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# Efeito dos modelos experimentais de hipertireoidismo e hipotireoidismo sobre aspectos bioquímicos e comportamentais em ratos adultos

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*Para a minha melhor e eterna amiga: minha Mãe Marlene*

"...You gave me wings and made me fly
You touched my hand I could touch the sky
I lost my faith you gave it back to me
You said no star was out of reach
You stood by me and I stood tall
I had your love I had it all
I'm grateful for each day you gave me
Maybe I don't know that much
But I know this much is true
I was blessed because I was loved by you..."

"...Você me deu asas e me fez voar
Você tocou minha mão e eu pude tocar o céu
Eu perdi minha fé, você devolveu-a para mim
Você disse que estrela nenhuma estava fora de alcance
Você me apoiou e eu fiquei de pé
Eu tive seu amor, eu tive isso tudo
Sou grata por cada dia que você me deu
Talvez eu não saiba muito
Mas eu sei que isto é verdade
Eu fui abençoada porque fui amada por você..."

Celine Dion

*Para o amor da minha vida: meu marido Fer*

"...My love
Whenever I was insecure
You built me up and made me sure
You gave my pride back to me
Precious friend
With you I'll always have a friend
You're someone who I can depend
To walk a path that never ends
Without you
My life has no meaning or rhyme
Like notes to a song out of time
How can I repay
You for having faith in me
God bless you..."

"...Meu amor
Quando eu estava insegura
Você me ergueu e me deu certeza
Você devolveu o meu orgulho devolta
Amigo precioso
Com você eu sempre terei um amigo
Você é alguém de quem eu posso depender
Para andar em um caminho que nunca termina
Sem você
Minha vida não tem sentido ou rima
Como notas para uma música fora do tempo
Como eu posso retribuir
Você por ter fé em mim
Deus abençoe você..." The Stylistics

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A Deus, Criador do Universo.

# Sumário

# PARTE I

**Resumo**

Os hormônios da tireóide, incluindo a triiodotironina (T3) e a tetraiodotironina (T4), são reconhecidos como hormônios metabólicos fundamentais do corpo. Os hormônios da tireóide são essenciais para a maturação e função do sistema nervoso central (SNC) de mamíferos e sua deficiência, durante um período crítico do desenvolvimento, profundamente afeta a função cognitiva.

A sódio potássio adenosina 5'-trifosfatase ($Na^+$,$K^+$-ATPase) é uma enzima crucial responsável pelo transporte ativo de íons sódio e potássio no SNC necessário para manter o gradiente iônico para a excitabilidade neuronal. Estudos sugerem que a $Na^+$,$K^+$-ATPase possa ter um papel na formação da memória. Além disso, os hormônios da tireóide parecem estimular a atividade da $Na^+$,$K^+$-ATPase no coração de algumas espécies.

Neste trabalho investigamos o efeito da administração crônica de L-tiroxina (L-T4) e propiltiouracil (PTU), uma droga antitireóide, sobre alguns paradigmas de comportamento: esquiva inibitória, campo aberto, plus maze e Y-maze, e sobre a atividade da $Na^+$,$K^+$-ATPase no córtex parietal e no hipocampo de ratos. Utilizando tratamentos que demonstraram induzir alterações nos hormônios da tireóide similares àquelas presentes nos pacientes com hiper e hipotireoidismo, nós buscamos compreender o efeito de um estado alterado por essas doenças sobre o aprendizado e a memória e sobre a atividade da $Na^+$,$K^+$-ATPase. Nossos resultados demonstraram que um estado hiper e hipotireóide altera o comportamento animal e também podem indicar um efeito dos hormônios da tireóide sobre o aprendizado e a memória.

Decidimos investigar também o efeito de um modelo animal agudo de hipertireoidismo previamente estabelecido. Utilizando um tratamento agudo para induzir esse modelo animal, nós intencionamos verificar o efeito de níveis alterados de hormônios da tireóide sobre o aprendizado e a memória e sobre a atividade da $Na^+$,$K^+$-ATPase no córtex parietal e no hipocampo de ratos.

Nossos resultados demonstraram que um tratamento agudo com L-T4 não alterou a evocação da memória da tarefa da esquiva inibitória, mas teve um efeito significativo no plus maze e no campo aberto em ratos. Sugerimos que animais tratados com L-T4 apresentam uma melhora na habituação a um ambiente novo, assim como uma melhor avaliação de um ambiente perigoso, respectivamente. A atividade da

$Na^+,K^+$-ATPase está aumentada no córtex parietal (30%), mas não está alterada no hipocampo no grupo tratado com L-T4. Essas duas estruturas cerebrais estão envolvidas nos processos de memória e já foi previamente demonstrado que existe uma dissociação dupla entre elas para informação de localização espacial, memória perceptual e episódica. Dessa forma, propomos a hipótese que esse aumento da $Na^+,K^+$-ATPase no córtex parietal pode estar correlacionado a nossos resultados nos testes comportamentais, que sugerem um papel dos hormônios da tireóide assim como da $Na^+,K^+$-ATPase nos processos cognitivos.

**Abstract**

Thyroid hormones, including triiodothyronine (T3) and tetraiodothyronine (T4), are recognized as key metabolic hormones of the body. Thyroid hormones are essential for normal maturation and function of the mammalian central nervous system (CNS) and its deficiency, during a critical period of development, profoundly affects cognitive function.

Sodium potassium adenosine 5'-triphosphatase ($Na^+,K^+$-ATPase) is a crucial enzyme responsible for the active transport of sodium and potassium ions in the CNS necessary to maintain the ionic gradient for neuronal excitability. Studies suggest that $Na^+,K^+$-ATPase might play a role on memory formation. Moreover, thyroid hormones were proposed to stimulate $Na^+,K^+$-ATPase activity in the heart of some species.

In this work we investigated the effect of a chronic administration of L-thyroxine (LT4) or propylthiouracil (PTU), an antithyroid drug, on some behavioral paradigms: inhibitory avoidance task, open field task, plus maze and Y-maze, and on the activity of $Na^+,K^+$-ATPase in the rat parietal cortex and hippocampus. By using treatments which have shown to induce alterations in thyroid hormones levels similar to those found in hyperthyroid and hypothyroid patients, we aimed to understand the effect of an altered hyperthyroid and hypothyroid state on learning and memory and on the activity of $Na^+,K^+$-ATPase. Our results showed that a hyper and hypothyroid state can alter animal behavior and they also might indicate an effect of thyroid hormones on learning and memory.

We also decided to investigate the effect of an previously established acute hyperthyroid animal model. By using an acute treatment to induce this hyperthyroid animal model, we aimed at investigating the effect of an altered thyroid hormones levels on learning and memory and on the activity of $Na^+,K^+$-ATPase in the parietal cortex and hippocampus.

Our results have shown that the acute treatment with L-T4 did not alter the retrieval of the inhibitory avoidance task, but had a significant effect on the elevated plus maze and on open-field performance in rats. We suggest that animals subjected to L-T4 administration improved the habituation to a novel environment as well as a better evaluation of a dangerous environment, respectively. $Na^+,K^+$-ATPase activity is increased in parietal cortex (30%), but it is not altered in hippocampus in L-T4 treated group. These both brain structures are involved in memory processing and it was previously demonstrated that there is a double dissociation between them for spatial location information, perceptual and episodic memory. We propose the hypothesis that this increase of $Na^+,K^+$-ATPase activity in parietal cortex may be correlated to our results in behavior tests, which suggest a role of thyroid hormones as well as of the $Na^+,K^+$-ATPase in the cognitive process.

**Lista de Abreviaturas**

ADP: 5'-difosfato de adenosina

ATP: 5'- trifosfato de adenosina

DNA: ácido desóxiribonucléico

LTM: Memórias de longa duração – *Long-Term Memory*

LTP:Potenciação de Longa Duração: *Long-Term Potentiation*

L-T4: levotiroxina

mRNA: RNA mensageiro

PPF:Facilitação de Pulso Pareado -*Pared-Pulse Facilitation*

PTU : propiltiouracil

SNC: sistema nervoso central

STM: Memórias de curta duração - *Short-Term Memory*

TAS: *Total Anti-oxidante Status*

TR: receptores tireoidianos

TRH: hormônio liberador da tireotrofina

TSH: hormônio estimulante da tireóide

T3: 3,5,3'-triiodotironina

T4: 3,5,3',5'-tetraiodotironina ou tiroxina

WM: Memória imediata ou de trabalho- *Working Memory*

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# 1. Introdução

## 1.1. Hormônios da Tireóide

### 1.1.1. Histórico

A glândula tireóide foi primeiramente descrita por Galen e denominada "glandulae thyroidaeae" por Wharton em 1656 (HARINGTON, 1935; FARWELL & BRAVERMAN, 2006).

*Século XIX*

- Químicos franceses e de outros grupos europeus sugeriram uma correlação entre a presença do iodo na glândula tireóide humana e doenças como bócio, cretinismo e mixedema.

- Médicos ingleses estabelecem uma associação entre a atrofia da tireóide, a deficiência de iodo e o bócio.

- Os irmãos cirurgiões Kocher e Reverdin da Suiça reportaram que a ressecção da glândula tireóide levava ao mixedema em seus pacientes. Impressionante na época, foi o aparecimento de sintomas semelhantes ao cretinismo em crianças que foram submetidas à tireoidectomia.

*Século XX*

- Experimentos com animais nos quais a glândula tireóide era removida e posteriormente enxertada novamente para reverter os efeitos da falta da glândula tireóide, colaboraram para demonstrar o efeito benéfico da administração do pó ou extrato de tireóide ovino, porcino ou bovino em pacientes com mixedema, cretinismo e retardo no crescimento, além de outras conseqüências da falta dos hormônios da tireóide.

- Em 1912, o biólogo alemão J. F. Gudernatsch observou que alimentando sapos com pedaços de glândula tireóide de mamíferos induzia metamorfose, dando ênfase ao papel pós-embrionário dos hormônios da tireóide no desenvolvimento dos vertebrados.

- No início do século XX apenas uma proteína contendo iodo tinha sido isolada da tireóide, a chamada tireoglobulina, e imaginava-se ser essa a representante ativa da glândula.

- Em 1915, Edward Kendall isolou, identificou, e trabalhou com uma nova substância com atividade hormonal chamada tiroxina (KENDALL, 1929). Kendall continuou identificando e isolando hormônios do córtex adrenal o que lhe garantiu o prêmio Nobel em 1929.

- Em 1929, Charles Harington determinou a síntese total da L-tiroxina, o isômero natural da tiroxina, que foi o primeiro hormônio sintético. A disponibilidade do hormônio sintético puro facilitou a determinação das múltiplas ações e a quantificação das atividades biológicas no homem e em espécies vertebradas por fisiologistas.

- Durante 30 anos a 3,5,3',5'-tetraiodotironina ou tiroxina (T4) foi considerada a forma ativa do hormônio da tireóide.

- Em 1952, dois grupos de pesquisa – um em Paris e outro em Londres – descobriram a 3,5,3'-triiodotironina (T3) na tireóide e no sangue. Entre as décadas de 1940 e 1950 surgem novas técnicas de dosagens desses hormônios usando isótopos radioativos e separação das proteínas ou aminoácidos por cromatografia ou eletroforese. O grupo francês foi capaz de identificar o radioiodo ($^{131}$I)T3 marcado como uma molécula constituinte da molécula de tireoglobulina da tireóide de ratos, da qual, juntamente com T4, seria liberado por proteólise e secretado na circulação. Ao mesmo tempo em Londres, o T3 foi isolado do sangue de um paciente ao qual o $^{131}$I tinha sido

administrado por razões terapêuticas. Eles demonstraram que parte do T3 havia sido derivada da deiodinação do T4. Eles também sintetizaram o T3, pois demonstrou ser mais potente que o T4 em todos os testes de biodisponibilidade, como prevenção de bócio, elevação do metabolismo basal, aumento da taxa do crescimento.

- Estudos posteriores confirmaram que a maioria do T3 nos tecidos e em grande parte no sangue é derivado do T4, o que nos leva a um conceito universal que T3 é a forma fisiologicamente ativa do hormônio da tireóide tendo como o T4 o seu precursor (TATA, 2007).

### 1.1.2. Síntese e Regulação dos Hormônios da Tireóide

Os hormônios da tireóide, que incluem a 3,5,3'-triiodotironina (T3) e a 3,5,3',5'-tetraiodotironina ou tiroxina (T4), requerem um raro elemento – o iodo - para apresentar bioatividade (GRANNER, 2003; REED, 2002). O iodo é essencial na síntese desses hormônios, com quatro iodos por molécula de T4, compreendendo 66% de seu peso e três iodos por molécula de T3, equivalente a 58% do seu peso.

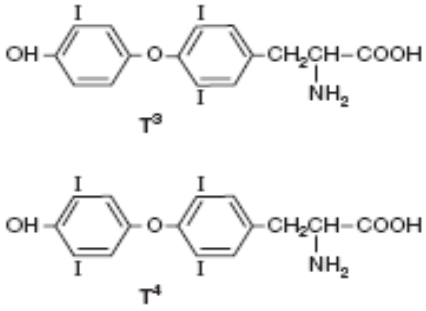


Figura 1 – Estrutura química dos hormônios da tireóide. Fonte: HARPER, 2003.

Normalmente, ~90 πg (~120 nmol) de T4 e ~6.5 πg (~10 nmol) de T3 são secretados diariamente pela glândula tireóide. Então 60 a 80 πg (~550 nmol) de iodo devem ser transportadas para a glândula para manter a produção diária de hormônio. O iodo nem sempre está presente em quantidades suficientes nas fontes dietéticas do meio ambiente (REED, 2002). Na maior parte do mundo, o iodo é um escasso componente do solo, e por esse motivo aparece em pequenas quantidades nos alimentos (GRANNER,2003).

Os hormônios da tireóide são formados dentro das células foliculares da glândula tireóide e são liberados na circulação sistêmica em resposta ao hormônio estimulante da tireóide (TSH) (SMITH et al., 2002). A síntese ocorre como parte de uma grande molécula precursora (a tireoglobulina); eles são armazenados em um reservatório intracelular chamado colóide; e existe uma conversão periférica de T4 para T3, que é a forma hormonal funcionalmente mais ativa (GRANNER, 2003; SMITH et al., 2002). A Tireoglobulina age como uma grande molécula pró-hormônio na síntese de T3 e T4. A precursora dos hormônios da tireóide é uma grande proteína iodinada glicosilada com massa molecular de 660 kDa (REED, 2002). Os carboidratos correspondem por 8-10% do peso da tireoglobulina e o iodo por aproximadamente 0.2-1%, dependendo da quantidade de iodo na dieta. A tireoglobulina é composta de duas grandes subunidades. Ela contém 115 resíduos de tirosina, cada qual sendo um potencial sítio de iodinação. Aproximadamente 70% do iodo na tireoglobulina existe em precursores inativos, monoiodotirosina (MIT) e diiodotirosina (DIT), enquanto 30% está nos resíduos iodotironil, T3 e T4. Quando as fontes de iodo são suficientes, a relação T4:T3 é aproximadamente 7:1. A tireoglobulina, uma grande molécula de aproximadamente 5000 aminoácidos, fornece a conformação necessária para o

acoplamento tirosil e organificação do iodo necessária na formação dos hormônios da tireóide diaminoácido. Eles são sintetizados na porção basal da célula e movem-se para o lúmem, onde ficam na forma de armazenamento do T3 e T4 no colóide; muitas semanas de suprimento desses hormônios existem em uma glândula tireóide normal. Alguns minutos após a estimulação da tireóide pelo TSH, a forma de armazenamento do T3 e T4 presente no colóide reentra na célula, aumentando a atividade fagolisossômica. Várias proteases ácidas e peptidases hidrolisam a tireoglobulina nos seus aminoácidos constituintes, incluindo o T3 e o T4, que são liberados na porção basal da célula (GRANNER, 2003).

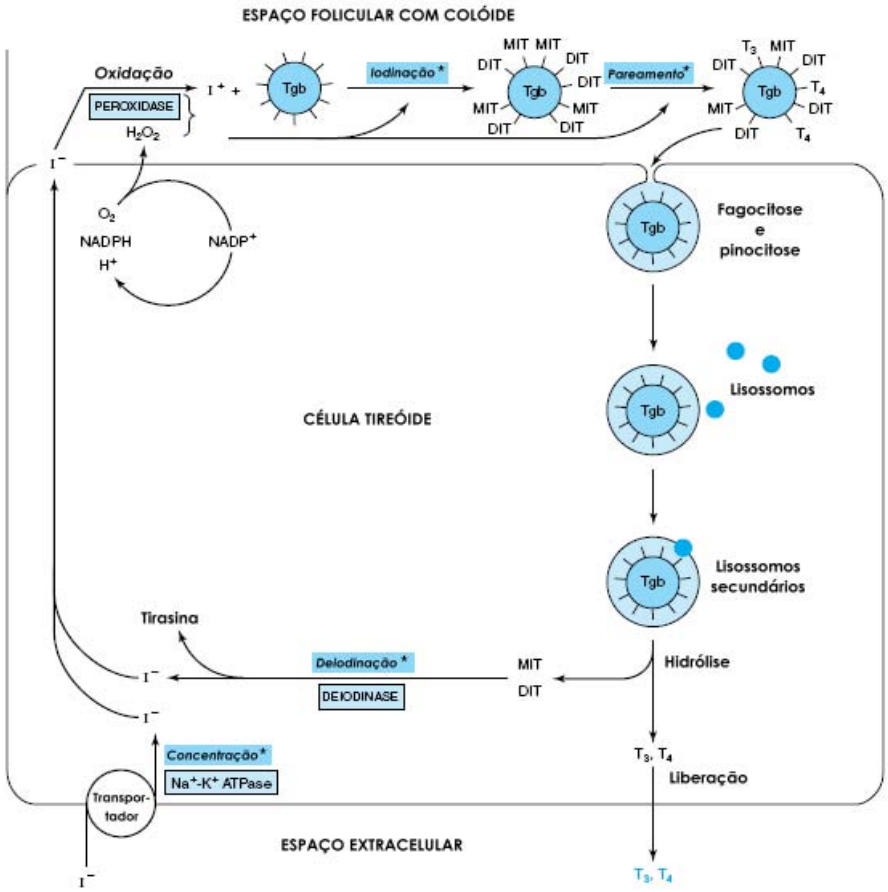


Figura 2 - Modelo esquemático do metabolismo do iodo no folículo tireóide. Fonte: HARPER, 2003.

Na circulação, os hormônios da tireóide ligam-se a uma variedade de proteínas plasmáticas, embora uma pequena percentagem de ambos - T3 e T4- permaneça na sua forma livre. Enquanto T4 é produzido inteiramente pela glândula tireóide, a maioria do T3 circulante é derivado da deiodinação do T4 nos tecidos não tireoidianos como o fígado e o rim. As enzimas deiodinases agem removendo de forma catalítica o iodo e podem fazê-lo em diferentes sítios para produzir igualmente T3 via a 5'-deiodinação ou os metabólitos inativos rT3 ou T2 através da 5-deiodinação (SMITH et al., 2002; BIANCO & KIM, 2006).

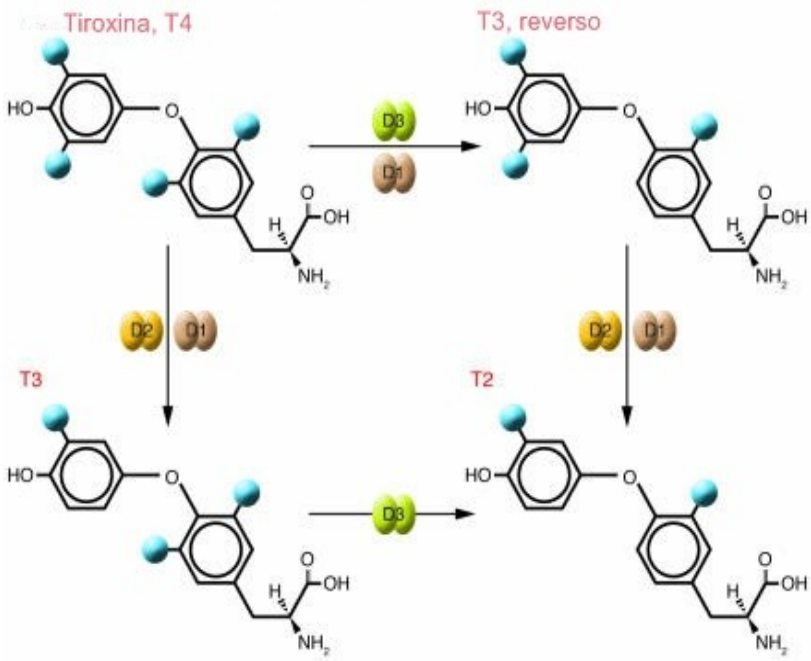


Figure 3 - Reações básicas das deiodinases. Fonte: Adaptado de BIANCO & KIM, 2006.

