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Francine Luciano Rahmeier

EFEITO DA TAURINA E DO AMBIENTE ENRIQUECIDO SOBRE O COMPORTAMENTO, A MEMÓRIA E O HIPOCAMPO DE RATOS ADULTOS, DIABÉTICOS E NÃO DIABÉTICOS

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Orientadora: Profª Dra. Marilda da Cruz Fernandes

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Dedico este trabalho à minha mãe,

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Ítaca

"Quando você partir, em direção a Ítaca,
que sua jornada seja longa,
repleta de aventuras, plena de conhecimento.
Não tema Laestrigones e Ciclopes, nem o furioso Poseidon;
você não irá encontrá-los durante o caminho, se o pensamento estiver elevado,
se a emoção jamais abandonar seu corpo e seu espírito.
Laestrigones e Ciclopes, e o furioso Poseidon
não estarão em seu caminho se você não carregá-los em sua alma,
se sua alma não os colocar diante de seus passos.
Espero que sua estrada seja longa.
Que sejam muitas as manhãs de verão,
que o prazer de ver os primeiros portos traga uma alegria nunca vista.
Procure visitar os empórios da Fenícia,
recolha o que há de melhor.
Vá às cidades do Egito,
aprenda com um povo que tem tanto a ensinar.
Não perca Ítaca de vista, pois chegar lá é o seu destino.
Mas não apresse os seus passos;
é melhor que a jornada demore muitos anos
e seu barco só ancore na ilha quando você já estiver enriquecido
com o que conheceu no caminho.
Não espere que Ítaca lhe dê mais riquezas.
Ítaca já lhe deu uma bela viagem;
sem Ítaca, você jamais teria partido.
Ela já lhe deu tudo, e nada mais pode lhe dar.
Se, no final, você achar que Ítaca é pobre,
não pense que ela o enganou.
Porque você tornou-se um sábio, viveu uma vida intensa,
e este é o significado de Ítaca".

KONSTANTINOS KAVÁFIS

SUMÁRIO

LISTA DE ABREVIATURAS

AE: Ambiente enriquecido

Apaf-1: Fator de ativação da apoptose 1

ATP: Adenosina tri-fosafato

BrdU: 5- bromo-2'-deoxiuridina

CA: Corno de Ammon

DM: Diabetes *mellitus*

DM1: Diabetes *mellitus* tipo 1

DM2: Diabetes *mellitus* tipo 2

EA: Enriquecimento ambiental

EI: Esquiva inibitória

EROs: Espécies reativas de oxigênio

GABA: Ácido gama-aminobutírico

GD: Giro denteado

GFAP: Proteína glial fibrilar ácida

IH: Imuno-histoquímica

LTP: Potenciais de longa duração

NCAM: Molécula de adesão celular neural

NeuN: Antígeno neuronal nuclear

RO: Reconhecimento de objetos

SN: Sistema nervoso

SNC: Sistema nervoso central

SNP: Sistema nervoso periférico

STZ: Etreptozotocina

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RESUMO

Introdução: O diabetes *mellitus* tem sido estudado nos últimos anos como um grande causador de danos neurodegenerativos, déficits de memória e cognitivos. A taurina e o ambiente enriquecido (AE) vêm se destacando por apresentarem efeitos neuroprotetores e estimulantes, que merecem estudos mais aprofundados. **Objetivos:** Nesse trabalho, objetivamos estudar a influência da taurina e do AE sobre o giro denteado do hipocampo de animais diabéticos e não diabéticos, especificamente sobre as células da glia e apoptose celular, além de avaliar o impacto desses fatores sobre o comportamento e memória desses animais. **Materiais de Métodos:** Para o experimento foram utilizados inicialmente 88 animais, divididos em dois grupos de 44 cada: um destes, submetido ao AE e outro à caixa de moradia padrão. Dentro de cada grupo, aproximadamente metade dos animais foram induzidos ao diabetes *mellitus* tipo 1, onde alguns desses foram tratados durante 30 dias, com taurina. Durante o experimento, foram avaliadas memórias de curta e longa duração. No 30º dia de tratamento, os animais foram eutanasiados por perfusão transcardíaca e retirados seus cérebros, que após processados e seccionados, foram submetidos a técnicas de imuno-histoquímica para GFAP e caspase-3 clivada. **Resultados:** Observamos que animais tratados com taurina apresentaram melhores desempenhos em tarefas comportamentais e de memória, e que o AE também demonstrou ter efeitos positivos, principalmente em animais não diabéticos. Ainda, a taurina e o AE aparentam ser capazes de interferir sobre a apoptose neuronal e a perda de células gliais, e em alguns momentos, esses dois fatores parecem ter efeitos sinérgicos. **Conclusões:** A partir desses dados, pode-se observar que a taurina e o AE podem possuir efeitos neuroprotetores e neuroestimulantes.

Palavras-chave: Taurina, ambiente enriquecido, diabetes, memória, hipocampo, neurodegeneração.

1 INTRODUÇÃO

Segundo dados da *International Diabetes Federation*, cerca de 382 milhões de pessoas no mundo tem diabetes *mellitus* (DM), e acredita-se que até o ano de 2035, este número aumente para 592 milhões de casos. Este mesmo estudo demonstra que no Brasil, o número de pessoas diabéticas chega a 11,9 milhões. Estima-se que o diabetes seja responsável, direta ou indiretamente, por cerca de 4 milhões de mortes por ano, e que a expectativa de vida esteja reduzida, em média, em 15 anos para pacientes com diabetes *mellitus* tipo 1 (DM1) e em 5 a 7 anos para pacientes com diabetes *mellitus* tipo 2 (DM2) (IDF Diabetes Atlas, 2013; Souza e cols., 2012). Esta alta incidência se deve a dietas altamente calóricas e estilos de vida sedentários (Reaven, 2005).

A hiperglicemia crônica é capaz de causar diversos efeitos negativos sobre o organismo, e o sistema nervoso central (SNC) é muito afetado. Podemos encontrar danos em regiões como o hipocampo, prejudicando funções de cognição e memória, o que pode levar a estados depressivos (Baynes, 1991; Bloomgarden, 1999; Ozkaya e cols., 2002; Greenwood e Winocur, 2005; Messier, 2005; Stranahan e cols., 2008; Caletti e cols., 2012; Mello e cols., 2012; Reagan, 2012).

Nos últimos anos, os cientistas vêm buscando mais explicações acerca do assunto, e estudando alternativas para melhorar ou reverter o quadro neurodegenerativo causado pela hiperglicemia crônica. A taurina é uma substância de baixa produção endógena, sendo obtida principalmente de fontes exógenas, como a alimentação ou por suplementação. Este aminoácido possui importantes efeitos para o desenvolvimento do SNC, e estudos demonstraram que em quadros de estresse metabólico, incluindo a hiperglicemia, ela apresenta efeitos neuroprotetores, antioxidantes, e até hipoglicemiantes (Suzuki e cols., 2001; Franconi e cols., 2006; Szymanski e Winiarska,

2008; Schaffer e cols., 2009; Ito e cols., 2012). Ainda, estudos têm mostrado que a taurina pode ser capaz de interferir nas vias de apoptose (Menzie e cols., 2013) e estimular a neurogênese (Shivaraj e cols., 2012).

Ainda, em busca da neutralização dos efeitos negativos causados pela neurodegeneração, vem-se estudando os efeitos positivos do enriquecimento ambiental (EA) em estudos com animais, que simularia a influência de exercícios físicos e estímulos sensoriais e de aprendizado, sendo relatada na literatura, uma melhoria de quadros de déficit cognitivo e de memória (Kempermann e cols., 1997; Piazza e cols., 2011; Ahmadalipour e cols., 2015).

Frente a isto, a proposta de unir em um único experimento, a suplementação com a taurina e a exposição ao ambiente enriquecido em um quadro hiperglicêmico, se torna útil para buscar mais resultados sobre este assunto.

1.1 Diabetes *mellitus*

Pode-se definir o DM como uma condição metabólica caracterizada pela deficiência total ou parcial da secreção e/ou funcionamento da insulina, resultante de uma destruição ou disfunção de células beta pancreáticas, fazendo com que os níveis de glicose intracelular fiquem diminuídos, e aumentados no meio extracelular, caracterizando um quadro de hiperglicemia (Kumar e cols., 2005; ADA, 2009).

O DM é classificado de acordo com a ação das células beta pancreáticas, produtoras de insulina: o DM1 é caracterizado pela degeneração das células beta, sendo dependente de insulina, enquanto a principal causa de DM2 é uma progressiva resistência insulínica com eventual deficiência insulínica, como resultado da constante hiperglicemia (Yi e cols., 2009).

Diversos órgãos e sistemas orgânicos são afetados por esta condição patológica, gerando uma série de complicações (Greenwood e Winocur, 2005; Messier, 2005). Devido à hiperglicemia crônica, pode haver o surgimento de complicações, como retinopatia, nefropatia, alterações cardiovasculares e vasculares, sendo comum observar em diabéticos, amputações não traumáticas de membros inferiores, cegueira irreversível e doença renal crônica (Souza e cols., 2012). Indivíduos adultos com DM possuem um risco de duas a quatro vezes maior de desenvolver doença cardiovascular, doença vascular periférica e acidente vascular cerebral em comparação com uma pessoa saudável (Greenwood e Winocur, 2005; Messier, 2005).

Diversas pesquisas com animais têm sido feitas, buscando-se entender cada vez mais os efeitos do diabetes sobre o organismo (Beauquis e cols., 2006; Lebed e cols., 2008; Revsin e cols., 2009). Algumas substâncias químicas são capazes de induzir o DM, como a estreptozotocina (STZ). A STZ é um antibiótico que destrói seletivamente as células beta-pancreáticas através da formação de radicais livres, acumulando-se na porção central das ilhotas, suprimindo a liberação de insulina. Devido a este fato, é utilizado para induzir modelos animais de DM1 (Lenzen, 2008). A STZ produz no animal muitas das alterações observadas em seres humanos, como: hiperglicemia, hipoinsulinemia, hiperfagia, polidipsia, perda de massa corporal, neuropatia periférica e déficit cognitivo, sendo assim, um modelo amplamente utilizado para estudar alterações periféricas e centrais promovidas pela hiperglicemia crônica (Serino e cols., 1998; Beauquis e cols., 2010).

Além de todos os danos já citados, decorrentes da hiperglicemia, esta, se não tratada, pode levar ao surgimento de alterações metabólicas no SNC, afetando sua estrutura, fisiologia, neuroquímica e citoarquitetura (Stranahan e cols., 2008).

1.2 O sistema nervoso central

Por definição, segundo Ross e Pawlina (2012) “o sistema nervoso capacita o corpo a responder a alterações contínuas em seu ambiente externo e interno. Ele controla e integra as atividades funcionais dos órgãos e sistemas orgânicos”. É graças ao sistema nervoso (SN) que é possível que um organismo funcione coordenadamente (Junqueira e Carneiro, 2013).

O SN é formado pelo SNC, composto pelo encéfalo e medula espinhal, e o sistema nervoso periférico (SNP), composto por nervos e gânglios. O SNC é revestido por meninges, que são compostas de tecido conjuntivo, e protegido mais externamente por revestimentos ósseos, nomeados de crânio e vértebras. O encéfalo está dividido em três partes: o cérebro, o cerebelo e o tronco encefálico. O cérebro constitui a parte mais larga do encéfalo, ocupando cerca de 80% da cavidade craniana. Possui dois hemisférios, um direito e outro esquerdo, conectados por axônios do corpo caloso, cada um dividido em quatro lobos: frontal, parietal, occipital e temporal (Machado, 2000; Bear e cols., 2008; Junqueira e Carneiro, 2013).

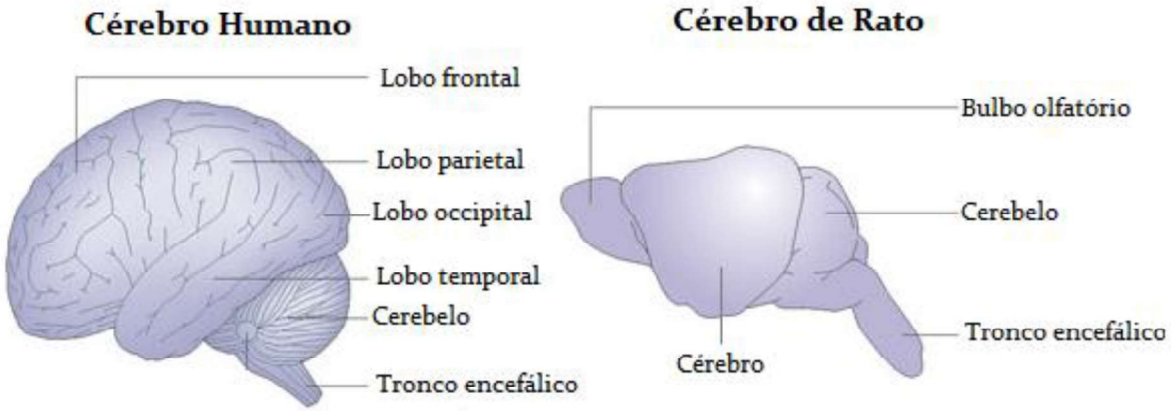

Figura 1. Desenho comparativo entre cérebro de humanos e de roedores (Extraído e adaptado de Cryan e Holmes, 2005).

