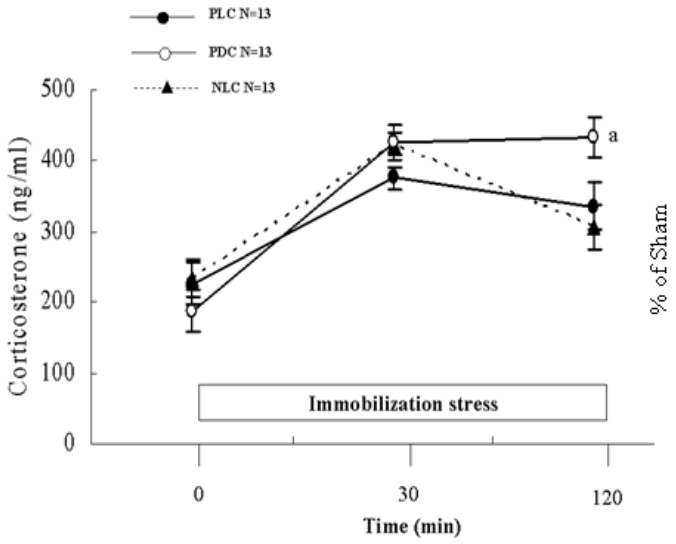

Fig. 9. Prolonged dark phase cycle attenuated negative feedback of corticosterone response to stress. Plasma corticosterone responses at 120 min after the start of stress were significantly higher in PDC than in NLC rats ($P < 0.05$) and tended to be higher compared with PLC rats ($P < 0.10$). Results are means ± SEM. [a] $P < 0.05$; NLC vs PDC.

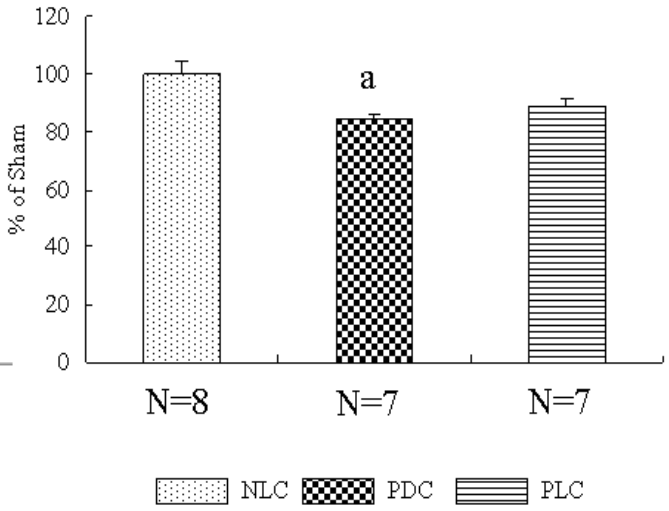

Fig. 10. Influence of ELC on glucocorticoid receptor mRNA expression in the hippocampus as determined by RT-PCR. Levels of GR were lower in PDC than in NLC offspring. Results are means ± SEM. [a] $P < 0.05$; NLC vs PDC.

that plasma corticosterone responses at 120 min after the start of stress were significantly higher in PDC than in NLC rats (P = 0.018) and tended to be higher compared with PLC rats (P = 0.085).

ANOVA revealed that ELC affected hippocampal mRNA levels of glucocorticoid receptor ($F_{2,19} = 6.19$, P = 0.0085) (Figure 10). Levels of GR were lower in PDC than in NLC offspring. Downregulation of GR mRNA expression is consistent with the finding of impaired negative feedback of corticosterone in PDC offspring.

Discussion

The study had 4 major findings. 1) PDC can alter both quality and quantity of maternal behavior. 2) Later in life, PDC affects emotionality of offspring, through underlying alterations of the HPA system. 3) PDC can also affect memory functioning of offspring by changing hippocampal NR2B receptor expression. 4) It is assumed that PDC-induced alterations of maternal care can contribute to their offspring's neurobehavioral phenotype. Since PLC did not induce a clear alteration in maternal care by dams and an active coping style in offspring in this study, these findings did not support our primary prediction. However, from the results it can be postulated that the altered levels of maternal care in response to different ELC, especially PDC, are involved in the development of the defensive phenotype of offspring. Thus, the effects of ELC are partially consistent with the "maternal mediation hypothesis" earlier formulated by Levine (Levine, 1967), and Smothermann & Bell (Smotherman & Bell, 1980) and enlarged upon by Champagne and Meaney (Champagne & Meaney, 2001; Champagne et al., 2003a).