Três enzimas deiodinases foram identificadas: iodotironina deiodinase tipo I, II, III (D1, D2, D3 respectivamente) que regulam a atividade do hormônio da tireóide removendo o iodo da molécula precursora T4. Essas três enzimas constituem um grupo de proteínas integrais de membrana diméricas contendo uma dobra tioredoxina (LEONARD et al., 2001; BIANCO & LARSEN, 2005; BIANCO & KIM, 2006) que pode ativar ou inativar o hormônio da tireóide, dependendo de onde elas agem nos anéis fenol ou tirosil das iodotironinas, respectivamente (KUIPER et al., 2005). D2 gera a forma ativa do hormônio da tireóide T3 via deiodinação do T4. D3 inativa T3 e previne a ativação do T4. D1 é a enzima cinéticamente menos eficiente, que pode ativar ou inativar T4 em uma base equimolar, e seu papel ainda não está muito claro. Geralmente, um tipo celular expressa uma deiodinase por vez, alguns tecidos não expressam nenhuma e todas as três estão expressas na glândula pituitária (BIANCO & KIM, 2006). A glândula tireóide é controlada pela secreção hormonal do eixo hipotálamo-pituitário. A síntese e a secreção do TSH do tirótrofo é estimulada pelo tripeptídeo chamado hormônio liberador da tireotrofina (TRH). Esses pequeno peptídeo, clivado de um grande pró-hormônio, é liberado de células neurosecretoras no hipotálamo, no interior dos capilares hipotálamo-hipofisários portais, onde eles são transportados para os tirótrofos pituitários. A secreção do TSH é inibida por outros hormônios (incluindo a somatostatina e a dopamina) e também citocinas, particularmente IL-1δ, IL-6 e TNF-γ (NUSSEY & WHITEHEAD, 2001). Manter a concentração normal dos hormônios da tireóide na circulação dependende do grupo de neurônios hipotalâmicos localizados no núcleo paraventricular que produzem o tripeptídeo, TRH. Esses neurônios regulam a síntese de TRH na pituitária anterior e, então, a liberação do hormônio da tireóide pela glândula tireóide (LECHAN & FEKETE, 2006).

A concentração dos hormônios da tireóide na circulação é regulada por um processo de retro-alimentação homeostático envolvendo o eixo hipotálamo-pituitário.

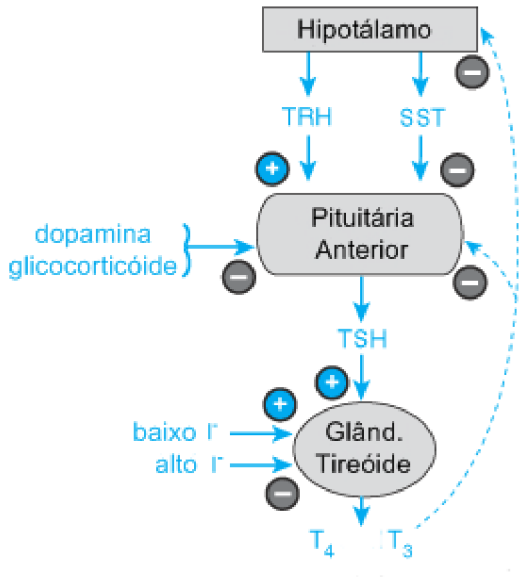


Figura 4 - Regulação da secreção dos hormônios da tireóide. Fonte:GOODMAN & GILMAN, 2006.

O principal efeito dos hormônios da tireóide é reduzir a resposta dos tirótrofos mais do que alterar a taxa de secreção do TRH pelo hipotálamo. A sensibilidade dos tirótrofos ao TRH depende da sua concentração intracelular de T3, 80% da qual é derivada da conversão intra-pituitária de T4 para T3. Quando a concentração de T4 circulante é baixa, há um aumento dos receptores de TRH e um aumento na síntese de TSH resultante, o que aumenta a resposta do TSH ao TRH. O contrário também é verdadeiro na presença de altas concentrações circulantes dos hormônios da tireóide (NUSSEY & WHITEHEAD, 2001).

O processo de retro-alimentação que regula a concentração dos hormônios tireoidianos é afetado por fatores internos e externos que podem alterar a taxa com a

qual o TSH é secretado (por exemplo, balanço energético, variação circadiana, temperatura do meio ambiente, algumas doenças - como anorexia nervosa, etc.) (NUSSEY & WHITEHEAD, 2001; REED, 2002; LECHAN & FEKETE, 2006).

### 1.1.3. Receptores Nucleares dos Hormônios da Tireóide

Os efeitos do T3 são mediados por receptores intracelulares chamados de receptores tiroidianos (TR) que são fatores de transcrição modulados pelo ligante com uma afinidade pelo T3 aproximadamente dez vezes maior do que pelo T4, o que ativa ou reprime a expressão gênica quando eles se acoplam ao seu ligante. Esses receptores são membros de uma grande superfamília que incluem receptores nucleares de outras moléculas hidrofóbicas pequenas como esteróides, prostaglandinas, vitamina D3, ácido retinóico, ácidos graxos, e então chamados receptores órfãos, com ligante não reconhecido. Essa superfamília, que parece ser muito antiga, está representada em todas as espécies animais (MANGELSDORF et al., 1995; SARLIEVE et al., 2004).

Os receptores dos hormônios da tireóide são produtos de dois genes, designados TRγ e TRδ que em humanos estão locados em diferentes cromossomas. TRγ dá origem a várias isoformas por um *splicing* alternativo do RNA mensageiro (mRNA) (SARLIEVE et al., 2004, CHASSANDE et al., 1997; FLAMANT & SAMARUT, 2003), incluindo duas isoformas TRγ1 e TRγ2, que diferem na sua porção carboxi terminal. Além disso, a expressão alternativa do DNA gera Rev-ErbAγ. Nem TRγ2, nem Rev-ErbAγ podem ser considerados receptores pois eles são incapazes de ligarem-se ao hormônio. Através da ativação de promotores alternados, a transcrição do gene TR

δ produz três receptores funcionais: TRδ1, TRδ2 e TRδ3, que diferem entre si na seqüência amino-terminal (SARLIEVE et al., 2004).

No clássico modelo de ação dos receptores tireoidianos, na ausência de T3 o receptor tireoidiano apresenta uma função transcricional intrínseca de repressão (FLAMANT & SAMARUT, 2003). A ligação do hormônio é seguida de mudanças conformacionais que afetam a interação do receptor com a maquinaria basal transcricional, promovendo estabilização e/ou recrutamento dos fatores transcricionais basais e estimulando a transcrição (BRENT et al., 1991). O sinal do hormônio da tireóide pode ser modulado por vários fatores incluindo as isoformas presentes dos receptores da tireóide, o elemento resposta do DNA no gene regulado, a disponibilidade dos receptores nucleares para hormônio da tireóide, a interação com co-ativadores e co-repressores, e a disponibilidade de ligantes (SARLIEVE et al., 2004).

### 1.1.4. Oxidação e Iodinação

Consistente com a condição necessária para a halogenação dos anéis aromáticos, a iodinação dos resíduos de tirosina requer espécies iodinadas estarem em um maior estado de oxidação que o ânion. A oxidação é efetuada pela tireóide peroxidase, uma enzima que contém um grupamento heme e que utiliza o peróxido de hidrogênio como oxidante (ARVAN P., 2005; FARWELL & BRAVERMAN, 2006). A peroxidase é uma proteína de membrana que parece concentrar-se próximo à superfície apical da célula tireóide. A reação resulta na formação dos resíduos monoiodotirosil e diiodotirosil em tireoglobulina anterior ao seu armazenamento extracelular no lúmem do folículo da tireoidiano. A formação do peróxido de hidrogênio que serve de substrato para a

peroxidase ocorre próximo a este sítio de utilização e é estimulado pelo aumento do cálcio citosólico (TAKASU et al., 1987; FARWELL & BRAVERMAN, 2006).

### 1.1.5. Efeitos Sistêmicos dos Hormônios da Tireóide

Os hormônios da tireóide são conhecidos como hormônios metabólicos chave do organismo, sendo o T3 a forma hormonal funcionalmente mais ativa (SMITH et al., 2002; STOICA et al., 2007) e o T4, o principal hormônio secretado pela glândula tireóide, considerado precursor ou pró-hormônio (CHOKSI et al., 2003; STOICA et al., 2007).O T3 liga-se a um de vários receptores nucleares (alfa e beta), que então modulam a expressão dos genes contendo seqüências ligadas (elementos de resposta ao T3) para o complexo hormônio-receptor. Nos tecidos periféricos, o T3 aumenta a síntese protéica, o metabolismo celular, a geração de calor e a função cardiovascular (LECHAN & FEKETE, 2006).

Os hormônios da tireóide apresentam muitas funções fisiológicas e essencialmente modulam todas as rotas metabólicas através de alterações no consumo de oxigênio e mudanças no metabolismo das proteínas, lipídios, carboidratos e vitaminas (SMITH et al., 2002). Além disso, através da direta manipulação da expressão de proteínas associadas às rotas metabólicas, os hormônios da tireóide também afetam a síntese e a degradação de outros hormônios e fatores de crescimento e, então, influenciam indiretamente na sinalização hormonal (SMITH et al., 2002).

A manutenção da temperatura corporal constante em situações fisiológicas diferentes (por exemplo em variações de temperatura, atividade física, ingestão de alimentos), requer um sistema regulatório sofisticado tanto a nível de órgão quanto a

nível celular. O mais importante fator endócrino que modula a termogênese em situações como estas são os hormônios da tireóide (JANSKÝ, 1995; LANNI et al., 2003; HAMPL et al., 2006). Do ponto de vista bioquímico, calor é a porção de energia liberada durante a fosforilação oxidativa através do transporte de elétrons através da cadeia respiratória, no interior da matriz mitocondrial. O interior da membrana mitocondrial, ao contrário da membrana externa muito permeável, contém muitas proteínas transmembrana que servem como um sistema anti-porta para a troca de ânions entre a matriz de ânions. Esses transportadores de ADP-ATP e fosfato apresentam significante homologia com outro grupo de proteínas transmembrana que translocam $H^+$, conhecidas como proteínas desemparelhadas (*uncoupling proteins* ou UCPs). Podemos considerar a produção de calor, ou seja, o salto de prótons pela cadeia respiratória e seu retorno à matriz, o principal evento (BRAND, 1990; HAMPL et al., 2006). Os hormônios da tireóide são os maiores reguladores dos processos metabólicos oxidativos (JANSKÝ, 1995; LANNI et al., 2003; HAMPL et al., 2006). O metabolismo oxidativo total em repouso (taxa metabólica basal) medida pelo consumo de oxigênio, é altamente sensível ao status dos hormônios tireoideanos (HAMPL et al., 2006).

A glândula tireóide e seus hormônios são centrais no desenvolvimento dos seres humanos e dos animais. O T3 exerce uma função essencial no metabolismo dos carboidratos e das proteínas em todas as células. Alterações no T3 podem afetar o corpo profundamente nos sistemas cardiovascular, nervoso, imune e reprodutor. Em mamíferos em desenvolvimento, o hormônio da tireóide regula o crescimento e tem um papel principal no desenvolvimento e diferenciação dos tecidos (CHOKSI et al., 2003; HARVEY & WILLIAMS, 2002; STOICA et al., 2007).

### 1.1.6. Hormônios da Tireóide e o Sistema Nervoso Central

Além dos efeitos sistêmicos dos hormônios da tireóide, pesquisas recentes têm se concentrado na atividade desses hormônios no sistema nervoso central (SNC) (SMITH et al., 2002; SUI et al., 2005; STOICA et al., 2007). O desenvolvimento do SNC nos mamíferos é precedido de eventos precisamente determinados em tempo e localização. A maioria desses eventos são delimitados por fatores genéticos. No entanto, outros fatores, como hormônios e fatores do crescimento, são também importantes porque agem controlando o tempo e a coordenação de processos mecanicamente não relacionados (BERNAL et al., 2003; STOICA et al., 2007). A deficiência dos hormônios da tireóide durante os períodos críticos do desenvolvimento do SNC nos mamíferos leva a danos profundos e potencialmente irreversíveis na maturação cerebral. Síndromes clínicas devido à falta do hormônio da tireóide durante os períodos fetal e pós-natal são conhecidas em humanos e em animais (PORTERFIELD & HENDRICH, 1993; DELANGE,1997; STOICA et al., 2007). Em ratos, a deficiência dos hormônios da tireóide causa uma série de anomalias no SNC das quais, alteração da migração celular, diferenciação neuronal e desmielinização são as principais (BERNAL et al., 2003; PORTERFIELD & HENDRICH, 1993; DELANGE,1997; LUCIO et al., 1997; OPPENHEIMER & SCHWARTZ, 1997, STOICA et al., 2007). Os hormônios da tireóide parecem regular tais processos associado com a diferenciação cerebral terminal como migração neuronal, dendrídica e crescimento axonal, sinaptogênese e mielinação (BERNAL, 2002; STOICA et al., 2007).

Foi demonstrado que existe alta afinidade entre o T3 e seus receptores no cérebro de ratos (SCHWARTZ & OPPENHEIMER, 1978; SMITH et al., 2002), distribuição espacial e regional dos receptores dos hormônios da tireóide e seus mRNA

foram descritos no cérebro tanto nos estágios de desenvolvimento como no estágio adulto (BRADLEY et al., 1989; COOK et al., 1992; GULLO et al., 1987; MELLSTROM et al., 1991; SMITH et al., 2002). Em humanos, a presença de receptores dos hormônios da tireóide pode ser observada por volta da décima semana de gestação (BERNAL & PEKONEN, 1984; SMITH et al., 2002).

Essa forte e dependente relação existente entre o SNC e os hormônios da tireóide não é restrita às células neuronais. Efeitos metabólicos que afetam o metabolismo do acetato (CHAPA et al., 1995; SMITH et al., 2002) foram demonstrados ocorrer no cérebro em resposta aos hormônios da tireóide. Em astrócitos, por exemplo, foi demonstrado: (1) apresentarem receptores para hormônios da tireóide (LUO et al., 1986; LUO et al., 1989), (2) possuir dependência dos hormônios da tireóide para o transporte de glicose (RODER et al., 1985), (3) depender dos hormônios da tireóide para expressar proteínas estruturais específicas (CHAUDHURY et al., 1985; GAVARET et al., 1991; SIEGRIST-KAISER et al., 1990) e (4) apresentarem uma correlação positiva entre os níveis de hormônios da tireóide e a expressão de δ-adrenoceptor (DAS & PAUL, 1994; KAPLAN & YASKOSKI, 1980; SMITH et al., 2002). A deficiência de hormônios da tireóide também provoca déficit na maturação das células gliais no hipocampo de ratos em estágio fetal e conseqüentemente afeta a migração de outras células para essa região (MARTINEZ-GALAN et al., 1997). Além disso, o *gyrus dentatus*, região cerebral que apresenta a capacidade de produzir novos neurônios durante a vida adulta em diversas espécies de mamíferos (ALTMAN & DAS, 1965; ERIKSSON et al., 1998; AMBROGINI et al., 2005), parece também sofrer a ação dos hormônios da tireóide na regulação da produção de novos neurônios no cérebro de ratos (AMBROGINI et al., 2005).

Os hormônios da tireóide são essenciais para a maturação normal e função do SNC de mamíferos (OPPENHEIMER & SCHWARTZ, 1997; SANTOS & PÉREZ-CASTILLO, 2000; DARBRA et al., 2003; SUI et al., 2005) e não é de surpreender que a disfunção no eixo da tireóide esteja relacionado a uma série de patologias relacionadas ao SNC (SMITH et al., 2002). A deficiência dos hormônios da tireóide durante um período crítico do desenvolvimento, afeta profundamente as funções cognitivas (VARA et al., 2002; DONG et al., 2005). Por outro lado, o excesso dos hormônios da tireóide também causa uma série de alterações neurológicas e comportamentais relacionadas ao aumento do ritmo-alfa (SMITH et al., 2002). Contudo, a relação entre os hormônios da tireóide e as doenças psiquiátricas e alterações cognitivas ainda está incerta e deve ser melhor compreendida (CONSTANT et al., 2001; SIESSER et al., 2005; DONG et al., 2005).

### 1.2. Hipotireoidismo

O hipotireoidismo, também conhecido como mixedema quando severo, é a mais comum doença da glândula tireóide. Geralmente, o hipotireoidismo resulta da deficiência de iodo, gerando a produção diminuída dos hormônios da tireóide e levando as manifestações clínicas características de insuficiência da glândula tireóide, tais como baixo índice metabólico, intolerância ao frio, fraqueza muscular, fadiga, bradicardia, mixedema, depressão e déficit cognitivo. (MENNEMEIER et al., 1993; STEDMAN, 1996; DUGBARTEY, 1998; NUSSEY & WHITEHEAD, 2001; BURMEISTER et al., 2001; ROBERTS & LADENSON, 2004; ZHU et al., 2006, FARWELL & BRAVERMAN, 2006).

Nas zonas não endêmicas, onde o iodo é suficiente, a tireoidite auto-imune crônica (tireoidite de Hashimoto) é responsável pela maioria dos casos de hipotireoidismo. As doenças auto-imunes da tireóide são as mais prevalentes dentre as demais doenças auto-imunes existentes, afetando 5% da população em geral (KAVVOURA et al., 2007). Os dois tipos mais comuns de doenças auto-imunes da tireóide são a tireoidite de Hashimoto e a doença de Graves (KAVVOURA et al., 2007). A doença de Hashimoto é caracterizada por altos níveis de anticorpos circulantes contra a tireóide peroxidase, ou menos comumente, contra a tireoglobulina (FARWELL & BRAVERMAN, 2006). Finalmente, a destruição da célula tireóide pode resultar da morte celular por apoptose devido à interação de Fas com o ligante Fas nos tirócitos (GIORDANO et al., 1997; FARWELL & BRAVERMAN, 2006).

A falha da tireóide de produzir suficiente hormônio é a mais comum causa de hipotireoidismo e chama-se *hipotireoidismo primário* (FARWELL & BRAVERMAN, 2006). O tratamento do hipotireoidismo primário é o pró-hormônio tiroxina (NUSSEY & WHITEHEAD, 2001). O *hipotireoidismo central* ocorre muito menos freqüentemente e resulta da diminuição da estimulação da tireóide pelo TSH por falha pituitária (*hipotireoidismo secundário*) ou falha hipotalâmica (*hipotireoidismo terciário*) (FARWELL & BRAVERMAN, 2006). Por ser menos freqüente, o hipotireoidismo causado pela diminuição da estimulação trófica da glândula devido a uma doença ou a uma falha no eixo hipotalâmico-pituitário faz com que na ausência do TRH e/ou TSH a função da tireóide esteja reduzida (NUSSEY & WHITEHEAD, 2001). O tratamento ainda é uma questão de debate, pois deve-se estudar em cada caso o quanto o paciente necessita de reposição hormonal. A combinação de T3 e T4 para

mimetizar a secreção da glândula está em pauta como uma solução para esses casos (NUSSEY & WHITEHEAD, 2001).

O hipotireoidismo presente ao nascimento (*hipotireoidismo congênito*) é a doença endócrina mais freqüente que ocorre na fase prematura da vida e a mais comum causa de retardo mental evitável no mundo (LA FRANCHI, 1999; OLIVIERI et al., 2007; FARWELL & BRAVERMAN, 2006). Nas fases do desenvolvimento que abrange as fases fetal, neonatal e início da infância; a disfunção da glândula se não apropriadamente tratada causa danos sérios e irreversíveis ao SNC que tem como conseqüência alteração das funções neurológicas, dos processos metabólicos, da capacidade mental o que resulta em retardo mental (OLIVIERI et al., 2007, NUSSEY & WHITEHEAD, 2001; DARBRA et al., 2003; DONG et al., 2005). Testes de *screening* para a doença são feitos no mundo todo de forma que o diagnóstico seja feito em tempo de iniciar o tratamento adequadamente (OLIVIERI et al., 2007). No Brasil, a Política de Triagem Neonatal visa fazer o diagnóstico precoce das seguintes doenças: fenilcetonúria, hipotireoidismo congênito, doença falciforme e outras hemoglobinopatias. Esta atividade é conhecida popularmente como "Teste do Pezinho". O tratamento do hipotireoidismo congênito consiste em fazer a reposição hormonal com tiroxina o mais breve possível após o nascimento (NUSSEY & WHITEHEAD, 2001).

O *hipotireoidismo subclínico* é caracterizado por taxas elevadas de TSH plasmáticas e níveis normais de hormônios da tireóide (OSTERWEIL et al., 1992; MANCIET et al.; 1995; NYSTROM et al., 1997; ROBERTS et al., 2006; SAMUELS et al., 2007). Muitos estudos apontam que o hipotireoidismo subclínico causa disfunções cognitivas mais amenas do que aquelas presentes no hipotireoidismo (BURMEISTER et al.,2001; ROBERTS & LADENSON, 2004; ZHU et al. , 2006). Déficits de memória

também foram descritos em alguns pacientes e foram parcialmente revertidos com a reposição com tiroxina (ZHU et al., 2006; BALDINI et al., 1997; HAGGERTY et al., 1990; NYSTROM et al., 1988).

### 1.3. Hipertireoidismo

A tireotoxicose é uma condição causada por elevadas concentrações de hormônios da tireóide livres na corrente circulatória. Várias doenças de diferentes etiologias podem resultar nesta síndrome. O termo hipertireoidismo é restrito àquelas no qual a produção dos hormônios da tireóide ou a liberação está aumentada devido à hiperfunção da glândula (FARWELL & BRAVERMAN, 2006). O hipertireoidismo é caracterizado pela secreção aumentada dos hormônios da tireóide e que não se encontra mais sob o controle regulador dos centros hipotalâmicos hipofisários levando as manifestações clínicas de um estado hipermetabólico, tais como perda de peso, fraqueza muscular, exoftalmia, irritabilidade, ansiedade, taquicardia, tremores, distúrbios do sono, paranóia, mania e depressão (STEDMAN, 1996; SMITH et al., 2002, FARWELL & BRAVERMAN, 2006).

