Maternal Hyperthyroidism in Rats Impairs Stress Coping of Adult Offspring

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Given the evidence that maternal hyperthyroidism (MH) compromises expression of neuronal cytoskeletal proteins in the late fetal brain by accelerated neuronal differentiation, we investigated possible consequences of MH for the emotional and cognitive functions of adult offspring during acute and subchronic stress coping. Experimental groups consisted of male rat offspring from mothers implanted with osmotic minipumps infusing either thyroxine (MH) or vehicle (Ctrl) during pregnancy. Body weight and T4 level were monitored during the first 3 postnatal months, and no differences were found with the controls. We analyzed hippocampal CA3 pyramidal neurons and dentate granular cell morphology during several postnatal stages and found increased dendritic arborization. On postnatal day 90 a modified subchronic mild stress (SCMS) protocol was applied to experimental subjects for 10 days. The Morris water maze was used before, during, and after application of the SCMS protocol to measure spatial learning. The tail suspension test (TST) and forced-swimming test (FST) were used to evaluate behavioral despair. The MH rats displayed normal locomotor activity and spatial memory prior to SCMS, but impaired spatial learning after acute and chronic stress. In both the FST and TST we found that MH rats spent significantly more time immobile than did controls. Serum corticosterone level was found to increase after 30 min of restraint stress, and corticotropin-releasing factor immunoreactivity was found to be increased in the central nucleus of the amygdala. Our results suggest that MH in rats leads to the offspring being more vulnerable to stress in adulthood. © 2007 Wiley-Liss, Inc.

Key words: depression; spatial learning; corticotropin releasing factor; corticosterone

Hormones markedly affect neuronal structure and function in various ways throughout life. During pregnancy, maternal thyroid hormones [triiodothyronine (T3) and thyroxine (T4)] cross the placenta in rat (Obregon et al., 1984; Morreale de Escobar et al., 1988) and human (Vulsma et al., 1989) and are postulated to regulate fetal brain development. The relatively inactive T4 is converted to the more active form, T3, in brain tissue by the action of the enzyme deiodinase type II (Tanaka et al., 1981; Courtin et al., 1988). Thyroid hormone (TH) appears to regulate those processes associated with terminal brain differentiation such as dendritic and axonal growth, synaptogenesis, neural migration, and myelination (for a review, see Oppenheimer and Schwartz, 1997). TH also plays a significant role in the proliferation and survival of brain cells. A deficiency of TH alters the expression of several members of the Bel-2 family, down-regulating proapoptotic genes and up-regulating antiapoptotic ones (Singh et al., 2003), and increases pro–nerve growth factor and p75 neurotrophin receptor levels associated with enhanced apoptosis (Kumar et al., 2006) in developing rat cerebellum and cerebral cortex, respectively.

Most studies of prenatal thyroid disorders, both in humans and in rats, have centered on hypofunction because of the high incidence of hypothyroidism and the severe consequences it has for offspring. Nevertheless, maternal hyperthyroidism (MH) is a significant endocrinologic disorder in pregnancy. The prevalence of thyrotoxicosis, predominantly Graves’ disease, has been reported to be 0.05–0.2%, with an additional 3% of mothers exhibiting gestational transient thyrotoxicosis (Burrow, 1993; Glinoer, 1997; Polak et al., 2006). However, according to clinical reports, only 1% of children born to women with Graves’ disease are described as having hyperthyroidism, and overt neonatal hyperthyroidism is rare and concerns only 1 of 50,000 neonates (Polak, 1998). On the other hand, studies in an animal model of MH have shown that this condition...
compromises expression of neuronal and astrocytic cytoskeletal proteins in the late fetal brain, suggesting accelerated neuronal differentiation (Evans et al., 2002). This aberrant timing of the central nervous system (CNS) development might lead to subtle but irreversible changes in its synaptic wiring, which could exert crucial influences on neural plasticity in adulthood, although the individual might be seen as normal in an unaltered situation. Despite numerous studies on the molecular and neurochemical effects of hyperthyroidism in the prenatal period, little is known about the long-term consequences of such postulated early-developmental acceleration of the CNS in the adulthood of offspring.