Effects of ELC on maternal behavior

In rodent studies, it was revealed that the dam's genetic background (Ahmadiyeh et al., 2004; Neumann et al., 2005), levels of maternal care received by the dam (Francis et al., 1999a; Francis et al., 2000; Champagne & Meaney, 2006), and various stress exposures during the peripartum period (Pardon et al., 2000; Darnaudery et al., 2004; Macri et al., 2004; Ruedi-Bettschen et al., 2004; Smith et al., 2004; Champagne & Meaney, 2006) modulate the characteristics of maternal behavior. In our present study, similar alterations of maternal behavior were observed by merely lengthening the duration of the dark or light phase even during the stress hyporesponsive period of dams (Neumann, 2001), indicating the robust influence of ELC.

Both acutely and continuously, PDC decreased active nursing throughout the observational period. However, the PLC-induced elevation of active nursing did not reach significance in the 24 h of intermittent observation. Those effects of ELC were so selective that ELC had no effect on total time spent with pups under PDC and PLC. Maternal behavior is regulated by the neural circuit that involves the medial preoptic area of the hypothalamus (Numan et al., 1977), and is mediated by several hormones, such as prolactin (Bridges et al., 1974; Bridges et al., 1985; Neumann, 2003), oxytocin (Pedersen & Prange, 1979; Pedersen et al., 1982; Francis et al., 2000; Bosch et al., 2005; Champagne & Meaney, 2006), estrogen (Siegel & Rosenblatt, 1975a; 1975b; Champagne et al., 2003b; Champagne et al., 2006) and corticosterone (Neumann, 2001, 2003). Classically, Schelstraete et al. reported that inverting the light/dark cycle and temperature every 72 h, designated as atypical zeitgeber, disrupted temporal distribution of maternal care (Schelstraete et al., 1992). Particularly in female rats, the duration of light exposure regulates the prolactin surge and subsequently controls reproduction (Pieper & Gala, 1979; Leadem, 1988; Nelson et al., 1994; Sterner & Cohen, 1995). It can be assumed that the manipulation of ELC changed the total amount and temporal distribution of maternal behavior by modulating these neural networks.

In general, changes in the light cycle are associated with stress and are commonly correlated with an increase in glucocorticoid secretion (Stephens, 1980; Munck et al., 1984). Since PDC not only lengthens the active period of the dam, but also demands a longer duration for entraining to a new lighting condition (Pittendrigh & Daan, 1976; Honma et al., 1985; Boon et al., 1997; Refinetti, 2004; Weinert et al., 2005), PDC dams are postulated to be under stressful situations resembling jet-lag, thus reducing provision of maternal care. On the other hand, in rodents, darkness increases activity while light suppresses it, so it could be first predicted that the low activity level induced by PLC, which was observed on PND 10-12, might lengthen the time on the nest and maternal interaction with pups in PLC mothers. But we found no clear increment in either active nursing or contact time with pups among PLC mothers. Although without significance, maternal care in PLC mothers transiently changed; it decreased first, then increased compared with NLC mothers. Repeated chronic circadian changes have been reported to apparently induce stressful effects on organisms (Stephens, 1980; Munck et al., 1984). However, a single phase-shift did not cause clear stress responses in rats (Sei et al., 2003). Based on these findings, the severity of stress derived from our paradigm of ELC may be lower than that in chronic circadian changes, but higher than in a single phase-shift to some extent. In this context, the reason we could not observe an alteration of maternal care in PLC mothers might be that the stressful influence of light-induced circadian change in the first few days might be antagonized by the positive effect of a long photoperiod on maternal behavior.

Effects of ELC on offspring's activity rhythm

In the neonatal period, maternal care and/or its absence affects diurnal rhythms of activity (Viswanathan & Chandrashekaran, 1985; Shimoda et al., 1986; Viswanathan, 1999; Ohta et al., 2003), corticosterone secretion (Hiroshige et al., 1982c; 1982b; 1982a; Yamazaki & Takahashi, 1983), and Clock gene expressions in the suprachiasmatic nucleus (SCN) of pups (Ohta et al., 2002, 2003). As early as PND 6, this maternal entrainment gradually decreased in parallel with the development of retinohypothalamic synaptogenesis of pups, getting directly entrained to light/dark cycle (Duncan et al., 1986). Our results indicated that the circadian rhythm of adolescent and adult offspring was almost completely synchronized with the normal light/dark cycle. It can be assumed that the altered distribution of the active/inactive cycle in the dam affected the pups' sleep/wake rhythm in the neonatal period, but that later these effects could no longer be observed as pups independently entrained to environmental lighting conditions.

Effects of ELC on offspring's fearfulness and HPA system

In the series of studies using high/low LG (licking and grooming) dams, the lower nursing activity in dams contributed to the decrement of an active and exploratory coping style and enhanced HPA reactivity to stress among the pups (Liu et al., 1997; Caldji et al., 1998; Francis et al., 1999b; Caldji et al., 2003; Menard et al., 2004; Zhang et al., 2005). This was later confirmed by other paradigms using 2 different strains that vary in the levels of active nursing (Ahmadiyeh et al., 2004; Priebe et al., 2006). Since maternal behavior retains the humidity and body temperature of the pup and controls catecholaminergic and HPA activity (Levine, 1967; Stanton et al., 1988; Stanton & Levine, 1990; van Oers et al., 1998; Schmidt et al., 2002), it is conceivable that early adversity, such as low maternal care, produces long-lasting adverse effects on the development of a defensive phenotype, even in adulthood (Liu et al., 1997; Francis et al., 1999b; Gonzalez et al., 2001; Ruedi-Bettschen et al., 2004; Yamazaki et al., 2005; Yoshihara et al., 2005; Priebe et al., 2006).

