Developmental Hypothyroidism Increases the Expression of Kainate Receptors in the Hippocampus and the Sensitivity to Kainic Acid-Induced Seizures in the Rat

Elena Giné, Jose Angel Morales-Garcia, Ana Perez-Castillo, and Angel Santos

Thyroid hormones are essential for normal brain development, and multiple alterations at behavioral, cognitive, cellular, and molecular levels have been described in animals made hypothyroid during development. Here we analyzed the effect of developmental hypothyroidism in the rat on the sensitivity to kainic acid-induced limbic seizures and the expression of kainate receptors in the hippocampus. Our results show that hypothyroid rats are extremely sensitive to the proconvulsant and neurotoxic effects of kainic acid (KA). Hypothyroid rats entered in status epilepticus at a dose of KA three times lower than that required to reach status epilepticus in control animals. In accordance with this, high levels of glial activation and neuronal loss after low KA dose injections were observed only in the hippocampus of hypothyroid rats. These effects correlated with an increased expression of kainate receptor subunits, excluding GluR5, in the hippocampus of hypothyroid animals. The concentrations of GluR6, GluR7, KAR1, and KAR2 (ionotropic glutamate receptor subunits of the kainic acid subtype) mRNAs were increased between 50 and 250% in hypothyroid animals relative to the values in controls. In agreement with these results, Western blot and immunohistochemical analysis showed a clear increase in the hippocampal content of GluR6/7 proteins in hypothyroid animals. (Endocrinology 151: 3267–3276, 2010)
quences of hypothyroidism are more dramatic and results in numerous alterations, such as reduction in dendritic arborization of cerebellar Purkinje cells; impairment of nerve process development; poor connectivity among neurons; changes in microtubule content; and impaired myelin deposition, cell migration, and synaptogenesis (5).

Many of these alterations become permanent unless an appropriate replacement therapy with thyroid hormones is started very soon after birth. Not all the areas in the brain are equally sensitive to the action of these hormones, and this sensitivity depends on the developmental stage of the animals. Therefore, marked differences, in the response to thyroid hormones, have been observed in different areas and at different developmental ages (8–10).

The hippocampus is indeed an area sensitive to the action of thyroid hormones, both during development and in adults, as shown by numerous clinical and experimental data. In humans, when hypothyroidism occurs during the developmental period, irreversible and severe cognitive deficits and morphological alterations in the hippocampus are observed (11, 12). These effects are less severe when hypothyroidism arises in the adult life, during which minor and reversible cognitive deficits and mood disorders are observed (13). Related with these symptoms, a decrease in glucose metabolism in diverse brain areas, including the hippocampus, has been described in adult hypothyroid patients (14). All these symptoms are reversed by an appropriate thyroid hormone therapy. In experimental animals, developmental hypothyroidism has been associated with morphological, electrophysiological, and biochemical alterations in the hippocampus. A reduction in the number and maturation of both glial and neuronal populations have been described in specific layers of the hippocampus of hypothyroid rats (4, 15, 16) together with marked alterations of synaptic transmission and plasticity and gene expression (17–20). Many of these changes become permanent unless an appropriate therapy is applied. In adult-onset hypothyroidism, diverse neurological alterations also have been described in the rat hippocampus. Memory deficits in hypothyroid animals have been associated with long-term potentiation impairment in CA1 (21) and decreased hippocampal neurogenesis could contribute to depressive-like behavior (22).

A possible role of thyroid hormones in rendering the brain more susceptible to alterations similar to epilepsies derives from clinical cases of dysthyroidism associated with seizures (23–28). Experimental evidence also indicates that dysthyroidism lowers the seizure threshold (29, 30). Additionally, genetically epilepsy-prone rats are hypothyroid (31), and mice prone to audiogenic seizures have postnatal levels of thyroid hormone higher than control mice; treatment with antithyroid drugs improves this condition and T3 worsens it (32).

The neurotoxin kainic acid (KA) is a potent agonist of the kainate receptors, although also can act through other ionotropic glutamate receptors. Systemic or brain administration of this toxin produces convulsion and can render animals susceptible to unprovoked recurrent seizures, resembling some of the features of human temporal lobe epilepsy such as hippocampal sclerosis (33). Besides KA, other pharmacological agents, frequently used to induce limbic seizures and extensive hippocampal damage, include the cholinergic agonist pilocarpine, probably the most frequently used model of temporal lobe epilepsy, and the secretion of γ-aminobutyric acid (GABAergic) antagonist pentylenetetrazole (PTZ) (34).

