Research article

Time–place learning is altered by perinatal low-protein malnutrition in the adult rat

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Malnutrition produces changes in the central nervous system (CNS) of mammals during development, related to the intensity and timing of the malnutrition insult during the pre- or postnatal period. Protein malnutrition produces irreversible changes in hippocampal formation and some brain stem nuclei. The suprachiasmatic nucleus (SCN) is dramatically altered by low-protein diets during the gestational and perinatal periods. Also, it is known that circadian oscillators regulate physiological, behavioral, and cognitive processes and there is evidence that the time–place learning process exhibits a daily temporal distribution. The aim of this study was to determine the effects of chronic, prenatal, or postnatal malnutrition on daily patterns of the time–place learning process in the adult rat. Forty Sprague–Dawley male 90-day-old rats, were divided into four groups: 10 well nourished controls (Co), 10 chronically (CM), 10 prenatally malnourished (PrM), and 10 postnatally malnourished (PtM) rats. Efficiency in time–place learning was tested by using a behavioral T-maze. Each rat was assayed for 10 trials before considering the final probe of efficiency. Each trial was 60 seconds long, final efficiency was measured by the amount of time the rat took to reach the end of an arm containing a water pot. Each rat was tested in 2-hour spans until completion of a full 24-hour cycle. A Cosinor analysis was used to evaluate acrophase and percentage of rhythmicity. The obtained results suggest that time–place learning process is influenced by the circadian clock. The severity and timing of prenatal or chronic protein malnutrition modifies the acrophase and rhythmicity of the learning circadian pattern, which can impact important cognitive functions.

Keywords: Time–place learning, Low-protein malnutrition, Rat, Suprachiasmatic nuclei, Circadian rhythm, Brain development

Introduction

There is considerable evidence indicating that physiological and behavioral functions are regulated by temporal cues. The biological clock is involved in processes ranging from genetic expression to displaying a specific behavior, including learning and performance of memory. Circadian or daily organization in mammals is mainly driven by the suprachiasmatic nucleus (SCN), which entrains and regulates phase and period of peripheral oscillators;[1] therefore, dysfunction on the integration of the input to SCN and its related output to other brain structures lead to disadvantages in the internal temporal order as well as the animal's interaction with the cyclic environment.[2]

It is well known that early protein malnutrition can damage the vulnerable central nervous system (CNS) and its functional development. The consequences of protein-deficiency or protein-calorie malnutrition are expressed as alterations during CNS maturation in the neuroanatomical, neurochemical, and behavioral functions. It has been reported that malnutrition reduces brain size, body cells, dendritic arborization, etc., resulting in functional, cognitive, or behavioral impairments. However, depending on the type, timing, duration, and severity of malnutrition, the effects can be either reversible or permanent. Malnutrition is a global problem affecting a large population in the world, mainly children (WHO, 2006) and nearly 24% of newborns are underweight,

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with its consequences including predisposition to neural and metabolic diseases, it is highly important to understand the effect of malnutrition on animal physiology.

Low-protein diets instituted during critical periods of brain development had been reported to negatively create an impact on some physiological and cognitive functions in the short and long term.[3–10] There is evidence that protein malnutrition (induced by 6% casein diets) produce long-term effects in the neural substrate of hippocampal formation,[11,12] and other brain areas involved in the sleep–wake cycle regulation;[4] also, it may affect the circadian organization in rats reducing the somatic size of SCN cells[13] and alter circadian behaviors.[14–18]

In humans and other mammals, attention processes exhibit changes during the day and it is quite possible that some of these processes are clock regulated.[19] A deficit in memory in water maze experiments was observed in continuous phase shifting protocols in rats[20] as well as other cognitive functions,[21,22] indicating that a deficit in circadian regulation may produce deficits in cognitive and social memory[23] attention processes and learning.[24] Also, there is evidence that time–place learning is based on the ability to integrate spatial information with temporal cues.[25,26] Although those studies explore only morning and afternoon performance, it suggests that this cognitive behavior can be driven by a circadian oscillator.

The current study was designed to explore if there is a temporal modulation of learning by evaluating rats in two phases of their circadian rhythms at 2-hour intervals in the T-maze paradigm. We also aim to determine the effects of chronic (pre- and postnatal), pre- or postnatal malnutrition (using 6% casein diets) in a time–place learning task using a T-maze where water was available as a reinforcer in the water-deprived adult rat.