Any physical or psychological stressor that threatens the homeostasis of an organism can initiate a set of behavioral and neuroendocrine responses intended to help the organism to adapt to the altered situation. In conducting this neuroendocrine response, the limbic-hypothalamic-pituitary-adrenal axis and the sympathoadrenal axis are two major pathways mediating the major components of the stress response (Gulpinar and Yegen, 2004). Recent opinions on stress emphasize that there are individual differences and several response patterns in coping with these challenges (McEwen and Sapolsky, 1995). The role of stress in induction, maintenance, and relapse of psychiatric dysfunction is well established, and there is good evidence that changes in glutamatergic and dopaminergic neurotransmission in the prefrontal cortex and hippocampus may be responsible for behavioral abnormalities seen in emotional disturbances (Moghaddam, 2002). A recent hypothesis on the pathophysiology of depressive disorders involves adaptive plasticity of the neural system. As proposed by Duman et al. (1999), depression could result from an inability to make the appropriate responses to stress, as a consequence of a dysfunction of the normal mechanisms underlying neural plasticity.

The goal of the present study was to investigate whether MH produces anatomocytotoxic relatively stable lifelong changes during development that could influence or alter the adulthood behaviors of offspring, especially their vulnerability to stress. We used the well-validated chronic mild stress protocol to produce mild and unpredictable daily stressful situations for the rats. This protocol is regarded as closely modeling the human situation, consisting more of daily hassles than traumatic events (for reviews, see Willner, 1997, 2005).

**MATERIALS AND METHODS**

**Animals**

Wistar rats from the animal house of our Faculty of Medicine were used in this study. All animal procedures were performed in accordance with the principles presented in the “Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research” by the National Research Council. Ten postnatal day 90 (P90) female rats were implanted subcutaneously with Alzet osmotic pumps (Model 2ML4, pumping rate 2.5 μL/hr for 28 days) infusing either thyroxine (to progenitors of MH subjects) or vehicle (to progenitors of control (Ctrl) subjects). The dose of thyroxine (T4; T2501, Sigma-Aldrich Inc.) was 1.5 μg/100 g of premat-

**Experimental Design and Stress Procedure**

Experimental procedures were performed when rats were in the young adult stage (starting at P90, body weight 350 ± 10 g) except for morphological analysis, in which brain samples were collected at P7, P30, and P75 and processed with the Golgi-Cox impregnation method (see detailed description below). A modified subchronic mild stress protocol (SCMS) lasting 10 days was used in this study (Table I). The regimen consisted of the application of a variety of unpredictable stressors, one per day, which included: 30 min of restraint stress (confinement into a cylinder 20 cm long and 7.5 cm in diameter), habitat changing with increased light and noise, one light cycle of sleep deprivation (four rats housed in cages 39 × 75 × 34 cm with a 3-cm water level and four islands made of cylinders 8 cm in height and 7.5 cm in diameter), 1 day of food and water deprivation, one 10-min period of electric foot-shock stressor (0.2 mA, 0.5-sec pulse, 2 per min over 10 min for a total of 20 shocks; Heinrichs and Koob, 2005). The behavioral and cognitive tests specified below also served as unpredictable stressors during the 10-day protocol. A summary of the experimental design is shown in Table I.

**Morris Water Maze**

The Morris water maze (MWM; Morris, 1984) assesses spatial learning and memory retention. A black circular pool (diameter 156 cm, height 80 cm) filled with 30 cm of water (25°C ± 1°C) with visual cues was used for this cognitive test. A circular black escape platform (diameter 12 cm) was submerged 1 cm below the water surface. Rats were allowed up to 120 sec to locate the escape platform. If the allowed time was finished and the experimental subjects had not found the platform, they were guided to the platform. Once on the platform, rats were permitted to stay for 5 sec to allow them
to observe place and location. To assess cognitive function in stress-naïve experimental subjects and any possible difference in response to this mild swimming stress, rats were not pre-trained for the first cognitive test (nonstress situation) on day 1 of the SCMS protocol. The second MWM test was performed on day 3 of the SCMS protocol, when the rats had undergone an immobilization stressor on day 2 and habitat changing stress with increased light and noise for 2 hr prior to the cognitive test during the endogenous activity period. This was defined as an acute stress situation. A third MWM test was performed on day 10 of the SCMS protocol and designated as a subchronic stress situation. The memory retention test of the MWM was performed on day 11, when the platform was removed, and the time spent on the platform quadrant was recorded. The starting position was in the quadrant opposite (IV) the platform quadrant (II) and was unchanged for the four tests.