In this work, we analyzed, in adult rats, the effect of developmentally induced hypothyroidism on hippocampal sensitivity to this neurotoxin and its possible causes. Our results show a higher sensitivity of hypothyroid animals to KA-induced limbic seizures and neurological damage together with a parallel increase in the amount of ionotropic glutamate receptor of the kainate type in the hippocampus. These effects were not reversed by thyroid hormone treatment because only minor improvements were observed in hypothyroid rats treated with physiological doses of thyroid hormones.

## Materials and Methods

### Materials

Methyl mercaptoimidazole, T4, T3, KA, pilocarpine, scopolamine, lithium chloride, and pentylenetetrazole were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent grade or molecular biology grade.

### Animal treatment

Female Wistar rats were mated and the day of appearance of the vaginal plug was considered as d 0 of fetal age. To induce fetal and neonatal hypothyroidism, dams were given 0.02% methyl mercaptoimidazole in the drinking water at d 12 of gestation. This protocol ensures that the animals are hypothyroid, as shown by the decrease growth rate and circulating levels of T3 (35). Methyl mercaptoimidazole treatment was continued throughout the whole experimental period, and all studies were conducted in adult-age rats. To determine the reversibility of the effects caused by hypothyroidism, hypothyroid rats were daily treated with a physiological combination of T4 (0.9 μg per 100 g body weight (BW)) and T3 (0.2 μg per 100 g BW) during 1 wk. The corresponding hypothyroid controls received an equivalent volume of physiological saline.

All animal related procedures were approved by the Laboratory Animal Care and Use Committee of the Universidad Complutense de Madrid and were conducted in accordance with the guidelines of the European Communities Council, Directive 86/609/EEC. All efforts were made to minimize animal suffering and reduce the number of animals used.
Seizure induction

KA was sc injected at the indicated doses and the animals were placed in individual plastic cages and monitored for 3 h. Video recordings were made with a black-and-white video camera. The convulsive behavior was classified according to the accumulative scale of Racine (36) and Sperk et al. (33), as follows: stage 0, no changes; stage 0.5, wet dog shakes; stage 1, mouth and facial movements; stage 2, head nodding; stage 3, forelimbs clonus; stage 4, rearing; stage 5, rearing and falling; stage 6, death. Status epilepticus (SE) was defined as continuous or intermittent behavioral seizure activity without recovery of complete consciousness (stages 4–5) for at least 30 min. Behavioral seizures were scored by their latency (minutes) to onset of SE after KA injection.

Seizure induction by lithium-pilocarpine was as follows: the day before the experiment the animals were ip injected with 128 mg/kg LiCl; 30 min before the first pilocarpine injection, the animals were ip injected with scopolamine (1 mg/kg), and pilocarpine (10 mg/kg) was injected every 30 min (up to four injections maximum) until the animal reached SE (37). SE was stopped after 1 h with ip injection of diazepam (4 mg/kg). Finally, the proconvulsant drug pentylenetetrazole was ip injected at a subthreshold dose (35 mg/kg) and the convulsive behavior of animals monitored as indicated.

Real-time PCR

Total RNA from the hippocampus was purified according to the method of Chomczynski and Sacchi (38), and samples (2 μg) were used for the synthesis of cDNA with the RETRoscript kit (Ambion, Austin, TX) using pd(N)6 random hexamer as primers. Real-time PCR was performed in ABI Prism equipment using the SYBR Green PCR master mix (Applied Biosystems, Warrington, UK) and 300-nm concentrations of specific primers. In all samples, each specific sequence was measured at least twice in triplicate. The primers used for the determination of the concentration of the different transcripts were as follows: GluR5: TCA AAA TCC GCC AGC TTC C and TGA GCA GAG TGT TGG CGT CT; GluR6: CAG TCC ACG TTC AGC ACG GTT CGT CT and TTC CAG CGG GTG TCT ATG TG; GluR7: CCG CAA GTC TGA TAG GAC CC and CAG TAG CCC TCC AAC CGG FT; KAR1 (ionotropic glutamate receptor subunits of the kainic acid subtype): CAG CCC AGT GTG TTT GTG A and AAC ACC CTG GCA ATT CCC TC; and KAR2: TCT TGG GCT TTT CCA GTG TCA and CAA ACT CCG AGT AGG GAT. All of them synthesized DNA fragments of 51 bp. Amplification of the 18S rRNA was used for normalization of cDNA loading in the PCR as previously described (39).