A doença de Graves é a causa mais comum de hipertiroidismo, abrange cerca de 60% a 90% dos casos dependendo da idade e da região geográfica estudada. Trata-se de uma doença auto-imune resultante da produção de auto-anticorpos que estimulam os receptores de TSH e produzem uma secreção excessiva de hormônios da tireóide (REED, 2002; FARWELL & BRAVERMAN, 2006). Geralmente, a doença de Graves aparece associada a outra doença auto-imune: a exoftalmia, por exemplo, presente em cerca de 50% dos casos ocorre devido a uma inflamação auto-imune mediada do tecido

conectivo periorbital e de músculos extraoculares (FARWELL & BRAVERMAN, 2006; RAPOPORT & MCLACHLAN, 2000).

Aproximadamente 1-2% dos pacientes com hipertireoidismo evoluem para um evento fisiologicamente estressante chamado 'tempestade tireóide', cujos sintomas são falhas cardíacas e arritmias, intolerância ao calor, perda de apetite, perda de peso, tremores e febre (NGO & CHEW, 2007).

Existem três métodos para tratar o hipertireoidismo: medicamentos anti-tireóide, cirurgia e radioisótopos. Nenhum deles é ideal, cada um tem suas vantagens e desvantagens e freqüentemente uma combinação de terapias é feita (NUSSEY & WHITEHEAD, 2001; FARWELL & BRAVERMAN, 2006).

## 1.4. Memória

> O aprendizado resulta do armazenamento da informação como conseqüência da prática, da experiência, da introspecção, resultando em uma alteração relativamente permanente do comportamento real ou potencial. A informação gerada pelo aprendizado é chamada memória, e sua expressão é chamada de evocação (CAMMAROTA et al., 2007; IZQUIERDO & MCGAUGH 2000; IZQUIERDO & MEDINA 1997; KANDEL & SQUIRE 2000)

Memória é a aquisição, a formação, a conservação e a evocação de informações. A aquisição é o novo padrão funcional criado pelo sistema nervoso como conseqüência de uma situação nova ou repetida. A aquisição é também chamada de aprendizagem: só se "grava" aquilo que foi *aprendido*. Evocar a memória significa recordar, lembrar, sendo definida como a emissão de determinada resposta quando o sujeito experimental

é recolocado na situação ambiental em que esta foi elaborada ou selecionada. Só *lembramos* aquilo que gravamos, aquilo que foi aprendido. (IZQUIERDO, 2002).

Para entender a cognição, é importante entender como uma resposta aprendida se torna uma memória de longa duração, por exemplo. Isso ocorre através do chamado processo de consolidação da memória (IZQUIERDO et al., 2006). Alguns neurocientistas vêem a consolidação como um processo que dura poucas horas no qual as memórias são transformadas de um estado mais lábil para um estado mais estável (BLISS & COLLINGRIDGE, 1993; MALENKA & NICOLL 1999; KANDEL & SQUIRE 2000, IZQUIERDO & MCGAUGH 2000; IZQUIERDO & MEDINA 1997; IZQUIERDO et al., 2006). Por outro lado, existem vários autores que acreditam na existência de um outro tipo de consolidação, que dura por vários dias ou meses, ou até mesmo a vida inteira (MCGAUGH, 2000). Não há dúvida que informações podem ser acrescentadas ou perdidas em memórias através de períodos prolongados (IZQUIERDO, I. & MEDINA J.H. 1997; IZQUIERDO & MCGAUGH 2000; IZQUIERDO et al., 2006). Neste trabalho, iremos nos referir a forma mais 'clássica' de consolidação, que dura algumas horas (IZQUIERDO, I. & MEDINA J.H. 1997; IZQUIERDO & MCGAUGH 2000; MCGAUGH, 2000; IZQUIERDO et al., 2006).

A memória é a mais básica e importante operação do cérebro. Poucos processos cognitivos, incluindo reconhecimento, linguagem, planejamento, resolução de problemas, tomada de decisões e criatividade, podem operar efetivamente sem a contribuição da memória. As memórias dos humanos e dos animais provêm de experiências. Por isso, é mais sensato falar em "memórias" e não em "memória", já que há tantas memórias possíveis quanto forem as experiências possíveis (IZQUIERDO, 2002).

Existe um processo de tradução entre a realidade das experiências e a formação da memória respectiva; e outro entre esta e a correspondente evocação. Os processos de tradução, na aquisição e na evocação, devem-se ao fato de que em ambas ocasiões, assim como durante o longo processo de consolidação, são utilizadas redes complexas de neurônios. Uma experiência visual penetra pela retina , é transformada em sinal elétrico, chega através de várias conexões neuronais ao córtex occipital e lá causa uma série de processos bioquímicos de formação da memória.

Ao converter a realidade em um complexo código de sinais elétricos e bioquímicos, os neurônios *traduzem*. Na evocação, ao reverter essa informação para o meio que nos rodeia, os neurônios reconvertem sinais bioquímicos ou estruturais em elétricos, de maneira que novamente nossos sentidos e nossa consciência possam interpretá-los como pertencendo a um mundo real (IZQUIERDO, 2002).

Podemos afirmar que *somos aquilo que recordamos*, literalmente (IZQUIERDO, 2002).

### 1.4.1. Tipos de Memória

As memórias podem ser classificadas:

De acordo com seu **conteúdo**:

- *Declarativas ou explícitas*: São aquelas memórias que podem ser descritas por meio de palavras em humanos. Subdividem-se em memória declarativa episódica, que abrange aquelas memórias que possuem uma referência temporal (ex. autobiográfica); e em memória declarativa semântica, que envolve conceitos atemporais (ex. conhecimentos, cultura) (SQUIRE, 1992; BONINI, 2006).

- *Não declarativas ou implícitas*: São aquelas memórias que não podem ser descritas por meio de palavras. Subdividem-se em memória não declarativa de representação perceptual, lida com representações sem significado aparentemente conhecido (imagens e sons), mas úteis como dicas facilitatórias da evocação (em inglês esse tipo de memória é chamado de *priming*) (IZQUIERDO, 2002). A de procedimento lida com hábitos, habilidades e regras (ex. andar de bicicleta, uso gramatical da língua materna). A memória não declarativa associativa lida com a associação de dois ou mais estímulos (condicionamento pavloviano ou clássico), ou de um estímulo a uma resposta (condicionamento operante) (IZQUIERDO & MCGAUGH 2000; BONINI, 2006; SQUIRE, 1992). Já a não associativa lida com a atenuação ou a intensificação de uma resposta (habituação ou sensibilização, respectivamente), através da repetição de um mesmo estimulo (ex. sonoro ou doloroso, respectivamente) (IZQUIERDO, 2002).

De acordo com sua **duração**:

- *Memórias de curta duração -Short-Term Memory (STM)*: O termo STM designa a memória que desenvolve-se em poucos segundos ou minutos e dura por várias horas enquanto ocorre lentamente a consolidação da LTM. Dura minutos ou horas. Garante o sentido de continuidade do presente. (IZQUIERDO et al., 1998a; IZQUIERDO et al., 1998b; IZQUIERDO et al., 1998c; McGAUGH, 1966; WOLFMAN et al.,1994; BONINI, 2006).

- *Memórias de longa duração – Long-Term Memory (LTM)*: O termo LTM é usado para designar memórias que duram pelo menos 24h. Dura horas, dias ou anos. Garante o registro do passado autobiográfico e dos conhecimentos do indivíduo. (IZQUIERDO et al, 1998a; IZQUIERDO & MEDINA, 1995; McGAUGH, 1966; IZQUIERDO et al., 1998d; WOLFMAN et al., 1994; IZQUIERDO et al., 1999). As

memórias de longa duração podem ser divididas em associativas e não associativas dependendo dos mecanismos requeridos para a sua formação. Memórias associativas são baseadas na aquisição de uma ligação entre um evento específico e um estímulo. Memórias não associativas são adquiridas quando exposições contínuas ou repetidas a um novo estímulo muda a resposta comportamental a este (VIANNA et al., 2000; IZQUIERDO & MEDINA, 1997; ZHU et al., 1997; THIEL et al., 1998; EICHENBAUM, 1999; McGAUGH 2000).

De acordo com sua **natureza de arquivo**:

- STM

- LTM

– Transitórias: de momento a momento, memória imediata ou de trabalho – *Working Memory* (WM). A memória imediata ou operacional que dura segundos ou alguns minutos (GOLD & McGAUGH, 1975; JACOBSEN, 1936; MARKOWITSCH, 1997; IZQUIERDO et al, 1999). Corresponde ao processamento contínuo das informações recém-adquiridas e/ou recém-evocadas permitindo o raciocínio e o planejamento do comportamento. Serve para gerenciar a realidade. Diferencia-se das demais por não armazenar arquivos (BONINI, 2006; IZQUIERDO, 2002).

### 1.4.2. Testes de Aprendizado e Memória

O *teste de esquiva inibitória* é um modelo bem estabelecido de memória aversiva motivada dependente do hipocampo que envolve não descer de uma plataforma para evitar um leve choque nas patas (IZQUIERDO & MEDINA, 1997; IZQUIERDO & MCGAUGH, 2000). O animal é submetido a uma sessão de treino, na qual é colocado sobre a plataforma do aparelho de esquiva inibitória e, ao descer desta com as quatro patas, recebe um leve choque (0,3-0,4 mA); após 24h é colocado novamente na plataforma (sessão de teste) e o comportamento de permanência na plataforma nos permite avaliar o aprendizado e a memória. Na sessão de teste, o choque não é usado e o tempo de permanência na plataforma (latência) é medido (com um teto de 180s) como medida de retenção da memória (BARROS et al., 2006; COITINHO et al., 2006; ROESLER et al., 2006; IZQUIERDO & MCGAUGH, 2000; IZQUIERDO & MEDINA, 1997).

Existem vários motivos pelos quais a esquiva inibitória é o teste mais amplamente utilizado para estudos de memória e considerado um paradigma do aprendizado (MCGAUGH, 1966, 2000; GOLD, 1986; ROSE, 1995a,b; IZQUIERDO & MEDINA, 1997). Podemos citar como exemplo a sua rápida aquisição (segundos), o que facilita a análise do tempo de ocorrência de eventos pós-treino (GOLD, 1986; IZQUIERDO & MEDINA, 1995; IZQUIERDO et al., 1997; O`CONNELL et al, 1997); e sua farmacologia e bases moleculares, que já foram extensamente estudadas (IZQUIERDO & MEDINA, 1995; IZQUIERDO & MEDINA, 1997).

O teste de esquiva inibitória corresponde a muitos exemplos de importantes tipos de aprendizados em humanos, como lembra de evitar colocar os dedos em uma tomada de luz, atravessar uma rua sem olhar para os lados, visitar lugares perigosos ou

entrar em um bairro suspeito (IZQUIERDO et al., 2006). Na realidade, este teste representa um dos maiores determinantes do comportamento utilizado para a sobrevivência de todas as espécies (GOLD, 1986), e a rapidez de sua aquisição não deve subentender que ele é inato ou implícito (IZQUIERDO & MEDINA, 1997). De fato, existem vários componentes implícitos mensuráveis nesse teste (bradicardia) que, diferentemente de seus componentes explícitos (aumento do tempo de latência), são insensíveis ao choque eletro-convulsivo (HINE & PAOLINO, 1970; IZQUIERDO & MEDINA, 1997).

Além disso, em estudos farmacológicos, o teste de esquiva inibitória permite avaliar, conforme o momento de administração de uma droga, seu efeito na aquisição, consolidação ou evocação da memória (MCGAUGH, 1966; IZQUIERDO & McGAUGH, 2000). Em ambos trabalhos que compõem esta tese, a administração das soluções foi crônica devido ao efeito metabólico desejado e, portanto, não pudemos avaliar esses três processos separadamente. Contudo, pudemos verificar o efeito sobre a memória de longa duração dessa tarefa.

O *teste de habituação em campo aberto* foi originalmente descrito por Hall (1934) e tinha como objetivo testar os efeitos de ambientes não familiares sobre a emocionalidade em ratos.

O teste consiste na mensuração dos comportamentos eliciados pela colocação do sujeito experimental em um espaço novo e aberto do qual a fuga é prevenida por uma parede circundante (NAHAS, 1999). Esse é um dos tipos de tarefas de aprendizado não-associativo mais elementares: o comportamento de habituar-se a um ambiente novo (VIANNA, 2000).

Nos trabalhos realizados utilizamos o campo aberto de duas formas diferentes. No primeiro trabalho, realizamos somente a sessão de teste, na qual os animais eram observados por 2,5 minutos e utilizamos como parâmetros o tempo de latência para sair do primeiro quadrado (timidez), número de bolos fecais (emocionalidade), número de cruzamentos (taxa de ambulação – atividade motora) e número de *rearings* (levantar-se em duas patas - habituação) (NETTO et al., 1986). No segundo trabalho, os animais eram observados por 5 minutos divididos em dois intervalos de 2,5 minutos, para avaliar a resposta do animal a um ambiente novo e utilizamos como parâmetros número de cruzamentos e o número de *rearings* (VIANNA, 2000; NETTO et al, 1986).

O *Teste de Plus Maze* é um dos mais populares testes *in vivo* para animais utilizados atualmente. Embora ele seja frequentemente utilizado como uma ferramenta para classificar drogas de efeitos ansiolíticos (HANDLEY & MITHANI, 1984; PELLOW et al., 1985; LISTER, 1990; CAROBREZ & BERTOGLIO, 2005), atualmente ele tem sido útil para compreender as bases biológicas da emocionalidade relacionadas com aprendizado, memória, dor, hormônios, dependência assim como vários tipos de doenças relacionadas com ansiedade, como ansiedade generalizada, fobia e estresse pós-traumatico (ADAMEC et al., 1998; FILE et al., 1998; LAMPREA et al., 2000; CAROBREZ et al., 2001; RASMUSSEN et al., 2001; BANNERMAN et al., 2004; CAROBREZ & BERTOGLIO, 2005).

O aparelho é formado de dois corredores com um ângulo de $90^0$ entre eles, um com braços abertos e outro com braços fechados. O teste consiste em colocar do sujeito experimental no centro desse aparelho para explorá-lo livremente por 10 minutos. O número de entradas e a percentagem de tempo nos braços abertos são medidas do estado de ansiedade do animal (IZQUIERDO et al., 2002; PELLOW et al., 1985).

Por outro lado, ainda é uma questão de debate se o comportamento exploratório dos animais depende apenas da aversão aos braços abertos ou das características contrastantes dos braços fechados e abertos no plus maze. Mais trabalho é necessário para entender o estímulo responsável que faz com que os roedores evitem os braços abertos. Nesse contexto, a existência de pelo menos dois ambientes com diferentes níveis de aversão, braços abertos e fechados, parece ser um requisito para o animal desenvolver aversão aos braços abertos no plus maze (BERTOGLIO & CAROBREZ, 2000; SALUM et al.,2003; CAROBREZ & BERTOGLIO, 2005). Esse teste foi aplicado em ambos estudos realizados.

O *teste de Y-Maze* é um teste de dupla alternativa em condições que requerem orientação espacial egocêntrica, ao mesmo tempo que excluem orientação espacial alocêntica. A orientação espacial egocêntrica envolve o aprendizado de procedimento, também conhecido como implícito ou aprendizado de hábito (KNOWLTON et al., 1996; GRAYBIEL et al. 2000), e a orientação espacial alocêntica requer aprendizado declarativo, ou aprendizado explícito (KNOWLTON et al., 1996).

O teste é realizado em um labirinto de oito braços (radial maze) onde existem portas estilo guilhotina no final proximal de cada braço. O estado das portas (abertas/fechadas) é arranjado de forma que um "Y" é sempre acessível e o início sempre é no ponto distal do Y. Um *pellet* (bolinha de ração) é colocado no final distal do Y e na primeira corrida ambos braços são recompensados. Cada rato realiza dez corridas nos braços dos Y-maze, de forma a aprender a alternância do teste: o braço recompensado anteriormente não será o mesmo que será o próximo recompensado. O número de erros (entrada nos braços errados) e o tempo para o consumo do *pellet* são medidos. Para excluir condições que permitam a orientação alocêntrica, a orientação do

Y é modificada todos os dias em uma ordem pseudo-randomizada. (MASON et al., 1985; PYCH et al., 2006). Para estimular a realização deste teste, os animais têm seu suprimento de ração diário reduzido.

Realizamos o Y-maze apenas no tratamento crônico de 28 dias, pois no tratamento de 3 dias não seria viável um experimento que dura 8 dias.

### 1.4.3. Memória, LTP e Hormônios da Tireóide

Desde que a LTP *(Long-Term Potentiation* – Potenciação de Longa Duração) foi definida por Bliss e Lomo (1973), muitos autores se referiram a esta como sendo o mecanismo de formação da memória (IZQUIERDO, 1994; MALENKA & NICOLL, 1999); especialmente na literatura anterior a 1995 ou 1996 (IZQUIERDO & McGAUGH, 2000). A LTP foi imediatamente reconhecida como uma mudança neuronal capaz de prover uma base para a formação da memória (IZQUIERDO & McGAUGH, 2000).

Atualmente, a LTP é vista como uma forma bem caracterizada de plasticidade sináptica que preenche muitos dos critérios de um correlato neural da memória (COOK & BLISS 2006). A LTP resulta de elementos pré- e pós- sinápticos fazendo com que aja a facilitação da transmissão química que dura por horas *in vitro*, e podem persistir por períodos de semanas ou meses *in vivo* (BLISS & GARDNER-MEDWIN 1973; ABRAHAM et al., 2002, COOK & BLISS 2006). Não se pode concluir definitivamente que a LTP provê um mecanismo para as bases neurais do aprendizado e memória, mas ela é certamente um modelo fisiológico eloqüente desses processos (COOK & BLISS 2006).

A LTP foi estudada em uma série de modelos animais de várias espécies iniciando por camundongos (NOSTEN-BERTRAND et al., 1996) até macacos (URBAN et al., 1996), e a um número de diferente de sinapses através do SNC, do neocórtex cerebral (FOX, 2002) até a medula espinhal (JI et al., 2003). Recentemente, Cook e Bliss (2006) demonstraram que a LTP pode ser induzida no SNC de humanos e que provavelmente compartilha as mesmas bases moleculares já descritas para esse fenômeno em roedores. Além disso, demonstraram que déficits em LTP estão correlacionados com déficits hipocampo-dependentes em humanos (COOK & BLISS 2006).

Alterações nos hormônios da tireóide causam mudanças na LTP (GERGES et al., 2001; SUI et al., 2006).Estudos eletrofisiológicos demonstraram que o hipotireoidismo induzido pela exposição a drogas anti-tireóide (propiltiouracil – PTU), altera a transmissão sináptica e a plasticidade na região CA1 do hipocampo de ratos neonatos (NIEMI et al., 1996; SUI & GILBERT, 2003; VARA et al., 2002; SUI et al., 2005). Recentemente, Sui e colaboradores (2006) demonstraram em um estudo eletrofisiológico em ratos que a LTP e a PPF (*Pared-Pulse Facilitation* – Facilitação de Pulso Pareado), uma outra forma de plasticidade sináptica de curta duração que se acredita ser um mecanismo importante para o aprendizado (ZUCKER & REGEHR, 2002; CHAPMAN et al., 1995; SILVA et al., 1996, SUI et al., 2006), estão ambas inibidas no hipotireoidismo induzido. Essas inibições de LTP e PPF são consistentes com as disfunções neurológicas e cognitivas características do hipotireoidismo em adultos (BALDINI et al., 1997; DUGBARTEY, 1998; JOFFE & SOKOLOV, 1994 , SUI et al., 2006). Além disso, nesse mesmo trabalho havia um grupo de animais em que o hipotireoidismo induzido era modificado ao estado eutireoidiano pela reposição

hormonal com hormônio da tireóide, o resultado foi a melhora dos déficits de LTP e PPF o que levou a conclusão de que isso pode estar correlacionado com a melhora no aprendizado e na memória observados nos casos de reposição hormonal em pacientes com hipotireoidismo (DUGBARTEY, 1998;SUI ET AL., 2006).

### 1.5. $Na^+,K^+$-ATPase (ATP fosfoidrolase, EC 3.6.1.3)

A $Na^+,K^+$-ATPase é uma proteína integral de membrana responsável pelo transporte ativo de íons $Na^+$ e $K^+$ através das membranas das células da maioria dos eucariotos (KAPLAN, 2002).

Ela transloca três íons $Na^+$ e dois íons $K^+$ contra os seus gradientes de concentração através da membrana celular, utilizando ATP como força motriz. Com isso, produz-se um gradiente eletroquímico através da membrana celular, que é essencial para a atividade elétrica nos tecidos excitáveis além de auxiliar no movimento de outros íons e nutrientes através da membrana como uma atividade secundária (BERTORELLO & KATZ, 1995). Além disso, este gradiente é usado como fonte de energia para a formação, despolarização e repolarização do potencial de membrana, para a manutenção e regulação do volume celular, transporte ativo glicose-dependente de $Na^+$, aminoácidos, neurotransmissores e para cotransporte/antiporte de outros íons (GEERING, 1990).