In examining corticosterone response to immobilization stress, we found that PDC offspring exhibited sustained HPA reactivity to stress, but PLC offspring exhibited HPA reactivity similar to that in NLC offspring. Additionally, in two independent behavioral experiments, PDC offspring exhibited a decrement in exploratory behavior both in frequency and duration; on the other hand, PLC offspring exhibited an increment in frequency but not in duration of exploratory behavior. The latter inconsistent results of PLC offspring in behavioral parameters might be due to their novelty preference and/or habituation. Although the severity of immobilization stress was quite different from stress induced by the elevated-plus maze or social interaction test, in accordance with the behavioral phenotype, PDC offspring had a sustained elevated corticosterone response to stress with decreased levels of hippocampal GR mRNA expression compared with NLC offspring. These results are similar to those in other reports of a positive correlation between amounts of active nursing in the dam and the level of active coping style with attenuated HPA response in offspring (Liu et al., 1997; Francis et al., 1999a; Macri et al., 2004; Priebe et al., 2006).

Because our early lighting manipulations affected both dam and litter, it cannot be denied that other factors except for active nursing, such as direct effects of the lighting condition, influenced the development of offspring. However, based on previous studies, it is natural to think at least in part that a PDC-induced decrement of maternal care contributed to fearfulness and a sustained HPA response in PDC offspring in our study.

Effects of ELC on memory and hippocampal NR2B mRNA expression

It is often reported that early rearing experience, such as high/low maternal care (Liu et al., 2000b; Bredy et al., 2003a; Bredy et al., 2003b; Bredy et al., 2004) and maternal separation (Francis et al., 2002; Roceri et al., 2002), alters offspring's memory functioning with hippocampal neuroplasticity. To investigate memory functioning in offspring, we used the object recognition test, which is usually considered to be associated with relatively low stress, to exclude as much as possible a stress response in different ELC-manipulated rats.

Our results revealed that PDC offspring exhibited impairment of short-term memory with decreased hippocampal NR2B mRNA expression. This correspondence between disordered memory and NMDA functioning was consistent with results in previous reports that suggested that object recognition memory was related to hippocampal NMDA receptor functioning (Tang et al., 1999; Rampon et al., 2000; Baker & Kim, 2002). PLC offspring showed neither any change in memory functioning nor hippocampal NMDA receptor expression compared with NLC offspring. Also, in terms of early rearing environment, these results were similar to previous results that reported impaired memory with decreased NMDA receptor mRNA expression in offspring reared by low LG mothers (Liu et al., 2000b; Bredy et al., 2003b; Bredy et al., 2004).

On the other hand, although there were no significant differences in hippocampal mRNA levels of the 3 NMDA receptor subunits examined and the GR between NLC and PLC offspring, the mRNA levels of NR2B and GR in PLC offspring tended to be downregulated to some extent (NR2B: $p = 0.15$, GR: $p = 0.07$, compared with NLC offspring), even though, there was no decrease in active nursing by their mothers. Based on the trend of those decreases in PLC offspring as well as a trend toward a decrease in NR1 ($p = 0.087$, compared with NLC offspring) and the significant decrease in NR2B and GR in PDC offspring, it can be speculated that changes in ELC may have partially affected hippocampal mRNA expression of these receptors in these offspring not only through the length of active nursing but other factors. Actually, Macri et al. suggested that the temporal distribution of maternal care may contribute to the phenotype of offspring with regard to early handling and maternal separation (Macri et al., 2004). In addition to the transient changes in daily amounts of maternal care and the altered diurnal distribution of maternal care in PLC mothers, the direct effects of lighting change on offspring might have additionally contributed to those PLC offspring's intermediate phenotypes. In this context, although the precise mechanism remains unknown, it is likely that the combination of low maternal care and light change-mediated stress may have induced the significant decrease both in NR2B and GR in the PDC group.

In natural conditions, energy-conserving, adaptive adjustments occur among individuals, especially in response to decreasing day length that are believed to promote survival during the harsh conditions of winter (Bronson, 1985). Laboratory rats are sensitive to reproductive inhibition by exposure to a short photoperiod alone and tend to avoid rearing pups under those harsh conditions that would decrease their chance of survival (Leadem, 1988; Heideman & Sylvester, 1997; Lorincz et al., 2001). Although our photoperiodic manipulation was artificial, the PDC-induced decrement of maternal behavior might be considered as one of those correspondent behavioral changes under harsh conditions, not suitable for rearing pups. From another point of view, according to the maternal mediation hypothesis, mothers mediate environmental information into the developing nervous system of pups in the neonatal period via maternal behavior, which in turn determines defensive responses to threatening situations in adult offspring (Cameron et al., 2005). Our observations partially support the hypothesis that a short photoperiod (PDC), which is an

environmental cue for harsh conditions, decreases maternal care and communicates the information into pup's neurodevelopment.