In control hippocampus, random primer cDNA (dilution 1:10) gave cycle threshold values of around 26, 21, 22, 23, and 24 for GluR5, GluR6, GluR7, KAR1, and KAR2 transcripts, respectively. In the case of 18S rRNA, a dilution of 1:1000 gave cycle threshold values between 20 and 21.

Western blot analysis

Hippocampal tissues were homogenized in radioimmunoprecipitation assay buffer and equal quantities of total protein separated by 10% SDS-PAGE and transferred to nitrocellulose membranes (Protran; Whatman, Dassel, Germany). Blots were probed with anti-GluR6/7 polyclonal antibody (Millipore, Bedford, MA) and monoclonal anti-α-tubulin (Sigma). Immunoreactive bands were visualized using the ECL detection kit (Amersham Biosciences, Inc., Buckinghamshire, UK) according to the manufacturer’s instructions and quantified by densitometry using ImagePro Plus software (Media Cybernetics, Carlsbad, CA).

Immunohistochemistry

The animals were anesthetized and perfused transcardially with 4% paraformaldehyde solution. The brains were removed, postfixed in the same solution at 4°C overnight, cryoprotected in the paraformaldehyde solution containing 30% sucrose, and kept at –70°C until used. Coronal sections (30 μm) were obtained in a cryostat and processed for cresyl violet (Nissl stain) or immunohistochemistry using the diaminobenzidine method. For the diaminobenzidine method, the sections were immersed for 15 min in 3% H₂O₂ to inactivate endogenous peroxidase and then blocked for 2 h at room temperature in 5% normal goat serum (Vector Laboratories, Burlingame, CA) in PBS containing 4% BSA, 0.1 ml lysine, and 0.1% Triton X-100. Afterward the sections were incubated overnight with the corresponding primary antibodies. After several rinses, sections were incubated for 1 h with a biotinylated secondary antibody. Finally, the sections were processed after the avidin-biotin protocol (Vectastain ABC kit; Vector Laboratories). Tissues were mounted onto gelatin-coated slides and were let to dry. Finally, the slides were dehydrated, cleared in xylene and mounted with DePeX (Serva, Heidelberg, Germany). The slides were examined with a Zeiss (Oberkochen, Germany) Axioshot microscope, equipped with an Olympus Optical (Tokyo, Japan) DP-50 digital camera and a Leica (Nussloch, Germany) MZ6 modular stereomicroscope. Neuronal integrity was assessed by counting the number of Nissl-positive cells in the CA3 region of the hippocampus in four independent well defined high-magnification (×400) fields per section and in five sections per animal using computer-assisted image analysis software (Soft Imaging System, Münster, Germany).

Astrogliosis was evaluated by quantifying the number of activated cells [high glial fibrillary acidic protein (GFAP) immunostaining] as described above and the intensity of GFAP staining in at least 100 cell bodies per animal. For this, randomly chosen cells were manually traced and their mean staining intensity was determined using computer-assisted image analysis software (Soft Imaging System).

The extent of microgliosis was quantified by counting the number of OX-42-positive cells in four independent well-defined high-magnification (×400) fields per section and in four sections per animal as described above.

The following primary antibodies were used: monoclonal anti-CD11b (OX-42) and anti-GFAP antibodies (Serotec, Düsseldorf, Germany) and Sigma, respectively, and polyclonal anti-GluR6/7 antibody. Before immunostaining with anti GluR6/7, tissue sections were boiled in 10 mM citrate buffer according to the manufacturer’s instructions.

Statistical analysis

Data were analyzed by ANOVA, followed by Newman-Keuls test as post hoc or Student t test. The threshold of statistical significance was set at P < 0.05.