Na parte mais externa do cérebro encontramos o córtex, também conhecido como substância cinzenta e que contém em sua maioria corpos celulares, enquanto a parte mais interna, chamada de substância branca, é formada principalmente por axônios, prolongamentos celulares e células da glia. (Machado, 2000; Bear e cols., 2008; Junqueira e Carneiro, 2013).

O tecido que compõe o SNC é formado por 2 classes principais de células, os neurônios e as células da glia, que abrangem os astrócitos, oligodendrócitos, micróglia e células ependimárias (Mello e cols., 2012; Ross e Pawlina, 2012; Junqueira e Carneiro, 2013).

Constituído por dendritos, corpo celular e axônio revestido ou não por bainha de mielina, os neurônios são classificados como a unidade funcional do SNC. Estes neurônios são capazes de responder aos diversos estímulos a que são submetidos através de impulsos nervosos, que vão desencadear reações fisiológicas, motoras, emocionais, entre outras. Os astrócitos fazem a sustentação do SNC, e com os pés vasculares, ligam-se aos capilares sanguíneos, auxiliando os neurônios na transmissão sináptica e promovem sua excitabilidade. Ainda, controlam as substâncias no nível extracelular dos neurônios. Os oligodendrócitos têm a função de isolar eletricamente os axônios dos neurônios dentro do SNC, produzindo em torno deste, a bainha de mielina. A micróglia representa as células fagocitárias do SNC, enquanto as células epiteliais ependimárias revestem as paredes dos ventrículos cerebrais (Mello e cols., 2012; Junqueira e Carneiro, 2013).

Figura 2. Células presentes no Sistema Nervoso Central (Adaptado de Moore e Persaud, 2003).

1.2.1 Hipocampo

O hipocampo é uma estrutura localizada no lobo temporal de cada hemisfério cerebral e relaciona-se diretamente com as funções de memória e aprendizagem. Também, faz parte do sistema límbico, tendo um papel integrativo, sendo essencial para os processos cognitivos e afetivos. É composto por dois grupos principais de células: células piramidais, que formam o Corno de Ammon (CA1, CA2, CA3, CA4) e as células granulares, que formam o giro denteado (GD). Essas estruturas, dispostas em camadas celulares, juntamente com outras regiões adjacentes, denominadas complexo subicular e córtex entorrinal, compõem a formação hipocampal (Bear e cols., 2008).

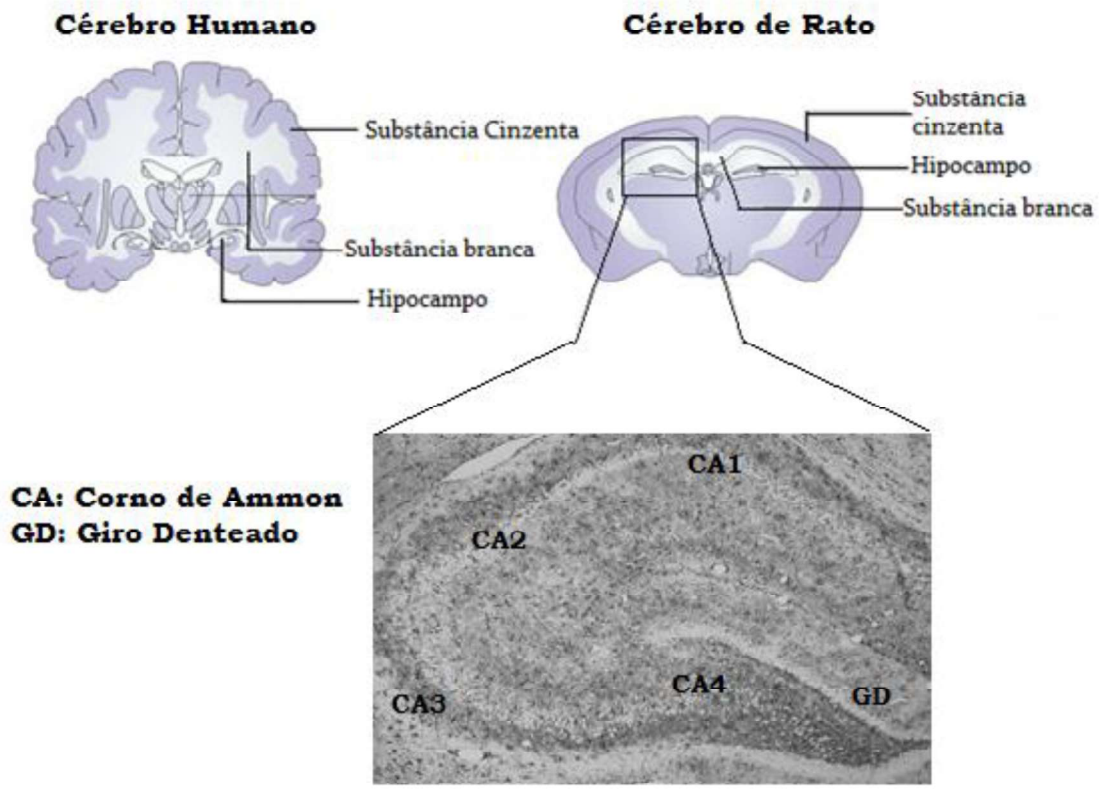

Figura 3. Ilustração mostrando a divisão interna de substância branca e cinzenta no cérebro de humanos e roedores, indicando também a posição do hipocampo, divisões do Corno de Ammon e giro denteado (Adaptado de Cryan e Holmes, 2005).

O GD possui funções muito importantes que o diferencia de outras sub-regiões dentro do hipocampo, sendo crucial para a formação de novos neurônios, em um processo chamado neurogênese adulta. Este processo envolve uma série de estágios distintos do desenvolvimento celular, dentre os quais se destacam: proliferação, sobrevivência e diferenciação celular (Kempermann e cols., 1998; Malberg e cols., 2000; Kempermann e cols., 2015). A neurogênese hipocampal, sob condições fisiológicas, gera um único tipo celular, que são os neurônios da camada granular do GD. Esses neurônios são excitatórios e recebem aferências do córtex entorrinal e enviam informações para as células piramidais do CA3 através de interneurônios. As

células precursoras de novos neurônios estão presentes em uma pequena faixa entre a camada granular e o hilo, chamada zona subgranular (Kempermann e cols., 2015).

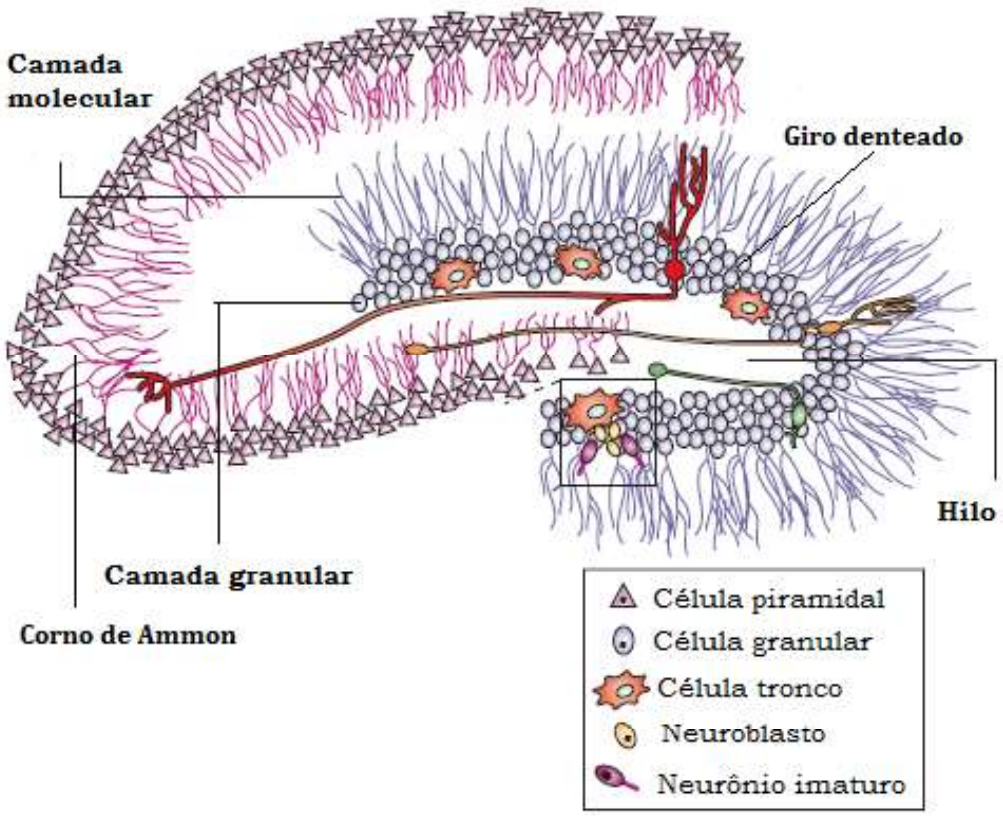

Figura 4. Desenho esquemático mostrando os tipos de células presentes na camada granular e molecular do GD, incluindo células precursoras de neurônios (adaptado de Vescovi e cols., 2006).

Dentro do hipocampo, as mudanças na força das sinapses entre grupos de neurônios, desempenham um papel crucial em funções cognitivas, de aprendizado e memória (Leuner e cols., 2006).

1.2.2 Memória

Através da aquisição de conhecimento, o processo pelo qual esses conhecimentos são armazenados, chamamos de memória. Em um primeiro momento, ocorre a exposição a determinadas experiências, onde ocorre a aquisição da informação; em um segundo momento, estas informações são processadas e armazenadas, por um mecanismo chamado consolidação da memória; por fim, as informações contidas na memória podem ser recordadas em algum momento, através da evocação. O processo de formação de memória envolve uma rede complexa de fatores bioquímicos, anatômicos, fisiológicos, comportamentais e ambientais (Izquierdo e cols., 1998; Zugno, 2007; Quillfeldt, 2010).

Podemos dividir a memória em dois tipos principais: a memória de curta duração e de longa duração. Na memória de curta duração, também chamada de memória operacional, ocorre o armazenamento de informações em contextos específicos por um curto período de tempo, que duram de minutos há poucas horas. Ainda, dentro da memória de curta duração, temos a memória de trabalho, que perdura por poucos segundos, para que, por exemplo, nossas atividades cotidianas possam ser feitas em continuidade, com nexo, sem que esqueçamos o que estamos fazendo naquele exato momento (Gazzaniga e cols., 2006).

A memória de longo prazo ocorre coletando-se informações em diferentes situações e não apenas em momentos e contextos específicos, que são armazenadas por longos períodos de tempo, até mesmo por toda a vida. Esta é dividida em memória declarativa (para eventos e fatos) e memória de não declarativa (para habilidades, principalmente, adquirida pela repetição e prática) (Okano e cols., 2000; Gazzaniga e cols., 2006; Valadares, 2006; Zugno, 2007; Deb e cols., 2015).