Since there are distinct differences in the daily rhythm of maternal behavior and photoperiodic responsiveness between nocturnal and diurnal animals, it is difficult to compare results from rodent studies to those from human studies. However, previous human infant studies indicated the benefits of structured care under a regular 12h:12h light/dark cycle on the somatic growth and development of the sleep/wake rhythm (Sander et al., 1972; Mann et al., 1986; Miller et al., 1995). In addition, Ohta and colleagues (Ohta et al., 2006) recently demonstrated that altered photoperiodic conditions induce acute and lasting effects on the developing biological clocks in mice. Considering findings from rodent studies, including our study, as well as human studies, the possibility that ELC play an important role in the neurodevelopment of offspring through mother-infant interactions is postulated.

In summary, we found that the early lighting environment altered maternal care and directly and/or indirectly affected the development of emotionality and memory functioning in their offspring, which underlies long-standing changes in neurobehavioral systems. These effects are quite similar to those induced by other early adverse paradigms such as maternal separation and low maternal care. Taken together, it is postulated that ELC are, at least in part, important factors controlling early mother-pup interactions and neurodevelopment of offspring. However, it cannot be ruled out that the effect of early lighting change is involved in our experimental paradigm. Therefore, further studies are required to elucidate whether the effect of photoperiodic duration or circadian change is more critical in early mother-pup interactions and neurodevelopment of emotionality and memory functioning in offspring.

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Abbreviations:

ANOVA, analysis of variance; ELC, early lighting conditions; GR, glucocorticoid receptor; HPA, hypothalamo-pituitary-adrenacortical; LG, licking and grooming; NLC, normal lighting conditions; NMDA, N-methyl d-aspartate; NR, N-methyl d-aspartate receptor; PDC, prolonged dark phase conditions; PLC, prolonged light phase conditions; PND, post natal day; RT-PCR, real-time quantitative polymerase chain reaction; SCN, suprachiasmatic nucleus;

References

Ader, R. & Grota, L.J. (1970) Rhythmicity in the maternal behaviour of Rattus norvegicus. Anim Behav, 18, 144-150.

Ahmadiyeh, N., Slone-Wilcoxon, J.L., Takahashi, J.S. & Redei, E.E. (2004) Maternal behavior modulates X-linked inheritance of behavioral coping in the defensive burying test. Biol Psychiatry, 55, 1069-1074.

Baker, K.B. & Kim, J.J. (2002) Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. Learn Mem, 9, 58-65.

Benstaali, C., Mailloux, A., Bogdan, A., Auzeby, A. & Touitou, Y. (2001) Circadian rhythms of body temperature and motor activity in rodents their relationships with the light-dark cycle. Life Sci, 68, 2645-2656.

Biagini, G., Pich, E.M., Carani, C., Marrama, P. & Agnati, L.F. (1998) Postnatal maternal separation during the stress hyporesponsive period enhances the adrenocortical response to novelty in adult rats by affecting feedback regulation in the CA1 hippocampal field. Int J Dev Neurosci, 16, 187-197.

Boon, P., Visser, H. & Daan, S. (1997) Effect of photoperiod on body mass, and daily energy intake and energy expenditure in young rats. Physiol Behav, 62, 913-919.

Bosch, O.J., Meddle, S.L., Beiderbeck, D.I., Douglas, A.J. & Neumann, I.D. (2005) Brain oxytocin correlates with maternal aggression: link to anxiety. J Neurosci, 25, 6807-6815.

Bredy, T.W., Grant, R.J., Champagne, D.L. & Meaney, M.J. (2003a) Maternal care influences neuronal survival in the hippocampus of the rat. Eur J Neurosci, 18, 2903-2909.

Bredy, T.W., Humpartzoomian, R.A., Cain, D.P. & Meaney, M.J. (2003b) Partial reversal of the effect of maternal care on cognitive function through environmental enrichment. Neuroscience, 118, 571-576.

Bredy, T.W., Zhang, T.Y., Grant, R.J., Diorio, J. & Meaney, M.J. (2004) Peripubertal environmental enrichment reverses the effects of maternal care on hippocampal development and glutamate receptor subunit expression. Eur J Neurosci, 20, 1355-1362.

Bridges, R.S., DiBiase, R., Loundes, D.D. & Doherty, P.C. (1985) Prolactin stimulation of maternal behavior in female rats. Science, 227, 782-784.