Methods

Animals and housing

Sprague–Dawley male rats were obtained from several breeders as previously published.[17] Animals were born and raised in a controlled environment that oscillated between 20 and 24°C, light photoperiod consisted of LD 12:12 (12 hours of light-12 hours of darkness, lights on 8:00, lights off 20:00) and during the dark phase of the cycle; a dim red light was used as the background (1–3 Lx) at the Instituto de Neurobiologia vivarium. Animals were organized in groups as described earlier.[5,27] Briefly, there were two groups of 10 nuliparous female rats (250 g), each were fed one of the two diets: adequate protein chow diet (5001 laboratory rodent, Purina, St. Louis, MO, USA) or low-protein diet (casein 6%, Teklad Wisconsin, Madison, WI, USA) 5 weeks before mating and thorough gestation, lactation, and until assayed. During mating they were caged with males in a proportion of 2:1 for a week or when sperm was present on vaginal smears. Mating males were fed with the diet corresponding to the assigned females. Fig. 1 summarizes the research design. At birth, litters were cross-fostered to a lactating mother receiving a 6% casein or chow diet. During lactation and after weaning, the PrM and CM rats received the low protein (6% casein) diet, whereas the Co and PtM rats were given the chow diet. All litters contained 8 pups (6 males and 2 females). Rats were pair-housed in hanging cages after weaning. We employed 90 120-day-old male rats, divided into four groups, consisting

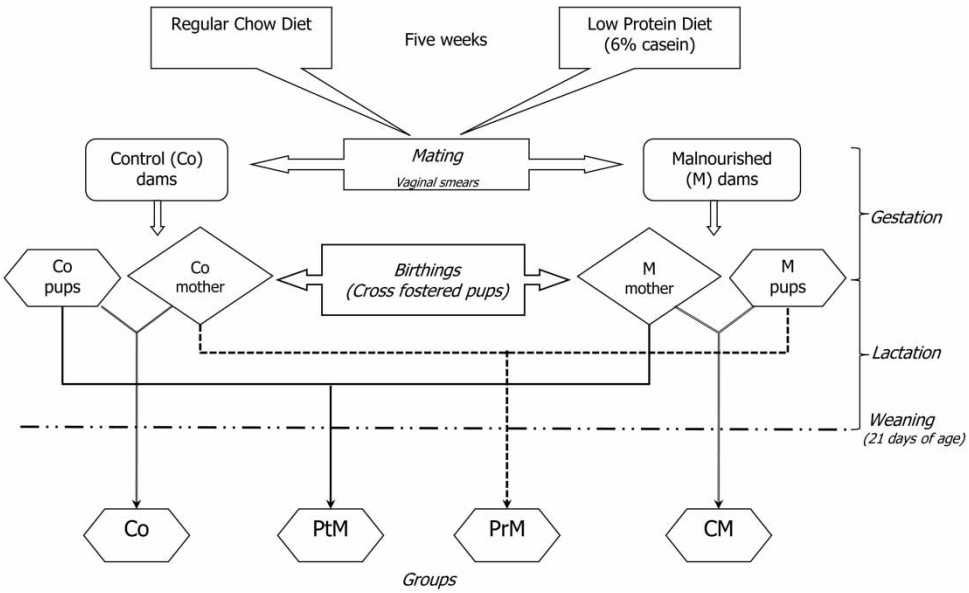

Figure 1 Malnutrition protocol.

of 10 rats per group: (a) controls (Co), (b) chronic (CM), (c) postnatal (PtM), and (d) prenatally (PrM) malnourished rats.

Behavioral procedure

T-maze test

The testing apparatus was a black, wooden T-maze. Two different T-maze measures were designed since body size in subjects differed according to the malnutrition experimental group. For Co and PrM (290–300 g body weight), the start arm was 60 cm long and each choice arm was 40 cm long; for PtM and CM (50–70 g), the start arm was 40 cm long and each choice arm was 25 cm long. All arms were 10 cm wide. Water was used as a reinforcer in a petri dish (4 cm), at the end of one arm. All subjects were water-deprived for 12 hours before recording.

All rats were handled for 10 minutes per day for at least 10 days before testing. All rats were individually trained in a T-maze. Rats were acquainted with the maze but not trained for choices, since that was not the objective of this study. Rats were given nine trials per training session.