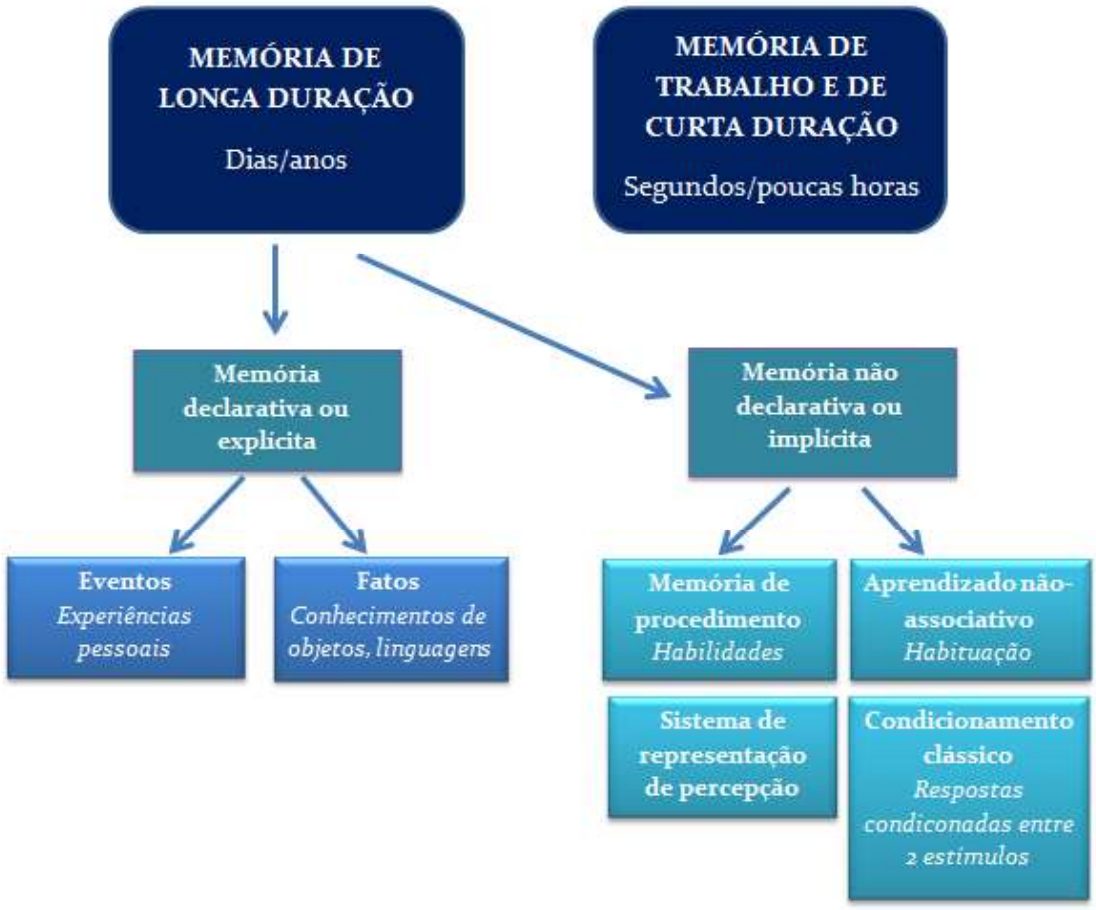

Figura 5. Esquema indicativo dos tipos de memória (Adaptada de Gazzaniga e cols, 2006).

Dentro do hipocampo, as memórias de curta duração são processadas e logo são esquecidas. Nas memórias de longa duração, o processo de aquisição e consolidação da memória, é bastante complexo. Resumidamente, um primeiro momento envolve a plasticidade sináptica e os potenciais de longa duração (LTP) entre neurônios pré-sinápticos e pós-sinapticos presentes no hipocampo, que interagem com outras regiões do cérebro, como a amigdala e os núcleos da base. Em seguida, ocorre a liberação de neurotransmissores, que ativam genes, neuroreceptores e a síntese de proteínas, que finalmente, terminam o processo de consolidação da memória. Após este processo, a memória fica armazenada em outras regiões do cérebro, principalmente no córtex

cerebral, e pode ser evocada a qualquer momento (Izquierdo e cols., 1998; Gazzaniga e cols., 2006).

1.3 Relação do Diabetes com SNC

O SNC, para seu bom funcionamento, necessita de glicose (Mello e cols, 2012). Porém, em situações em que há hiperglicemia crônica, pode haver sérios prejuízos ao SNC (Baynes, 1991; Reagan, 2012).

O estresse metabólico causado pela hiperglicemia é capaz de afetar o SNC de tal forma, que acaba resultando em dano neuronal e degenerativo, levando a diversos efeitos crônicos e adversos sobre o cérebro, como a diminuição da plasticidade sináptica, neurotoxicidade, déficits de memória e aprendizado (Stranahan e cols., 2008).

1.3.1 Dano tecidual

Em estudos envolvendo modelos experimentais de DM2, já foram observados alguns danos na região do hipocampo em decorrência da hiperglicemia crônica, como a perda e atrofia de espinhos dendríticos (Malone e cols., 2008), diminuição na neurogênese adulta (Kempermann e cols., 1998; Malberg e cols., 2000) interferência na proliferação celular (Beauquis e cols., 2006; Zhang e cols., 2008; Balu e Lucki, 2009), alterações de metabolismo mitocondrial, interferindo na respiração celular (Choi e cols., 2014) e principalmente, o aumento da apoptose neuronal (Hawkins e Davies, 2001).

O processo de apoptose ocorre por um mecanismo natural para desencadear a morte programada da célula. Este processo pode ocorrer por duas vias: a via extrínseca,

que é ativada por fatores de necrose tumoral, e a via intrínseca, que é ativada por algum tipo de estresse no meio extracelular ou intracelular, enviando sinais principalmente para a mitocôndria, que libera fatores pró-apoptóticos (Hengartner, 2000; Grivicich e cols., 2007). Quando a mitocôndria é estimulada por esses fatores, aumenta sua permeabilidade de membrana, o que faz com que a água do espaço intermembrana entre para seu interior, ocasionando sua ruptura. Essa ruptura, ocasiona a liberação de Citocromo-c, que juntamente com o fator de ativação de apoptose 1 (Apaf-1) e Caspase-9, forma um apoptossomo. Este apoptossomo leva a ativação da Caspase-9, que por sua vez, ativa a Caspase-3, finalizando o processo de apoptose, ocasionando a morte da célula. Além de provocar o rompimento da mitocôndria, os fatores pró-apoptóticos interferem na cadeia de produção de ATP, aumentando a produção de espécies reativas de oxigênio (EROs) (Grivicich e cols. 2007; Menzie e cols.2013). As EROs são moléculas que possuem um elétron desemparelhado em sua eletrosfera, altamente reativo, e que podem reagir com outras moléculas. Elas são produzidas naturalmente como um mecanismo de defesa a agressores, mas quando em excesso, exercem um efeito pró-oxidante, desencadeando um fenômeno que chamamos de estresse oxidativo (Hawkins e Davies, 2001; Warnholtz e cols., 2004; Dröse e Brandt, 2012). No estresse oxidativo, as EROs são capazes de reagir com lipídios de membrana de qualquer tecido, levando a morte celular. Se esta reação não for neutralizada por antioxidantes, ocorre uma inflamação crônica tecidual, aumentando ainda mais a morte celular, o que leva a destruição do tecido que for afetado (Valko e cols., 2007). Todo esse mecanismo de indução a apoptose pela liberação de fatores pró-apoptóticos e pelo estresse oxidativo pode ser induzido pela hiperglicemia crônica, e há indícios de que ela seja capaz de sozinha, induzir a ativação de caspase-3 e caspase-9 (Hawkins e Davies, 2001; Zeng e cols., 2010).

A hiperglicemia crônica também estimula um fenômeno que chamamos de reatividade astrocitária, que indica o sofrimento de células da glia em resposta ao estresse gerado por esta hiperglicemia, capaz de prejudicar seu bom funcionamento (Saravia e cols, 2002). Esta reatividade é uma reação normal a lesões, caracterizada pela proliferação intensa de astrócitos e expressão aumentada de proteína glial fibrilar ácida (GFAP), que é uma proteína de diferenciação de células da glia expressa por astrócitos (Verkhratsky e cols, 2015). Considerando que as células da glia desempenham um papel crítico no desempenho de diversas atividades neurais, um dano a estas células pode ocasionar diversos prejuízos, entre eles, o déficit cognitivo (Saravia e cols., 2002; Revsin e cols., 2005; Beauquis e cols., 2006; Lebed e cols., 2008). A hiperglicemia crônica também afeta a secreção da proteína S100B pelos astrócitos, que além de neuroprotetora, é responsável pela viabilidade e diferenciação neural (Lebed e cols., 2008; Mello e cols., 2012; Tramontina e cols, 2012). Ainda, o quadro hiperglicêmico é capaz de alterar o metabolismo do glutamato (Reagan, 2012). Uma vez que o astrócito já está com sua função comprometida, ele não consegue fazer a captação de glutamato na fenda sináptica. Isto gera um efeito excitotóxico, que irá aumentar o influxo de Ca^{2+} para o interior da célula neural, iniciando assim a produção de EROs desenfreadamente. Este aumento na produção de EROs não pode ser controlado pela atividade antioxidante normal, que se torna insuficiente, e assim, este estresse oxidativo causa sérios prejuízos ao tecido nervoso (Nardin, 2006; Mello e cols.,2012; Reagan, 2012).

1.3.2 Memória, aprendizado e déficit cognitivo

Alguns estudos já avaliaram os danos que a hiperglicemia crônica pode causar sobre a memória (Malone e cols., 2008; Revsin e cols., 2009; Piazza e cols, 2011).

A diminuição na taxa de neurogênese e o aumento na taxa de apoptose, são fatores que levam a neurodegeneração e diminuem a capacidade funcional das células. Estes fatores são muito influentes, e podem explicar a diminuição na capacidade cognitiva, de formação e evocação de memória em animais diabéticos (Kempermann e cols., 1998; Malberg e cols., 2000; Hawkins e Davies, 2001).

Além disso, evidências recentes sugerem que a hiperglicemia crônica é capaz de causar defeitos na transmissão e plasticidade sináptica hipocampal, interferindo nas sinapses entre neurônios, tanto na fase pré como na pós-sináptica, prejudicando a propagação da informação. É capaz de afetar a estrutura e a morfologia de elementos importantes envolvidos nas sinapses, gerando danos funcionais que podem resultar em comprometimento do aprendizado, da consolidação da memória e aumento do déficit cognitivo. Entre estes elementos estruturais, observam-se modificações estruturais nos terminais sinápticos, defeitos nas vesículas secretoras de neurotransmissores e retração dos dendritos, que não desempenham suas funções adequadamente (Reisi e cols, 2008; Revsin e cols., 2009; Reagan, 2012).

Sendo assim, cada vez mais buscam-se alternativas para tentar reduzir os efeitos negativos da hiperglicemia sobre o SNC (Reisi e cols, 2008; Stranahan, 2008; Yi e cols., 2009).

1.4 Taurina e o SNC

A taurina (ácido 2-aminoetanossulfônico) é um dos aminoácidos livres mais abundantes em alguns tecidos de mamíferos, principalmente humanos, como músculo esquelético, cardíaco e cérebro. Considerada como um aminoácido semi-essencial, possui diversas funções importantes, como antioxidante, anti-inflamatório,

antiarrítmico, regulador dos canais iônicos, formação de ácidos biliares, entre outros (Oja e Saransaari, 2007; Puerta e cols., 2010; Shivaraj e cols., 2012; Nóbrega, 2013; De Luca e cols., 2015). A taurina pode ser sintetizada no fígado a partir de dois aminoácidos, a metionina e cisteína, que sofrem ação de algumas enzimas, sendo transformada, ao final, em hipotaurina e finalmente, a taurina (Vitvitsky e cols., 2011; Menzie e cols., 2013).

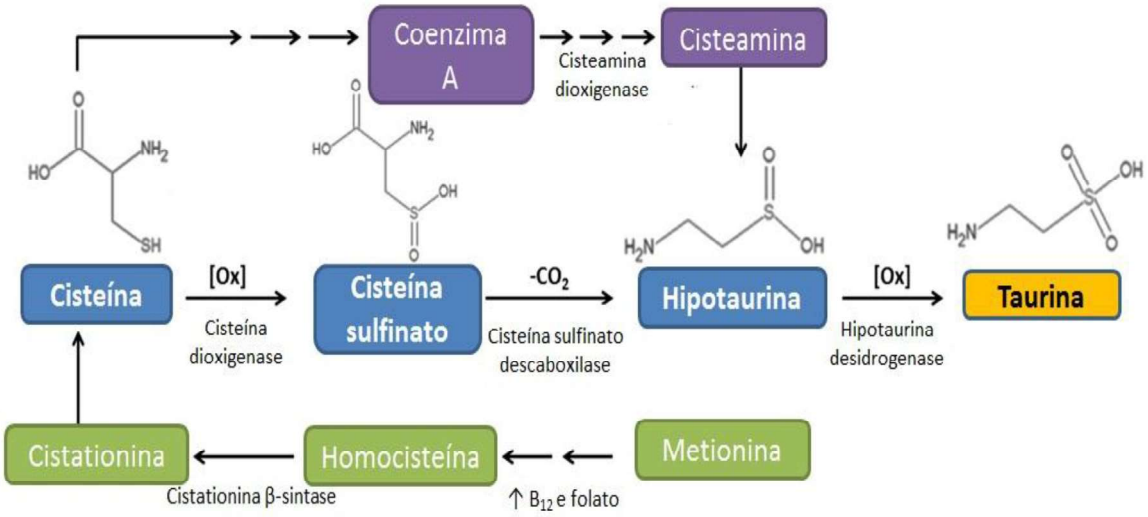

Figura 6. Rota de biossíntese da Taurina a partir de metionina e cisteína (adaptado de De Luca e cols., 2015).