Bridges, R.S., Goldman, B.D. & Bryant, L.P. (1974) Serum prolactin concentrations and the initiation of maternal behavior in the rat. Horm Behav, 5, 219-226.

Bronson, F.H. (1985) Mammalian reproduction: an ecological perspective. Biol Reprod, 32, 1-26.

Caldji, C., Diorio, J. & Meaney, M.J. (2003) Variations in maternal care alter GABA(A) receptor subunit expression in brain regions associated with fear. Neuropsychopharmacology, 28, 1950-1959.

Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P.M. & Meaney, M.J. (1998) Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proc Natl Acad Sci U S A, 95, 5335-5340.

Cameron, N.M., Champagne, F.A., Parent, C., Fish, E.W., Ozaki-Kuroda, K. & Meaney, M.J. (2005) The programming of individual differences in defensive responses and reproductive strategies in the rat through variations in maternal care. Neurosci Biobehav Rev, 29, 843-865.

Champagne, F. & Meaney, M.J. (2001) Like mother, like daughter:

evidence for non-genomic transmission of parental behavior and stress responsivity. Prog Brain Res, 133, 287-302.

Champagne, F.A., Francis, D.D., Mar, A. & Meaney, M.J. (2003a) Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiol Behav, 79, 359-371.

Champagne, F.A., Weaver, I.C., Diorio, J., Sharma, S. & Meaney, M.J. (2003b) Natural variations in maternal care are associated with estrogen receptor alpha expression and estrogen sensitivity in the medial preoptic area. Endocrinology, 144, 4720-4724.

Champagne, F.A. & Meaney, M.J. (2006a) Stress During Gestation Alters Postpartum Maternal Care and the Development of the Offspring in a Rodent Model. Biol Psychiatry. (in press).

Champagne, F.A., Weaver, I.C., Diorio, J., Dymov, S., Szyf, M. & Meaney, M.J. (2006b) Maternal care associated with methylation of the estrogen receptor alpha 1b promoter and estrogen receptor alpha expression in the medial preoptic area of female offspring. Endocrinology. (in press).

Coble, P.A., Reynolds, C.F., 3rd, Kupfer, D.J., Houck, P.R., Day, N.L. & Giles, D.E. (1994) Childbearing in women with and without a history of affective disorder. II. Electroencephalographic sleep. Compr Psychiatry, 35, 215-224.

Darnaudery, M., Dutriez, I., Viltart, O., Morley-Fletcher, S. & Maccari, S. (2004) Stress during gestation induces lasting effects on emotional reactivity of the dam rat. Behav Brain Res, 153, 211-216.

Duncan, M.J., Banister, M.J. & Reppert, S.M. (1986) Developmental appearance of light-dark entrainment in the rat. Brain Res, 369, 326-330.

Ennaceur, A. & Delacour, J. (1988) A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav Brain Res, 31, 47-59.

Francis, D., Diorio, J., Liu, D. & Meaney, M.J. (1999a) Nongenomic transmission across generations of maternal behavior and stress responses in the rat. Science, 286, 1155-1158.

Francis, D.D., Champagne, F.A., Liu, D. & Meaney, M.J. (1999b) Maternal care, gene expression, and the development of individual differences in stress reactivity. Ann N Y Acad Sci, 896, 66-84.

Francis, D.D., Champagne, F.C. & Meaney, M.J. (2000) Variations in maternal behaviour are associated with differences in oxytocin receptor levels in the rat. J Neuroendocrinol, 12, 1145-1148.

Francis, D.D., Diorio, J., Plotsky, P.M. & Meaney, M.J. (2002) Environmental enrichment reverses the effects of maternal separation on stress reactivity. J Neurosci, 22, 7840-7843.

Gonzalez, A., Lovic, V., Ward, G.R., Wainwright, P.E. & Fleming, A.S. (2001) Intergenerational effects of complete maternal deprivation and replacement stimulation on maternal behavior and emotionality in female rats. Dev Psychobiol, 38, 11-32.

Gray, S.J. (1994) Comparison of effects of breast-feeding practices on birth-spacing in three societies: nomadic Turkana, Gainj, and Quechua. J Biosoc Sci, 26, 69-90.

Gray, S.J. (1995) Correlates of breastfeeding frequency among nomadic pastoralists of Turkana, Kenya: a retrospective study. Am J Phys Anthropol, 98, 239-255.

Grota, L.J. & Ader, R. (1974) Behavior of lactating rats in a dual-chambered maternity cage. Horm Behav, 5, 275-282.

Heideman, P.D. & Sylvester, C.J. (1997) Reproductive photoresponsiveness in unmanipulated male Fischer 344 laboratory rats. Biol Reprod, 57, 134-138.

Hiroshige, T., Honma, K. & Watanabe, K. (1982a) Ontogeny of the circadian rhythm of plasma corticosterone in blind infantile rats. J Physiol, 325, 493-506.