After training, rats were water-deprived with food *ad libitum* for at least 12-hours. Every rat was assayed for nine trials before the probe trial (where water tap was taken). Each trial was a maximum of 60 seconds long and if the animal did not reach the water pot, it was withdrawn from the maze for 30 seconds before the next trial. Although the choice was correct, drinking water was not allowed. At the end, animals were re-located in their home cages at least 24 hours before the next test. The order of the trials was counterbalanced by alternating the correct choice arm to be on either the right or the left. After each testing period, the rats were returned to their home cage and allowed to drink freely. After each trial, the T-maze surface was wiped with a diluted chlorine solution (3%), to avoid any animal's odor remaining from previous trials.

Each session test consisted of 2-hour spans a day until completing the full 24 hours. When the test had to be run in the dark phase, a dim red-light background was used; therefore, animals were never exposed to bright light during the dark phase of the cycle.

During each trial, the following were considered: start latency, which indicates the alternation strategy in time–place learning; ambulation score (escape latency), considered as the time spent exploring the start arm of the maze; and mean latency as the time used for the rat to pick the correct entry in the arm of the maze (where the water pot was located). Also, parameters such as spontaneous alternation and feces and urine were observed.

Data analysis

In order to test daily rhythmicity, a Cosinor analysis was used (COSANA software, A. Benedito-Silva, GMDRB, Departamento de Fisiologia e Biofísica, ICB/USP, Brazil). Parameters including acrophase and percentage of rhythmicity were considered. Statistical differences between groups ($P < 0.05$) were obtained by means of student *t*-test analysis. Data on the present work are indicated as average ± SEM, unless specified. The performance of the time–place learning (learning efficiency) of Co rats was used as a reference to be compared among malnourished groups.

Results

Learning efficiency

Fig. 2 shows four daily profiles of average values (±SE) of learning efficiency assayed in a T-maze. Bars on graphs indicate LD 12:12 conditions. At the top, the Co group shows clear daily variations with maximal learning efficiency during the first half of

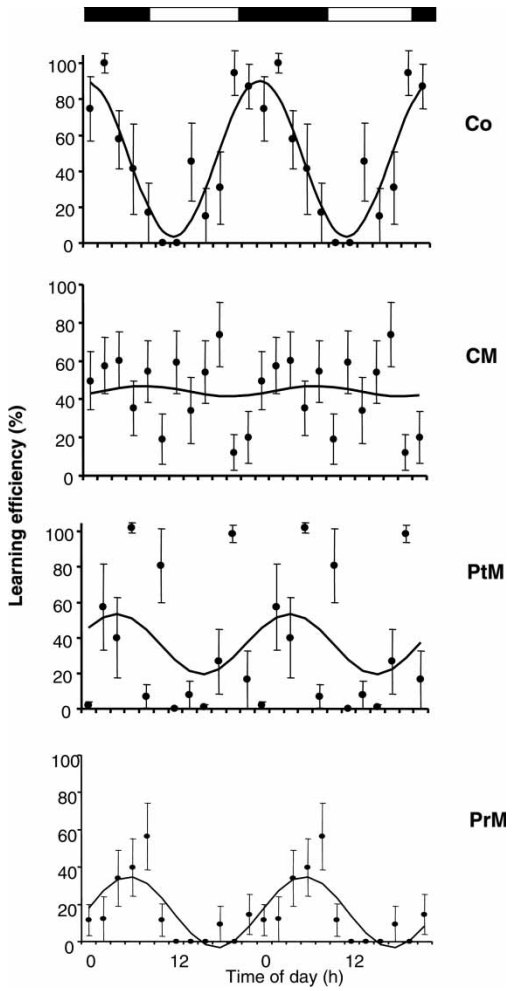

Figure 2 Average values (±SE) of final efficiency after 10 trials at different times of the day. Groups are indicated with initials: control (Co), chronic (CM), postnatal (PtM), and prenatal malnutrition (PrM).