Como a produção endógena é insuficiente, a taurina é absorvida através da alimentação, com alimentos ricos em aminoácidos, como o leite e derivados, carne, nozes, feijão e frutos do mar (Nóbrega, 2013; De Luca e cols., 2015).

Possui também um importante papel no funcionamento do SNC dos mamíferos, atuando em diversos processos, como osmorregulação, neuromodulação, estabilização da membrana, neuroproteção e proliferação celular, tendo ainda um grande potencial antioxidante (Franconi e cols., 2006; Oja e Saransaari, 2007; Ito e cols., 2012;

Kumari e cols., 2013; Menzie e cols., 2013). A taurina só não pode ser considerada um neurotransmissor por não possuir um receptor próprio, sendo assim, ela atua como um inibidor através dos receptores de GABA e outros receptores potenciais (Ripps e Shen, 2012; Menzie e cols., 2013).

Recentemente, alguns trabalhos demonstraram o papel benéfico da taurina no DM1 e DM2, resistência à insulina e suas complicações, incluindo nefropatia, retinopatia, neuropatia, aterosclerose e cardiomiopatia (Garcia e cols., 2003; Ito e cols., 2012; Nóbrega, 2013; De Luca e cols.,2015).

Este aminoácido tem demonstrado desempenhar um papel preventivo e terapêutico através dos seus efeitos antioxidantes, previamente comprovados, que são principalmente exercidos nas mitocôndrias, demonstrando ter uma ação benéfica relevante no contexto da diabetes (Suzuki e cols., 2001; Szymanski e Winiarska, 2008; Schaffer e cols., 2009). A taurina parece neutralizar os efeitos excitotóxicos do glutamato, diminuindo o influxo de Ca^{+2} para o interior da célula, controlando assim a produção de EROs (Schaffer e cols., 2009; Menzie e cols., 2013).

Recentes estudos sustentam a hipótese de que a suplementação dietética de aminoácidos como substâncias nutracêuticas pode ser benéfica para a saúde de indivíduos diabéticos, de maneira a controlar a hiperglicemia, evitando alterações neuronais importantes no SNC. Especula-se que ao nível central a taurina possa aumentar a atividade motora e motivação via estimulação da dopamina (Garcia e cols., 2003; Caletti e cols., 2012). Ainda, ela é capaz de agir na tríade de sintomas apresentados pela doença mal controlada (polifagia, polidipsia e hiperglicemia), indicando a proteção que o aminoácido oferece frente às complicações apresentadas pelo diabete. Sugere-se também que a taurina seja capaz de influenciar na saciedade,

porém, os mecanismos pelos quais esta saciedade ocorre ainda não foram elucidados (Veldhorst e cols., 2009).

A taurina exerce um número notável de efeitos positivos sobre os processos celulares em algumas doenças. No cérebro, representa a molécula mais relevante osmoticamente ativa e se especula que ela atue como um neuroprotetor, interferindo nas vias de apoptose e neutralizando danos decorrentes de estresse oxidativo no tecido, prevenindo assim a morte de células nervosas (Puerta e cols., 2010; Kumari e cols., 2013). Estudos também têm demonstrado a importância da taurina no processo de neurogênese e sinaptogênese, sendo capaz de estimular o desenvolvimento de células tronco em regiões do GD do hipocampo, proliferação de novos neurônios e neurotransmissão, melhorando a memória e déficits cognitivos (Shivaraj e cols., 2012). A taurina também parece ter um efeito protetor contra a apoptose de células gliais (Zeng e cols., 2010).

1.5 Ambiente enriquecido e SNC

O AE é definido como uma combinação de interação social, exposição continuada a "possibilidades de aprendizagem" e exercícios físicos. Um ambiente enriquecido experimental pode ser composto por uma gaiola mais ampla, com várias vias de acesso, rodas de exercício, opções de esconderijos e brinquedos de vários tamanhos e texturas. Desse modo, os artifícios presentes no ambiente, devem proporcionar a aquisição de novas habilidades e experiências, e que segundo alguns autores podem alterar a estrutura e função do encéfalo de roedores, por meio de estímulos excitatórios, o que aumenta a capacidade de plasticidade neuronal. Isso faz

com que o animal se torne mais curioso, facilitando o aprendizado e melhorando processos de memória (Krech e cols., 1960; Biernaskie e Corbett, 2001).

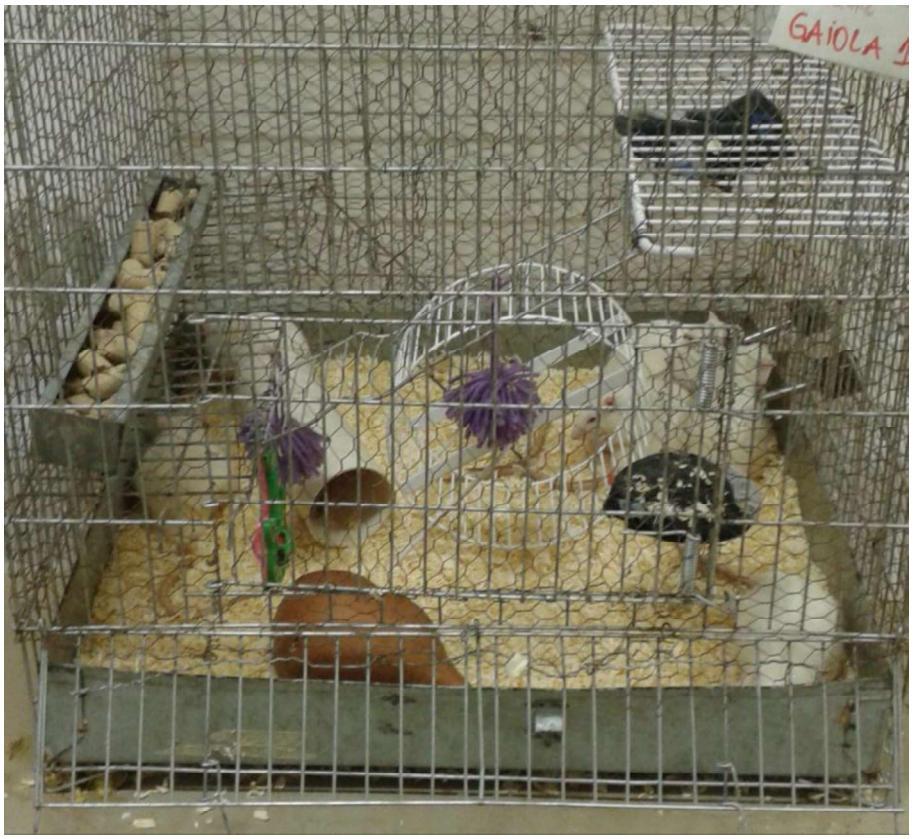

Figura 7. Exemplo de gaiola de enriquecimento ambiental (56x56x56), com 3 andares, contendo rampas, brinquedos e objetos para esconderijo (material do pesquisador).

Em estudos com animais com dano encefálico, a exposição ao enriquecimento ambiental (EA) pode desempenhar um papel importante na plasticidade neuronal hipocampal, bem como, melhorar as funções afetadas (van Praag e cols., 2000, Ahmadalipour e cols., 2015).

Algumas pesquisas demonstram que o AE é capaz de influenciar na neurogênese hipocampal e aumentar o número de astrócitos (van Praag e cols., 2000; Komitova e cols., 2005; Segovia e cols., 2006). Além disso, foi descrito que o AE é capaz de estimular o aumento dos prolongamentos dendríticos e proteger a rede vascular

do hipocampo de animais diabéticos (Beauquis e cols., 2010). Ainda, é possível observar sua influência sobre habilidades, aprendizado e memória (Beauquis e cols., 2010; Piazza e cols., 2011; Ahmadalipour e cols., 2015).

1.6 A imuno-histoquímica na avaliação do dano ao tecido nervoso

Existem diversas técnicas que são capazes de evidenciar alterações em células do SNC e possíveis danos neurais. Estudos recentes utilizam diversas técnicas para estudar o comportamento de astrócitos (GFAP), proliferação celular e neurogênese (5-bromo-2'-deoxyuridine (BrdU); Neuronal Nuclei (NeuN); Neural cell adhesion molecule (NCAM)) e apoptose neuronal (Caspase-3), frente a desordens metabólicas e doenças em nível cerebral. Estas técnicas compreendem a imunocitoquímica, imunofluorescência, *western blot* e também a imuno-histoquímica (IH), que detecta componentes celulares no tecido através de marcadores, gerando ligações antígeno-anticorpo capazes de ser observadas e quantificadas (Kandratavicius e cols., 2007; Revsin e cols., 2009; Beauquis, 2010; Piazza, e cols., 2011; Hsiao e cols., 2014).

A GFAP é uma proteína de filamentos intermediários encontrada no citoplasma dos astrócitos. Sabendo-se que em quadros estressores, como na hiperglicemia crônica, há o aumento da reatividade astrocitária, é muito útil para avaliar a dimensão do dano neural pela técnica de IH (Saravia e cols, 2002; Revsin e cols, 2005; Lebed e cols., 2008).

Para detecção de células em apoptose podemos utilizar como marcador a Caspase-3, que é uma protease da família das caspases, que está intimamente ligada à cascata de eventos que termina em apoptose (Chen e cols., 2015). Na hiperglicemia crônica, sabe-se que há a ativação de Caspase-3 em decorrência do dano mitocondrial, e

por isso, células em apoptose podem ser observadas pela técnica de IH (Hawkins e Davies, 2001; Zeng e cols., 2010; Menzie e cols, 2013).

1.7 Avaliação do comportamento animal e déficits de memória

Existem diversas formas pelas quais se pode avaliar o comportamento, a memória e o aprendizado de animais. Através de testes bem estabelecidos, é possível avaliar estados emocionais e a capacidade do animal de aprender e conseguir evocar uma memória (Quillfeldt, 2010).

1.7.1 Teste do campo aberto

Esse teste permite avaliar a atividade locomotora de animais, a atividade exploratória, o estado de ansiedade, de depressão e medo. Conhecendo-se o comportamento normal do animal, é possível avaliar comportamentos patológicos (Oliveira e cols., 2008; Quillfeldt, 2010). O teste consta de um aparato, chamado arena, que é cercado por paredes, onde o animal não tem visão para o exterior. O assoalho desta arena possui marcações, em quadrantes, que delimitam o centro e a periferia da arena, para que a partir destes, seja possível avaliar em qual região o animal permanece por mais tempo.

O animal é solto no centro da arena, e seus comportamentos são filmados por um período pré-determinado, de acordo com cada protocolo. Na análise dos vídeos, podem ser avaliadas: a distância que o animal percorreu dentro da arena; o tempo gasto na periferia do aparato; o número de quadrantes atravessados em cada região, que irá

indicar o nível de deambulação do animal; o tempo que o animal permaneceu imóvel (FREEZING), farejando (SNIFFING) e explorando em pé (REARING), que irão indicar a atividade locomotora e exploratória; o tempo gasto no centro e o número de quadrantes atravessados; tempo de autolimpeza (GROOMING) e quantidade de defecação, que são parâmetros que indicam o nível de ansiedade do animal (Gould e cols., 2009; Quillfeldt, 2010; Guilhermitti, 2011). Muitos estudos utilizam esse teste para avaliar o efeito de drogas, doenças e tratamentos (Carola e cols., 2002; Cryan e Holmes, 2005; Hsiao e cols., 2014), incluindo aqueles para avaliar efeitos da diabetes, enriquecimento ambiental e da administração de taurina (Piazza e cols., 2011; Caletti e cols., 2012).

1.7.2 Reconhecimento de Objetos

A tarefa de reconhecimento de objetos (RO) avalia a capacidade do animal de relembrar um objeto que lhe foi apresentado, e ainda, perceber a troca do objeto antigo por um novo e diferente. Essa tarefa envolve memória espacial e a memória para itens, além disso, envolve processos cognitivos de identificação, percepção e discriminação, por exemplo (Quillfeldt, 2010).