Results

Behavioral effects of KA in hypothyroid rats

We first analyzed in the three groups of rats (control, hypothyroid, and hypothyroid treated with T4/T3) the be-
behavioral response to KA administration. As shown in Fig. 1A, the hypothyroid animals showed an increased sensitivity to KA-induced limbic seizures, and almost all the animals entered in SE at a dose as low as 3.75 mg/kg BW. On the contrary, at that dose none of the control animals entered in SE, and only some animals showed early symptoms of KA action such as wet dog shakes. A time-course response to a dose of 3.75 mg/kg of KA is shown in Fig. 1B, in which the difference between control and hypothyroid animals is clearly observed. To test whether this higher sensibility of hypothyroid animals to KA could be reversed by the administration of thyroid hormones, hypothyroid rats were treated with T4/T3 during 1 wk as indicated in Materials and Methods. This treatment has been previously shown to revert the effects of hypothyroidism on different biochemical and behavioral parameters in these hypothyroid adult rats (39). As can be observed in Fig. 1, this treatment had no effect on the sensitivity to KA, as determined by the dose-response curve (Fig. 1A). However, a longer latency to reach SE was observed in the T4/T3-treated hypothyroid rats (76 ± 7.5 min in hypothyroid and 97 ± 7.0 min in treated animals, P < 0.01), indicating a higher resistance to entrance in SE in these animals.

Effect of hypothyroidism on KA-induced neurological damage in the hippocampus

The KA-induced neurodegeneration was analyzed 72 h after injection of the excitotoxin at a dose of 3.75 mg/kg. Our results clearly show a dramatic reduction in the number of neurons, particularly in the CA3 subfield of the hippocampus, in the hypothyroid animals (Fig. 2). In hypothyroid animals an 80% reduction in the number of neurons was observed in CA3 (Fig. 2B). In contrast, at this concentration, KA did not cause any neuronal damage in the control animals. In hypothyroid animals treated with T4/T3, the loss of neurons after KA injection was slightly lower (69%) than the one observed in the hypothyroid group. These results are essentially in accordance with the behavioral results described in Fig. 1. In rats not injected with KA, the same number of neurons were observed in the CA3 field among the three groups of animals analyzed (data not shown), indicating that there are no differences in the number of CA3 hippocampal neurons due to the thyroidal state of the animal, which is in agreement with previously reported data (16).

Effect of hypothyroidism on KA-induced glial activation in the hippocampus

Glia activation was analyzed 72 h after KA (3.75 mg/kg) injection by determining the number and intensity of GFAP-positive cells and the number of cells stained with anti-CD11b (OX-42). As can be seen in Fig. 3A, a
high number of cells became intensively stained with GFAP antibody as a consequence of the injection of KA in the hypothyroid animals. This strong astrogliosis was absent in the control group. Previous treatment of the hypothyroid animals with T4/T3 significantly reduced the number of GFAP-positive cells after KA injection (Fig. 3A). In addition to the decrease in the number of GFAP-positive cells, also a decrease in the intensity of staining of individual astrocytes was observed after T4/T3 treatment. In the treated group, the intensity of GFAP staining was 65% of the average value observed in untreated hypothyroid animals. Similar results were observed for OX-42 staining (Fig. 3B). In control animals no OX-42-positive cells were observed after the injection of KA. In contrast, hypothyroid rats showed a high number of OX-42-positive cells. This dramatic increase in the number of activated microglial cells was clearly reduced when hypothyroid animals were previously treated with T4/T3. The number of OX-42-positive cells in the treated groups was 45% of the number found in the untreated group.

**Effect of hypothyroidism on the expression of kainate receptor subunits**

Because the action of KA is mainly mediated through its binding to ionotropic glutamate receptors of the kainate
subtype, we analyzed the possible effect of congenital hypothyroidism on the expression of genes coding for subunits of these receptors. As shown in Fig. 4A, with the exception of GluR5, the amount of all the transcripts of kainate receptor subunits were increased in the hippocampus of hypothyroid animals. This increase was more patent for subunits GluR7 and KAR2 (Fig. 4A). The treatment with T4/T3 did not normalize transcript concentration, suggesting that the effect of hypothyroidism cannot be reversed by this late treatment with thyroid hormones. Only a partial decrease in GluR7 transcript was observed after treatment. In line with the increase in kainate receptor transcripts, an increase in the amount of GluR6/7 protein was observed by Western blot and immunohistochemistry in the hippocampus of hypothyroid animals (Fig. 4, B and C). After quantification of the Western blot data (Fig. 4C), a 5-fold increase in the amount of GluR6/7 protein was observed, which was partially reverted by thyroid hormone treatment. No major differences in the localization of these proteins were observed among the three groups of animals.