Repetitive Forced-Swimming Stress

Animals from the MH and Ctrl groups were assessed for depression-like behavior using a modified version of the forced-swimming test (FST; Porsolt et al., 1978; Wellman et al., 2007), in which experimental subjects were exposed repeatedly to the swimming stressor during the dark period of the artificial light–dark cycle. The repeated FST allowed us to evaluate depression-like behavior after both acute and repetitive exposure to the test while acting as a stressor and helped to rule out the possibility of any motor impairment. The test consisted of exposure to a vertical Plexiglas cylinder 45 cm in height and 30 cm in diameter containing water to a height of 25 cm kept at 25°C ± 1°C. On P60, the rats underwent the first FST for 6 min to assess this behavioral parameter prior to the SCMS protocol. We designated this as the baseline test. On P96, day 1 of the test, rats received a single 15-min FST. On day 2 of the test, the rats received four consecutive 6-min exposures with a 12- to 15-min interval between trials. Passive immobility (cessation of spatial displacement with or without minor involuntary movements of the hind limbs) during min 2–6 was analyzed off-line. The observer scored the rats as either “swimming” or “immobile” every 5 sec (sampling frequency criterion described by Detke et al., 1995), and the percentage of immobility episodes was calculated as immobility episodes observed/total number of observations × 100. Immobility counts in the FST are regarded as a measure of behavioral despair.

Tail Suspension Test

In the tail suspension test (TST; Chermat et al., 1986), rats are suspended by the tail for 6 min during which they show periods of agitation and immobility. Duration of immobility is measured as an indicator of behavioral despair as an additional test to confirm the depression-like behavior displayed in the FST. The tail suspension apparatus consisted of a wooden cubicle with inside dimensions of 25 × 25 × 55 cm. A cylinder (5 cm in diameter, 25 cm in length) was fixed inside the cubicle (5 cm from the top) as a tail hanger. Rats were suspended from the cylinder using adhesive tape placed at about half the total tail length. Compared with the traditional design, in which a hook is used as a tail hanger, the TST apparatus used in this study avoided possible harm to the rats’ tails. The entire test was video-recorded, and total immobility time was analyzed off-line.

Corticosterone Measurement

Pre- and postrestraint stress (30 min) serum corticosterone (CORT) level during the rats’ late activity period was determined. Rats from the MH and Ctrl groups were anesthetized with an overdose of sodium pentobarbital and cardiac blood samples were collected and allowed to coagulate at room temperature for 3 hr. Samples were subsequently centrifuged at 2,000 g for 15 min. Serum was removed and stored at −20°C until analysis. Serum CORT concentration was determined by a commercially available ELISA kit according to the manufacturer’s instructions (Assay Designs Inc., Ann Arbor,

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*MWM at day 1 was not applied to these animals.
Histological Procedures for Corticotropin-Releasing Factor Immunoreaction

After the MWM memory retention test, rats were treated with an overdose of sodium pentobarbital and perfused transcardially with 0.9% saline followed by cold fixative containing 4% paraformaldehyde in 0.1M sodium phosphate buffer (PB; pH 7.4) plus 15% (v/v) saturated picric acid for 15 min. Brains were removed, blocked, and then thoroughly rinsed with PB. Coronal sections (50 μm) of amygdala and hypothalamus were obtained using a vibratome (Leica VT 1000, Heidelberg, Germany). Free-floating sections were quenched by incubating brain sections in 3% hydrogen peroxide in PB for 10 min. All the sections for immunoreaction were then incubated with PBST: PB + 0.9% NaCl + 0.3% Triton X-100 (T-7878, Sigma-Aldrich, Inc.) and 20% normal goat serum (NGS; 005-000-121, Jackson ImmunoResearch Laboratories, Inc.) for 1 hr at room temperature (RT). Rabbit antiserum anti-corticotropin-releasing factor (CRF; AP1760, Chemicon International Inc., CA) was used as primary antibody diluted 1:1,000 in PBST + 1% NGS and incubated overnight in a cold room. Afterwards, sections were rinsed for 10 min three times with PBST and then incubated for 4 hr at RT with biotinylated goat antirabbit secondary antibodies (1:200; Vector Labs, Burlingame, CA). Finally, sections were incubated in avidin-biotin-peroxidase complex (Elite ABC kit, Vector Labs) for 1 hr at RT. Peroxidase was detected using diaminobenzidine (DAB) as chromogen. Sections were developed using a Liquid DAB-Plus Substrate Kit (00-2020; Zymed Laboratories, San Francisco, CA). For indirect immunofluorescence Alexa Fluor 488 goat antirabbit IgG was used as a secondary antibody (A11008, Molecular Probes, Eugene, OR).