O teste consiste em submeter o animal a uma sessão de treino, dentro de uma arena, contendo 2 objetos idênticos. Durante alguns minutos, o animal pode explorar a vontade a arena e os objetos. O animal é retirado desta arena por um tempo, que pode variar de minutos até um dia, de acordo com cada protocolo, e em seguida, retorna para a sessão de teste. No teste, o animal é apresentado novamente a dois objetos, sendo que um destes é o mesmo que já lhe foi apresentado na sessão de treino, e o outro é trocado por um objeto novo, de cores, dimensões e formato diferentes (Hsiao e cols, 2014).

Alguns estudos utilizam esta técnica para avaliar danos à memória causados por diversas doenças, incluindo a diabetes, e também, para observar a influência de medicamentos sobre a aquisição, consolidação e evocação destas memórias (Dix e Aggleton, 1999; Revsin e cols, 2009; Piazza e cols., 2011; Hsiao e cols, 2014; Zuloaga e cols., 2015).

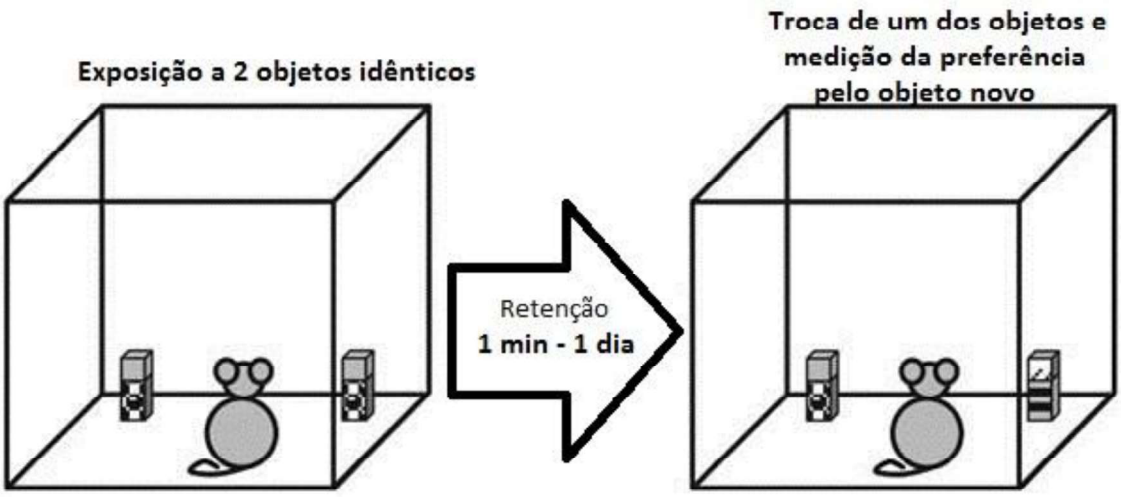

Figura 8. Teste de reconhecimento espontâneo de objetos para avaliação de memória espacial e de curto prazo em ratos. O tempo total de exploração é de geralmente 5 minutos, e o tempo de intervalo entre o treino e o teste pode variar de 1 minuto a 1 dia, conforme o protocolo (adaptado de Dix e Aggleton, 1999).

1.7.3 Esquiva inibitória

A esquiva inibitória (EI), ou esquiva passiva, avalia a capacidade do animal de adquirir, consolidar e evocar memórias aversivas através de estímulos com choque elétrico, e também é capaz de avaliar memória espacial e de trabalho. Diversos estudos

utilizam este sistema de choque para avaliar déficits de memória nas mais diversas patologias (Zugno, 2007; Ahmadalipour e cols., 2015; Tabrizian e cols., 2015).

No presente teste, o animal, em uma sessão de treino, é colocado dentro de uma caixa fechada, sobre uma plataforma com uma das paredes transparente, para melhor observação. A tendência é que após algum tempo de exploração, o animal desça da plataforma para explorar o restante do ambiente. Quando o animal desce da plataforma e toca com as quatro patas o assoalho do aparato, que é composto por barras metálicas conectadas a uma fonte elétrica, recebe um choque intermitente que pode variar até 1mA (miliampère), durante 3-5 segundos. Neste momento espera-se que o animal adquira uma memória aversiva a este choque. Na sessão de teste, que pode ocorrer 24 horas após o treino, o animal é recolocado no aparato sob as mesmas condições, e é medido o tempo que o animal leva para descer da plataforma, chamado de tempo de latência. Desta vez, quando o animal tocar as barras com as patas, não levará outro choque. Teoricamente, um animal que possui uma boa memória consegue evocar a memória aversiva ao choque, apresentando assim um tempo de latência maior do que animais que possuem déficits de memória (Zugno, 2007; Quillfeldt, 2010; Ogren e Stiedl, 2013).

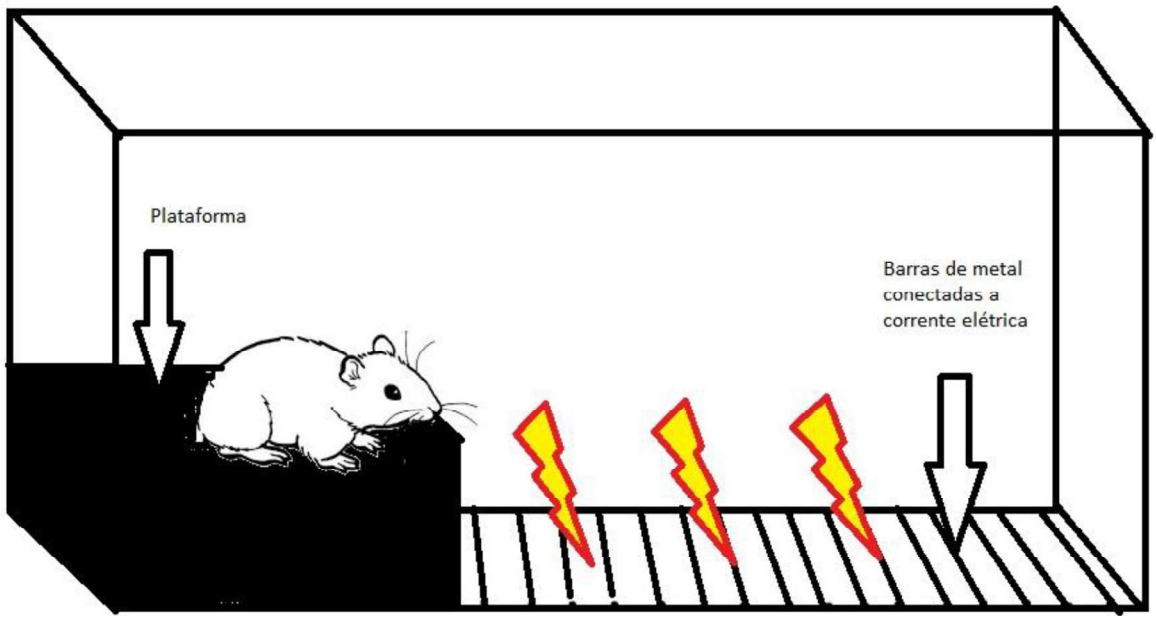

Figura 9: Desenho representativo do teste de esquiva inibitória, mostrando a plataforma e as barras de metal conectadas a fonte elétrica, que irá liberar o choque a medida que o animal tocá-las com as 4 patas (adaptado de Quillfeldt, 2010).

1.8 Referências bibliográficas

Ahmadalipour A, Sadeghzadeh J, Vafaei AA, Bandegi AR, Mohammadkhani R, Pour AR. Effects of environmental enrichment on behavioral deficits and alterations in hippocampal BDNF induced by prenatal exposure to morphine in juvenile rats. Neuroscience. 2015 Oct; 1(305):372-83.

ADA. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2009; 32:62–67.

Balu DT, Lucki I. Adult hippocampal neurogenesis: regulation, functional implications, and contribution to disease pathology. Neurosci Biobehav Rev. 2009; 333:232-52.

Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes. 1991; 404:405-12.

Bear MF, Connors BW, Paradiso MA. Neurociências: desvendando o sistema nervoso. Porto Alegre: Artmed. 2008; 3ª ed, 896p.

Beauquis J, Roig P, De Nicola A, Saravia F. Short-term environmental enrichment enhances adult neurogenesis, vascular network and dendritic complexity in the hippocampus of type 1 diabetic mice. Plos One. 2010; 5(11).

Beauquis J, Roig P, Delarche FH, De Nicola A, Saravia F. Reduced hippocampal neurogenesis and number of hilar neurones in streptozotocin-induced diabetic mice: reversion by antidepressant treatment. Eur J Neurosci. 2006; 236:1539-46.

Biernaskie J, Corbett D. Enriched rehabilitative training promotes improved forelimb motor function and enhanced dendritic growth after focal ischemic injury. J Neurosci. 2001; 2114:5272-80.

Bloomgarden ZT. Nephropathy and retinopathy. Diabetes Care. 1999; 224:640-4.

Caletti G, Olguins DB, Pedrollo EF, Barros HM, Gomez R. Antidepressant effect of taurine in diabetic rats. Amino Acids. 2012.

Carola V, D'Olimpio F, Brunamonti E, Mangia F, Renzi, P. Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behav Brain Res. 2002; 134: 49-57.

Chen H, Yang X, Feng Z, Tang R, Ren F, Wei K, *et al*. Prognostic value of Caspase-3 expression in cancers of digestive tract: a meta-analysis and systematic review. Int J Clin Exp Med. 2015; 8(7):10225–10234.

Choi J, Chandrasekaran K, Demarest TG, Kristian T, Xu S, Vijaykumar K, *et al.* Brain diabetic neurodegeneration segregates with low intrinsic aerobic capacity. Ann Clin Transl Neurol. 2014; 1(8):589–604.

Cryan JF, Holmes A. The ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discov. 2005; 775-790.

Deb D, Bairy KL, Nayak V, Rao M. Comparative effect of lisinopril and fosinopril in mitigating learning and memory deficit in scopolamine-induced amnesic rats. Adv Pharmacol Sci. 2015.

De Luca A, Pierno S, Camerino DC. Taurine: the appeal of a safe amino acid for skeletal muscle disorders. J Transl Med. 2015 Jul; 13:243.

Dix SL, Aggleton JP. Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. Behav Brain Res. 1999; 992: 191-200.

Dröse S, Brandt U. Molecular mechanisms of superoxide production by the mitochondrial respiratory chain. Adv Exp Med Biol. 2012; 748:145–169.

Franconi F, Loizzo A, Ghirlanda G, Seghieri G. Taurine supplementation and diabetes mellitus. Curr Opin Clin Nutr Metab Care. 2006; 91:32-6.

Garcia VL, Bragança E, Burini RC. A taurina como ergogênico. Nutrição em Pauta. 2003; 1161.

Gazzaniga MS, Ivry RB, Mangun GR. Neurociência cognitiva: a biologia da mente. 2ª ed. Porto Alegre: Artmed, 2006.

Gould T, Dao D, Kovacsics C. The open field test. In: Gould TD, editor. Mood and anxiety related phenotypes in mice: characterization using behavioral tests. Neuromethods, v.42. Human Press. 2009.

Greenwood CE, Winocur G. High-fat diets, insulin resistance and declining cognitive function. Neurobiol Aging. 2005; 26(1): 42-5.

Grivicich I, Regner A. Rocha AB. Morte celular por apoptose. Revista Brasileira de Cancerologia. 2007; 53(3):335-343.

Guilhermitti, A.C. Comportamento de filhotes de rato (*Rattus norvegicus*) em um campo aberto na presença e na ausência de animais adultos [dissertação]. São Paulo. Universidade de São Paulo, 2011.

Hawkins CL, Davies MJ. Generation and propagation of radical reactions on proteins. Biochim Biophys Acta. 2001; 15042-3:196-219.

Hengartner MO. The biochemistry of apoptosis. Nature. 2000; 407:770-76.

Hsiao, YH, Hung, HC, Shen SH, Gean PW. Social interaction rescues memory deficit in an animal model of Alzheimer's disease by increasing BDNFD dependent hippocampal neurogenesis. J Neurosci. 2014 Dec; 34(49):16207–16219.

International Diabetes Federation. IDF Diabetes Atlas. 2013. 6ª ed, 160 p.

Ito T, Schaffer SW, Azuma J. The potential usefulness of taurine on diabetes *mellitus* and its complications. Amino Acids. 2012; 425: 1529-39.