Table 1 Acrophase values (Cosinor analysis) during daytime are shown for the controls (Co) and malnourishment protocols used

	Co	CM	PtM	PrM
Start latency	6.27(±1.3)[a]	9.82(±1.9)	9.67(±2.2)	12.33(±0.5)[A]
Escape latency	12.65(±2.0)	12.86(±2.5)	11.86(±2.5)	12.7(±3.7)
Mean latency	15.47(±1.5)	18.17(±1.1)	14.43(±2.0)	9.97(±2.8)
Learning efficiency	9.45(±0.9)[a]	11.20(±1.8)	14.14(±2.1)	13.31(±0.9)[A]

CM, chronic; PrM, prenatal; and PtM, postnatal. Capital letter indicates statistical differences with its corresponding lower case.

the dark phase, while its lower values are near lights on and during the photic phase of the cycle.

In the Co group, 70% of the animals tested presented a significant Cosinor fit (CF, $P < 0.05$). CM rats have a low-efficiency profile throughout the LD cycle compared with Co and the CF where the data was not significant. PtM rats displayed high scores after lights were on and off reaching the control 100% of efficiency; nevertheless just two animals fit to the Cosinor curve.

PrM rats better fit to Cosinor analysis; however, the peak of performance was not enough to reach Co levels. The only groups that showed more than 50% of animals with significant fit to Cosinor analysis were Co and PrM.

Acrophases analysis

Table 1 shows the average values and standard error of acrophase for each group tested. Statistical differences were found between Co and PrM when start latency was compared (indicated with capital and lower-case letters). Escape latency and mean latency were not different among groups. Learning efficiency was different between Co and PrM. A delay of nearly 4 hours was found in the prenatally malnourished group.

Percentage of rhythmicity

Table 2 displays average values (±SE) from the Cosinor analysis. Larger values or percentages were found in Co group at learning efficiency, which correspond to the fitted curved shown in Fig. 2. Significant differences in this parameter were observed in the PrM group when analyzing start latency.

Discussion

In the present study, we show a daily cycle in a behavioral parameter associated with the LD conditions in control animals as well as the variation in rhythm observed according to the type of malnutrition. The place learning tested by using the T-maze shows differential response regarding the malnutrition protocol used (chronic, pre- or postnatal).

In order to achieve the learning aim, we used a pot of water as a reward in animals that were 12-hours-deprived of water animals. The paradigm used involves spatial memory that depends on the hippocampus and pre-frontal brain areas.[28,29] The aim of the test was to determine whether the rats were capable of learning which arm contained the water pot with regard to the clues present in the test room and the time of day (time– place learning).

The learning efficiency indicated in this work considers the frequency with which animals selected the arm containing the water pot as well as the time employed for this task (latency), at several hours during the 24-hour LD cycle. The results obtained indicate a clear daily rhythm on the task efficiency, differentially affected by the kind of malnutrition protocol used, as shown in Fig. 1. Of particular interest, PtM shows rhythmicity in the task, but its acrophase is delayed with regard to that observed in controls. Such delay has also been observed in other behaviors and sleep cycles in the same kind of malnutrition.[15,16] With regard to the effect on simple learning, all experimental groups presented alterations., i.e., PtM rats took latencies during exploration ('stem' latency) of under 20 seconds decreasing the time at rest, while CM increased the latencies. PrM rats had similar time between activity and rest latency, while Co rats spend more time in rest latency than that observed in CM.

The effect of malnutrition on cognitive behavior has been studied in relation to neuro-anatomical findings. The effects of malnutrition have produced contradictory

Table 2 Percentage of rhythmicity (Cosinor)

	Co	CM	PtM	PrM
Start latency	33.4(±8.0)[a]	17.3(±2.8)[a]	27.9(±4.3)[a]	51.4(±6.1)[A]
Escape latency	24.6(±6.8)	16.8(±3.0)[a]	17.9(±2.9)[a]	35.9(±6.3)[A]
Mean latency	30.9(±8.2)	14.4(±1.9)	18.7(±3.3)	17.0(±4.7)
Learning efficiency	59(±6.4)[A]	13.0(±3.4)[a b]	9.3(±2.0)[a b]	29.0(±3.9)[a B]

Higher percentage values are mainly Co during learning efficiency and PrM during start latency. Capital letter indicates statistical differences with its corresponding lower case.

results according to the kind of malnourishment protocol used, i.e., the duration or development stage in which it is studied.[3,30]

The diverse studies in relation to low-protein malnutrition, high lipids, or protein-caloric malnutrition during the perinatal period, indicate a reduction in the number of neurons as well as the cell size, less dendrite growing, and therefore reduced synapses density; however, such effects seems to depend on the phase of development when the malnutrition was initiated.[30–32]