Image Analysis

The central nucleus of the amygdala (CeA) and paraventricular nuclei (PVN) were analyzed in regions spanning bregma −1.60 and −3.30 mm and −1.80 and −2.12 mm, respectively, according to the Paxinos and Watson brain atlas (Paxinos and Watson, 1998). Immunoreactive (IR) cell counting was performed using a Nikon Eclipse 50i microscope with a 40× objective lens. The IR-positive cells were projected and marked into a defined area (being circles 50 μm in diameter for CeA and 100 μm in diameter for PVN) through a drawing tube (Nikon Y-IDT) at ×8 magnification. Counting was performed manually, and data were then normalized.

Golgi-Cox Impregnation

For the Golgi-Cox impregnation procedures, on P7, P30, and P75 two rats from each group received an overdose of anesthesia and were decapitated. Brains were removed from the skulls quickly, and their central thirds (along the antero-posterior axis) were cut with a sharp blade into blocks approximately 5 mm thick. Tissues were briefly rinsed with 0.1M PB (pH 7.4) and then immersed in sequenced impregnation solutions (FD Rapid GolgiStain kit, FD Neuro Technologies, Ellicott City, MD) for 2 weeks in the dark. Sections (100 μm) were sliced using a vibratome and were dried naturally at RT in the dark and then stained with a solution provided with the stain kit. Dendritic patterns of dentate granule cells and CA3 pyramidal neurons were reconstructed using a drawing tube at ×400 magnification. Dendritic arborization areas in two dimensions were estimated using Adobe Photoshop (Adobe Systems Inc.) and Fovea Pro (an image tool kit from Reindeer Graphics, Asheville, NC).

Statistical Analyses

Quantitative results were expressed as mean ± standard error of mean (SEM). Groups were tested for differences by analysis of variance followed by the Student-Newman-Keuls test, using InStat (GraphPad Software, San Diego, CA). Differences were considered statistically significant at P < 0.05 (*P < 0.05, **P < 0.01, and ***P < 0.001, versus the control group).

RESULTS

Spontaneous Locomotor Activity, Body Weight, and T4 Serum Level Are Unaffected in MH Rats

MH rats displayed normal locomotor activity during the manipulations and different tests. Their body weight was similar to that of the Ctr rats and on P90 was 350 ± 10 g. The measured mean serum T4 concentration was 1.26 ± 0.14 ng/dL, which was not significantly different from that of the control (1.17 ± 0.06 ng/dL).

Altered Serum CORT Level in MH Rats after Acute Stress

To monitor the HPA axis response to an acute restraint stress, serum CORT levels were assessed by comparing MH animals with controls. First, we measured serum CORT level in the rats’ late-activity period before any stressor was applied. The mean serum CORT concentrations without restraint stress of the control and MH groups—3.303 ± 0.416 and 3.029 ± 0.617 μg/dL (n = 4), respectively—did not differ significantly. Rats were subjected to acute restraint stress (30 min) in their late activity period, when the intrinsic circadian amplitude of CORT was expected to reach its minimum (Fig. 1). The average CORT level of MH rats was 18.3 ± 5 g/dL, whereas that of the controls was 9.4 ± 3.0 μg/dL. This increase was consistent with previously reported levels in response to such a stressor (Viau and Sawchenko, 2002).