Izquierdo I, Barros DM, Souza TM, de Souza MM, Izquierdo LA, Medina JH. Mechanisms for memory types differ. Nature. 1998; 3936686: 635-6.

Junqueira LC, Carneiro J. Histologia básica: texto e atlas. Rio de janeiro: Guanabara Koogan. 12ª ed., 2013.

Kandratavicius L, Monteiro MR, Romcy-Pereira RN, Arisi GM, Cairasco NG, Leite JP. Neurogênese do cérebro adulto e na condição epilética. J Epilepsy Clin Neurophysiol. 2007; 13(3):119-123.

Kempermann G, Kuhn HG, Gage FH. Experience-induced neurogenesis in the senescent dentate gyrus. J Neurosci. 1998; 189:3206-12.

Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. Nature.1997; 386: 493-495.

Kempermann G, Song H, Gage FH. Neurogenesis in the adult hippocampus. Cold Spring Harb Perspect Biol. 2015.

Komitova M, Mattson B, Johansson B, Erikson PS. Enriched environment increases neural stem/progenitor cell proliferation and neurogenesis in the subventricular zone of stroke-lesioned adult rats. Stroke. 2005 Jun; 36(6):1278-82.

Krech D, Rosenzweig MR, Bennett EL. Effects of environmental complexity and training on brain chemistry. J Comp Physiol Psychol. 1960; 53:509-19.

Kumar V, Abbas AK, Fausto N. Robbins & Cotran: Patologia: bases patológicas das doenças. 7ª ed. Rio de Janeiro: Elsevier, 2005.

Kumari N, Prentice H, Wu JY. Taurine and its neuroprotective role. Adv Exp Med Biol. 2013; 775: 19–27.

Lebed YV, Orlovsky MA, Nikonenko AG, Ushakova GA, Skibo GG. Early reaction of astroglial cells in rat hippocampus to streptozotocin-induced diabetes. Neurosci Lett. 2008; 4442:181-5.

Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008; 512: 216-26.

Leuner B, Gould E, Shors TJ. Is there a link between adult neurogenesis and learning? Hippocampus. 2006;163: 216-24.

Machado A. Neuroanatomia funcional. 2ªed. São Paulo: Atheneu, 380p, 2000.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci. 2000; 2024:9104-10.

Malone JI, Hanna S, Saporta S, Mervis RF, Park CR, Chong L*, et al.* Hyperglycemia not hypoglycemia alters neuronal dendrites and impairs spatial memory. Pediatr Diabetes. 2008; 96:531-9.

Mello AS, Santos AQ, Funchal C. Correlação entre hiperglicemia e células do SNC, com enfoque na atividade glial. Revista de Neurocienciência. 2012; 20(2):294-301.

Menzie J, Prentice H, Wu JY. Neuroprotective mechanisms of taurine against ischemic stroke. Brain Sci. 2013 Jun; 3(2): 877–907.

Messier C. Impact of impaired glucose tolerance and type 2 diabetes on cognitive aging. Neurobiol Aging. 2005; 26(1): 26-30.

Moore KL, Persaud TVN. The developing human: clinically oriented embryology. 7ª ed, Saunders. 2003, 560p.

Nardin P. Avaliação dos parâmetros bioquímicos e morfológicos em células gliais expostas ao um meio com alto conteúdo de glicose [dissertação]. Porto Alegre: Universidade Federal do Rio Grande do Sul, 2006.

Nobrega, MP. Efeito da suplementação de taurina na oxidação de substratos energéticos e no desempenho de atletas nadadores [dissertação]. São Paulo: Universidade Estadual de São Paulo, 2013.

Ogren SO, Stiedl O. Passive Avoidance. In: Stolerman IP. Encyclopedia of Psychopharmacology. Springer 2013:960-967.

Oja SS, Saransaari P. Pharmacology of taurine. Proc West Pharmacol Soc. 2007; 50: 8-15.

Oliveira RB, Nascimento MVM, Valadares MC, de Paula JR, Costa EA , da Cunha LC. Avaliação dos efeitos depressores centrais do extrato etanólico das folhas de *Synadenium umbellatum pax* e de suas frações em camundongos albinos. Revista Brasileira de Ciências Farmacêuticas. 2008 Jul/Set; 44(3).

Okano H, Hirano T, Balaban E., "Learning and memory," Proceedings of the National Academy of Sciences of the United States of America. Proc Natl Acad Sci. 2000; 97(23): 12403–12404.

Ozkaya YG, Agar A, Yargicoglu P, Hacioglu G, Bilmen-Sarikcioglu S, Ozen I*, et al.* The effect of exercise on brain antioxidant status of diabetic rats. Diabetes Metab. 2002; 285:377-84.

Piazza FV, Pinto GV, Trott G, Marcuzzo S, Gomez R, Fernandes MC. Enriched environment prevents memory deficits in type 1 diabetic rats. Behav Brain Res. 2011; 217: 16-20.

Puerta, CD, Arrieta FJ, Balsa JA, Botella-Carretero JI, Zamarrón I *et al.* Taurine and glucose metabolism: a review. Nutrición Hospitalaria. 2010; 6(25): 910-919.

Quillfeldt JA. Behavioral Methods to Study Learning and Memory in Rats. In: Andersen ML, Tufik S, editors. Animal models as tools in ethical biomedical research. São Paulo, Universidade Federal de São Paulo. 2010:227-269.

Reagan LP. Diabetes as a chronic metabolic stressor: causes, consequences and clinical complications. Exp Neurol. 2012; 2331:68-78.

Reaven GM. The insulin resistance syndrome: definition and dietary approaches to treatment. Annu Rev Nutr. 2005; 25:391-406.

Reisi P, Babri S, Alaei H, Sharifi MR, Mohaddes G, Lashgari R. Effects of treadmill running on short-term pre-synaptic plasticity at dentate gyrus of streptozotocin-induced diabetic rats. Brain Res. 2008; 1211:30-6.

Revsin Y, Rekers NV, Louwe MC, Saravia FE, De Nicola AF, de Kloet ER*, et al.* Glucocorticoid receptor blockade normalizes hippocampal alterations and cognitive impairment in streptozotocin-induced type 1 diabetes mice. Neuropsychopharmacology. 2009; 343:747-58.

Revsin Y, Saravia F, Roig P, Lima A, de Kloet ER, Homo-Delarche F*, et al.* Neuronal and astroglial alterations in the hippocampus of a mouse model for type 1 diabetes. Brain Res. 2005; 10381: 22-31.

Ripps H, Shen W. Review: Taurine: A “very essential” amino acid. Molecular Vision. 2012; 18:2673-2686.

Ross MH, Paulina W. Histologia: texto e atlas, em correlação com Biologia celular e molecular. 6ª ed. Rio de Janeiro: Guanabara Koogan, 2012.

Saravia FE, Revsin Y, Gonzalez Deniselle MC, Gonzalez SL, Roig P, Lima A*, et al.* Increased astrocyte reactivity in the hippocampus of murine models of type 1 diabetes: the nonobese diabetic (NOD) and streptozotocin-treated mice. Brain Res. 2002; 9572:345-53.

Schaffer SW, Azuma J, Mozaffari M. Role of antioxidant activity of taurine in diabetes. Can J Physiol Pharmacol. 2009; 872: 91-9.

Segovia G, Yague AG, Garcia-Verdugo JM, Mora F. Environmental enrichment promotes neurogenesis and changes the extracellular concentrations of glutamate and GABA in the hippocampus of aged rats. Brain Res Bull. 2006; 701: 8-14.

Serino R, Ueta Y, Tokunaga M, Hara Y, Nomura M, Kabashima N*, et al.* Upregulation of hypothalamic nitric oxide synthase gene expression in streptozotocin-induced diabetic rats. Diabetologia. 1998; 416:640-8.

Shivaraj MC, Marcy G, Low G, Ryu JR, Zhao X, Rosales FJ, *et al*. Taurine induces proliferation of neural stem cells and synapse development in the developing mouse brain. Plos One, 2012 Aug; 7(8):1-12.

Souza CF, Gross JL, Gerchman F, Leitão CB. Pré-diabetes: diagnóstico, avaliação de complicações crônicas e tratamento. Arquivos Brasileiros de Endocrinologia e Metabologia. 2012; 56:275-284.

Stranahan AM, Arumugam TV, Cutler RG, Lee K, Egan JM, Mattson MP. Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. Nat Neurosci. 2008; 113:309-17.

Suzuki T, Wada T, Saigo K, Watanabe K. Novel taurine-containing uridine derivatives and mitochondrial human diseases. Nucleic Acids Res Suppl. 2001; 1: 257-8.

Szymanski K, Winiarska K. Taurine and its potential therapeutic application. Postepy Hig Med Dosw. 2008; 62: 75-86.

Tabrizian K, Yaghoobi NS, Iranshahi M, Shahraki J, Rezaee R, Hashemzaei M. Auraptene consolidates memory, reverses scopolamine-disrupted memory in passive avoidance task, and ameliorates retention deficits in mice. Iran J Basic Med Sci. 2015; 18(10):1014-9.

Tramontina AC, Nardin P, Santos AQ, Tortorelli L, Wartchow KM, Andreazza AC, *et al.* High-Glucose and S100B Stimulate Glutamate Uptake in C6 Glioma Cells. Neurochem Res. 2012; 37:1399-408.

Valadares CT, Efeitos da desnutrição protéica pós-natal do desempenho de ratos em diferentes tarefas de aprendizagem e memória [tese]. São Paulo: USP, 2006.

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007 Aug; 39(1):44-84.

van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. Nat Rev Neurosci. 2000; 13:191-8.

Veldhorst MA, Nieuwenhuizen AG, Hochstenbach-Waelen A, Westerterp KR, Engelen MP, Brummer RJ*, et al.* Effects of high and normal soyprotein breakfasts on satiety and subsequent energy intake, including amino acid and 'satiety' hormone responses. Eur J Nutr. 2009; 482: 92-100.

Verkhratsky A, Steardo L, Parpura V, Montana V. Translational potential of astrocytes in brain disorders. Prog Neurobiol. 2015.

Vescovi AL, Galli R, Reynolds BA. Brain tumour stem cells. Nat Rev Cancer. 2006; 6: 425-436.

Vitvitsky V, Garg SK, Banerjee R. Taurine biosynthesis by neurons and astrocytes. J Biol Chem. 2011; 286:32002-32010.

Warnholtz A, Wendt M, August M, Münzel T. Clinical aspects of reactive oxygen and nitrogen species. Biochem Soc Symp. 2004; (71):121-133.

Yi SS, Hwang IK, Yoo KY, Park OK, Yu J, Yan B*, et al.* Effects of treadmill exercise on cell proliferation and differentiation in the subgranular zone of the dentate gyrus in a rat model of type II diabetes. Neurochem Res. 2009; 346: 1039-46.

Zeng K, Xu H, Mi M, Chen K, Zhu J, Yi L, *et al.* Effects of taurine on glial cells apoptosis and taurine transporter expression in retina under diabetic conditions. Neurochem Res. 2010 Oct; 35: 1566-1574.

Zhang WJ, Tan YF, Yue JT, Vranic M, Wojtowicz JM. Impairment of hippocampal neurogenesis in streptozotocin-treated diabetic rats. Acta Neurol Scand. 2008; 1173: 205-10.

Zugno, AI. Guanidino acetato altera parâmetros bioquímicos e comportamentais em ratos: efeito neuroprotetor da taurina e antioxidantes [tese]. Porto Alegre: Universidade Federal do Rio Grande do Sul, 2007.

Zuloaga KL, Johnson LA, Roese NE, Marzulla T, Zhang W, Nie X, *et al*. High fat diet-induced diabetes in mice exacerbates cognitive deficit due to chronic hypoperfusion. J Cereb Blood Flow Metab. 2015.

2 OBJETIVOS

1.1. Objetivo geral

Avaliar a influência da taurina e do ambiente enriquecido sobre o giro denteado do hipocampo de ratos diabéticos e não diabéticos.

1.2. Objetivos específicos

- Avaliar o impacto do uso da taurina e exposição ao AE sobre a memória (de curta e de longa duração) e comportamento nos diferentes grupos experimentais;
- Avaliar a influência da taurina e do AE sobre a densidade dos filamentos intermediários (GFAP+) dos astrócitos no GD do hipocampo de animais diabéticos e não diabéticos;
- Avaliar a influência da taurina e do AE sobre a apoptose, observando a variação no número de células marcadas com caspase-3 clivada no GD do hipocampo de animais diabéticos e não diabéticos;
- Verificar em todas as análises, se a taurina e o AE, juntos, são capazes produzir efeitos sinérgicos.