No SNC a $Na^+,K^+$-ATPase tem um papel fundamental, pois o gradiente transmembrana permite o transporte $Na^+$-dependente de cálcio e aminoácidos e a recaptação de neurotransmissores (SWEADNER, 1989; BLANCO & MERCER, 1998; LINGREL et al., 2003; TAGUCHI et al., 2007), está implicada na excitabilidade neural

(SASTRY & PHILLIS, 1977), na produção metabólica de energia (MATA et al.,1980), na captação e liberação das catecolaminas (BOGDANSKI et al., 1968) e da serotonina (SCHULPIS et al., 2007). Na sinapse, a atividade desta ATPase contribui diretamente para a excitabilidade da membrane através da manutenção dos gradientes de $Na^+$ e $K^+$ e indiretamente por processos de transporte de muitos solutos incluindo o $Ca^{2+}$(TAGUCHI et al., 2007). Algumas evidências demonstram que essa enzima pode ter um papel nos processos de formação e consolidação da memória (REIS et al., 2002; WYSE et al., 2004). Além disso, a $Na^+$,$K^+$-ATPase consome de 40 a 50% do ATP gerado no cérebro e está em alta concentração neste tecido(ERECISKA & SILVER, 1994).

A diminuição da atividade da $Na^+$,$K^+$-ATPase parece estar relacionada com disfunções neurológicas de algumas neuropatologias (LEES, 1993; ERECISKA & SILVER, 1994), como a epilepsia (GRISAR et al. 1992), a doença de Alzheimer (LIGURI et al., 1990; HATTORI et al., 1998; SANTOS & PÉREZ-CASTILLO 2000), além de estar envolvida na patogênese de doenças do humor e doenças depressivas (GOLDSTEIN et al., 2006).

Os hormônios da tireóide estimulam a atividade da $Na^+$,$K^+$-ATPase no coração de algumas espécies (SHAO et al., 2000; BOOK et al., 1997). Essa enzima sofre importante ação dos hormônios da tireóide durante o desenvolvimento cerebral (SCHMITT & MCDONOUGH, 1988), além disso, a falha de sua atividade no cérebro de ratos está relacionada a um dos possíveis mecanismos bioquímicos que permitem a disfunção neurológica presente no hipotireoidismo neonatal (BILLIMORIA et al., 2006). Recentemente, foi demonstrado o efeito de modelos experimentais de hipo e

hipertireoidismo sobre a atividade da $Na^+,K^+$-ATPase em algumas estruturas cerebrais de ratos (CARAGEORGIOU et al., 2005 e 2007).

## 2. Objetivos

Considerando que:

a) Os hormônios da tireóide são fundamentais ao SNC seu excesso ou sua deficiência estão relacionados com uma série de disfunções neurológicas e comportamentais;

b) Estes hormônios parecem estar envolvidos nos mecanismos de formação da memória, pois sua falta está relacionada ao déficit cognitivo e de memória geralmente presentes como sintomas no hipotireoidismo;

c) A $Na^+,K^+$-ATPase é uma enzima que tem especial importância no SNC por estar envolvida no transporte de íons e neurotransmissores e parece apresentar um papel nos mecanismos de formação de consolidação da memória;

d) Esta enzima sofre ação dos hormônios da tireóide em diferentes tecidos e estruturas cerebrais; o presente trabalho tem como objetivos verificar:

1- O efeito da administração crônica de L-T4 ou PTU sobre alguns paradigmas do comportamento, de forma a verificar o efeito dos modelos de hiper e hipotireoidismo crônicos sobre a memória dessas tarefas.

2- O efeito da administração crônica de L-T4 ou PTU sobre a atividade da $Na^+,K^+$-ATPase de membrana plasmática sináptica de hipocampo e de córtex parietal de ratos adultos.

3- O efeito da administração aguda de L-T4 sobre alguns paradigmas do comportamento, de forma a verificar o efeito do modelo de hipertireoidismo agudo sobre a memória dessas tarefas.

4- O efeito da administração aguda de L-T4 sobre a atividade da $Na^+,K^+$-ATPase de membrana plasmática sináptica de hipocampo e de córtex parietal de ratos adultos.

**PARTE II**

## CAPÍTULO I

Efeitos dos hormônios da tireóide sobre a memória e sobre a atividade da $Na^+,K^+$-ATPase no cérebro de ratos.

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# Effects of Thyroid Hormones on Memory and on $Na^+$, $K^+$-ATPase Activity in Rat Brain

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**Abstract:** Thyroid hormones (THs), including triiodothyronine (T3) and tetraiodothyronine (T4), are recognized as key metabolic hormones of the body. THs are essential for normal maturation and function of the mammalian central nervous system (CNS) and its deficiency, during a critical period of development, profoundly affects cognitive function. Sodium-potassium adenosine 5'-triphosphatase ($Na^+$, $K^+$-ATPase) is a crucial enzyme responsible for the active transport of sodium and potassium ions in the CNS necessary to maintain the ionic gradient for neuronal excitability. Studies suggest that $Na^+$, $K^+$-ATPase might play a role on memory formation. Moreover, THs were proposed to stimulate $Na^+$, $K^+$-ATPase activity in the heart of some species. In this work we investigated the effect of a chronic administration of L-thyroxine (L-T4) or propylthiouracil (PTU), an antithyroid drug, on some behavioral paradigms: inhibitory avoidance task, open field task, plus maze and Y-maze, and on the activity of $Na^+$, $K^+$-ATPase in the rat parietal cortex and hippocampus. By using treatments which have shown to induce alterations in THs levels similar to those found in hyperthyroid and hypothyroid patients, we aimed to understand the effect of an altered hyperthyroid and hypothyroid state on learning and memory and on the activity of $Na^+$, $K^+$-ATPase. Our results showed that a hyper and hypothyroid state can alter animal behavior and they also might indicate an effect of THs on learning and memory.

**Key words:** Thyroid hormones, $Na^+$, $K^+$-ATPase, L-thyroxine, propylthiouracil, learning, memory.

## INTRODUCTION

Thyroid hormones (THs), including triiodothyronine (T3) and tetraiodothyronine (T4), are recognized as key metabolic hormones of the body, with T3 being the most functionally active form. THs have many physiological actions, and essentially modulate all metabolic pathways through alterations in oxygen consumption and changes in protein, lipid, carbohydrate, and vitamin metabolism. Alterations in structure, function and behavior as a consequence of thyroid dysfunction have highlighted the importance of these hormones, especially for central nervous system (CNS) development and maintenance (Smith *et al.*, 2002).

THs are essential for normal maturation and function of the mammalian CNS (Oppenheimer & Schwartz, 1997; Santos & Pérez-Castillo, 2000; Darbra *et al.*, 2003; Sui *et al.*, 2005) and its deficiency, during a critical period of development, profoundly affects cognitive functions (Vara *et al.*, 2002; Dong *et al.*, 2005). Hypothyroidism during human perinatal development results in profound alterations of mental capacities, neurological functions and metabolic processes (Darbra *et al.*, 2003; Dong *et al.*, 2005). The possibility of cognitive dysfunction, even in minor degrees of thyroid failure, has been pointed by some authors on the basis of neuropsychiatric consequences of myxedema in humans (Sher, 2001; Baldini *et al.*, 1997; Monzani *et al.*, 1993; Haggerty *et al.*, 1990). Several studies with hypothyroid patients usually reported cognitive impairments (Burmeister *et al.*, 2001; Roberts & Ladenson, 2004; Zhu *et al.*, 2006). Impairment of memory performance was also described in patients with subclinical hypothyroidism, which was partially reversed by replacement with L-thyroxine (L-T4) (Zhu *et al.*, 2006, Baldini *et al.*, 1997; Haggerty *et al.*, 1990; Nystrom *et al.*, 1988). Cognitive difficulties may be protean, but severe memory deficit is frequent in Hashimoto's encephalopathy, a steroid-responsive encephalopathy of unknown etiology associated with high titers of serum antithyroid (usually anti-TPO) antibodies (Mocellin *et al.*, 2006).

However, hyperthyroidism is characterized by the excess of THs secretion, which also causes many neurological and

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behavioral symptoms, including an increase in the frequency of alpha-rhythm, irritability, anxiety and restlessness progressing to nervousness, tremulousness, tachycardia, sleep disturbances, whilst paranoia and, in most severe patients, symptoms of mania and depression (Smith *et al.*, 2002). Even so, the relationship between thyroid status and cognitive or psychiatric disturbances remains unclear (Constant *et al.*, 2001; Siesser *et al.*, 2005; Dong *et al.*, 2005).

Sodium-potassium adenosine 5'-triphosphatase ($Na^+$, $K^+$-ATPase) is a crucial enzyme responsible for the active transport of sodium and potassium ions in the CNS necessary to maintain the ionic gradient for neuronal excitability. It is present at high concentrations in the brain, consuming about 40-50% of the ATP generated in this tissue (Ericinska & Silver, 1994). Evidence suggests that $Na^+$, $K^+$-ATPase might play a role on memory formation (Wyse *et al.*, 2004; Reis *et al.*, 2002). THs were proposed to stimulate $Na^+$, $K^+$-ATPase activity in the heart of some species (Shao *et al.*, 2000; Book *et al.*, 1997). It was also demonstrated that $Na^+$, $K^+$-ATPase suffers an important action of THs during brain development (Schmitt & McDonough, 1988).

Considering that (a) THs have a relevant action on the CNS and its deficiency or excess can cause neurological and behavior abnormalities, (b) $Na^+$, $K^+$-ATPase can suffer alterations on its activity by THs action in different tissues, (c) and this enzyme seems to play a role on memory formation, we decided to investigate the effect of a chronic administration of L-T4 or propylthiouracil (PTU) - an antithyroid drug - on some behavioral paradigms: inhibitory avoidance task, open field task, plus maze and Y-maze, and the activity of $Na^+$, $K^+$-ATPase in the rat parietal cortex and hippocampus. By using treatments which have shown to induce alterations in THs levels similar to those found in hyperthyroid and hypothyroid patients, we aimed to understand the effect of an altered hyperthyroid and hypothyroid state on learning and memory and on the activity of $Na^+$, $K^+$-ATPase.

## MATERIAL AND METHODS

### Animals and Reagents

Male Wistar 45-day-old rats were obtained from the Central Animal House of the Biochemistry Department at the Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil. Animals were maintained on a 12:12 h light/dark cycle in an air-conditioned constant temperature, colony room, with free access to water and a 20% (w/w) protein commercial chow (Germani, Porto Alegre, RS, Brazil). All chemicals were purchased from Merck (Darmstadt, Germany).

### Drug Administration Procedure

*Experiment 1:* Seventy animals were divided into four groups (A, B, C and D). Group A animals were treated for 14 days with daily intraperitoneal (i.p.) administration of L-T4 (25 µg/100g of body weight) to induce the hyperthyroidism animal model (Hu *et al.*, 2003) and group B received saline (0.9 % NaCl) as a control ($n$ = 20 animals per group). After this pre-treatment, which was important to elevate THs levels in the animals' blood, rats were submitted to behavior procedures. During the experimental days rats still received daily i.p. injections of L-T4 or saline, which lasted for 14 more days: the treatment lasted for 28 days in total. Group C was also treated daily with i.p. administration of L-T4 (25 µg/100g of body weight) and group D received saline (0.9 % NaCl) as a control ($n$ = 15 animals per group). Groups C and D were also treated for 28 days using the same drug administration procedure, but they were not assessed behaviorally.

*Experiment 2:* To induce the hypothyroidism animal model we used a protocol previously established (Duarte *et al.*, 2000), with some modifications. Sixty animals were divided into four groups (F, G, H and I). Group F animals were treated for 14 days with daily oral administration of PTU (50 mg/200g of body weight) and group G with water by gavage ($n$ =15 animals per group). As in the hyperthyroidism model, after the pre-treatment, which was important to lower THs levels in the animals' blood, rats were submitted to behavioral procedures. During experimental days rats still received daily oral administration of PTU or water, which lasted for 14 more days: the treatment lasted for 28 days in total. Group H was also treated daily with oral administration of PTU (50 mg/200g of body weight) and group I received water by gavage as control ($n$ =15 animals per group). Groups H and I were also treated for 28 days using the same drug administration procedure, but they were not assessed behaviorally.

### Experimental Protocols

After the pre-treatment of 14 days, 24h after the last administration, groups A and B animals (experiment 1) or groups F and G animals (experiment 2) were subjected to a step-down inhibitory avoidance task, open field and plus maze in that order. After that, for 2 days, water was continuously available but the amount of food was reduced (12g of standard lab chow per animal per day) in order to increase motivation to perform the Y-maze task. During the 8 experimental days in Y-maze, water was continuously available and food was provided in the late afternoon of each day after the experiment (12g of standard lab chow per animal per day). Table **1a** shows the experimental protocol used for the groups of animals assessed behaviorally in both experiments.

After 28 days of treatment, 24h after the last administration, blood samples were collected by cardiac puncture from groups C and D animals (experiment 1) or from groups H and I animals (experiment 2) ($n$ =10 animals of each group) and serum was separated by centrifugation and stored at -$20^0$C for T3, T4 and TSH determination. Animals randomly selected from groups C and D (experiment 1) or from groups H and I (experiment 2) ($n$ = 5 animals of each group) were killed by decapitation and the brains rapidly extracted. The brain was dissected on ice-cooled glass plates, and the parietal cortex and hippocampus were removed for the determination of $Na^+$, $K^+$-ATPase activity. Table **1b** shows the experimental protocol used for the groups of animals not assessed behaviorally in both experiments.

### Behavioral Procedures

*Step-down inhibitory avoidance task*: Animals were subjected to training and test sessions in a step-down inhibitory

**Table 1a. Experimental Protocol Used for Groups of Animals Assessed Behaviorally in Both Experiments. Rats Received the Treatment During 28 Days in Total**

| | |
|---|---|
| From day 1 to 14 | Groups A, B / F, G<br>Pre-treatment period to induce hyper or hypothyroidism animal model. |
| Days 15 and 16 | Animals were subjected to a step-down inhibitory avoidance task. |
| Day 17 | Animals were subjected to open field. |
| Day 18 | Animals were subjected to plus maze. |
| Days 19 and 20 | The amount of food was reduced (12g of standard lab chow per animal per day) in order to increase motivation to perform the Y-maze task. Water was continuously available. |
| From day 21 to 29 | Animals were subjected to 8 experimental days in Y-maze. Food was provided in the late afternoon of each day after the experiment (12g of standard lab chow per animal per day). Water was continuously available. |

**Table 1b. Experimental Protocol Used for Groups of Animals Not Assessed Behaviorally in Both Experiments. Rats Received the Treatment During 28 Days in Total**

| | |
|---|---|
| From day 1 to 28 | Groups C, D / H, I<br>Animals received the treatment to induce hyper or hypothyroidism |
| Day 29 | **-Blood samples**: Blood samples were collected by cardiac puncture and serum was separated by centrifugation and stored at -20$^0$C for T3, T4 and TSH determination.<br>- **$Na^+$, $K^+$-ATPase activity assay**: Animals were killed by decapitation and the brains rapidly extracted. The brain was dissected on ice-cooled glass plates, and the parietal cortex and hippocampus were removed for the determination of $Na^+$, $K^+$-ATPase activity. |

avoidance task, with an interval of 24h in between (Izquierdo & Medina, 1997; Barros *et al.*, 2004). This task is an established model of aversively motivated, hippocampus-dependent memory that involves learning not to step down from a platform in order to avoid a mild foot shock (Izquierdo & Medina, 1997; Izquierdo & McGaugh, 2000). The task was carried out in an automatically operated, brightly illuminated box. The left extreme of the grid was covered by a 7.0 cm wide, 2.5 cm high formic platform. Animals were placed on the platform and their latency to step down placing their four paws on the grid (42.0 X 25.0 cm grid of parallel 0.1 cm caliber stainless steel bars spaced 1.0 cm apart) was measured. In test sessions, no foot shock was used and step-down latency (with a ceiling of 180 s) was used as a measure of memory retention as described in previous reports (Barros *et al.*, 2006; Coitinho *et al.*, 2006; Roesler *et al.*, 2006; Izquierdo & McGaugh, 2000; Izquierdo & Medina, 1997).

*Open-field habituation*: This task was run in a wooden box measuring 60 x 40 x 50 cm with a frontal glass wall, whose floor was divided with black lines into 12 squares. The animals were gently placed facing the rear left corner of the arena and observed for 2.5 min. The latency to leave the first square (timidity) and defecation (number of stools) were taken as measures of the rats' emotionality. The number of crossings from one square to another is indicative of motor activity and the number of rearing responses is a measure of habituation (Netto *et al.*, 1986).

*Plus maze*: The elevated plus maze consisted of four 9 cm wide, 45 cm long corridors placed at 90° angles and 88 cm above the room floor level. The middle crossing between the four corridors was 9 cm$^2$ large and was open. Two opposite corridors were surrounded by 26 cm high plywood walls (closed arms), and the other two were not (open arms). Rats were placed in the middle of the four arms and left to explore the apparatus freely for 10 min. The number of entries and percentage of time in the open arms was a measure of the state of anxiety of the animals (Izquierdo *et al.,* 2002; Pellow *et al.*, 1985).

*Y-maze*: Experiments were done with the aid of an eight-arm-labyrinth, which consisted of one central platform with a diameter of 40 cm that was connected to eight arms of 55.5 cm length, 15 cm width and 22.5 cm height. The arms were arranged radially round the platform with 45º between each. Guillotine-doors existed at the proximal end of each arm. The state of the doors (open/closed) was arranged in a manner that a "Y" was always accessible and the starting point always laid in the distal end of the Y. A pellet was placed at the distal end of each arm and the starting was never rewarded. During the experiment, a single animal had to perform up to ten delayed two-alternative-choice runs on the Y arms. The rats had to leave the starting arm and enter one of two other possible arms. Only one arm was rewarded with a food pellet in alternate trials, with the exception of the first run, where both arms were rewarded. Time to pellet con-

sumption was measured. Time limit was set to 180 sec. In order to exclude conditions that allowed allocentric orientation, the orientation of the Y was changed every experimental day in a pseudo-randomized order (Mason *et al.*, 1985; Pych *et al.*, 2006). During the eight experimental days, the animals received only 12g of food each per day.

### Preparation of Synaptic Plasma Membrane from the Hippocampus and the Parietal Cortex

Membranes from the hippocampus and the parietal cortex were prepared according to a well-established method (Jones & Matus, 1974), with some modifications (Wyse *et al.*, 2000). The structures were homogenized in 10 volumes of a 0.32M sucrose solution containing 5 mM HEPES and 1 mM EDTA. The homogenate was centrifuged at 1000Xg for 20min and supernatant removed and centrifuged at 12.000g for further 20 min. The pellet was then resuspended in hypotonic buffer (5.0 mM Tris-HCl buffer, pH 8.1), incubated at $0^{o}$C for 30 min, and applied to a discontinuous sucrose density gradient consisting of sucrose layers of 0.3, 0.8 and 1.0 M. After centrifugation at 69.000Xg for 2h, the fraction at the 0.8-1.0 M sucrose interface was taken as a membrane enzyme preparation.

### $Na^+$, $K^+$-ATPase Activity Assay

The reaction mixture for the $Na^+$, $K^+$-ATPase assays contained 5.0 mM $MgCl_2$, 80.0 mM NaCl, 20.0 mM KCl, 40.0 mM Tris-HCl buffer, pH 7.4, in a final volume of 200 µL. The reaction was started by the addition of ATP (disodium salt, vanadium free) to a final concentration of 3.0 mM. Control assays were carried out under the same conditions with the addition of 1.0 mM ouabain. $Na^+$, $K^+$-ATPase activity was calculated by the difference between the two assays (Wyse *et al.*, 2000). Released inorganic phosphate (Pi) was measured (Chan *et al.*, 1986). Enzyme specific activities were expressed as nmol Pi released per min per mg of protein. All assays were performed in duplicate and the mean was used for statistical analysis.

### Protein Determination

Protein was measured according to a well-described method (Bradford, 1976) using a bovine serum albumin as standard.

### Determination of Thyroid Hormones in Serum

To evaluate the treatment we measured the THs serum levels. Total T3, T4 and TSH in serum were measured by a chemiluminescent immunoassay (Immulite®, DPC, CA, USA), according to suppliers' instructions.

### Statistical Analysis

Training-test latency differences of inhibitory avoidance task were assessed by Wilcoxon test and test latency scores were analyzed among different groups by individual Mann-Whitney U-test (two tailed). Open field and plus maze behaviors were analyzed by unpaired Student t-test. In the Y-maze task, running-time and amount of errors were measured on each experimental day. For analysis of an effect over time, the data from day one to eight were analyzed using the Kruskal-Wallis One-Way Analysis of Variance (ANOVA), for each group separately. To test for differences between the test and the control group, Wilcoxon Rank Sum and Mann-Whitney U-Test were done. The Kruskal-Wallis One-Way Analysis of Variance (ANOVA) was used for measurement of the amount of errors over time. For testing group differences, Wilcoxon Rank Sum and Mann-Whitney U-Test were chosen. Data from $Na^+$, $K^+$-ATPase activity were analyzed by unpaired Student t-test, and data from determination of THs in serum were also analyzed by unpaired Student t-test.