Increased Depressive-Like Behavior during SCMS Application

The speed of development of behavioral despair under the SCMS paradigm was assessed using two well-validated behavioral tests, the FST and the TST. Both tests are based on rats having an immobility response to an inescapable adverse situation. Duration of immobility, reflected as frequency of immobility episodes of total observations in the FST, is understood to be a measure...
of behavioral despair with a direct relationship to it. A month before application of the SCMS protocol, rats were subjected to the first FST for 6 min in order to have a baseline reference for immobility (Fig. 2A, baseline). The MH rats had fewer immobility episodes than did the rats from the Ctrl group. On day 7 of the SCMS protocol animals underwent 2 consecutive days of the forced-swimming stress test (FSST; Wellman et al., 2007). Rats from both experimental groups were observed to have a greater number of immobility episodes during this repeated forced-swimming test. The immobility count of the MH rats was much more pronounced (Fig. 2A, days 1 and 2). However, immobility counts of the MH and Ctrl rats did not differ significantly during the last exposure to the FST on the second day. This lack of difference may suggest that both the control and MH rats were approaching their physical effort limits. On the ninth day of the SCMS, the rats were exposed to the TST for 6 min (Fig. 2B). Again, the mean duration of immobility of the MH animals was longer (205.2 ± 13.79 sec) than that of the Ctrl rats (128.6 ± 37.10 sec).

Impaired Rat Spatial Learning and Memory Retention during SCMS Application

The effects of the SCMS on spatial learning and memory retention of the MH rats were tested using the hidden-platform water maze (Morris water maze, MWM). As shown in Figure 3A–C, the latency to escape to the platform of the Ctrl group decreased while advancing the trials. The slopes of the time sequences of the first three trials were relatively steep, approaching the shortest latency at the third trial, regardless of the duration of application of the SCMS protocol. In contrast, MH rats displayed progressively impaired spatial learning while continuing the SCMS protocol. Figure 3A shows the performance of naive MH rats in stress and swimming. The time sequence of the first four trials revealed a steep slope matching that of the control, suggesting normal spatial learning ability. However, the escape latency in the fifth and sixth trials peaked in the sequence and decreased again in the seventh and eighth trials. This may be related to an overreaction of the...
HPA axis to the novelty of swimming and handling generated in the first trial of MH rats, although this novelty did not seem to be a significant stressor for Ctrl rats. Figure 3B shows the performance of rats in an acute mild stress situation, which included habitat changing and increased noise and light 2 hr prior to the MWM. The slope of the MH rats was much smoother than that of the control rats, and clear differences between the MH and Ctrl groups were observed. After the 10 days of the SCMS protocol, the MH rats showed a MWM time sequence with a totally different form than that of the naive and acute stress MH groups, with several peaks and almost no reduction in latency at the end of the test (Fig. 3C). Latency reduction in the Ctrl group was delayed in comparison with that in the naive and acute stress Ctrl rats but reached a similar level after the third trial. In the quadrant preference test for memory retention (Fig. 3D), the MH rats showed no preference for the quadrant where the platform was. During the experimental periods, no rats showed any apparent sign of discomfort or locomotor disability.

Increased Number of CRF-IR Neurons in CeA

Immunocytochemical detection for CRF revealed an increased number of CRF-IR cells in the CeA of MH rats subjected to the SCMS scheme (Fig. 4A–C). Positive labeling for CRF was detected in neurons in the dorsal and ventral subdivisions of the medial parvo-cellular part of the caudal PVN. Also, a few cells were observed in the dorsal and lateral subdivisions of the magnocellular part (PaLM; Fig. 4D–F). These locations correspond with the typical areas of the PVN in which CRF neurons have been described (Swanson and Sawchenko, 1983). We found no significant differences in the number of CRF-IR cells in these hypothalamic regions (Fig. 4F), although the expression pattern in MH rats seemed to be more scattered toward lateral hypothalamic regions (Fig. 4E,F). It is worth noting that this staining was performed in tissue from animals without previous treatment with colchicine, which is known to exert an axonal transport blocking effect and helps the detection of CRF or any other peptide by accumulation of antigens in the cell body.
Increased Dendritic Receptive Field in Hippocampal Projection Neurons during Development and Adulthood

To elucidate the possible mechanisms underlying the behavioral changes, hippocampal CA3 pyramidal neurons and dentate gyrus (DG) granule cell dendritic arborization were analyzed through reconstruction of Golgi-Cox-impregnated neurons on postnatal days P7, P30, and P75. A general observation is that the dendritic receptive fields (2-D projection of 3-D arborization) were larger in the MH rats in all three stages, as shown by representative morphology of these neurons in Figure 5. Using Adobe Photoshop and FoveaPro, we estimated the 2-D dendritic receptive fields, finding that at P75 the value of the MH rats of 92.73 ± 3.56 µm² was significantly different from that of the Ctrl rats of 48.9 ± 2.66 µm² in CA3 pyramidal neuron apical receptive fields. Regarding DG granule cell dendritic receptive fields, no significant differences were found. However, thicker dendrite shafts were observed in samples from the MH rats. The inserts in Figure 5-P7 illustrate examples of primary and secondary dendrite ramifications.