3 ARTIGO CIENTÍFICO REDIGIDO EM INGLÊS

O artigo intitulado, “The effect of taurine and enriched environment on behaviour, memory and hippocampus of diabetic rats”, encontra-se a seguir, e está de acordo com as normas de publicação, que seguem em ANEXO, da revista Neuroscience Letters.

THE EFFECT OF TAURINE AND ENRICHED ENVIRONMENT ON BEHAVIOUR, MEMORY AND HIPPOCAMPUS OF DIABETIC RATS

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Abstract

Diabetes mellitus (DM) has been studied recently as a major cause of cognitive deficits, memory and neurodegenerative damage. Taurine and enriched environment have stood out for presenting neuroprotective and stimulating effects that deserve further study. In this paper, we examined the effects of taurine (T) and enriched environment in the context of diabetes, evaluating effects on behaviour, memory, death and cellular activity. Eighty-eight Wistar rats were divided into 2 groups (E=enriched environment; C=standard housing). Some animals (24/group) underwent induction of diabetes, and within each group, some animals (half of diabetics (D) and half of non-diabetics (ND)/group) were treated for 30 days with taurine. Untreated animals received saline (S). In total, there were eight subgroups: DTC, DSC, NDTC, NDSC, DTE, DSE, NDTE and NDSE. During the experiment, short and long-term memories were evaluated. On the 30th day from the beginning of treatment, the animals were euthanized by transcardiac perfusion for removal of brains. All brain sections were made for immunohistochemistry procedures for GFAP and cleaved caspase-3. As a result, we observed that animals treated with taurine showed better performance in behavioural and memory tasks, and the enriched environment had positive effects, especially in non-diabetic animals. Furthermore, taurine and enriched environment seemed to be able to interfere with neuronal apoptosis and loss of glial cells, and in some instances, these two factors seemed to have synergistic effects. From these data, taurine and enriched environment may have important neurostimulant and neuroprotective effects.

Keywords: Taurine, enriched environment, diabetes, memory, dentate gyrus, neurodegeneration.

1. Introduction

According to the *International Diabetes Federation*, approximately 382 million people worldwide have diabetes *mellitus* (DM), and it is believed that by the year 2035, this number will increase to 592 million cases [1].

Chronic hyperglycaemia may cause various effects on the body and central nervous system (CNS). Several chronic effects on the brain have been described, such as decreased synaptic plasticity [2, 3], neurotoxicity, neuroinflamation, decreased cell proliferation, increased neuronal apoptosis [4-6], astrocyte damage, excitotoxicity, changes in glutamatergic neurotransmission [3, 7] and mainly cognitive, memory and learning deficits [2, 3, 8-10].

The effects of environmental enrichment (EE) in animal studies have been observed to simulate the influence of physical exercises, sensory stimulation and learning, with positive effects on skills, memory and cognitive deficits [11-13]. Studies have shown that the EE is able to influence hippocampal neurogenesis, also increase dendritic extensions and the number of astrocytes [11-16].

Other studies have shown the positive effects of taurine. Taurine, considered as a semi-essential amino acid, it has several important functions, especially on the mammalian CNS acting in various processes such as osmoregulation, neuromodulation, membrane stabilization and cell proliferation [17, 18]. In metabolic stress conditions, such as DM, taurine has neuroprotective and antioxidants effects [19-21]. It interferes with the apoptosis pathways [22-24], helps in cell proliferation, neurogenesis, improves cognitive deficits [25] and neutralises excitotoxicity [26].

Faced with so many beneficial effects, this study seeks to unite in a single experiment the effects of taurine and exposure to an EE in a hyperglycaemic state, to obtain more results on this subject.

2. Material and methods

2.1. Animals

Eighty-eight male adult Wistar rats weighing 300g on average, from the local breeding colony (Universidade Federal de Ciências da Saúde de Porto Alegre, Brazil - UFCSPA) were used. All procedures were approved by the Ethics Committee of Universidade Federal de Ciências da Saúde de Porto Alegre – UFCSPA (Protocol 134/13). The animals were manipulated according to international and national laws for the ethical care and manipulation of laboratory animals (European Communities Council Directive of 22 September 2010, 2010/63/EU and Lei 11.794/08).

2.2. Experimental groups

The animals were divided into 2 main groups, according to the environment: group C were housed in standard housing and group E, were housed in cages with EE. Each group (C and E) was divided into four subgroups, according to the induction of diabetes and the treatment, totalling eight subgroups: diabetic animals treated with taurine in group C (DTC, n=12); diabetic animals untreated with taurine in group C (DSC, n=12); non-diabetic (ND) animals treated with taurine in group C (NDTC, n=10); non-diabetic animals untreated with taurine in group C (NDSC, n=10); diabetic

animals treated with taurine in group E (DTE n=12); diabetic animals untreated with taurine in group E (DSE, n=12); non-diabetic animals treated with taurine in group E (NDTE, n=10); non-diabetic animals untreated with taurine in group E (NDSE, n=10) (Figure 1).

2.3. Environmental conditions

Forty-four animals were housed in cages with EE (group E), divided proportionally between 6 cages (56x56x56cm) in order to promote social interaction. Each cage had three floors connected by ramps, promoting physical exercise and movement. Various elements of different shapes and textures were placed, including: balls, stairs, cubes, tunnels, wool pompoms, chopped paper, sand paper, swings, burrows, rattles and wheels exercises, which were available to the animals during the 30 days of the experiment. They were always 5 different objects in each cage and once a week, some of them were replaced by different ones, with the intention to stimulate sensory, motor and cognitive functions. The remaining forty-four animals were housed in standard housing (group C) (Plexiglas® cages, 41x34x16cm), with two animals per cage in order to minimise the social interaction and without any kind of EE.

All animals in C and E groups, until the beginning of the experiment, went through a period of adaptation to new environments, for about 10 days. They were kept under ideal conditions of temperature (22 ± 2°C) and humidity (55%), with a light:dark cycle of 12 hours. All animals had free access to food and water.

2.4. Induction and confirmation of diabetes

After the habituation and handling period (10 days), 24 animals from each group were induced with diabetes. After 16 hours of fasting, animals received intraperitoneal (i.p.) injection of 50 mg/kg of streptozotocin (Sigma®), dissolved in citrate buffer (pH 4.5). Non-diabetic control animals were injected only with citrate buffer (1 mL/kg i.p.). The hyperglycaemia was confirmed 48 hours after administration. Animals who showed blood glucose above 200 mg/dL were considered diabetics. No animal had to be excluded. During the 30 days of the experiment, glucose levels were measured once a week, until the day before euthanasia.

2.5 Administration of taurine

Taurine (Pharmanostra®) was administered at a dose of 100 mg/kg i.p., dissolved in saline, with the first dose given 1 day after diabetes confirmation and applied daily for 30 days. Taurine was administered in half of the diabetic animals and half of the non-diabetic animals from each group (C and E). The animals untreated with taurine received only saline (1 mL/kg).

2.6. Open field task (OF)

The open field task evaluates the animal's behaviour in a different environment. On the 25th day of the experiment, 1 hour after administration of taurine or saline, the animals were individually placed in an empty arena (Insight®, 60x60x60cm), surrounded by walls and the floor were divided into 12 quadrants; their behaviours were

recorded for 5 minutes. Then, the videos were watched by two observers, and the following behaviours were analysed and measured: (1) input frequency in the periphery area (CROSSINGp); (2) time in the centre area (TIMEc); (3) time sniffing (SNIFFING) and (4) number of "rearing" (REARING). These analysed parameters were able to express tranquillity, anxiety, fear and exploratory behaviour.

2.7. Object recognition task

The animals were placed individually in the same arena used for the open field task (60x60x60cm) for 5 minutes for 2 consecutive days. On the third day (27th day of treatment) the animals underwent a training session for 5 minutes, with two identical objects (A1 and A2) being presented to them. After training, the animals returned to the cages for 1 minute. Meanwhile, one object (A2) was replaced by another unfamiliar object (B). The animals returned to the arena and the exploratory behaviours were recorded for 3 minutes, for frequency analysis and time of sniffing and exploration of objects, considering a minimum distance of 2cm from them. Behaviours such as climbing up on objects were not considered exploratory.

The object recognition index (RI) is calculated using the formula: (B)/(B+A1)*100, which express the percentage of preference for the new object, where B is the new object and A1 is the familiar object [13].

2.8. Histological procedures

The animals were euthanized, transcardially perfused after the 30th day of the experiment. They were anaesthetised with ketamine (80-100 mg/kg i.p.) and xylazine

(5-10 mg/kg i.p.), heparinized with 0.1mL heparin and perfused with 0.9% saline for 10 minutes, followed by 4% paraformaldehyde diluted in phosphate buffer (PBS) 0.1M (pH 7.2-7.4) for 30 minutes. Then the brains were removed, which were maintained for 24 hours in 4% buffered paraformaldehyde, and then in 70% ethanol for at least 24 hours. The samples embedded in paraffin. Coronal sections were made 4μm in the dentate gyrus of the hippocampus, as stereotactic atlas coordinates [27] (2.8 to 4.3 coordinates of bregma) with a rotatory microtome. Nine coronal sections per animal were selected for analysis, with a distance of 80μm between each cut. The experimental design is shown in Figure 2.

2.9. Immunohistochemistry

After deparaffinization, the slices to GFAP were allowed to stand in PBS (pH 7.4) with 0.5% Triton X-100 (PBS-tx) for 15 minutes, while the slices to cleaved caspase-3 were allowed to stand in citrate buffer (pH 6.00) at 98°C for 20 minutes for antigen retrieval. After, the blocking of endogenous peroxidase was made with hydrogen peroxide (30V) 5% in methanol for 10 minutes (3 times). Nonspecific proteins were blocked with bovine serum albumin 1% (BSA) (Sigma®) in PBS-tx for at least 1 hour at room temperature (RT). Then, the sections were incubated with primary monoclonal antibody (GFAP: Dako® 1:500; Cleaved caspase-3: Cell Signalling Technology® 1:500) overnight at 4°C. After, the sections to GFAP were incubated with the secondary and tertiary antibody (HRP® Advance kit, Dako®) for 40 minutes each at RT, while the section to cleaved caspase-3 were incubated with the secondary antibody (SignalStain® Apoptosis Kit, Cell Signaling Technology®) also for 40 minutes. Finally, immunohistochemical reaction was revealed with a 0.06% 3.3'-diaminobenzidine

(DAB) (Dako®) in PBS-tx for at least 3 minutes, counterstained with haematoxylin (only cleaved caspase-3) and mounted on slides using Entellan® (Merck®). For GFAP, the samples were photographed and analysed by optical densitometry using the software Image Pro Plus® 6.3 (Media Cybernetics®). For cleaved caspase-3, the samples were analysed by two researchers, in Olympus BX-40 microscope (Olympus®). Immunoreactive cells for cleaved caspase-3 were quantified in the granular layer of the dentate gyrus of the hippocampus.

2.10. Statistical analysis

The results were expressed as mean±standard deviation. Statistical analysis was performed by a three-way analysis of variance (ANOVA) followed by Bonferroni's *post-hoc* test. Difference was considered significant if $p<0.05$.

3. Results

3.1. Open field task

The results that CROSSINGp showed between animals of group C, DTC (17.7±5.07) did not differ in relation to its control DSC (18±2.79) but NDTC (16.6±2.63) differ significantly from NDSC (25.1±1.91) with $p<0.001$. In the group E, DTE (22.2±3.88) showed lower average in relation to DSE (26.9±1.91) with $p=0.004$ and NDTE (44.2±5.37) showed higher average in relation to NDSE (35.7±2.43) and the others subgroups with $p<0.001$. Animals of group C showed lower number of CROSSINGp in relation to animals of group E ($p<0.005$).

The results that REARING showed that DTC (16.70±2.75) did not differ in relation to DSC (14.00±2.17), already, NDTC (12.70±2.26) showed lower number of REARING in relation to NDSC (22.00±4.55), with $p<0.001$. In the group E, DTE (14.5±3.24) did not differ from DSC (15.8±2.20), but NDTE (25.80±3.15) showed higher average in relation to NDSE (22.25±4.77) and the others subgroups with $p=0.02$.

As for SNIFFING, DTC (61±3.93) showed higher average in relation to DSC (56.1±3.98) with $p=0.02$, and NDTC (43.5±2.63) differ from NDSC (55.7±4.52) with $p<0.001$. In the group E, diabetic animals (DTE 61.5±6.43 and DSE 65.3±5.20) did not differ among themselves, but NDTE (68.7±6.02) differ from animals NDSE (60.8±5.55) and also differ from NDTC, with $p<0.001$.