**Effect of hypothyroidism on behavioral effects and neurological damage induced by pilocarpine and PTZ**

Finally, we analyzed the effect of lithium-pilocarpine and PTZ on seizure induction and neuronal damage in control and hypothyroid rats. Our results show that hypothyroid animals are less sensitive to the proconvulstent effect of pilocarpine than the control ones. As shown in Fig. 5A, 40% of the control rats entered in SE after two injections of pilocarpine; in contrast, none of the hypothyroid rats entered in SE at that dose. When the latency time (the time from the first injection of pilocarpine to SE) was calculated, a clear difference was observed between both groups. Control rats needed 79 ± 4.5 min and a mean of 2.7 injections of pilocarpine to reach SE, whereas hypothyroid animals required 110 ± 7.5 min and an average of 3.7 injections (P < 0.001). In the case of PTZ, the hypothyroid rats were more sensitive to the action of this drug. As shown in Fig. 5B, all the hypothyroid rats reached convulsive stage 4–5 after a single injection of a subthreshold dose of PTZ (35 mg/kg); in contrast, none of the control animals showed convulsive behavior. Regarding neuronal loss in the hippocampus, lithium-pilocarpine-induced SE caused extensive damage of pyramidal neurons in both control and hypothyroid animals (Fig. 5C). On the other hand, a single subthreshold dose of PTZ caused some disorganization in pyramidal cells layers only in the hypothyroid animals, and no effect was observed in controls (Fig. 5C). This is in agreement with the fact that only hypothyroid animals showed convulsive behavior after a single subthreshold dose of PTZ and that this effect was brief and transitory and therefore insufficient to cause major neuronal damage.

**Discussion**

In this work, we have shown that hypothyroid rats are more sensitive to the KA-induced limbic seizures. This was accompanied by a higher expression, in the hippocampus, of genes coding for kainate receptor subunits. Both effects
are basically irreversible because the administration of physiological amounts of thyroid hormones to these animals has only a modest effect on the sensitivity to KA and the expression of kainate receptor subunits. These results suggest that an increased expression of kainate receptors in the hippocampus could be the cause, or at least play an important role, of the increased sensitivity to KA observed in developmental hypothyroid rats.

Multiple pharmacological, biochemical, and electrophysiological data implicate kainate receptors, particularly those present in the mossy fibers terminal, in the induction of limbic seizures. KA facilitates excitatory synaptic transmission at the mossy fibers (40), and this facilitation is GluR6 dependent, being absent in GluR6−/− mice (41). Forced overexpression of the fully edited GluR6 subunit in the hippocampus of the rat induces limbic seizures (42). Conversely, the lesion of mossy fibers in rats prevents the development of electrographic SE in response to KA administration (43). In addition, other kainate receptor subunits could also play an important role in the epileptogenic action of KA (34).

Our results, indicating that an increase in the expression of kainate receptors in the hippocampus could decrease the threshold of seizure induction, are in agreement with results previously reported by other authors. It has been broadly described that antipsychotic medications can reduce seizure threshold in some patients (44). Interestingly, Meador-Woodruff et al. (45) have shown that treatment of rats with clozapine, the second-generation antipsychotic drug most frequently associated with seizures in humans, notably increases the expression of GluR6 in all areas of the hippocampus. Also, Rangel et al. (46) have shown that mice lacking the cellular prion protein expressed higher levels of GluR6 and GluR7 in the hippocampus and showed enhanced susceptibility to KA-induced damage.

The induction of limbic seizures is a complex process, and, besides ionotropic GluRs, numerous genes have been implicated in their development. Marked differences in KA sensitivity have been described among different mouse strains (47), and multiple genes have been suggested to play a facilitatory or inhibitory effect on KA-induced limbic seizures (48, 49). One of these genes is neuropilin 2, the semaphorin 3 receptor. The null mutant mice for this gene have a reduced hippocampal population of GABAergic interneurons and are more prone to limbic seizure induction (50). In this regard, it is interesting to note that the hippocampus of rats deprived of thyroid hormones from gestational day 6 until postnatal day 30 have a decreased density of the parvalbumin subpopulation of GABAergic interneurons and that this deficit persisted in adulthood (4). Thus, it is likely that both effects, a decreased population of parvalbumin neurons and an increased expression of kainate receptors, could contribute to the increased sensitivity to KA observed in hypothyroid rats.