DISCUSSION

It is well established that thyroid hormone (TH) action early in life plays a key role in determining the normal timing of neural development. An abundant number of studies focusing on excessive or insufficient TH during early development have revealed that this endocrine imbalance leads to abnormalities in brain structure. For instance, neonatal transient elevation of TH, from postnatal day 1 to postnatal day 4 in rats causes hypertrophic development of CA3 pyramidal neurons in the hippocampus and of astroglial cells in the hippocampus and basal forebrain (Gould et al., 1990a, 1990b). Our data describing an increase in the dendrite receptive fields in hippocampal CA3 pyramidal neurons at three postnatal ages, P7, P30, and P75, are analogous to those...
seen in the rodent neonatal hyperthyroidism model and extend existing observations.

The present data are the first to describe the emotional and cognitive consequences of mild maternal hyperthyroidism in adult rat offspring under subchronic mild stress. Earlier studies concerning neonatal hyperthyroidism have suggested that thyroxin accelerates the maturation of the pituitary-adrenal response to electric shock (Schapiro and Norman, 1967) and the onset of physiological and behavioral bioenvironmental interaction in general (Schapiro, 1968). It has also been reported that neonatal thyroxin stimulation of albino rats disrupts hippocampal LTP, and despite hypertrophied hippocampal CA3 pyramidal neurons and elevated density of cholinergic neurons in the basal forebrain, they are actually less efficient in learning a spatial maze (Pavlides et al., 1991). The present finding of cognitive impairment in the MH rats, shown by their greater number of mistakes and increased latency in the Morris water maze, is compatible with previous observations in neonatal hyperthyroid rats. Moreover, our data make an important addition to previous observations in several areas. The relatively mild degree of maternal hyperthyroidism used in this experimental design apparently did not produce alterations in the adulthood of the offspring under normal conditions, as we reported in the results. However, when the subchronic mild stress scheme was applied, their spatial learning capacity was progressively impaired along the protocol. Furthermore, the display of depression-like behaviors by the MH adult offspring was accelerated, as results of both the forced-swimming stress test (FSST) and the tail suspension test (TST) showed. It is worth mentioning that depression-like behaviors have multiple dimensions and that analysis of such behaviors in an animal model is always difficult to sort out. Normally, a battery of tests is recommended to assess such behaviors. The forced-swimming test (FST) is probably the most widely and frequently used protocol. In the present experimental design, we used a modified version of the Porsolt test (Porsolt et al., 1978), mainly because repeated exposure to the test in a short time is a stressor for rats. Hence, the depression-like behavior could be more quickly observed. On the other hand, the FSST demands a major physical effort by the animal under study. A motor-impaired rat could be easily distinguished during this repeated swim test. Both the FST and TST have immobility as a parameter to be measured. However, the actual parameter being measured is the response of the animal to the specific condition of each test, that is, the adoption of floating behavior in the FST or of stationary behavior in the TST. It has been suggested that the observed behaviors might be produced by different biological substrates, and different hypotheses have been proposed to explain the observed immobility in FST and TST (Cryan et al., 2005). To summarize these observations, our data suggest that maternal hyperthyroidism, however moderate or without apparent alteration in the adult offspring under normal conditions, could bring about severe consequences because of the enhanced stress response.