## RESULTS

Fig. (**1a**) demonstrates the effect of a chronic treatment of L-T4 on training and test sessions of a step-down inhibitory avoidance task. Latency differences in training were not significant among control and L-T4 groups in Mann-Whitney U test (U= 243.5, $p$ = 0.3694) followed by Wilcoxon test ($p$ = 0.4337). Latency differences in test performance were significant among control and L-T4 groups according to Mann-Whitney U test (U= 88, $p$<0.0001), followed by Wilcoxon test ($p$ = 0.0002). Fig. (**1b**) demonstrates the effect of a chronic treatment of PTU on training and test sessions of a step-down inhibitory avoidance task. Latency differences in training were not significant among control and PTU groups in Mann-Whitney U test (U= 67, $p$=0.5679) followed by Wilcoxon test ($p$= 0.6772). Latency differences in test performance were significant among control and PTU

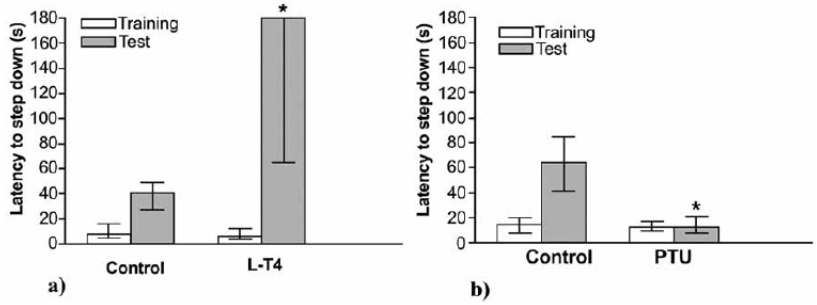


**Fig. (1).** Effect of chronic administration of (a) LT4 and (b) PTU on test session performance of step-down inhibitory avoidance task. (a) Data are median (interquartile range) of 20 animals in each group. *Different from the control group (Mann-Whitney: p<0.0001; Wilcoxon: p=0.0002). (b) Data are median (interquartile range) of 15 animals in each group. *Different from the control group (Mann-Whitney: p=0.0001; Wilcoxon: p= 0.0005).

groups according to Mann-Whitney U test (U= 7.0, $p< 0.001$), followed by Wilcoxon test ($p< 0.001$).

Table **2a** and **b** shows the effect of a chronic treatment of L-T4 or PTU, respectively, on open field behavior. Neither L-T4-treated rats nor PTU-treated rats display changes in any measures taken in the arena when compared to controls using unpaired Student t-test. These results can suggest that the effects obtained in the inhibitory avoidance task are not due to any impairment in locomotor activity.

**Table 2a. Effect of L-T4 Administration on Open Field Behavior in Adult Rats**

| | Treatment | |
|---|---|---|
| | **Saline** | **L-T4** |
| Latency to leave the first square (s) | 5.84 ±0.56 | 6.61 ± 0.81 |
| Number of crossings | 44.04 ± 2.4 | 42.41 ± 2.89 |
| Number of rearings | 16.10 ± 1.29 | 17.76 ± 1.57 |
| Number of fecal boli | 5.76 ± 0.56 | 5.65 ± 0.49 |

Data are mean ± S.E.M. ($n$=20 animals per group).

**Table 2b. Effect of PTU Administration on Open Field Behavior in Adult Rats**

| | Treatment | |
|---|---|---|
| | **Saline** | **PTU** |
| Latency to leave the first square (s) | 7.06 ± 1.03 | 8.26 ± 0.98 |
| Number of crossings | 45.60 ± 3.51 | 35.60 ± 3.96 |
| Number of rearings | 23.53 ± 2.32 | 19.13 ± 2.45 |
| Number of fecal boli | 5.67 ± 0.69 | 4.60 ± 0.51 |

Data are mean ± S.E.M. ($n$=15 animals per group).

Table **3** shows the effect of a chronic treatment of L-T4 or PTU on elevated plus maze in order to investigate a pro- or anti-conflict behavior. The performance of L-T4-treated rats as well as PTU-treated rats was not affected when compared to controls using unpaired Student t-test. These results can suggest that the effects obtained in the inhibitory avoidance task are not due to the state of anxiety of the animals.

Fig. (**2a** and **b**) demonstrates the effects of a chronic treatment of L-T4 on a delayed two-alternative-choice-task in a Y-maze during eight experimental days. (a) Running Time: Kruskal-Wallis One-Way ANOVA revealed a highly significant decrease ($p<0.0001$) in running time in L-T4-treated rats as well as in controls from day one to day eight, which means that both groups decreased the time up to consumption of the pellet during the experimental days. According to Mann-Whitney U-Test, there are significant differences between both groups from day 1 to day 8. These results suggest that L-T4-treated rats were able to find the pellet quickly. (b) Errors: Kruskal-Wallis One-Way ANOVA revealed a significant decrease in errors in the control group from day one to day eight ($\chi^2= 17.71$, $p= 0.0133$), although it revealed a non-significant decrease in errors in the L-T4 group from day one to day eight. Mann-Whitney U-Test showed significant differences between both groups only on day 7 ($p$=0.0046) (Fig. **2**: see legend for statistical details).

**Table 3. Effect of L-T4 or PTU Administration on Plus Maze Behavior in Adult Rats**

| Treatment | *n* | % Time in open arms | Open arms entries |
|---|---|---|---|
| Saline | 20 | 33.94 ± 3.65 | 6.43 ± 0.67 |
| L-T4 | 20 | 28.04 ± 3.29 | 6.61 ± 1.03 |
| | | | |
| Saline | 15 | 48.53 ± 7.60 | 2.47 ± 0.35 |
| PTU | 15 | 29.71 ± 5.62 | 1.80 ± 0.26 |

Data are mean ± S.E.M.

Fig. (**3a** and **b**) demonstrates the effects of a chronic treatment of PTU on a delayed two-alternative-choice-task in a Y-maze during eight experimental days. (a) Running Time: Kruskal-Wallis One-Way ANOVA revealed a significant decrease ($p<0.0001$) in running time in controls from day one to day eight, which means that only this group decreased the time up to consumption of the pellet during the experimental days. Mann-Whitney U-Test showed significant differences between both groups on all experimental days. (b) Errors: Kruskal-Wallis One-Way ANOVA revealed a non-significant decrease in errors in both groups from day one to day eight. Mann-Whitney U-Test showed non-significant differences between both groups on each experimental day (Fig. **3**: see legend for statistical details).

The effect of L-T4 or PTU on $Na^+$, $K^+$-ATPase activity in the rat brain is demonstrated on Table **4**. The results show that L-T4 chronic administration caused a 57% inhibition of $Na^+$, $K^+$-ATPase activity in the synaptic plasma membrane from the hippocampus of rats (Student t-test, $p<0.001$) and did not cause alteration of $Na^+$, $K^+$-ATPase activity in the synaptic plasma membrane from the parietal cortex of rats (Student t-test, $p= 0.8344$). PTU chronic administration caused a 70% inhibition of $Na^+$, $K^+$-ATPase activity in the synaptic plasma membrane from the hippocampus of rats (Student t-test, $p<0.05$) and did not cause alteration of $Na^+$, $K^+$-ATPase activity in the synaptic plasma membrane from the parietal cortex of rats (Student t-test, $p= 0.2645$).

The serum profile of THs of the animals is demonstrated in Table **5**. In the L-T4-treated group alterations in total T3, T4 and TSH were significantly different from the control group according to Student t-test ($p<0.01$ in all cases). The results showed an elevation of total T3 serum levels, which is the most functionally active form of THs. We can also observe a decrease of TSH and total T4 serum levels, which demonstrates that this treatment simulated a chemical hyperthyroid state that caused a feed-back negative response on the release of the THs into the systemic circulation. In the

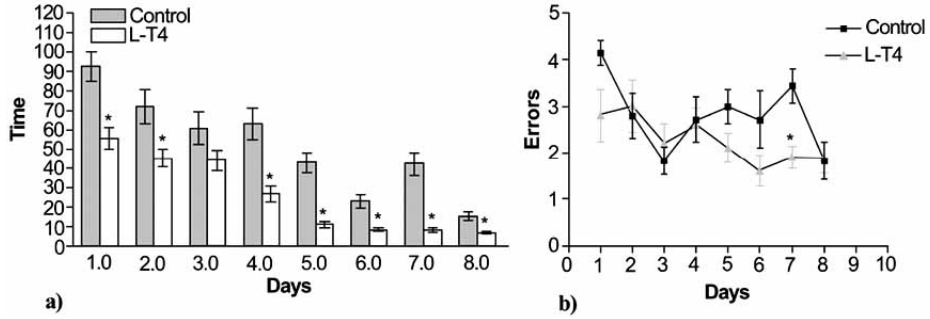


**Fig. (2).** Effect of daily administration of L-T4 treatment on a delayed two-alternative- choice-task in a Y-maze, under conditions that required egocentric spatial orientation. Rats had to perform up to 10 runs per experimental day ('Days'). **(a)** For running time ('Time'), time up to consumption of the pellet was measured; runs that exceeded 180 seconds were excluded. Data shown are means ± S.E.M. Kruskal-Wallis One-Way ANOVA revealed a highly significant ($p<0.0001$) decrease in running time in both groups from day one to day eight (data not shown in graphic). Mann-Whitney U-Test showed significant (*) differences of both groups on day 1 ($p<0.0001$), day 2 ($p= 0.0063$), day 4 ($p<0.0001$), day 5 ($p<0.0001$), day 6 ($p<0.0001$), day 7 ($p<0.0001$) and day 8 ($p<0.0001$). **(b)** A run into a not rewarded arm was counted as an error ('Errors'). Data shown are means ± S.E.M. Kruskal-Wallis One-Way ANOVA revealed a significant decrease in errors in the control group from day one to day eight ($\chi^2= 17.71$, $p= 0.0133$), although it revealed a non-significant decrease in errors in the L-T4 group from day one to day eight ($\chi^2=8.60$, $p=0.2829$) (data not shown in graphic). Mann-Whitney U-Test showed significant (*) differences of both groups only on day 7 ($p=0.0046$).

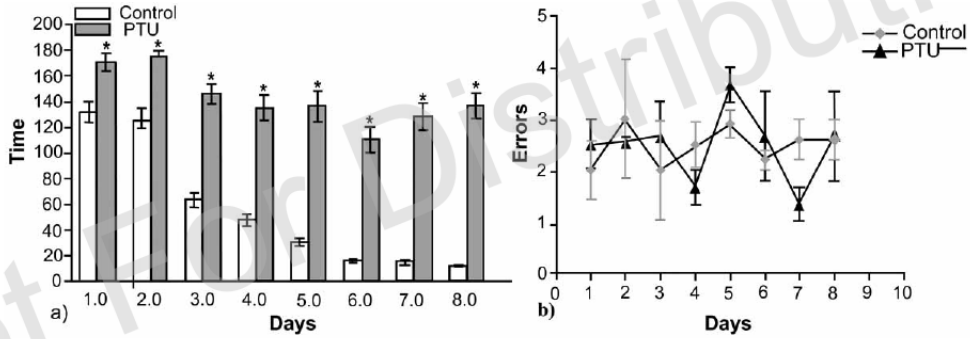


**Fig. (3).** Effect of daily administration of PTU treatment on a delayed two-alternative- choice-task in a Y-maze, under conditions that required egocentric spatial orientation. Rats had to perform up to 10 runs per experimental day ('Days'). **(a)** For running time ('Time'), time up to consumption of the pellet was measured; runs that exceeded 180 seconds were excluded. Data shown are means ± S.E.M. Kruskal-Wallis One-Way ANOVA revealed a significant ($p<0.0001$) decrease in running time in both groups from day one to day eight (data not shown in graphic). Mann-Whitney U-Test showed significant (*) differences of both groups on day 1 ($p= 0.0046$), day 2 ($p<0.0001$), day 3 ($p<0.0001$), day 4 ($p<0.0001$), day 5 ($p<0.0001$), day 6 ($p<0.0001$), day 7 ($p<0.0001$), day 8 ($p<0.0001$). **(b)** A run into a not rewarded arm was counted as an error ('Errors'). Data shown are means ± SEM. Kruskal-Wallis One-Way ANOVA revealed a non-significant decrease in errors in both groups from day one to day eight (Control: $\chi^2= 3.13$, $p= 0.8722$; PTU: $\chi^2=8.82$, $p=0.2661$). Mann-Whitney U-Test showed non-significant differences between both groups on each experimental day.

PTU-treated group alterations in total T3, T4 and TSH were significantly different from the control group according to Student t-test ($p<0.01$ in all cases). The results showed a decrease of total T3 and T4 serum levels. The results also demonstrated an elevation of TSH serum levels, which showed that this treatment simulated a chemical hypothyroid state.

## DISCUSSION

THs mediate important effects within the CNS throughout life and alterations of the thyroid axis seem to contribute to a variety of CNS-related pathologies. Hyper and hypothyroidism have been associated with many neurological symptoms, including mania and memory impairment, respectively. In elderly hypothyroid patients, dementia seems to be a dominant secondary characteristic (Loosen, 1992; Landi *et al.*, 2007). Adult-onset thyroid dysfunction is associated with both neurological and behavioral abnormalities, emphasizing the importance of THs for normal brain function (Smith *et al.*, 2002; Dong *et al.*, 2005; Sui *et al.*, 2005).

The aim of the present study was to investigate the effects of alteration of thyroid axis on learning and memory in experimental animal models. We used the experimental animal model of chronic hyperthyroidism (Hu *et al.*, 2003) and

hypothyroidism (Duarte *et al.*, 2000) in two distinct experiments with the same experimental protocols. By doing so, we aimed to compare these two pathological conditions in several behavioral tasks. We also investigated the effect of these two experimental models on $Na^+$, $K^+$-ATPase activity in the hippocampus and parietal cortex.

**Table 4. Effect of L-T4 or PTU on $Na^+$, $K^+$-ATPase Activity in the Synaptic Plasma Membrane from the Hippocampus and the Parietal Cortex of Rats**

| Treatment | *n* | Hippocampus | Parietal Cortex |
|---|---|---|---|
| Saline | 5 | 1088.42 ± 96.57 | 830.60 ± 138.14 |
| L-T4 | 5 | 620.65 ± 116.22* | 856.30 ± 190.49 |
| | | | |
| Saline | 5 | 1246.00 ± 278.80 | 1261.00 ± 236.00 |
| PTU | 5 | 877.50 ± 54.72* | 1510.00 ± 328.40 |

Data are mean ± S.D. for five independent experiments performed in duplicate and it is expressed by nmol Pi/min.mg protein. * Different from the control group, $p<0.05$.

Our results indicated that a chronic treatment with L-T4 for 14 days can improve the performance of an inhibitory avoidance task when compared to controls (Fig. **1a**). On the other hand, a chronic treatment with PTU also for 14 days impaired the test performance of the same task when compared to controls (Fig. **1b**). These results are in agreement with other studies showing that a hyper and hypothyroid state can alter animal behavior (Redei *et al.*, 2001; Smith *et al.*, 2002; Darbra *et al.*, 2003; Wilcoxon *et al.*, 2007). Furthermore, perturbations in thyroid hormone function are associated with behavioral impairments in tasks requiring integrity of the hippocampus (Akaike *et al.*, 1991; Darbra *et al.*, 2004; Guadano-Ferraz *et al.*, 2003; Sui *et al.*, 2005). Thyroid hormone alterations may also cause changes in long-term potentiation (LTP) (Gerges *et al.*, 2001; Sui *et al.*, 2006), a widely accepted cellular model for learning and memory which crucially involves the hippocampus (Bliss & Collingridge, 1993). Electrophysiological studies have demonstrated that hypothyroidism induced by PTU exposure alters synaptic transmission and plasticity in area CA1 of the neonatal rat hippocampus (Niemi *et al.*, 1996; Sui & Gilbert, 2003; Vara *et al.*, 2002; Sui *et al.*, 2005). It has been previously demonstrated that pyramidal cells of the hippocampal CA1 region show altered dendritic spine density in response to THs in adult subjects (Gould *et al.*, 1990). Additionally, treatment of hormonal replacement involving THs in age-related neurodegenerative disease, such as Alzheimer's disease, and in subclinical hypothyroidism has shown to significantly improve some aspects of memory performance (Haggerty *et al.*, 1990; Smith *et al.*, 2002). Sui and colleagues (2006) have demonstrated that infusion of T3 into the dorsal hippocampus of rats improved the long-term fear memory in the trace cued and delayed contextual fear conditioning procedures compared to their control groups. These might suggest a possible role of THs on memory processes.

We also reported the effect of these two chronic treatments, from day 21 to 29, on a delayed two-alternative-choice-task in a Y-maze, under conditions that required egocentric spatial orientation and, at the same time, excluded allocentric spatial orientation. Egocentric spatial orientation seems to involve procedural learning, also known as implicit or habit learning (Knowlton *et al.*, 1996; Graybiel *et al.*, 2000), and allocentric spatial orientation requires declarative learning, or explicit learning (Knowlton *et al.*, 1996). Our results showed that L-T4-treated animals were not able to learn delayed two-alternative-choice-task that required egocentric orientation under hyperthyroid condition, since the number of errors was not reduced over time. In contrast, errors were significantly reduced in the control group over time, which demonstrated that the animals were able to learn the task (Fig. **2b**). However, Fig. (**2b**) noticeably shows a trend for the L-T4 group to learn the task. On the $7^{th}$ experimental day, both groups were significantly different. The phenomenon demonstrated in Fig. (**2a**) at first glance seems striking: running time for the L-T4 group does lay below running time for the control group – significantly on the $1^{st}$, $2^{nd}$, $4^{th}$, $5^{th}$, $6^{th}$, $7^{th}$ and $8^{th}$ days. Subjects from the L-T4 group explored the maze quickly, anxious for food. On the other hand, animals from the control group explored the maze slowly, but concentrated on learning the task to be rewarded. L-T4 group subjects explored the maze faster, although they permanently made mistakes in choosing the right arm of the Y-maze, because they were unable to learn the rule of alternation of the rewarded arm. *In vivo* and *in vitro* studies indicate that thyroid hormones have a considerable impact on oxidative stress, and a similar treatment to induce hyperthyroidism animal model (Mogulkoc *et al.*, 2006) showed that hyperthyroidism increased oxidative damage in the cerebral tissue of rats. Recent findings demonstrated that T3 is associated with oxidative stress through a

**Table 5. Serum Thyroid Hormones Concentrations**

| Treatment | Total T3 (mg/dL) | Total T4 (μg/dL) | Total TSH (μUI/mL) |
|---|---|---|---|
| Saline | 33.74 ± 5.29 (8) | 4.93 ± 0.15 (7) | 0.1270 ± 0.0274 (7) |
| L-T4 | 75.77 ± 8.27 (7)* | 2.77 ± 0.47 (7)* | 0.0150 ± 0.0017 (8)* |
| | | | |
| Saline | 47.79 ± 2.11 (7) | 3.44 ± 0.50 (8) | 0.0719 ± 0.0201 (8) |
| PTU | 30.57 ± 2.46 (7)* | 0.75 ± 0.06 (8)* | 3.780 ± 0.5011 (7)* |

Data are expressed as mean ± S.E.M. Numbers in parentheses indicate number of animals per group. * Different from control, $p<0.01$.

negative regulation of superoxide dismutase-1 (SOD-1), a key enzyme in the metabolism of oxygen-free radicals (Santos *et al.*, 2006), showing that this effect may be important in T3 induction of oxidative stress in thyroid hormone excess. Moreover, an experimental hyperthyroid animal model conducted for four weeks with Sprague-Dawley rats caused higher levels of lipid peroxidation in the plasma, liver, heart and brain of L-T4-treated animals when compared to controls (Mohamadin *et al.*, 2006). Other studies have demonstrated the correlation between oxidative stress and learning and cognition. Kumar and colleagues (2006) have demonstrated that intracerebroventricular administration of colchicine - a microtubule-disrupting agent - causes free radical generation characterized by alterations in oxidative stress markers and poor retention of memory in Morris water maze and elevated plus maze task paradigms. They also demonstrated that a chronic administration of two cyclooxigenase inhibitors (naproxen or valdecoxib) may act like a free radical scavending and have a neuroprotective role against cognitive dysfunction, since it improves colchicine-induced cognitive impairment. It has been reported that aged male rats chronically treated with Deprenyl, an irreversible monoamine-oxidase B (MAO –B) inhibitor which has antioxidant and neuroprotective effects, increase spacial memory performance in Morris water maze - probably thru a suppression of lipid peroxidation in the prefrontal cortex, striatrum and hippocampus regions, and alleviation of the age-related decrease of the number of neurons in the hippocampus (Kiray *et al.*, 2006). Furthermore, an appropriate animal model for Alzheimer's disease, described by intracerebroventricular injections of streptozotocin, is associated with glucose metabolism, oxidative stress and impairment cholinergic neurotransmission and was tested with Pioglitazone, an insulin sensitizer and also an antioxidant, or without it. Animals treated with Pioglitazone improved cognitive performance in Step-through passive avoidance and Morris water maze, lowered oxidative stress markers and improved cerebral glucose utilization when compared to those not treated, which demonstrated a severe deficit in learning and memory associated to high levels of oxidative stress markers and impaired cerebral glucose utilization (Pathan *et al.*, 2006). Thus, the oxidative stress of the brain tissue caused by T3 excess could be an explanation, at least in part, why L-T4-treated animals were unable to learn the rule of alternation position of the rewarded arm in the Y-maze task.

PTU-treated animals were unable to learn the Y-maze task since the first experimental day when compared to controls (Fig. **3a**), mostly because, even in deprivation of food, they did not seem interested in exploring the maze. One reason that could explain this behavior was the low metabolism induced by hypothyroidism. Sui and cols (2006b) showed that adult-onset hypothyroidism did not change the basal synaptic transmission but significantly reduced paired-pulse facilitation and LTP of postsynaptic potentials. These inhibitions can be restored by thyroid hormone replacement. Another study demonstrated that iodine deficiency and hypothyroidism during critical periods of brain development impair LTP induction and decrease the expression of c-fos and c-jun proteins, which are important substracts to learning and memory, in the hippocampus (Dong *et al.*, 2005). It was recently demonstrated that working memory is impaired in both hypothyroid and subclinical hypothyroid patients, although the results obtained from the hypothyroid patients showed severe working memory deficits. Interestingly, they suggested that subclinical hypothyroid patients be treated with L-T4, which may prevent the cognitive impairment in the later stage of the disease, that is, hypothyroidism (Zhu *et al.*, 2006). However, we can also associate this result to the inhibition of $Na^+$, $K^+$-ATPase activity. Our results showed a 70% inhibition of $Na^+$, $K^+$-ATPase activity in the synaptic plasma membrane from the hippocampus of rats, but the treatment did not cause alteration of $Na^+$, $K^+$-ATPase activity in the synaptic plasma membrane from the parietal cortex in this group. It was recently demonstrated that there is a selective decrease of $Na^+$, $K^+$-ATPase activity in the brain of hypothyroid rats. Their results also showed that $Na^+$, $K^+$-ATPase activity was lower in the hippocampus but not in the cerebellum in a hypothyroid group (Pacheco-Rosado *et al.*, 2005). It was previously demonstrated that the inhibition of $Na^+$, $K^+$-ATPase activity causes edema and cell death in the central nervous system, and impairment of learning and memory (Sato *et al.*, 2004), which could explain, in part, our results in inhibitory avoidance task and in Y-maze.