Hiroshige, T., Honma, K. & Watanabe, K. (1982b) Possible zeitgebers for external entrainment of the circadian rhythm of plasma corticosterone in blind infantile rats. J Physiol, 325, 507-519.

Hiroshige, T., Honma, K. & Watanabe, K. (1982c) Prenatal onset and maternal modifications of the circadian rhythm of plasma corticosterone in blind infantile rats. J Physiol, 325, 521-532.

Honma, K., Honma, S. & Hiroshige, T. (1985) Response curve, free-running period, and activity time in circadian locomotor rhythm of rats. Jpn J Physiol, 35, 643-658.

Hoshino, K., Wakatsuki, Y., Iigo, M. & Shibata, S. (2006) Circadian clock mutation in dams disrupts nursing behavior and growth of pups. Endocrinology, 147, 1916-1923.

Jilge, B. (1993) A feeding-entrainable circadian oscillator system in the rabbit. Ann Ist Super Sanita, 29, 511-520.

Jilge, B. (1995) Ontogeny of the rabbit's circadian rhythms without an external zeitgeber. Physiol Behav, 58, 131-140.

Jutapakdeegul, N., Casalotti, S.O., Govitrapong, P. & Kotchabhakdi, N. (2003) Postnatal touch stimulation acutely alters corticosterone levels and glucocorticoid receptor gene expression in the neonatal rat. Dev Neurosci, 25, 26-33.

Kalinichev, M., Easterling, K.W., Plotsky, P.M. & Holtzman, S.G. (2002) Long-lasting changes in stress-induced corticosterone response and anxiety-like behaviors as a consequence of neonatal maternal separation in Long-Evans rats. Pharmacol Biochem Behav, 73, 131-140.

Leadem, C.A. (1988) Photoperiodic sensitivity of prepubertal female Fisher 344 rats. J Pineal Res, 5, 63-70.

Lee, M.H. & Williams, D.I. (1977) A longditudinal study of mother-young teraction in the rat: the effects of infantile stimulation, diurnal rhythms, and pup maturation. Behaviour, 63, 241-261.

Levine, S. (1967) Maternal and environmental influences on the adrenocortical response to stress in weanling rats. Science, 156, 258-260.

Levine, S. (2005) Developmental determinants of sensitivity and resistance to stress. Psychoneuroendocrinology, 30, 939-946.

Liu, D., Caldji, C., Sharma, S., Plotsky, P.M. & Meaney, M.J. (2000a) Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinepherine release in the hypothalamic paraventricular nucleus. J Neuroendocrinol, 12, 5-12.

Liu, D., Diorio, J., Day, J.C., Francis, D.D. & Meaney, M.J. (2000b) Maternal care, hippocampal synaptogenesis and cognitive development in rats. Nat Neurosci, 3, 799-806.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P.M. & Meaney, M.J. (1997) Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science, 277, 1659-1662.

Lorincz, A.M., Shoemaker, M.B. & Heideman, P.D. (2001) Genetic variation in photoperiodism among naturally photoperiodic rat strains. Am J Physiol Regul Integr Comp Physiol, 281, R1817-1824.

Macri, S., Mason, G.J. & Wurbel, H. (2004) Dissociation in the effects of neonatal maternal separations on maternal care and the offspring's HPA and fear responses in rats. Eur J Neurosci, 20, 1017-1024.

Mann, N.P., Haddow, R., Stokes, L., Goodley, S. & Rutter, N. (1986) Effect of night and day on preterm infants in a newborn nursery: randomised trial. Br Med J (Clin Res Ed), 293, 1265-1267.

Menard, J.L., Champagne, D.L. & Meaney, M.J. (2004) Variations of maternal care differentially influence 'fear' reactivity and regional patterns of cFos immunoreactivity in response to the shock-probe burying test. Neuroscience, 129, 297-308.

Miller, C., White, R. & Whitman, T. (1995) The effect of cycled versus non-cycled lighting on the growth and development of preterm infants. Infant Rehav Dev, 18, 95.

Morinobu, S., Fujimaki, K., Kawano, K., Tanaka, K., Takahashi, J., Ohkawa, M., Yamawaki, S. & Kato, N. (2003) Influence of immobilization stress on the expression and phosphatase activity of protein phosphatase 2A in the rat brain. Biol Psychiatry, 54, 1060-1066.

Munck, A., Guyre, P.M. & Holbrook, N.J. (1984) Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr Rev, 5, 25-44.

Myers, M.M., Brunelli, S.A., Squire, J.M., Shindeldecker, R.D. & Hofer, M.A. (1989) Maternal behavior of SHR rats and its relationship to offspring blood pressures. Dev Psychobiol, 22, 29-53.

Nelson, R.J., Moffatt, C.A. & Goldman, B.D. (1994) Reproductive and nonreproductive responsiveness to photoperiod in laboratory rats. J Pineal Res, 17, 123-131.