Relative to the possible effect of thyroid hormones on the expression of kainate receptors, previous studies have shown that an excess of thyroid hormones during the neonatal period induced an aberrant growth of mossy fiber projections, in both the CA3 subfield and dentate gyrus of the hippocampus, with a parallel increase in the number of high-affinity binding sites for KA (51). Conversely, neonatal hypothyroidism reduced 43% high-affinity binding of KA in the stratum lucidum, specifically in the ventral hippocampus (52). These results are clearly in contrast with ours because we observed an increase in the protein levels of GluR6/7 in the hippocampus of hypothroid animals, including the stratum lucidum. The reasons for
this discrepancy are unclear, but it has to be pointed out that in the study by Savage et al. (52), the rats were made hypothyroid from gestational d 18 until postnatal d 31 and then allow to recover until adulthood, whereas our animals were permanently hypothyroid since gestational d 12.

Our results show that hypothyroidism induces the expression of all kainate receptor subunits with the exception of GluR5. This is certainly an interesting observation, and further detailed studies to assess the molecular mechanisms by which thyroid hormones specifically regulate the expression of kainate receptor subunits are warranted. This generalized induction of kainate receptors in the hippocampus of hypothyroid rats is quite specific for this subfamily of ionotropic GluRs because it was not observed when N-methyl-D-aspartate and 2-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid receptor subunits were analyzed, and only the expression of a few subunits was altered (data not shown). Regarding these results, it is important to mention that GluR5, the only subunit not affected by hypothyroidism, has a distinct cellular expression in the hippocampus because it is expressed only in GABAergic neurons, in contrast with the other kainate receptor subunits, which are mainly expressed in glutamatergic neurons (53).

The cholinergic agonist, pilocarpine, and the GABAergic antagonist, pentylentetrazole, are drugs frequently used to induce limbic seizures. Here we show that congenital hypothyroid rats are more sensitive to PTZ, and consequently, subthreshold doses of this drug, which have no effect on control animals, are able to induce seizure activity in hypothyroid rats. In contrast, hypothyroid animals are less sensitive to pilocarpine administration, as shown by an increase in latency. This lower sensitivity to pilocarpine in hypothyroid animals could be the consequence of the well-established depressing effect of developmental hypothyroidism on cholinergic activity (54, 55).

Interestingly, Grigorenko et al. (56) suggested an implication of GluR6 in human epilepsy. An increase in the expression of GluR6 and the percentage of the edited form of this protein have been described in the hippocampus of patients with refractory epilepsy (57). Also, it has been shown that GluR6 mRNA is increased in the temporal cortex of patients with hemimeganencephaly, which is characterized by unilateral hemispheric enlargement, severe cytoarchitectural abnormalities, and intractable epilepsy (58). Moreover, in children with the rare Sturge-Weber neurological syndrome, which courses with seizure activity, a high incidence of central hypothyroidism and a positive response to T₄ therapy have been described (59).

In summary, our results suggest that hypothyroidism, through the regulation of kainate receptors, could favor KA-induced limbic seizures and neurological damage.

Acknowledgments

Address all correspondence and requests for reprints to: A. Santos, Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad Complutense, 28040 Madrid, Spain. E-mail: piedras3@med.ucm.es; or A. Perez-Castillo, Instituto de Investigaciones Biomédicas, Consejo Superior de Investigaciones Científicas, Universidad Autónoma, Arturo Duperier 4, 28029 Madrid, Spain. E-mail: aperez@ibb.uman.es.

This work was supported by Ministerio de Educacion y Ciencias Grants SAF2004-06263-CO2-02 (to A.S.), SAF2004-06263-CO2-01, and SAF2007-62811 and Comunidad de Madrid Grant GR/SAL/0033/2004 (to A.P.-C.). Centro de Investigación Biomédica en Red Sobre Enfermedades Neurológicas is funded by the Instituto de Salud Carlos III.

Disclosure Summary: The authors have nothing to disclose.

References


40. Gilbert ME, Sui L 2006 Dose-dependent reductions in spatial learning and synaptic function in the dentate gyrus of adult rats following developmental thyroid hormone insufficiency. Brain Res 1069:10–22


55. Gould E, Butcher LL 1989 Developing cholinergic basal forebrain neurons are sensitive to thyroid hormone. J Neurosci 9:3347–3358
57. Grigorenko EV, Bell WL, Glazier S, Pons T, Deadwyler S 1998 Editing status at the Q/R site of the GluR2 and GluR6 glutamate receptor subunits in the surgically excised hippocampus of patients with refractory epilepsy. Neuroreport 9:2219–2224