A stressful event, either physical or psychological, activates neural circuits involved in emotional responses, cognition, and homeostatic regulation. The hippocampal formation and the amygdala form the central axis of the limbic system and are highly responsible for cognitive and emotional responses to a stressful situation. The hippocampal formation receives major input from the entorhinal cortex through the perforant pathway, where highly processed sensory information from associational, perirhinal, and parahippocampal cortices as well as the prefrontal cortex is transmitted. The proximal segments of the apical dendrites of CA3 pyramidal neurons are covered with complex spines or excrescences that receive mossy fiber input from granule neurons of the dentate gyrus (Blackstad and Kjaerheim, 1961). The dentate gyrus–CA3 pathway provides the major excitatory afferent to the hippocampal regio inferior, and each CA3 neuron excites multiple pyramidal neurons (Ishizuka et al., 1990). Our finding of augmentation of CA3 pyramidal neuron dendrite receptive fields most likely indicates that these excitatory pathways to the hippocampus could be strengthened at the beginning of a stressful situation, which amplifies sensory inputs from the entorhinal cortex. Therefore, the stressors could have a more profound effect on hippocampal function. Ultrastructural studies showed that chronic stress alters the synaptic terminal structure in the hippocampus, especially the mossy fiber synapses boutons en passant from granule neurons to the proximal segments of apical dendrites of CA3 pyramidal neurons (Magariños et al., 1997), indicating a possible reorganization driven by hyperactivation of this pathway. Chronic exposure to higher levels of corticosterone (CORT) can cause irreversible hippocampal dysfunction and increase depression-like behavior in rats in a dose-dependent manner and disrupt normal HPA axis function (Johnson et al., 2006). Moreover, clinical evidence has shown that medically healthy patients with recurrent major depression can show decreased hippocampal volume and impaired cognition (Sheline et al., 1999). Using the subchronic mild stress paradigm, we observed faster development of depressive-like behaviors as well as impairment of cognitive functioning in MH rats under mildly stressful conditions.

The neurohormone CRF activates both hormonal and behavioral responses to a variety of stressors. Stress leads to CRF release from the hypothalamic PVN, resulting in increased plasma corticotrophin (ACTH) and adrenal steroid concentration. CRF-containing cells constitute a significant neuronal population in the central nucleus of the amygdala (CeA). The CeA has been shown to be a key regulator of the stress response mediated by CRF release from the PVN (for a review, see Gray and Bingman 1996). Discrete lesions of the CeA exacerbate the acute stress response (Carter et al., 2004). Our observation of an increased population of CRF-IR cells in the CeA but not in the PVN after being sub-
jected to chronic mild stress might indicate a stronger attempt to limit the stress overreaction in MH animals.

The observations from this study raise the question of the underlying mechanism by which mild maternal hyperthyroidism produces enhanced vulnerability to stress in adulthood. According to the current hypothesis of neural plasticity in some areas of the neural system, once neuronal connections are established during this critical period of brain development, the networks tend to be relatively stable (“rigid” synaptic connections), whereas other types of synaptic connections (“flexible” synaptic connections) undergo lifelong self-optimization processes, so-called use-dependent neural plasticity. The cognitive, behavioral, and emotional reactivity of an individual derived from this kind of connection is stepwise remodeled to meet environmental demands. Although the presence of rigid synaptic connections ensures stability of the principal characteristics of function, variable configuration of flexible synaptic connections determines the unique, nonrepeatable character of an experienced mental act (Arendt, 2001; Gulpinar and Yegen, 2004). There are many possible causes of aberrant synaptic connections. One possible cause is hyper- or hypothyroid neuronal dendritic arborization. In addition, neuronal migration aberrations can, in fact, alter physiological synaptic connections, resulting in loss of neural plasticity and consequently of adaptive capacity. Ausó et al. (2004) recently reported that a moderate and transient deficiency in maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. The opposite could also alter neuronal migration, although experimental data are required to confirm this hypothesis.

In conclusion, the results from this animal model analyzing the consequences of maternal hyperthyroidism in the adulthood of offspring suggest that this delicate prenatal endocrine modification could alter the neuronal structure of specific brain areas and influence the cognition and emotionality of offspring in adulthood. These modifications were demonstrated, letting the experimental subjects undergo subchronic mild stressful situations. Sufficient to say, the morphological and immunocytochemical changes we have reported represent only a part of the numerous and complex anatomical and physiological abnormalities in adulthood as a result of maternal hyperthyroidism. However, this kind of animal models allow us to gain insight into normal development and to evaluate integrative aspects of the long-term influences of neuroendocrine disorders during development.

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