Neumann, I.D. (2001) Alterations in behavioral and neuroendocrine stress coping strategies in pregnant, parturient and lactating rats. Prog Brain Res, 133, 143-152.

Neumann, I.D. (2003) Brain mechanisms underlying emotional alterations in the peripartum period in rats. Depress Anxiety, 17, 111-121.

Neumann, I.D., Kromer, S.A. & Bosch, O.J. (2005) Effects of psycho-social stress during pregnancy on neuroendocrine and behavioural parameters in lactation depend on the genetically determined stress vulnerability. Psychoneuroendocrinology, 30, 791-806.

Numan, M., Rosenblatt, J.S. & Komisaruk, B.R. (1977) Medial preoptic area and onset of maternal behavior in the rat. J Comp Physiol Psychol, 91, 146-164.

Ohta, H., Honma, S., Abe, H. & Honma, K. (2002) Effects of nursing mothers on rPer1 and rPer2 circadian expressions in the neonatal rat suprachiasmatic nuclei vary with developmental stage. Eur J Neurosci, 15, 1953-1960.

Ohta, H., Honma, S., Abe, H. & Honma, K. (2003) Periodic absence of nursing mothers phase-shifts circadian rhythms of clock genes in the suprachiasmatic nucleus of rat pups. Eur J Neurosci, 17, 1628-1634.

Ohta, H., Mitchell, A.C. & McMahon, D.G. (2006) Constant light disrupts the developing mouse biological clock. Pediatr Res, 60, 304-308.

Pardon, M., Gerardin, P., Joubert, C., Perez-Diaz, F. & Cohen-Salmon, C. (2000) Influence of prepartum chronic ultramild stress on maternal pup care behavior in mice. Biol Psychiatry, 47, 858-863.

Pedersen, C.A., Ascher, J.A., Monroe, Y.L. & Prange, A.J., Jr. (1982) Oxytocin induces maternal behavior in virgin female rats. Science, 216, 648-650.

Pedersen, C.A. & Prange, A.J., Jr. (1979) Induction of maternal behavior in virgin rats after intracerebroventricular administration of oxytocin. Proc Natl Acad Sci U S A, 76, 6661-6665.

Pieper, D.R. & Gala, R.R. (1979) Influence of the pineal gland, olfactory bulbs and photoperiod on surges of plasma prolactin in the female rat. J Endocrinol, 82, 279-285.

Pihoker, C., Owens, M.J., Kuhn, C.M., Schanberg, S.M. & Nemeroff, C.B. (1993) Maternal separation in neonatal rats elicits activation of the hypothalamic-pituitary-adrenocortical axis: a putative role for corticotropin-releasing factor. Psychoneuroendocrinology, 18, 485-493.

Pittendrigh, C.S. & Daan, S. (1976) A functional analysis of circadian pacemakers in nocturnal rodents. Journal of Comparative Physiology, 106, 291-331.

Priebe, K., Brake, W.G., Romeo, R.D., Sisti, H.M., Mueller, A., McEwen, B.S. & Francis, D.D. (2006) Maternal influences on adult stress and anxiety-like behavior in C57BL/6J and BALB/CJ mice: A cross-fostering study. Dev Psychobiol, 48, 95-96.

Pryce, C.R., Ruedi-Bettschen, D., Dettling, A.C., Weston, A., Russig, H., Ferger, B. & Feldon, J. (2005) Long-term effects of early-life environmental manipulations in rodents and primates: Potential animal models in depression research. Neurosci Biobehav Rev, 29, 649-674.

Rampon, C., Tang, Y.P., Goodhouse, J., Shimizu, E., Kyin, M. & Tsien, J.Z. (2000) Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. Nat Neurosci, 3, 238-244.

Refinetti, R. (2004) Daily activity patterns of a nocturnal and a diurnal rodent in a seminatural environment. Physiol Behav, 82, 285-294.

Roceri, M., Hendriks, W., Racagni, G., Ellenbroek, B.A. & Riva, M.A. (2002) Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. Mol Psychiatry, 7, 609-616.

Rosenfeld, P., Wetmore, J.B. & Levine, S. (1992) Effects of repeated maternal separations on the adrenocortical response to stress of preweanling rats. Physiol Behav, 52, 787-791.

Ruedi-Bettschen, D., Feldon, J. & Pryce, C.R. (2004) Circadian- and temperature-specific effects of early deprivation on rat maternal care and pup development: short-term markers for long-term effects? Dev Psychobiol, 45, 59-71.

Sander, L.W., Julia, H.L., Stechler, G. & Burns, P. (1972) Continuous 24-hour interactional monitoring in infants reared in two caretaking environments. Psychosom Med, 34, 270-282.

Schelstraete, I., Knaepen, E., Dutilleul, P. & Weyers, M.H. (1992) Maternal behaviour in the Wistar rat under atypical Zeitgeber.