On the other hand, light effects of malnutrition may not be observed at the anatomical level but may be observed at the behavioral level. Behavior in animals depends on the balance of the synthesis and recapture of neurotransmitters in different brain areas.[33] Diet can influence these factors, particularly biogenic amines.[34] In the present study, we used different models of malnutrition and therefore its effect on neurotransmitter synthesis may be different. In PrM, it is evident that serotonin, dopamine, and tryptophan are increased in diverse cerebral regions during early development and decreased in adults,[35] while the reverse was noted for norepinephrine with low levels early and increases in adulthood, receptors to opioids are increased,[36] and gamma aminobutyric acid (GABA) sub-units are altered.[37] Diminished glutamatergic activity as well as glutamate receptors density in brain cortex has been observed in chronic malnutrition with 8% protein diets.[38]

All of these changes could potentially related to the differences found in behavioral and electrophysiological studies in animal models of malnutrition, particularly in studies related with physiology of sleep[4,8,15,17] circadian rhythms,[4,14,16,18] spontaneous behavior such as play, exploration, parental care[9,38] and cognition processes.[39,40]

The temporal pattern in place-learning behavior tested in this study pertains to changes in an LD cycle of 24 hours. Daily and circadian changes are mainly regulated by the SCN. It is known that this structure in hypothalamus is also affected by deficient protein malnutrition. SCN is present in cyclic physiology in altricial mammals from before birth, and connects different structures during the development of CNS regulation of circadian outputs.[41–44] In rats, SCN presents rhythms from day 13 of gestation, while synaptogenesis continues until postnata day 17.[41,45,46] Therefore, the way in which each malnutrition protocol affects the rhythmic output depends on how reversible the damage is, being more severe when SCN is being formed, which may explain why PrM rats do not display cyclic learning in the test. PtM, however, displayed a daily cycle less robust than CO. Differences observed in these groups may be due to the possible effects on SCN output more than in its development, since the ptM embryos were on a standard nutritional protocol and possibly the outputs were affected by the postnatal insult.

Studies in behavior are essential to understand and thus correlate anatomical and physiological effects of malnutrition. This is an important problem in developing countries and such studies increase our understanding about how reducing adverse effects will contribute to better programs of education.

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References

1 Yamazaki S, Numano R, Abe M, Hida A, Takahashi R, Ueda M, *et al.* Resetting central and peripheral circadian oscillators in transgenic rats. Science 2000;288(5466):682–5.
2 Moore RY, Leak RK. Suprachiasmatic nucleus. In: Takahashi JS, Turek FW, Moore RY (eds.) Handbook of behavioral neurobiology. Circadian clocks. Vol. 12. New York: Kluwer Academic Plenum Pubs; 2000. p. 141–71.
3 Morgane PJ, Austin-LaFrance RJ, Brozino JD, Tonkiss J, Díaz-Cintra S, Cintra L, *et al.* Prenatal malnutrition and development of the brain. Neurosci Biobehav 1993;17(1):91–128.
4 Cintra L, Díaz-Cintra S, Galván A, Morgane P. Circadian rhythm of sleep in normal and undernourished rats. Bol Est Med Biol 1988;36:3–17.
5 Duran P, Galván A, Granados L, Aguilar-Roblero R, Cintra L. Effects of protein malnutrition on vigilance state and their circadian rhythms in 30-day old rats submitted total sleep deprivation. Nutr Neurosci 1999;2:127–38.
6 Cintra L, Durán P, Guevara MA, Aguilar A, Castañon-Cervantes O. Pre- and post-natal protein malnutrition alters the effect of rapid eye movements sleep-deprivation by the platform-technique upon the electrocorticogram of the circadian sleep-wake cycle and its frequency bands in the rat. Nutr Neurosci 2002;5(2):91–101.
7 Segura B, Guadarrama JC, Pratz G, Mercado V, Merchant H, Cintra L, *et al.* Conduction failure of action potentials in sensory sural nerves of undernourished rats. Neurosci Lett 2004;354(3):181–84.
8 Durán P, Galler JR, Cintra L, Tonkiss J. Prenatal malnutrition and sleep states in adult rats: effects of restraint stress. Physiol Behav 2006;89(2):156–63.
9 Tonkiss J, Galler JR. Prenatal protein malnutrition and working memory performance in adult rats. Behav Brain Res 1990;40(2): 95–107.
10 Tonkiss J, Bonnie KE, Hudson JL, Shultz PL, Durán P, Galler JR. Ultrasonic call characteristics of rat pups are altered following prenatal malnutrition. Dev Psychobiol 2003;43(2): 90–101.
11 Cintra L, Aguilar A, Grandos L, Galván A, Kempert T, DeBassio W, *et al.* Effects of prenatal protein malnutrition on hippocampal CA! pyramidal cells in rats of four age groups. Hippocampus 1997;7(2):192–203.
12 Cintra L, Granados L, Aguilar A, Kemper T, DeBassio W, Galler J, *et al.* Effects of prenatal protein malnutrition on mossy fibers of the hippocampal formation in rats of four age groups. Hippocampus 1997;7(2):184–91.
13 Cintra L, Galván A, Díaz-Cintra S, Escobar C. Brain Res. protein malnutrition on suprachiasmatic nucleus cells in rats of three ages. Brain Res 1990;532(1–2):271–7.
14 Aguilar-Roblero R, Salazar-Juarez A, Rojas-Castañeda J, Escobar C, Cintra L. Organization of circadian rhythmicity and suprachiasmatic nuclei in malnourished rats. Am J Physiol 1997;273(4 Pt 2):R1321–31.
15 Cintra L, Durán P, Guevara MA, Aguilar A, Castañón-Cervantes O. Pre- and post-natal protein malnutrition alters the effect of rapid eye movements sleep-deprivation by the platform-technique upon the electrocorticogram of the circadian