We also observed a 57% inhibition of $Na^+$, $K^+$-ATPase activity in the synaptic plasma membrane from the hippocampus of rats in the L-T4 group, but no alteration of $Na^+$, $K^+$-ATPase activity in the synaptic plasma membrane from the parietal cortex. Carageorgiou and cols (2005) also observed an inhibition of brain $Na^+$, $K^+$-ATPase in hyperthyroidism by using a similar hyperthyroidism animal model and proposed that it may be due to the increase of intracellular calcium by L-T4 (Ernest, 1989; Carageorgiou *et al.*, 2005). They also evaluated TAS (total antioxidant status) as an oxidative stress marker and observed that hyperthyroid animals seem to have a shorter lifespan and, at advanced age, present a myelin deficit and that this may be due to the damage produced by the oxidative stress generated by an excess of THs (Pasquini and Adamo, 1994). However, this brain oxidative stress (inhibited TAS) was not found in the group of hyperthyroid animals in their study (Carageorgiou *et al.*, 2005). On the other hand, their animals were treated only for 14 days, while ours were treated for 28 days. Then, this phenomenon observed by us could be due to the oxidative stress. A recent publication demonstrated that oxidative stress, caused by excess of morphine, totally inhibited $Na^+$, $K^+$-ATPase activity in adult rat brain (Guzman *et al.*, 2006). As we mentioned earlier, thyroid hormones have a considerable impact on oxidative stress in the rat brain (Mogulkoc *et al.*, 2006), and this chronic treatment to induce hyperthyroidism carried out by us succeeded well to increase T3 serum levels in the animals when compared with controls, as shown in Table **5**. We also mentioned that excess of T3 could induce oxidative stress (Santos *et al.*, 2006). Then, considering the hypothesis that the inhibition of $Na^+$, $K^+$-ATPase activity in the synaptic plasma membrane from the hippocampus was due to thyroid hormone excess, which caused an oxidative stress in the rat brain; and considering that the inhibition of $Na^+$, $K^+$-ATPase activity caused impairment of learning and memory (Reis *et al.*, 2002), that could explain, at least in part, the learning deficit of the hyperthyroid group in Y-maze. Additionally, it was previously shown that $Na^+$, $K^+$-ATPase activity is selectively reduced in patients with cogni-

tive deficits, such as Alzheimer's disease (Hattori *et al.*, 1998; Liguri *et al.*, 1990; Reis *et al.*, 2002), and that oxidative stress is involved in these disorders.

However, we demonstrated an improvement on learning and memory of the inhibitory avoidance task before the pretreatment in the L-T4 group when compared with controls. Considering the metabolic effect that THs have on the CNS and that in some diseases, such as subclinical hypothyroidism and hypothyroidism, in which treatment with L-T4 significantly improves some aspects of memory performance, we may presume that these hormones have an important role on memory formation. Even so, the improvement of memory only occurs as a consequence of these hormones reposition if their blood levels are low in the patients. In our experiments we demonstrated that, in elevated levels, thyroid hormones can improve the learning and memory of an inhibitory avoidance task, but in animals exposed to these higher levels for a longer period (for 14 more days) it had the opposite effect on Y-maze, probably due to oxidative stress of the brain tissue. It seems there is a thin line between the beneficial dose of the THs, which could improve memory formation and retrieval, and the excessive dose, which can cause brain damage probably due to oxidative stress. Of course, these hypotheses merit further investigation.

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## CAPÍTULO II

Efeitos de um tratamento agudo com L-tiroxina sobre a memória, habituação, esquiva inibitória e sobre a atividade da $Na^+,K^+$-ATPase no cérebro de ratos.

Título:Effects of an acute treatment with L-thyroxine on memory, habituation, danger avoidance, and on $Na^+,K^+$-ATPase activity in rat brain
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**Effects of an acute treatment with L-thyroxine on memory, habituation, danger avoidance, and on $Na^+$, $K^+$-ATPase activity in rat brain**

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**ABSTRACT**

Thyroid hormones (THs) have a relevant action on brain development and maintenance. By using an acute treatment to induce a hyperthyroid animal model, we aimed at investigating the effect of an altered THs levels on learning and memory and on the activity of $Na^+$, $K^+$-ATPase in the rat brain. Our results have shown that the acute treatment with L-T4 did not alter the retrieval of the inhibitory avoidance task, but had a significant effect on the elevated plus maze and on open-field performance in rats. We suggest that animals subjected to L-T4 administration improved the habituation to a novel environment as well as a better evaluation of a dangerous environment, respectively. $Na^+$, $K^+$-ATPase activity is increased in parietal cortex (30%), but it is not altered in hippocampus in L-T4 treated group. These both brain structures are involved in memory processing and it was previously demonstrated that there is a double dissociation between them for spatial location information, perceptual and episodic memory. We propose the hypothesis that this increase of $Na^+$, $K^+$-ATPase activity in parietal cortex may be correlated to our results in behavior tests, which suggest a role of THs as well as of the $Na^+$, $K^+$-ATPase in the cognitive process.

**Key words:** hyperthyroidism, inhibitory avoidance, open-field, plus maze, $Na^+$,$K^+$-ATPase, hippocampus, parietal cortex.

## INTRODUCTION

Hyperthyroidism is an endocrine disease caused by excessive secretion of thyroid hormones (THs): triiodothyronine (T3) and tetraiodothyronine (T4). The symptoms include an increase frequency of alpha-rhythm, irritability, anxiety and restlessness progressing to nervousness, tremulousness, tachycardia, sleep disturbances, whilst paranoia and, in most severe patients, symptoms of mania and depression (Hargreaves et al., 1988; Smith et al., 2002). Thyroid crisis is an acute manifestation of thyrotoxicosis. Approximately 1-2% of hyperthyroid patients progress to a thyroid storm, often precipitated by a physiologically stressful event. Thyroid storm is a life-threatening state of severe hyperthyroidism and some of its symptoms are heart failure and arrhythmias, heat intolerance, loss of appetite, weight loss, tremors and fever (Ngo & Chew, 2007).

THs are essential for normal maturation and function of mammalian central nervous system (CNS) (Oppenheimer & Schwartz, 1997; Santos & Pérez-Castillo 2000; Darbra et al., 2003; Sui et al., 2006) and its deficiency, during a critical period of development, profoundly affects cognitive functions (Vara et al., 2002). Adult-onset thyroid dysfunction is associated with both neurological and behavior abnormalities, emphasizing the importance of THs for normal brain function and maintenance (Sher, 2001; Smith et al., 2002; Mogulkoc et al., 2006). However, the relationship between thyroid status and cognitive or psychiatric disturbances remains unclear (Smith et al., 2002; Wiens & Trudeau, 2006).

Sodium-potassium adenosine 5'-triphosphatase ($Na^+$, $K^+$-ATPase) is a crucial enzyme responsible for the activity transport of sodium and potassium ions in CNS. This pump is necessary for maintaining the sodium and potassium ion gradients across the cell membrane, and this sodium ion

gradient drives the sodium-dependent transport of calcium and amino acids as well as the reuptake of neurotransmitters (Sweadner, 1989; Blanco & Mercer, 1998; Lingrel et al., 2003; Taguchi et al., 2007). It is implicated in neural excitability(Sastry & Phillis, 1977) metabolic energy production, (Mata et al., 1980) uptake and release of catecholamines (Bogdanski et al., 1968) and serotonin (Schulpis et al., 2007). In the synapse, the ATPase activity contributes directly to the membrane excitability through the maintenance of $Na^+$ and $K^+$ gradients and indirectly to the many solute transport processes including $Ca^{2+}$ (Taguchi et al., 2007).Evidences suggest that $Na^+$, $K^+$-ATPase might play a role on memory formation and consolidation (Reis et al., 2002; Wyse et al., 2004). It was recently demonstrated that $Na^+$, $K^+$-ATPase have been implicated in the pathogenesis of mood disorders and may participate in the pathogenesis of depressive disorders (Goldstein et al., 2006); and that its activity is selectively reduced in patients with cognitive deficits, such as Alzheimer's disease (Lauder & Mugnaini, 1977; Santos & Pérez-Castillo 2000). Additionally, it was shown that the impairment of $Na^+$, $K^+$-ATPase activity in the rat brain could be one of the underlying biochemical mechanism leading CNS dysfunctions as a consequence of neonatal hypothyroidism (Billimoria et al., 2006), which shows that it suffers an important action of THs during the brain development (Billimoria et al., 2006; Santos & Pérez-Castillo 2000).

In the present study, we decided to investigate the effect of an acute administration of L-thyroxine (L-T4) on some behavioral paradigms: inhibitory avoidance task, open-field task, plus maze, and on the activity of $Na^+$, $K^+$-ATPase in the rat hippocampus and parietal cortex. By using a L-T4 treatment, which have been shown to induce increased THs levels similar to those found in hyperthyroid patients, we aimed at understanding the effect of an altered hyperthyroid acute state on learning, memory and on the activity of $Na^+$, $K^+$-ATPase.

## MATERIAL AND METHODS

*Animals and Reagents*

Male Wistar, 60-day-old rats obtained from the Central Animal House of Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. Animals were maintained on a 12:12 h light/dark cycle in an air-conditioned constant temperature, colony room, with free access to water and a 20% (w/w) protein commercial chow (Germani, Porto Alegre, RS, Brazil). All chemicals were purchased from Merck (Darmstadt, Germany).

*Drug Administration Procedure*

To induce the acute hyperthyroidism animal model we used a protocol previously established (Honda et al., 2000) with some modifications. Seventy animals were divided into four groups (A, B, C and D). Group A animals were treated for three days with daily intraperitoneal (i.p.) administration of L-T4 (500 μg/Kg/day) and group B received saline (0.9 % NaCl) as a control ($n$ = 20 animals per group). After this treatment for three days, which was important to elevate THs levels in the animals' blood, rats were submitted to behavior procedures. Group C was also treated daily with i.p. administration of L-T4 (500 μg/Kg/day) and group D received saline (0.9 % NaCl) as a control ($n$ =15 animals per group). Groups C and D were also treated for three days using the same drug administration procedure, but they were not assessed behaviorally.

*Experimental Protocols*

After the three-day treatment, 24 hours after the last administration, all animals from groups A and B were subjected to a step-down inhibitory avoidance task, open-field and plus maze in that order. The behavioral procedures were conducted since the early morning, with intervals of four hours among them.

In the same day, also 24 hours after the last administration, blood samples were collected by cardiac puncture from groups C and D animals randomly selected ($n$ =10 animals per group) and serum was separated by centrifugation and stored at -20$^0$C for T3, T4 and TSH determination. Animals randomly selected from groups C and D ($n$ = 5 animals per group) were killed by decapitation and the brains rapidly extracted. Brain was dissected on ice-cooled glass plates, and parietal cortex and hippocampus were removed for the determination of $Na^+$, $K^+$-ATPase activity. Animals used for blood samples and for $Na^+$, $K^+$-ATPase activity assay were not assessed behaviorally.

*Behavioral Procedures*

*Step-down inhibitory avoidance task*: Animals were subjected to training and test sessions in a step-down inhibitory avoidance task, with an interval of 24 hours in between (Izquierdo & Medina, 1997; Barros et al., 2004). This task is an established model of aversively motivated, hippocampus-dependent memory that involves learning not to step down from a platform in order to avoid a mild foot shock (Izquierdo & Medina, 1997; Izquierdo & McGaugh, 2000). The task was carried out in an automatically operated, brightly illuminated box. The left extreme of the grid was covered by a 7.0 cm

wide, 2.5 cm high formic platform. Animals were placed on the platform and their latency to step down placing their four paws on the grid (42.0 X 25.0 cm grid of parallel 0.1 cm caliber stainless steel bars spaced 1.0 cm apart) was measured. In test sessions, no foot shock was used and step-down latency (with a ceiling of 180 s) was used as a measure of memory retention as described in previous reports (Barros et al., 2006; Coitinho et al., 2006; Izquierdo & McGaugh, 2000; Izquierdo & Medina, 1997).

*Open-field habituation*: This task was run in a wooden box measuring 60 x 40 x 50 cm with a frontal glass wall, whose floor was divided by black lines into 12 squares. The animals were gently placed facing the rear left corner of the arena and observed during 5 min. The time used for observation of the animals' behavior was divided into two 2.5 min intervals to evaluate the animals' response to a novel environment. The number of crossings from one square to another is indicative of motor activity and the number of rearing responses is a measure of habituation (Netto et al., 1986).

*Plus maze*: The elevated plus maze consisted of four 9 cm wide, 45 cm long corridors placed at 90° angles and 88 cm above the room floor level. The middle crossing between the four corridors was 9 $cm^2$ large and was open. Two opposite corridors were surrounded by 26 cm high plywood walls (enclosed arms), and the other two were not (open arms). Rats were placed in the middle of the four arms and left to explore the apparatus freely for 10 min. The number of entries and percentage of time in the open arms was a measure of the state of anxiety of the animals (Izquierdo et al., 2002; Pellow et al., 1985).

*Preparation of Synaptic Plasma Membrane from the Hippocampus and the Parietal Cortex*

Membranes from the hippocampus and the parietal cortex were prepared according to a well-established method (Jones & Matus, 1974), with some modifications (Wyse et al., 2000). The structures were homogenized in 10 volumes of a 0.32M sucrose solution containing 5 mM HEPES and 1 mM EDTA. The homogenate was centrifuged at 1000Xg for 20min and supernatant removed and centrifuged at 12.000g for further 20 min. The pellet was then resuspended in hypotonic buffer (5.0 mM Tris-HCl buffer, pH 8.1), incubated at 0°C for 30 min, and applied to a discontinuous sucrose density gradient consisting of sucrose layers of 0.3, 0.8 and 1.0 M. After centrifugation at 69.000Xg for 2h, the fraction at the 0.8-1.0 M sucrose interface was taken as a membrane enzyme preparation.

*$Na^+$, $K^+$-ATPase Activity Assay*

The reaction mixture for the $Na^+$, $K^+$-ATPase assays contained 5.0 mM $MgCl_2$, 80.0 mM NaCl, 20.0 mM KCl, 40.0 mM Tris-HCl buffer, pH 7.4, in a final volume of 200 µL. The reaction was started by the addition of ATP (disodium salt, vanadium free) to a final concentration of 3.0 mM. Control assays were carried out under the same conditions with the addition of 1.0 mM ouabain. $Na^+$, $K^+$-ATPase activity was calculated by the difference between the two assays (Wyse et al., 2000). Released inorganic phosphate (Pi) was measured (Chan et al., 1986). Enzyme specific activities were expressed as nmol Pi released per min per mg of protein. All assays were performed in duplicate and the mean was used for statistical analysis.

*Protein Determination*

Protein was measured according to a well-described method (Bradford, 1976) using a bovine serum albumin as standard.

*Determination of Thyroid Hormones in Serum*

To evaluate the treatment, we measured the THs serum levels. Total T3, T4 and TSH in serum were measured by a chemiluminescent immunoassay (Immulite®, DPC, CA, USA), according to suppliers' instructions.

*Statistical Analyzes*

Training test latency differences of inhibitory avoidance task were assessed by Wilcoxon test and test latency scores analyzed among different groups by individual Mann-Whitney U-test (two tailed). Open-field and Plus Maze behaviors were analyzed by unpaired Student t-test. Data from $Na^+$,$K^+$-ATPase activity were analyzed by unpaired Student t-test as well as data from determination of THs in serum.

## RESULTS

Fig. 1 demonstrates the effects of an acute treatment of L-thyroxine on training and test sessions of a step-down inhibitory avoidance task. Latency differences in training were not significant between control and L-T4 groups in Mann-Whitney U test (U = 90.00, $p$ = 0.9805) followed by Wilcoxon test ($p$ = 0.9097). Latency differences in test performance were not significant according to Mann-Whitney U test (U = 80.50, $p$ = 0.6531) nor to Wilcoxon test ($p$ = 1.0).

The effects of L-T4 acute treatment on locomotor activity ('crossings') and exploratory behavior ('rearings') on open-field task is demonstrated in Fig.2 (a) and (b) respectively. Fig 2 (a) shows that there was no significant difference between groups in the number of crossings during the first 2.5 min interval ($p$ = 0.2707, df = 26). Although there was a significant difference in the second interval, the number of crossings in L-T4 group were reduced when compared to control group according to Student unpaired t-test ($p$ < 0.001, df = 26). Fig 2 (b) shows a significant difference between groups related to the number of rearings: L-T4 group performed a lower number of rearings when compared to controls during the two intervals according to Student unpaired t-test (first 2.5 min interval: $p$ <0.05 /second 2.5 min interval: $p$ <0.05, df = 26 in both intervals).

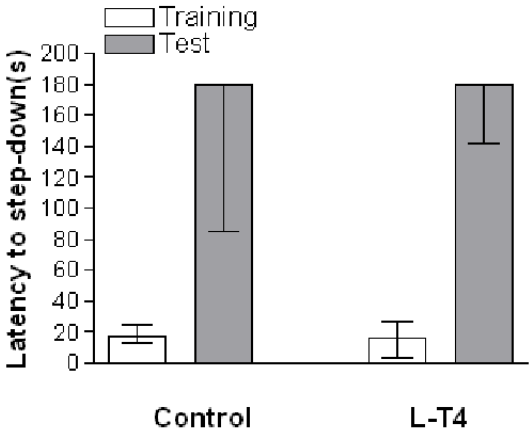


**Fig. 1** Effect of acute administration of LT4 on test session performance of step-down inhibitory avoidance task. Data are median (interquartile range) of at least 15 animals in each group. There was no difference between groups (Mann-Whitney: $p = 0.6531$; Wilcoxon: $p = 1.0$).

Table 1 shows the effect of L-T4 acute treatment on the elevated Plus Maze. The percentage of time in open arms was not significant between L-T4 and control group according to Student unpaired t-test ($p = 0.3868$, df = 26), which indicates there were no significant differences between the groups in basal locomotion and exploration. However, the total number of open arms entries was significantly different between the groups according to Student unpaired t-test ($p < 0.05$, df = 28) and to Mann-Whitney U test (U= 59.50, $p < 0.05$), probably indicating an increased of fear/anxiety in acute hyperthyroidism.

Fig. 3 (a) and (b) shows the effects of L-T4 acute treatment on $Na^+,K^+$-ATPase activity in the synaptic plasma membrane from rat hippocampus and parietal cortex. Fig. 3 (a) demonstrates that

there was no significant difference between groups: L-T4 (1471 ± 165, $n = 4$) and control (1502 ± 277, $n = 4$) (Student unpaired t, $p > 0.05$), respectively.Fig. 3 (b) shows that the L-T4 acute treatment caused a 40% increase of the enzyme activity (1307 ± 225, $n = 4$) when compared to controls (924 ± 177, $n = 4$) (Student unpaired t, $p < 0.05$).

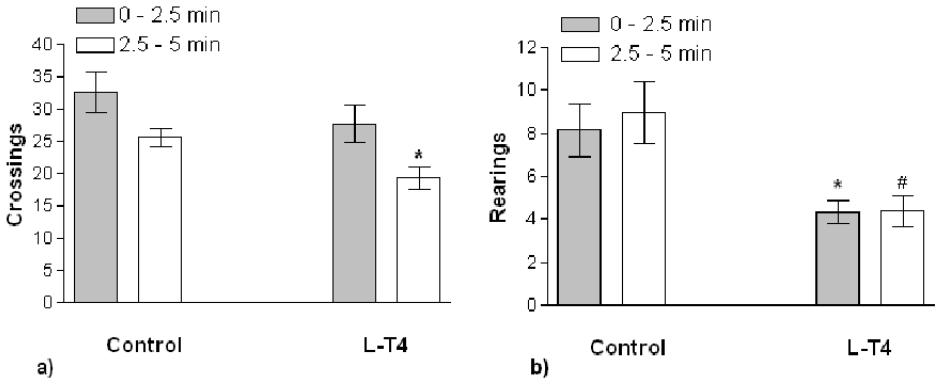


**Fig. 2** Effect of L-T4 acute administration on open-field behavior in adult rats**. (a)** For number of crossings ('crossings'), ambulation was counted. Data are given in mean ± S.E.M of at least 15 animals in each group. There was no difference between the groups during the first 2.5 min interval. *Different from the control group during the second 2.5 min interval ($p$ <0.01). **(b)** For number of rearings ('rearings'), this exploratory behavior was counted. Data are given in mean ± S.E.M of at least 15 animals in each group. *Different from the control group during the first 2.5 min interval ($p$ <0.05) and #different from the control group during the second 2.5 min interval ($p$< 0.05).