Physiol Behav, 52, 189-193.
Schmidt, M., Okimoto, D.K., Dent, G.W., Gordon, M.K. & Levine, S. (2002) Maternal regulation of the hypothalamic-pituitary-adrenal axis in the 20-day-old rat: consequences of laboratory weaning. J Neuroendocrinol, 14, 450-457.
Sei, H., Fujihara, H., Ueta, Y., Morita, K., Kitahama, K. & Morita, Y. (2003) Single eight-hour shift of light-dark cycle increases brain-derived neurotrophic factor protein levels in the rat hippocampus. Life Sci, 73, 53-59.
Shimoda, K., Hanada, K., Yamada, N., Takahashi, K. & Takahashi, S. (1986) Periodic exposure to mother is potent zeitgeber of rat pups' rhythm. Physiol Behav, 36, 723-730.
Siegel, H.I. & Rosenblatt, J.S. (1975a) Estrogen-induced maternal behavior in hysterectomized-overiectomized virgin rats. Physiol Behav, 14, 465-471.
Siegel, H.I. & Rosenblatt, J.S. (1975b) Latency and duration of estrogen induction of maternal behavior in hysterectomized-ovariectomized virgin rats: effects of pup stimulation. Physiol Behav, 14, 473-476.
Smith, J.W., Seckl, J.R., Evans, A.T., Costall, B. & Smythe, J.W. (2004) Gestational stress induces post-partum depression-like behaviour and alters maternal care in rats. Psychoneuroendocrinology, 29, 227-244.
Smotherman, W. & Bell, R. (1980) maternal mediation of early experience. In Bell RW, Smotherman WP (eds), Maternal influences and early behavior., 201-210.
Stanton, M.E., Gutierrez, Y.R. & Levine, S. (1988) Maternal deprivation potentiates pituitary-adrenal stress responses in infant rats. Behav Neurosci, 102, 692-700.
Stanton, M.E. & Levine, S. (1990) Inhibition of infant glucocorticoid stress response: specific role of maternal cues. Dev Psychobiol, 23, 411-426.
Stephens, D.B. (1980) Stress and its measurement in domestic animals: a review of behavioral and physiological studies under field and laboratory situations. Adv Vet Sci Comp Med, 24, 179-210.
Sterner, M.R. & Cohen, I.R. (1995) Steroid treatment fails to induce an afternoon luteinizing hormone or prolactin surge in rats exposed to short-term constant light at the time of ovariectomy. Neuroendocrinology, 62, 231-237.
Suenaga, T., Morinobu, S., Kawano, K., Sawada, T. & Yamawaki, S. (2004) Influence of immobilization stress on the levels of CaMKII and phospho-CaMKII in the rat hippocampus. Int J Neuropsychopharmacol, 7, 299-309.
Tang, Y.P., Shimizu, E., Dube, G.R., Rampon, C., Kerchner, G.A., Zhuo, M., Liu, G. & Tsien, J.Z. (1999) Genetic enhancement of learning and memory in mice. Nature, 401, 63-69.
van Oers, H.J., de Kloet, E.R., Whelan, T. & Levine, S. (1998) Maternal deprivation effect on the infant's neural stress markers is reversed by tactile stimulation and feeding but not by suppressing corticosterone. J Neurosci, 18, 10171-10179.
Viswanathan, N. (1999) Maternal entrainment in the circadian activity rhythm of laboratory mouse (C57BL/6J). Physiol Behav, 68, 157-162.
Viswanathan, N. & Chandrashekaran, M.K. (1985) Cycles of presence and absence of mother mouse entrain the circadian clock of pups. Nature, 317, 530-531.
Weinert, D., Freyberg, S., Touitou, Y., Djeridane, Y. & Waterhouse, J.M. (2005) The phasing of circadian rhythms in mice kept under normal or short photoperiods. Physiol Behav, 84, 791-798.
Yamazaki, A., Ohtsuki, Y., Yoshihara, T., Honma, S. & Honma, K. (2005) Maternal deprivation in neonatal rats of different conditions affects growth rate, circadian clock, and stress responsiveness differentially. Physiol Behav, 86, 136-144.
Yamazaki, J. & Takahashi, K. (1983) Effects of change of mothers and lighting conditions on the development of the circadian adrenocortical rhythm in blinded rat pups. Psychoneuroendocrinology, 8, 237-244.
Yoshihara, T., Otsuki, Y., Yamazaki, A., Honma, S., Yamasaki, Y. & Honma, K. (2005) Maternal deprivation in neonatal rats alters the expression of circadian system under light-dark cycles and restricted daily feeding in adulthood. Physiol Behav, 85, 646-654.
Zhang, T.Y., Chretien, P., Meaney, M.J. & Gratton, A. (2005) Influence of naturally occurring variations in maternal care on prepulse inhibition of acoustic startle and the medial prefrontal cortical dopamine response to stress in adult rats. J Neurosci, 25, 1493-1502.