sleep-wake cycle and its frequency bands in the rat. Nutr Neurosci 2002;5(2):91–101.
16 Durán P, Cintra L, Galler JR, Tonkiss J. Prenatal protein malnutrition induces a phase shift advance of the spontaneous locomotor rhythm and alters the rest/activity ratio in adult rats. Nutr Neurosci 2005;8(3):167–72.
17 Durán P, Miranda-Anaya M, Mondragón-García I, Cintra L. Protein malnutrition and aging affects entraining and intensity of locomotor activity and body temperature circadian rhythms in rats. Nutr Neurosci 2008;11(6):263–8.
18 Castanon-Cervantes O, Cintra L. Circadian rhythms of occipital-cortex temperature and motor activity in young and old rats under chronic protein malnutrition. Nutr Neurosci 2002;5(4):279–86.
19 Waterhouse JM, Minors DS, Akerstedt T, Reilly T, Atkinson G. Rhythms of human performance. In: Takahashi JS, Turek FW, Moore RY (eds.) Handbook of behavioral neurobiology. Circadian clocks. Vol. 12. New York: Kluwer Academic Plenum Pubs; 2000. p. 571–94.
20 Craig LA, McDonald RJ. Chronic disruption of circadian rhythms impairs hippocampal memory in the rat. Brain Res Bull 2008;76:141–51.
21 Tapp WN, Holloway FA. Phase shifting circadian rhythms produces retrograde amnesia. Science 1981;211(4486):1056–58.
22 Devan BD, Goad EH, Petri HL, Antoniadis EA, Hong NS, Ko CH, *et al.* Circadian phase-shifted rats show normal acquisition but impaired long-term retention of place information in the water task. Neurobiol Learn Mem 2001;75:51–62.
23 Moura Paula J, Gimenes-Junior Joao A, Valentinuzzi Veronica S, Xavier Gilberto F. Circadian phase and intertrial interval interfere with social recognition memory. Physiol Behav 2009; 96:51–6.
24 Zhou W, Crystal JD. Evidence for remembering when events occured in a rodent model of episodic memory. PNAS 2009; 106(23):9525–9.
25 Lukoyanov NV, Pereira PA, Mesquita RM, Andrade JP. Restricted feeding facilitates time-place learning in adult rats. Behav Brain Res 2002;134(1–2):283–90.
26 Pizzo MJ, Crystal JD. Evidence for an alternation strategy in time-place learning. Behav Process 2004;67(3):533–7.
27 Rojas JC, Vigueras RM, Reyes G, Rojas P, Cintra L, Aguilar-Roblero R. Morphological changes produced by acute prenatal exposure to ethanol on the immunoreactive vasoactive intestinal polypeptide cells of the suprachiasmatic nucleus of the rat. Proc West Pharmacol Soc 1999;42:75–6.
28 Jones MW, Wilson MA. Theta rhythms coordinate hippocampal-prefrontal interactions in a spatial memory task. PLoS Biol 2005;3(12):e402. Epub 2005 Nov 15.
29 Hyman JM, Zilli EA, Paley AM, Hasselmo ME. Medial prefrontal cortex cells show dynamic modulation with the hippocampal theta rhythm dependent on behavior. Hippocampus 2005;15(6):739–49.
30 Morgane PJ, Miller M, Kemper T, Stern W, Forber W, Hall R, *et al.* The effects of protein malnutrition on the developing central nervous system in the rat. Neurosci Biobehav Rev 1978; 2(3):137–239.