**Table 1 - Test session performance on plus maze of adult rats.**

| Treatment | *n* | % Time in open arms | Open arms entries |
|---|---|---|---|
| Saline | 15 | 41.53 ± 3.01 | 6.80 ± 0.69 |
| L-T4 | 15 | 46.25 ± 4.60 | 4.73 ± 0.49 * |

**Data are mean ±S.E.M. *Different from the control group, $p < 0.05$.**

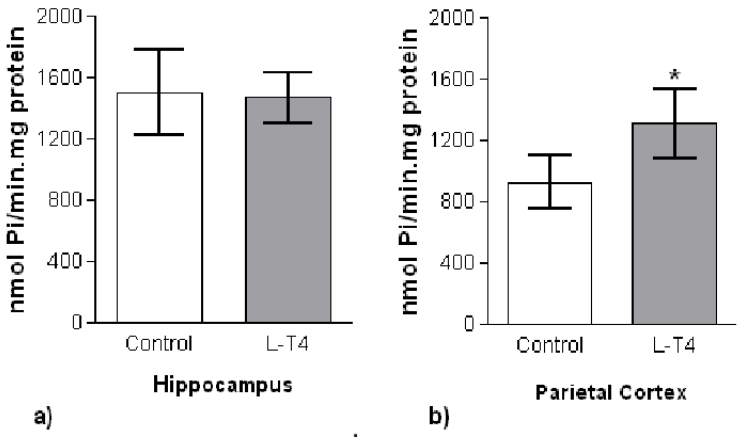


**Fig. 3** Effect of an acute treatment of L-T4 on $Na^+,K^+$-ATPase activity in the synaptic plasma membrane from the hippocampus and parietal cortex of rats. Data are mean ± S.D. for five independent experiments performed in duplicate and it is expressed by nmol Pi/min.mg protein.**(a)** There was no difference on $Na^+,K^+$-ATPase activity in the synaptic plasma membrane from the hippocampus between the groups. **(b)** * Different from the control group, $p < 0.05$.

Table 2 demonstrates THs serum profile in the hyperthyroid acute animal model. Alteration in total T3 serum levels was not significant when compared to controls according to Student unpaired t-test ( $p = 0.4325$, df = 16). However, the result noticeably showed a trend for the increase of total T3 levels in L-T4 group. The T4 and TSH serum levels were significant different from the control group according to Student t-test ($p < 0.01$ in both cases). The results demonstrated a trend for the increase of

total T3 serum levels, which is the most functionally active form of THs. We can also observe a decrease of TSH and total T4 serum levels, which demonstrates that this treatment simulates, in part, a chemical hyperthyroid acute state that caused a feed-back negative response on the release of the THs into the systemic circulation.

**Table 2- Serum thyroid hormones concentrations**

| Treatment | Total T3 (mg/dL) | Total T4 (μg/dL) | Total TSH (μUI/mL) |
|---|---|---|---|
| Saline | 44.42 ± 1.69 (9) | 3.70 ± 0.33 (9) | 0.0934 ± 0.0169 (9) |
| L-T4 | 53.29 ± 10.88 (9) | 2.37 ± 0.34 (8)* | 0.0268 ± 0.0050 (9)* |

**Data are expressed as mean ±S.E.M. Numbers in parentheses indicate the number of animals per group. * Different from control, $p < 0.01$.**

In addition to these results, we also measure the animals' body weight during the 3 days of the treatment. There was no difference between animals' body weight on the first day ($p = 0.8221$, df = 28) and on the second day ($p = 0.1934$, df = 28). However, there was a significant difference between L-T4 and control groups in the last day of injection according to Student t-test ($p < 0.05$, df = 28), which is also an evidence that we induced the expected metabolic effect of the THs in acute hyperthyroid animal model. These results are shown in table 3.

**Table 3- Animals' body weight**

| Days | Control (g) | L-T4 (g) |
|---|---|---|
| **1** | 224.9 ± 4.68 | 226.2 ± 3.56 |
| **2** | 226.3 ± 4.54 | 219.1 ± 2.92 |
| **3** | 227.8 ± 4.82 | 211.4 ± 3.76 * |

Data are expressed as mean ± S.E.M ($n$ =15 per group). *Different from the control group, $p< 0.05$.

## DISCUSSION

THs play a pivotal role in the development of the CNS (Bernal, 2002; Ambrogini et al., 2005). Inadequate supply of THs to developing brain leads to severe structural alterations with irreversible effects on behavior (Bernal, 2002; Ambrogini et al., 2005).THs mediate important effects within the CNS throughout life and alterations of the thyroid axis appear to contribute to a variety of CNS-related pathologies (Loosen, 1992) Previous studies in hypothyroid patients usually reported cognitive impairments (Mennemeier et al., 1993; Dugbartey, 1998; Burmeister et al., 2001; Roberts & Ladenson, 2004, Zhu et al., 2006) as well as patients with subclinical hypothyroidism were also associated with cognitive decline (Monzani et al., 1993; Baldini et al., 1997; del Ser Quijano et al., 2000; Zhu et al., 2006). Na+, K+-ATPase activity maintains and restores the resting potential of neuron and control the ionic gradient for neuronal excitability. It is already demonstrated that Na+, K+-ATPase activity is selectively reduced in patients with cognitive deficits (Hattori et al., 1998; Liguri et al., 1990) and that it suffers an important action of THs during the brain development (Smith et al., 2002). It was previously demonstrated that Na+, K+-ATPase activity was found reduced in the hyperthyroid rat whole brain (Carageorgiou et al., 2005) and recent findings have shown that Na+, K+-ATPase activity was decreased in the cerebellum, but it was not altered in the hypothalamus (Carageorgiou et al., 2007) in chronic hyperthyroid animal model in both cases. Wyse and colleagues (2004) have demonstrated that learning causes a transient alteration of hippocampal Na+, K+-ATPase activity, one marker of neuronal membrane function, and suggested an association between this enzyme activity and the process of memory consolidation. The present study has demonstrated the effects of an acute hyperthyroid animal model previously established (Honda et al., 2000) on learning, memory and on Na+, K+-ATPase activity in the hippocampus and parietal cortex of rats.

Our results demonstrated that an acute treatment with L-T4 carried out during three days did not alter the performance of an inhibitory avoidance task when compared to controls, as shown in Fig. 1. We may suggest that the THs high levels obtained in this acute treatment did not alter the formation and retrieval of step-down inhibitory avoidance aversively motivated, hippocampus-dependent memory (Izquierdo & Medina, 1995; Izquierdo & Medina, 1997; McGaugh, 2000). We may also correlate these results to the $Na^+$,$K^+$-ATPase activity, which was unaltered in the synaptic plasma membrane from the hippocampus, as demonstrated in Fig. 3 (a). There are evidences suggesting that $Na^+$, $K^+$-ATPase might play a role on the memory process of this task (Reis et al., 2002; Wyse et al., 2004) which crucially involves the hippocampus. This enzyme also suffers THs actions in the rat brain (Carageorgiou et al., 2005; 2007). Perhaps the immediate effects of elevated circulating T3 are merely discrete and may not interfere or alter the memory mechanisms involved in the inhibitory avoidance task as well as in the activity of $Na^+$, $K^+$-ATPase in the rat hippocampus.

A preferential role for dorsal hippocampus in spatial learning and memory is consistent with the fact that the major input of visual and spatial information to the hippocampus from primary sensory cortical areas, via association cortex, and perirhinal and entorhinal areas, it is mainly to the dorsal hippocampus (Amaral & Witter, 1995; Dolorfo & Amaral, 1998; Bertoglio et al., 2006). However, other types of sensory input, such as olfactory cues, appear to be more equally distributed along the hippocampus poles (Moser & Moser, 1998; Bertoglio et al., 2006). A mechanism by which the ventral hippocampus may regulate unconditioned defense behavior is through its connections with the hypothalamus and amygdaloid complex (Pentkowski et al., 2006; Bertoglio et al., 2006). Functional imaging, behavioral, electrophysiological, and neurochemical findings implicate the hippocampus in novelty processing (Knight 1996; Zhu et al., 1997; Honey et al., 1998; Thiel et al., 1998; Manahan-Vaughan & Braunewell, 1999; Strange et al., 1999; Vianna et al., 2000). The detection of novelty

depends on the activation of a distributed network involving the hippocampus (Knight & Nakata 1998; Vianna et al., 2000), and it is a memory-dependent process because the novel stimulus has to be compared with stored information to judge its novelty. However, novelty per se might not be the primary factor responsible for activation in the hippocampus. In this regard, it has been postulated that the hippocampus is necessary to record new events in any given situation (Eichenbaum et al., 1999; Martin, 1999; Vianna et al., 2000). In a previous work from our lab it was demonstrated for the first time that the detection of a spatial novelty results in the activation of several intracellular signaling pathways in the hippocampus that include a rapid increase in PKA activity followed by an activation of p42 and p44 MAPKs and CaMKII. Coinciding with the activation of MAPKs and CaMKII there is an increased phosphorylation of CREB. These changes are all transient, as no alterations in PKA activity or phospho-MAPKs, phospho-_CaMKII, or phospho-CREB levels were observed 3 hours after novelty (Vianna et al., 2000). Therefore, the detection of novelty is associated with a rapid and reversible activation of some signaling cascades in the hippocampus exhibiting time courses that differ substantially from those associated with inhibitory avoidance training (Izquierdo & Medina, 1997; McGaugh, 2000; Vianna et al., 2000). However, it is interesting to note that the time course of activation of hippocampal MAPKs and CaMKII after spatial novelty is quite similar to those observed after cue and contextual fear conditioning (Atkins et al., 1998;Vianna et al., 2000).On the other hand, the control of spatial orienting through either attention or saccades depends on a network of dorsal stream areas that includes the lateral intraparietal area, the frontal eye field, and the superior colliculus (Bisley & Goldberg, 2003; Goldberg et al., 2002; Snyder et al., 2000; Gottlieb, 2007). All three areas contain neurons that have spatially restricted visual receptive fields and respond selectively to conspicuous or behaviorally relevant objects. These areas are thought to provide topographic representations of the environment, which encode the salience of different objects and directly specify the attentional weight, or priority, associated with these objects (Gottlieb et al., 1998; Thompson & Bichot, 2005;

Gottlieb, 2007). Although the hippocampus and parietal cortex are not directly connected anatomically, (Burwell, 2000; Witter et al., 2000), behavioral data clearly indicate that the hippocampus and parietal cortex process spatial information. What is not clear, however, is whether or not the hippocampus and parietal cortex interact during spatial information processing. Recent findings indicate that the dorsal hippocampus and posterior parietal cortex interact during spatial tasks that require multiple training days, but not during the detection of novelty within a single day (Rogers & Kesner, 2007).Then, the data showed in the present study on the open-field task and on the plus maze task may not be due to the interaction of these two brain structures, but due to the spatial information processing by only one of them. Of course, this hypothesis requires further investigation.

We have observed an interesting alteration of performance on open-field task: the number of crossings in the first 2.5 min interval was similar in L-T4 group and control group. However, in the second 2.5 min interval there was a significant reduction of number of crossings in the L-T4 group as demonstrated in Fig. 2 (a). This phenomenon can also be observed in Fig 2(b), which showed that the number of rearings were significantly different in both intervals of the 5 min. These data suggests an increase in habituation capacity of the L-T4 treated animals to a novel environment. It was reported that sub-chronic and chronic treatments with L-T4 increase the cognitive function measured by a spatial learning task (Guadano-Ferraz et al., 1999). It has been proposed that approximately 80% of T3 is formed in the brain locally from T4 through the activity of the 5'-deiodinase type 2 (D2), an enzyme that is expressed mostly by glial cells, tanycytes in the third ventricle, and astrocytes throughout the brain. D2 activity is an important point of control of thyroid hormone action because it increases in situations of low T4, thus preserving brain T3 concentrations. Their results also demonstrated that D2 mRNA concentration was increased severalfold over normal levels in relay nuclei and cortical targets of the primary somatosensory and auditory pathways, suggesting that T3 has a role in the development

of these structures (Guadano-Ferraz et al., 1999). Considering that parietal cortex seems to participate in memory processes and may be related to spatial processing (Ardenghi et al., 1997), we propose the hypothesis that these results in the open-field task reported by us may be due to THs action in the cortical targets of the somatosensory pathways, which include the parietal cortex.

The plus maze (PM) stands as one of the most popular in vivo animal tests currently in use. Although it has been frequently used as a tool to screen anxioselective effects of drugs (Handley & Mithani, 1984; Pellow et al., 1985; Lister, 1990; Carobrez & Bertoglio, 2005), nowadays its usefulness has spread towards the understanding of the biological basis of emotionality related to learning and memory, pain, hormones, addiction and withdrawal, as well as of the various sub-types of anxiety disorders, such as generalized anxiety, phobia and post-traumatic stress (Adamec et al., 1998; File et al., 1998; Lamprea et al., 2000; Carobrez et al., 2001; Rasmussen et al., 2001; Bannerman et al., 2004; Carobrez & Bertoglio, 2005). The PM has also been successfully used to define brain areas related to fear/anxiety (Jinks & McGregor, 1997; Treit & Menard, 1997; File et al., 1998; Andrade et al., 1999; Lacroix et al., 2000; Adamec et al., 2001; Carobrez et al., 2001; Jardim & Guimarães, 2001; Adamec et al., 2003; Carobrez & Bertoglio, 2005). It is still a matter of debate whether rodents' exploratory behavior depends on the aversiveness of the open arms only or the contrasting characteristics of the open and the enclosed arms in the PM. More work is needed to clarify the stimuli responsible for rodent avoidance of the open arms of the PM. In this context, the existence of at least two environments with different levels of aversion, open and enclosed arms, appears to be required for animals to develop open arm avoidance in the PM test (Bertoglio & Carobrez, 2000; Salum et al., 2003; Carobrez & Bertoglio, 2005). Defensive behavior in mammals refers to any behavior which reduces the chances of an animal being harmed (McFarland, 1987). Defensive strategies encompass a plethora of behavioral manifestations, including the reactions to predators, conspecifics, certain

situations and inanimate objects (Rodgers & Dalvi, 1997). As such, flight, freezing, startle, and defensive threat and attack usually appear in response to proximal danger whereas environmental features can evoke a different set of reactions including risk assessment and avoidance behavior, more subtle defensive adaptations (Blanchard & Blanchard, 1989; Carobrez & Bertoglio, 2005). Of relevance to the present discussion are avoidance and the risk assessment (RA) behaviors. The primary indices of PM anxiety comprise spatiotemporal measures of open arm avoidance (% of entries and of time spent in), RA is a significant behavioral dimension, closely related to fear/anxiety (Rodgers et al., 1997; Carobrez & Bertoglio, 2005). The biological function of RA acts and postures is to inform behavioral strategies in potentially dangerous situations (Blanchard et al., 1991; Blanchard et al., 1993; Carobrez & Bertoglio, 2005). In fact, it is interesting to note that rodents continue to display enhanced RA behaviors even after ceasing to avoid, for example, an unprotected area, suggesting that this defensive pattern may even be more sensitive to anxiety modulating drugs than avoidancerelated measures (Rodgers & Cole, 1994; Griebel et al., 1997; Rodgers, 1997; Setem et al., 1999; Carobrez & Bertoglio, 2005).Our results have demonstrated that the acute treatment with L-T4 can alter the animal behavior on the plus maze: treated animals had a significant different performance when compared to controls, as demonstrated in Table 1. L-T4-treated animals spent almost the same percentage of time in open arms as controls, although the number of total entries in open arms were significantly different between groups and confirmed an overall preference for enclosed arms over open arms by L-T4 group. This result may represent an anxiolytic effect caused by the acute alteration of THs and are in agreement with clinical findings which have shown that in the acute phase of Graves' thyrotoxicosis many patients have also been found to have affective symptoms, including emotional lability, irritability, restlessness, agitation and anxiety (Whybrow et al., 1969; Schlote et al., 1992; Demet et al., 2002, Vogel et al., 2007). However, we may correlate these behavior results to the effect of THs on brain structures involving the cortical targets of the somatosensory pathways, which have an important

role in spatial processing. Perhaps the effect of THs improved spatial information processing, which made animals observe and evaluate better a novel risk situation in the open arms of the maze than the control group, once they avoided staying outside the enclosed arms. This hypothesis also demands further investigation.

For the Na+, K+-ATPase activity assay animals were not assessed behaviorally because it was previously demonstrated that training session of step-down inhibitory avoidance can alter this enzyme activity in the rat hippocampus (Wyse et al., 2004) and perhaps the other behavior experiments may also interfere in the final enzymatic results of our treatment. The hypothalamo-hypophyseal-thyroid system play a major role in the physiological processes controlling the internal milieu of the body and adapting it to the changing conditions of the external environment and in producing coordinated body responses to stress-inducing influences (Herman & Cullinau, 1997; Jocko, 1996; Pacak et al., 1998; Belyakova & Mendzheritskii, 2006).In addition, it was recently reported that male Wistar rats under an acute state of stress condition suffered rearrangements of thyroid status, which were biphasic: involved a marked increase in plasma thyroxine and triiodothyronine, while the second involved a selective decrease in the thyroxine concentration to normal levels (Belyakova & Mendzheritskii, 2006). Thus, we chose not to use animals assessed behaviorally for THs serum analysis either since the behavior tasks may represent or a stress situation or a novelty and perhaps these may also interfere in the thyroid hormone serum levels.

Our results shown in Figure 3 (a) and (b) demonstrated an alteration of $Na^+$, $K^+$-ATPase activity related to the acute treatment with L-T4: $Na^+$, $K^+$-ATPase activity is increased in parietal cortex (30%), but it is not altered in hippocampus in L-T4 treated group. Evidences suggest that that both the hippocampus and parietal cortex subserve spatial representations required for optimal learning and

performance of spatial tasks (Kesner et al., 1991), although it was subsequently demonstrated that there is a double dissociation between parietal cortex and hippocampus for spatial location information, perceptual and episodic memory (Chiba et al., 2002). Recently, rats lesioned in the dorsal hippocampus and parietal cortex were tested on both a metric and topological task and the results suggest that the hippocampus is necessary for metric representations, whereas the parietal cortex is necessary for topological representations (Goodrich-Hunsaker et al., 2005). Sui and colleagues (2006b) have demonstrated that infusions of T3 intrahippocampus enhances hippocampus-dependent long-term fear conditioning memory and suggested that trace contextual fear conditioning is not exclusively hippocampally mediated, at least in rats. However, the neural mechanism through which T3 acts, even though T3 can facilitate the neural processes involved in synaptic plasticity and learning and memory, the exact contribution of these processes to the enhancement of fear conditioning remains unknown. Furthermore, whether the same cellular processes mediate the effects of T3 on both types of fear conditioning is as yet unclear. Parietal cortex is known to be responsible for two different intellectual functions: attention and space surrounding. Considering that our results have demonstrated that the acute L-T4 treatment have an increasing effect on $Na^+$, $K^+$-ATPase activity in the parietal cortex, promoted a better performance of animals' habituation to a novel environment in the open-field task and probably increased animals' evaluation of a novel and dangerous environment in the plus maze task; we suggest that these present findings may be correlated and may indicate a role for THs as well as for the $Na^+$, $K^+$-ATPase in the cognitive process. Further studies are necessary to clarify the mechanisms underlying such effects of THs and its relevance to the understanding of learning and memory mechanisms.

THs have many physiological actions, and essentially modulate all metabolic pathways through alterations in oxygen consumption and changes in protein, lipid (Mogulkoc et al., 2006), carbohydrate

and vitamin metabolism (Smith et al., 2002). THs are essential for normal maturation and function of mammalian CNS (Darbra et al., 2003; Oppenheimer & Schwartz, 1997; Santos & Pérez-Castillo, 2000) and its deficiency, during a critical period of development, profoundly affects cognitive functions (Vara et al., 2002). Thyroid hormones are very important for human beings, not only for maintaining normal physiological functions, they are also thought to be related to high-level cognition (Timiras & Nzekwe, 1989; Loosen, 1992). Epidemiological surveys using the revised Wechsler Adult Intelligence Scale (WAIS-R) and the Mini-Mental State Examination (MMSE) suggest a positive correlation between plasma thyroid hormone level and cognitive function in people with normal plasma thyroid levels (Prinz et al., 1999; Volpato et al., 2002). Abnormal plasma concentrations of the thyroid hormone are thought to be a risk factor for dementia (Bulens, 1981; Smith & Granger, 1992; Kalmijn et al., 2000; Zhu et al., 2006).Cognitive decline often appears concomitant with aging, and it is particularly associated with many age-related neurodegenerative diseases along with various psychiatric and demyelinating disorders. Whilst alterations in specific components of the thyroid axis have been associated with this condition (Mogulkoc et al., 2006b), and treatment strategies involving THs provide benefit to some of the symptomatology in such cases, notably for psychiatric symptoms (Constant et al., 2001; Guzman et al., 2006). It was recently demonstrated that there are evidences in working memory function as well as in its neural substrate, thus patients with subclinical hypothyroidism would benefit from clinical treatment with L-T4 (Zhu et al., 2006).The important interaction between THs and CNS and, particularly, their effects on learning and cognition may represent one aspect that should be explored, better understood and perhaps more targeted therapeutically in the future.

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# PARTE III

## 3. Discussão

Os hormônios da tireóide exercem importantes efeitos no SNC e alterações no eixo tireoidiano parecem contribuir para uma série de doenças relacionadas ao mesmo. O hiper e o hipotireoidismo estão associados a uma série de sintomas neurológicos, incluindo mania e perda de memória respectivamente. Em pacientes idosos, a demência parece ser uma das principais características secundárias (LOOSEN, 1992; LANDI et al., 2007). A disfunção da tireóide em adultos é associada com anormalidades neurológicas e comportamentais, enfatizando a importância dos hormônios da tireóide para o funcionamento normal do cérebro (SMITH et al., 2002; DONG et al., 2005; SUI et al., 2005).

Em nosso estudo, avaliamos os efeitos dos modelos animais crônicos de hipertireoidismo (HU et al., 2003) e hipotireoidismo (DUARTE et al., 2000) em diversas tarefas comportamentais: esquiva inibitória, campo aberto, plus maze e Y-maze. Outro enfoque que decidimos investigar foi o efeito desses dois modelos experimentais sobre a atividade da enzima $Na^+,K^+$-ATPase de membrana plasmática sináptica de hipocampo e de córtex parietal.

Nossos resultados demonstraram que um tratamento crônico com L-T4 por 14 dias melhorou a performance da tarefa de esquiva inibitória quando comparado com o grupo controle. Por outro lado, o tratamento crônico com PTU também por 14 dias piorou a performance da mesma tarefa. Esses resultados estão em concordância com outros estudos mostrando que o hiper e o hipotireoidismo alteram o comportamento animal (REDEI et al., 2001; SMITH et al., 2002; DARBRA et al., 2003; WILCOXON et al., 2007). Além disso, perturbações na função tireoidiana estão associadas com falhas no comportamento de tarefas que requerem a integridade do hipocampo

(AKAIKE et al., 1991; DARBRA et al., 2004; GUADANO-FERRAZ et al., 2003; SUI et al., 2005). Alterações nos hormônios da tireóide podem também causar alteração na LTP (GERGES et al., 2001; SUI et al., 2006), um modelo celular bem aceito de aprendizado e memória que envolve crucialmente o hipocampo (BLISS & COLLINGRIDGE, 1993). Estudos eletrofisiológicos demonstraram que o hipotireoidismo induzido pelo PTU altera a transmissão sináptica e a plasticidade na região CA1 do hipocampo de ratos neonatos (NIEMI et al., 1996; SUI & GILBERT, 2003; VARA et al., 2002; SUI et al., 2005). Sui e colaboradores (2006) também demonstraram que a infusão de T3 no hipocampo dorsal de ratos melhora a memória aversiva de longa duração quando comparado aos controles. Esses dados sugerem um possível papel dos hormônios da tireóide nos processos de memória.

No presente trabalho investigamos também o efeito destes tratamentos crônicos nas tarefas de campo aberto e plus maze. Em ambas tarefas verificamos que não houve diferenças significativas entre os grupos tratados e os controles. Podemos concluir que os resultados obtidos na tarefa de esquiva inibitória não ocorreram devido a dificuldades na atividade motora dos animais e/ou devido ao estado de ansiedade dos animais, respectivamente.

O efeito da administração crônica de L-T4 e PTU também foi avaliada no labirinto de 8 braços através do teste de dupla escolha no Y-maze. Nossos resultados demonstraram que os animais tratados com L-T4 não foram capazes de aprender a tarefa, uma vez que o número de erros cometidos não foi reduzido ao longo do tempo. Contudo, o tempo de corrida foi menor no grupo tratado com L-T4 do que o grupo controle. Observamos que o grupo tratado com o L-T4 explorou o aparelho rapidamente, mas não aprendeu a regra do braço recompensado. Estudos *in vivo* e *in*

*vitro* mostram que os hormônios da tireóide têm um impacto considerável no estresse oxidativo e em um tratamento similar para induzir um modelo animal de hipertireoidismo (MOGULKOC et al., 2006) demonstrou que este induz dano oxidativo no cérebro de ratos. Além disso, o T3 é associado à regulação do estresse oxidativo através da superóxido desmutase-1 (SOD-1), uma enzima chave no metabolismo dos radicais livres de oxigênio (SANTOS et al., 2006), demonstrando que esse efeito pode ser importante na indução do estresse oxidativo pelo T3 no excesso de hormônios da tireóide. Além disso, vários estudos comportamentais em modelos animais demonstram uma estreita correlação entre o estresse oxidativo e o aprendizado e a cognição (KUMAR et al., 2006; KIRAY et al., 2006; PATHAN et al., 2006) e demonstram que em circunstâncias onde o estresse oxidativo é induzido no SNC, há déficit de aprendizado e de memória. Então, o estresse oxidativo do tecido cerebral causado pelo excesso de T3 poderia explicar, pelo menos em parte, a razão pela qual os animais não foram capazes de aprender a regra de alternância da posição do braço recompensado no Y-maze.

Os animais tratados com PTU não foram capazes de aprender a tarefa do Y-maze durante todo o período experimental, pois mesmo sofrendo deprivação alimentar eles não pareciam interessados em explorar o aparato. Sui e colaboradores (2006b) demonstraram que o hipotireoidismo em ratos adultos não muda a transmissão sináptica, mas significativamente reduz a facilitação de pulso pareado e a LTP dos potenciais pós-sinápticos e que essas inibições podem ser restauradas pela reposição do hormônio da tireóide. Outro estudo demonstrou que a deficiência de iodo e o hipotireoidismo durante períodos críticos do desenvolvimento cerebral prejudica a indução de LTP e diminui a expressão das proteínas c-fos e c-jun, que são importantes

substratos para o aprendizado e para a memória no hipocampo (DONG et al., 2005). Um recente estudo demonstrou que a memória de trabalho está diminuída em pacientes com hipotireoidismo e hipotireoidismo subclínico, contudo pacientes com hipotireoidismo apresentaram os resultados mais severos. Nesse mesmo estudo os autores sugerem o tratamento preventivo com L-T4 no hipotireoidismo subclínico, para evitar os danos cognitivos nos estágios mais avançados da doença, que é o próprio hipotireoidismo (ZHU et al., 2006). Por outro lado, podemos associar esse resultado à inibição da atividade da $Na^+,K^+$-ATPase. Nossos resultados demonstraram uma redução de 70% na atividade da $Na^+,K^+$-ATPase na membrana plasmática sináptica de hipocampo de ratos *in vivo*, mas o mesmo tratamento não causou alteração na atividade da $Na^+,K^+$-ATPase na membrana plasmática sináptica de córtex parietal *in vivo*. Pacheco-Rosado e colaboradores (2005) demonstraram que há uma diminuição seletiva da atividade da $Na^+,K^+$-ATPase no cérebro de ratos com hipotireoidismo e que a atividade da enzima está reduzida no hipocampo, mas não no cerebelo. A inibição dessa enzima também causa edema, morte celular no SNC e déficit de aprendizado e memória (SATO et al., 2004). Dessa forma, poderíamos explicar, pelo menos em parte, nossos resultados na esquiva inibitória e no Y-maze.

Também observamos uma redução de 57% na atividade da $Na^+,K^+$-ATPase na membrana plasmática sináptica de hipocampo *in vivo* nos animais tratados com L-T4, mas nenhuma alteração na atividade da $Na^+,K^+$-ATPase na membrana plasmática sináptica de córtex parietal. Carageorgiou e colaboradores (2005) observaram uma inibição da $Na^+,K^+$-ATPase cerebral usando um modelo de hipotireoidismo semelhante ao nosso e propuseram que isso aconteceria devido ao aumento do cálcio intracelular devido ao L-T4 (ERNEST, 1989; CARAGEORGIOU et al., 2005). Os referidos autores

também avaliaram o TAS (Total Anti-oxidante Status) como um marcador de estresse oxidativo, porém o marcador de estresse oxidativo (TAS inibido) não foi encontrado nos animais com hipertireoidismo nesse estudo (CARAGEORGIOU et al., 2005). Contudo, Carageorgiou e colaboradores trataram seus animais somente por 14 dias, enquanto os nossos animais foram tratados por 28 dias. Dessa forma, a redução da atividade da enzima observada em nosso trabalho pode ser devido ao estresse oxidativo. Uma recente publicação demonstrou que o estresse oxidativo causado pelo excesso de morfina inibiu completamente a atividade da $Na^+,K^+$-ATPase no cérebro de ratos adultos (GUZMAN et al., 2006). Como os hormônios da tireóide apresentam um impacto considerável no stress oxidativo do cérebro (MOGULKOC et al., 2006) e nosso tratamento foi bem sucedido em aumentar os níveis séricos de T3 quando comparados aos controles, podemos considerar as seguintes hipóteses: que a redução da atividade da enzima na membrana plasmática sináptica de hipocampo foi devido ao excesso de hormônio da tireóide, que causou estresse oxidativo no cérebro dos animais; e que redução da atividade dessa enzima causou dano no aprendizado e na memória da tarefa de Y-maze. Além disso, podemos acrescentar que foi previamente descrito que a atividade da $Na^+,K^+$-ATPase está reduzida em pacientes com déficit cognitivo, como na doença de Alzheimer (HATTORI et al., 1998; LIGURI et al., 1990) e que o estresse oxidativo está envolvido nessas doenças.

Nesse mesmo trabalho demonstramos uma melhora no aprendizado e na memória da tarefa de esquiva inibitória depois de um tratamento de 14 dias com L-T4 quando comparado aos controles. Considerando o efeito metabólico que os hormônios da tireóide têm no CNS em algumas doenças como o hipotireoidismo e o hipotireoidismo subclínicos, nas quais o tratamento com L-T4 significativamente

melhora a memória, podemos presumir que esses hormônios apresentam um papel importante na formação da memória. Porém, a melhora da memória ocorre como conseqüência da reposição hormonal nesses pacientes, quando os níveis normais de seus hormônios estão baixos. Em nossos experimentos demonstramos que em níveis elevados os hormônios da tireóide são capazes de melhorar o aprendizado e a memória da tarefa de esquiva inibitória, mas esses animais expostos a níveis altos por períodos maiores (por mais 14 dias) apresentaram dificuldade de aprendizado no Y-maze, provavelmente devido ao estresse oxidativo do tecido cerebral. Parece existir uma fina linha entre a dose benéfica dos hormônios da tireóide, que poderiam melhorar a formação da memória e a sua evocação e a dose excessiva, que pode causar dano cerebral provavelmente através do estresse oxidativo. Contudo, estudos subseqüentes deverão ser realizados para provar esta hipótese.

No segundo estudo, utilizamos um modelo experimental agudo de hipertireoidismo descrito por Honda e colaboradores (2000) e decidimos avaliar seu efeito sobre algumas tarefas comportamentais: esquiva inibitória, campo aberto e plus maze. Outro enfoque que decidimos investigar foi o efeito desse modelo animal sobre a atividade da enzima $Na^+,K^+$-ATPase de membrana plasmática sináptica de hipocampo e córtex parietal.

Nossos resultados demonstraram que o tratamento agudo com L-T4 não alterou a performance da tarefa de esquiva inibitória quando comparado aos controles. Sugerimos que os níveis de hormônios da tireóide obtidos nesse tratamento agudo não alteraram a formação e a evocação da memória aversiva e hipocampo dependente da tarefa de esquiva inibitória. Da mesma forma, não houve alteração na atividade da $Na^+,K^+$-ATPase de membrana plasmática sináptica de hipocampo *in vivo*. Esses

resultados podem estar correlacionados, pois há evidências na literatura sugerindo que a $Na^+,K^+$-ATPase possa desempenhar um papel no processo de memória desta tarefa (REIS et al., 2002; WYSE et al., 2004), que envolve crucialmente o hipocampo. Talvez o efeito imediato do T3 circulante seja discreto e não interfira ou altere os mecanismos de memória envolvidos na tarefa de esquiva inibitória ou a atividade da $Na^+,K^+$-ATPase no hipocampo de ratos.

O hipocampo tem um papel fundamental no aprendizado espacial e na memória que é consistente com o fato que a maior entrada de informações visuais e espaciais para o hipocampo é através das regiões corticais sensoriais primárias, via associação com o córtex e regiões perirrinal e entorrinal, é principalmente pelo hipocampo dorsal (AMARAL & WITTER, 1995; DOLORFO & AMARAL, 1998; BERTOGLIO et al., 2006). O hipocampo ventral parece regular um comportamento de defesa não condicionado através de conexões com o hipotálamo e o complexo amilóide (PENTKOWSKI et al., 2006; BERTOGLIO et al., 2006). Achados em imagem funcional, comportamentais, eletrofisiológicos e neuroquímicos demonstram o papel do hipocampo no processamento de um evento ou ambiente novo (KNIGHT 1996; ZHU et al., 1997; HONEY et al., 1998; THIEL et al., 1998; MANAHAN-VAUGHAN & BRAUNEWELL, 1999; STRANGE et al., 1999; VIANNA et al., 2000). A detecção de uma novidade depende da ativação de uma rede envolvendo o hipocampo (KNIGHT & NAKATA 1998; VIANNA et al., 2000), e é um processo dependente da memória, pois o estímulo novo deve ser comparado com a informação armazenada para ser julgado. Contudo, um evento novo *per se* pode não ser o primeiro fator de ativação no hipocampo. A esse respeito, ficou postulado que o hipocampo é necessário para gravar cada novo evento em qualquer situação apresentada (EICHENBAUM et al., 1999;

MARTIN, 1999; VIANNA et al., 2000). Por outro lado, o controle da orientação espacial através da atenção ou do movimento rápido dos olhos depende de uma rede que inclui a região intraparietal lateral, o campo ocular frontal e o colículo superior (BISLEY & GOLDBERG, 2003; GOLDBERG et al., 2002; SNYDER et al., 2000;GOTTLIEB, 2007). Todas essas três regiões contêm neurônios que possuem campos de recepção visual espacialmente restrita e respondem seletivamente a objetos evidentes ou comportamentalmente relevantes. Acredita-se que essas regiões fornecem uma representação topográfica do meio ambiente, que codificam a saliência de objetos diferentes e diretamente especificam a atenção relacionada ao peso, ou a prioridade associada a esses objetos (GOTTLIEB et al., 1998; THOMPSON & BICHOT, 2005; GOTTLIEB, 2007). Embora o hipocampo e o córtex parietal não estejam diretamente conectados anatomicamente (BURWELL, 2000; WITTER et al., 2000), dados comportamentais claramente indicam que o hipocampo e o córtex parietal processam informações espaciais. O que não está claro, no entanto, é se o hipocampo e o córtex parietal interagem durante o processamento das informações espaciais. Foi recentemente publicado que o hipocampo dorsal e o córtex parietal posterior interagem durante tarefas comportamentais que requerem múltiplos dias de treino, mas não durante a detecção de uma novidade em um único dia (ROGERS & KESNER, 2007). Então, os resultados apresentados em nosso estudo nas tarefas de campo aberto e de plus maze podem não terem sido devido à interação dessas duas estruturas cerebrais, mas devido ao processamento da informação espacial por apenas uma delas. Essa hipótese deve ser melhor investigada.

Nossos resultados demonstraram uma alteração no comportamento da tarefa de campo aberto: o número de cruzamentos no primeiro intervalo dos 2,5 min foi similar

no grupo controle e no grupo tratado com L-T4, contudo no segundo intervalo dos 2,5 min houve uma redução no número de cruzamentos no grupo tratado. O número de rearings foi significativamente menor no grupo tratado com L-T4 em ambos intervalos dos 5 minutos. Esses dados sugerem um aumento na capacidade de habituação a um ambiente novo nos animais tratados com L-T4. Considerando que um tratamento sub-crônico e crônico com L-T4 aumentou a função cognitiva de uma tarefa de aprendizado espacial (GUADANO-FERRAZ et al., 1999) e que 80% do T3 é formado no cérebro localmente à partir do T4 pela atividade de uma enzima chamada 5'-deiodinase tipo 2 (D2), que parece ter um papel fundamental em manter as concentrações cerebrais de T3 e também parece estar aumentada nos alvos corticais das rotas somatosensórias primárias e rotas auditórias, sugerindo que o T3 tem um papel no desenvolvimento dessas estruturas (GUADANO-FERRAZ et al., 1999). Além disso, considerando que o córtex parietal participa dos processos de memória e está relacionado aos processos espaciais (ARDENGHI et al., 1997), propusemos a hipótese de que esses resultados obtidos no campo aberto possam ser devido a ação dos hormônios da tireóide nos alvos corticais das rotas somatosensórias, que incluem o córtex parietal.

O tratamento agudo com L-T4 também alterou o comportamento dos animais na tarefa de plus maze: os animais tratados dispenderam quase a mesma percentagem de tempo nos braços abertos que os controles, contudo o número total de entradas nos braços abertos foi significativamente diferente entre os grupos e confirmou a preferência pelos braços fechados aos braços abertos pelo grupo L-T4. Esse resultado pode representar o efeito ansiolítico causado por alterações dos hormônios da tireóide e está de acordo com achados clínicos que mostram que na fase aguda da doença de Graves muitos pacientes também apresentam sintomas afetivos como labilidade

emocional, irritabilidade, agitação e ansiedade (WHYBROW et al., 1969; SCHLOTE et al., 1992; DEMET et al., 2002; VOGEL et al., 2007). Todavia, podemos correlacionar esses resultados comportamentais aos efeitos dos hormônios da tireóide nas estruturas cerebrais envolvidas nos alvos corticais das rotas somatosensórias, que apresentam um importante papel no processamento espacial. Talvez o efeito dos hormônios da tireóide tenha melhorado o processamento da informação espacial, o que fez os animais observarem e avaliarem melhor uma situação nova de risco nos braços abertos do aparelho do que o grupo controle, uma vez que eles evitaram sair dos braços fechados. Essa hipótese também requer uma melhor investigação.

Observamos uma alteração na atividade da enzima $Na^+,K^+$-ATPase relacionada ao tratamento agudo com L-T4: houve um aumento na atividade da $Na^+,K^+$-ATPase de membrana plasmática sináptica de parietal córtex (30%), mas não houve alteração na atividade da enzima no hipocampo do grupo tratado com o L-T4. Evidências da literatura mostram que ambos, hipocampo e córtex parietal,são úteis para representações espaciais que requerem aprendizado e performance ótimos de tarefas espaciais (KESNER et al., 1991), contudo foi subseqüentemente demonstrado que existe uma dissociação dupla entre o córtex parietal e o hipocampo para informação de localização espacial, memória perceptual e memória episódica (CHIBA et al., 2002). Recentemente, ratos lesionados no hipocampo dorsal e no córtex parietal foram ambos testados em tarefas métricas e topológicas, o resultado sugeriu que o hipocampo é necessário para representações métricas, enquanto que o córtex parietal é necessário para representações topológicas (GOODRICH-HUNSAKER et al., 2005). Sui e colaboradores (2006b) demonstraram que infusões de T3 intra-hipocampo aumenta a memória hipocampo dependente de longa duração condicionada pelo medo e sugeriram que o

condicionamento contextual pelo medo não é exclusivamente mediado pelo hipocampo, pelo menos em ratos. Contudo, o mecanismo neural através do qual o T3 age, ou até mesmo que ele pode facilitar os processos neurais envolvidos na plasticidade sináptica e no aprendizado e na memória, a sua exata contribuição para esses processos permanece desconhecido. O córtex parietal é responsável por duas diferentes funções intelectuais: atenção e localização espacial. Considerando que nossos resultados demonstraram que ratos agudamente tratados com tiroxina apresentaram um aumento na atividade da $Na^+,K^+$-ATPase no córtex parietal, apresentaram uma melhor habituação a um ambiente novo no campo aberto e provavelmente avaliam melhor um ambiente novo e perigoso na tarefa de plus maze; sugerimos que esses achados possam estar correlacionados e indicar um papel para os hormônios da tireóide assim como para a $Na^+,K^+$-ATPase nos processos cognitivos. Mais estudos serão necessários para certificar os mecanismos que causam os efeitos dos hormônios da tireóide e sua relevância, para que possamos entender as formas pelas quais eles agem no aprendizado e na memória.

Os hormônios da tireóide são muito importantes para os seres humanos não apenas por manter as funções fisiológicas, mas também por estarem relacionados com altos níveis de cognição (TIMIRAS & NZEKWE, 1989; LOOSEN, 1992). Um estudo epidemiológico usando a Escala Wechsler de Inteligência Adulta Revisada (WAIS-R) e o Exame Estadual Mini-Mental (MMSE), que sugeriram uma correlação positiva entre os níveis de hormônios da tireóide e as funções cognitivas em pessoas com níveis plasmáticos normais de hormônios da tireóide (PRINZ et al., 1999; VOLPATO et al., 2002). Concentrações plasmáticas anormais de hormônios da tireóide é um conhecido fator de risco para demência (BULENS, 1981; SMITH & GRANGER, 1992; KALMIJN et al., 2000; ZHU et al., 2006). O decline cognitivo geralmente aparece

concomitante com a idade e está particularmente associado com muitas doenças neurodegenerativas relacionadas à idade junto com várias doenças psiquiátricas e desmielinizantes. Enquanto alterações em componentes específicos no eixo da tireóide têm estado associado com essas condições (MOGULKOC et al., 2006b), e tratamentos envolvendo os hormônios da tireóide promovem benefícios a algumas das sintomatologias em alguns casos, principalmente os sintomas psiquiátricos (CONSTANT et al., 2001; GUZMAN et al., 2006). Além disso, foi recentemente reportado que pacientes com hipotireoidismo subclínico beneficiam-se com o tratamento com L-T4 em relação a sua memória de trabalho (ZHU et al., 2006).

A importante interação entre os hormônios da tireóide e o SNC e, particularmente, seus efeitos no aprendizado, na memória e na cognição podem representar um aspecto que deve ser explorado, melhor compreendido e talvez utilizado terapeuticamente no futuro, quando sua ação no SNC for completamente conhecida.

# PARTE IV

## 4. Bibliografia


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