31 Brock JW, Prasad C. Alterations in dendritic spine density in the rat brain associated with protein malnutrition. Brain Res Dev Brain Res 1992;66(2):266–9.
32 Andrade JP, Madeira MD, Paula-Barbosa MM. Effects of long-term malnutrition and rehabilitation on the hippocampal formation of the adult rat. A morphometric study. J Anat 1995; 187(Pt 2):379–93.
33 Adams W, Kulsjic S, Van der Buuse M. Serotonin depletion in the dorsal and ventral hippocampus. Effects on locomotor hyperactivity, prepulse inhibition and learning and memory. Neuropharmacology 2008;55:1048–55.
34 Wurtman RJ, Fernstrom JD. Control of brain monoamine synthesis by diet and plasma amino acids. Am J Clin Nutr 1975; 28(6):638–47.
35 Chen JC, Turiak G, Galler J, Volicer L. Postnatal changes of brain monoamine levels in prenatally malnourished and control rats. Int J Dev Neurosci 1997;15(2):257–63.
36 Fiacco TA, Rosene DL, Galler JR, Blatt GJ. Increased density of hippocampal kainate receptors but normal density of NMDA and AMPA receptors in a rat model of prenatal protein malnutrition. J Comp Neurol 2003;456(4):350–60.
37 Steiger JL, Galler JR, Farb DH, Russek SJ. Prenatal protein malnutrition reduces beta(2), beta(3) and gamma(2L) GABA(A) receptor subunit mRNAs in the adult septum. Eur J Pharmacol 2002;446(1–3):201–2.
38 Rotta LN, Leszczinski DN, Brusque AM, Pereira P, Brum LF, Nogueira CW, *et al.* Effects of undernutrition on glutamatergic parameters in the cerebral cortex of young rats. Physiol Behav 2008;94(4):580–5. Epub 2008 Apr 3.
39 Tonkiss J, Galler J, Morgane PJ, Bronzino JD, Austin-LaFrance RJ. Prenatal protein malnutrition and postnatal brain function. Ann N Y Acad Sci 1993;678:215–27.
40 Tonkiss J, Calderwood SK. Regulation of heat shock gene transcription in neuronal cells. Int J Hyperthermia 2005;21(5): 433–44.
41 Antle MC, LeSauter J, Silver R. Neurogenesis and ontogeny of specific cell phenotypes within the hamster suprachiasmatic nucleus. Brain Res Dev Brain Res 2005;157(1):8–18. Epub 2005 Apr 9.
42 Seron-Ferre M, Valenzuela GJ, Torres-Farfan C. Circadian clocks during embryonic and fetal development. Birth Defects Res C Embryo Today 2007;81(3):204–14.
43 Kabrita CS, Davis FC. Development of the mouse suprachiasmatic nucleus: determination of time of cell origin and spatial arrangements within the nucleus. Brain Res 2008;1195:20–7. Epub 2007 Dec 23.
44 Mohawk JA, Menaker M. A new (and different) circadian pacemaker. Cell Cycle. 2009 Sep 15;8(18):2861–2
45 Moore RY. Development of the suprachiasmatic nucleus. In: Klein D, Moore RY, Reppert SM (eds.) Suprachiasmatic nucleus. The mind's clock. New York: Oxford University Press; 1991.
46 Kennawaqy DJ. Programming of the fetal suprachiasmatic nucleus and subsequent adult rhythmicity. Trends Endocrinol Metab 2002;13(9):398–402.