Finally, as for TIMEc in the group C, DTC (24.8±2.93) and NDTC (17±1.56) showed higher averages compared to their controls DSC (18.16±3.18) and NDSC (20.09±1.93) respectively, with $p<0.001$. In the group E, DTE (12.2±2.74) and NDTE (8.4±1.25) did not differ in relation to their controls DSE (12.10±2.55) and NDSE (8±1.41) respectively. Still, animals of group C showed higher averages when compared with animals of group E ($p<0.001$), except for NDSC and NDSE, which did not differ. All results can be seen in Figure 3.

3.2. Object recognition task

In this task, the RI values did not differ among animals of group C (DTC 66.7±5.98; DSC 63.5±5.88; NDTC 71.8±9.72; NDSC 65.8±3.19). In the group E, DTE (88.24 ± 10.18) compared to DSE (64.6±15.15) showed significant difference ($p<0.001$), but NDTE (84.82±11.67) did not differ from NDSE (80.4±3.80). Animals of

group E had better RI in comparison to group C (DTE/DTC; NDTE/NDTC; NDSE/NDSC), with $p<0.005$ (Figure 4).

3.3. Densitometry Optical GFAP

In the group C, DTC (0.245±0.020) had higher optical density (OD) of GFAP in comparison to DSC (0.218±0.018), with $p<0.001$. Animals NDTC (0.242±0.010) in relation to NDSC (0.2341±0.004), showed no difference. In group E, diabetic animals (DTE 0.247±0.013 and DSE 0.246±0.017) did not differ among themselves, however, NDTE (0.295±0.029) had higher OD in relation to NDSE (0.236±0.013) and all subgroups ($p<0.001$) (Figure 5).

3.4. Cleaved caspase-3

Comparing the mean quantifying immunoreactive cells to cleaved caspase-3, DTC (40.96±2.54) had lower average in relation to DSC (45.45±1.32), with $p<0.001$, but between NDTC (33.28±2.86) and NDSC (34.50±3.88) did not showed difference. In the group E, DTE (43.74±1.95) and NDTE (34.77±2.48) in comparison to their controls DSE (48.10±2.46) and NDSE (40.71 ± 2.84) respectively, showed difference with $p<0.001$. Curiously, animals of group E in comparison to subgroups of group C (DTE/DTC; DSE/DSC; NDSE/NDSC), showed a higher number of apoptotic cells and although not a very big difference, it was significant ($p<0.05$) (Figure 6).

4. Discussion

Some results found in this study are new and showed positive effects of environmental enrichment and treatment with taurine. In the literature, there are few studies with diabetic animal models investigating the influence of taurine or EE on memory, behaviour or expression of immunohistochemical markers such as GFAP and caspase. However, when we searched for studies linking all these elements, data were not found.

Some studies have discussed how DM can adversely influence animal behaviour, reducing locomotor and exploratory activity, and increasing anxiety [3, 28]. In the OF task, the taurine in diabetic group C was not able to improve locomotor activity (CROSSINGp and REARING), but seems to have helped in improving the exploratory ability (SNIFFING) and also increasing TIMEc compared to the untreated diabetic controls. In theory, by instinct, animals tend to avoid the centre of an unknown location, performing thigmotactic behaviour. When the animal spends more time in the centre of arena, this animal apparently is less anxious [29]. In non-diabetic animals of group C, the taurine acts decreasing the locomotor and exploratory activity and increasing the TIMEc, showing an effect similar to anxiolytic, which was previously described by Caletti et al. [28]. In diabetics of group E, taurine acts decreasing the locomotor and exploratory activity showing effect anxiolytic. In non-diabetic animals of group E, the taurine act increasing the locomotor and exploratory activity in relation to NDSE and all subgroups, apparently showing a possible stimulating effect. The EE alone can improve locomotor and exploratory activity in comparison to standard housing animals (NDSE/NDSC) and potentiated to stimulant effect of taurine in NDTE suggest a synergistic effect. The EE did not able to improve the TIMEc in relation to

animals of group C, possibly because these animals were habituated to a wide and rich environment. Therefore, in front of an empty and new environment, they easily become more anxious, probably due to modulation in interoception and exteroception. The beneficial effects of EE have already been observed in other studies [13; 30], by stimulating curiosity and making them more willing and smart animals. This suggests that the physical and interactive activities are able to improve physical and locomotor states as well as states that can cause depression.

Another important complication of DM are memory deficits [10, 31] that can be improved when treated with taurine [32, 33] and positively affected by EE [13]. In the object recognition task, the taurine, in the group E, appeared to influence memory, possibly associated with EE since we observed a significant improvement in the performance of the animals, both diabetic and non-diabetic when compared to their controls and in relation to the equivalent subgroups of group C (DTC and NDTC). However, the animals of group C did not show any influence. When observing the performances of NDSE we show that they were better than those in NDSC, suggesting that the EE alone might have a positive influence on memory. Studies have shown that EE is able to improve memory [34] and the same effect was seen in animals with brain damage [30], diabetic [13] and senescent animals [35] confirming our results.

In immunohistochemistry for GFAP we did not observe astrocyte reactivity (AR) in diabetic animals. This reactivity, indicating a cell response to stress [2], has already been observed in the study by Saravia et al. [36]. This characterizes a physiological response to injury that can evolve to a pathophysiological condition, with hypertrophy-hyperplasia of astrocytes and increased expression of GFAP [37]. However, other study found that the AR has a sudden increase after the onset of hyperglycaemia, but after 4 weeks the reactivity begins to decline with decreased GFAP

expression [38] initiates a neurodegeneration process. Taking into account that the treatment of this experiment lasted 4 weeks, our animals would already be in this phase of decline, and thus their values were close to the controls. Moreover, taurine in DTC seemed to contain the decline observed in the DSC, keeping the GFAP levels above untreated animals, demonstrating a possible neuroprotective effect already observed by Zeng et al. [23]. In the group E, taurine did not have the same effect on the diabetic animals. However, untreated diabetic animals (DSE) did not have the same decrease in OD of GFAP seen in DSC, maintaining similar levels to the NDSE control, showing that EE may be able to interfere with neural damage processes, as described in another study [9].

Taurine appeared to influence NDTE animals, increasing the immunoreactivity of GFAP above all subgroups, which possibly in conjunction with EE presented higher OD of GFAP, giving the impression of a synergistic effect. We could see that this effect was not harmful to the animals in this group, since we observed that they performed better on memory tasks, had higher exploratory and locomotor activity and smaller cells in apoptosis values, compared to the others. This event has been described in other studies [35, 39] observing that healthy animals subjected to EE had increased immunoreactivity for GFAP in the hippocampal DG region, suggesting a response to the new cell formation and modulation of synapses due to various sensory stimuli and social interaction that EE offers, supporting our findings, which can be further enhanced by the stimulating effect of taurine.

Also, the taurine showed no effect on the physiological apoptosis mechanism in non-diabetic animals of group C, already observed in other study [19]. However, taurine appeared to be able to prevent the increasing number of apoptotic cells in diabetic animal in both groups (C and E), demonstrating a potential neuroprotective

effect, possibly playing an antioxidant role by reducing the damage caused by hyperglycaemia, as explained by other authors [19, 22, 24]. The same effect was observed in NDTE. Surprisingly, when we compared the subgroup averages in group E with the equivalent of C group, especially among controls (NDSC and NDSE), a small increase in the apoptosis were observed in group E compared to group C, contradicting some studies that did not show this pattern in healthy animals [40]. This small increase was not harmful to the behavioural and memory tasks because EE animals had excellent performances on such tasks as compared with the standard housing animals. This increase of apoptosis may be due to various sensory, emotional and physical stimuli that occur at EE that increases the consumption of ATP and oxygen, which cause a physiological increase in the production of reactive oxygen species (ROS). Previous studies have described that the increase in ROS production triggers oxidative stress [4, 5] and stimulates the release of pro-apoptotic factors. Also, studies have shown that EE is able to increase neurogenesis processes [11, 12, 14]. Therefore, an increase in neurogenesis can compensate this increase in apoptosis and this would be important data to analyse in future studies.

5. Conclusion

We conclude that the taurine is able to improve memory deficits induced by diabetes, increase the exploration activity and interfere with apoptosis and neurodegeneration process. The EE also modulate behaviour and animal physiology, increasing the curiosity and exploration. Thus, improved mechanisms of acquisition and memory consolidation, both in diabetic and non-diabetic animals. In some instances, the taurine and EE showed a beneficial synergistic effect on behaviour, memory, protection

against cell death and stimulating glial adaptive responses to treatment and environment rich in information. So, the positive results presented confirm that these factors can be very beneficial to the quality of life of individuals with DM.

Acknowledgment

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6. References

[1] IDA. International Diabetes Federation. IDF Diabetes Atlas, sixth ed,2013, 160 p.

[2] Y. Revsin, N.V. Rekers, M.C. Louwe, F.E. Saravia, A.F. De Nicola, E.R. de Kloet, et al., Glucocorticoid receptor blockade normalizes hippocampal alterations and cognitive impairment in streptozotocin-induced type 1 diabetes mice, Neuropsychopharmacology Feb 34 (3) (2009), 747-58.

[3] L.P. Reagan, Diabetes as a chronic metabolic stressor: causes, consequences and clinical complications, Exp. Neurol. 233 (1) (2012), 68-78.

[4] C.L. Hawkins, M.J. Davies, Generation and propagation of radical reactions on proteins, Biochim. Biophys. Acta 1504 (2-3) (2001), 196-219.

[5] G. Verdile, S.J. Fuller, R.N. Martins, The role of type 2 diabetes in neurodegeneration, Neurobiol. Dis. 84 (2015) 22-38.

[6] W.J. Zhang, Y.F. Tan., J.T. Yue, M. Vranic, J.M. Wojtowicz, Impairment of hippocampal neurogenesis in streptozotocin-treated diabetic rats, Acta Neurol. Scand. 117 (3) (2008), 205-10.

[7] A.M. Stranahan, Models and mechanisms for hippocampal dysfunction in obesity and diabetes, Neuroscience 309 (2015), 125-139.

[8] O.Ojo, J. Brooke, Evaluating the association between diabetes, cognitive decline and dementia, Int. J. Environ. Res. Public Health 12 (7) (2015), 8281-94.

[9] A.M. Stranahan, T.V. Arumugam, R.G. Cutler, K. Lee, J.M. Egan, M.P. Mattson, Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons, Nat. Neurosci. 11 (3) (2008), 309-17.

[10] E.R. Vieira, A. Mendy, C.M. Prado, J. Gasana, A.N. Albatineh, Falls, physical limitations, confusion and memory problems in people with type II diabetes,

undiagnosed diabetes and prediabetes, J. Diabetes Complications 29 (8) (2015), 1159-64.

[11] H. van Praag, G. Kempermann, F.H. Gage, Neural consequences of environmental enrichment, Nat. Rev. Neurosci. 1 (3) (2000) 191-8.

[12] J. Beauquis, P. Roig, A. De Nicola, F. Saravia, Short-term environmental enrichment enhances adult neurogenesis, vascular network and dendritic complexity in the hippocampus of type 1 diabetic mice, Plos One 5 (11) (2010), 1-12.

[13] F.V. Piazza, G.V. Pinto, G. Trott, S. Marcuzzo, R. Gomez, M.C. Fernandes, Enriched environment prevents memory deficits in type 1 diabetic rats, Behav. Brain Res. 217 (1) (2011), 16-20.

[14] M. Komitova, B. Mattson, B. Johansson, P.S. Erikson, Enriched environment increases neural stem/progenitor cell proliferation and neurogenesis in the subventricular zone of stroke-lesioned adult rats, Stroke 36 (6) (2005), 1278-82.

[15] G. Segovia, A.G. Yague, J.M. Garcia-Verdugo, F. Mora, Environmental enrichment promotes neurogenesis and changes the extracellular concentrations of glutamate and GABA in the hippocampus of aged rats, Brain Res. Bull. 70 (1) (2006), 8-14.

[16] M. Nilsson, M. Penki, Enriched environment and astrocytes in central nervous system regeneration, J. Rehabil. Med. 39 (5) (2007), 345-352.

[17] S.S Oja, P. Saransaari, Pharmacology of taurine, Proc. West. Pharmacol. Soc. 50 (2007), 8-15.

[18] A. De Luca, S. Pierno, D.C. Camerino, Taurine: the appeal of a safe amino acid for skeletal muscle disorders, J. Transl. Med. 13 (2015), 243.

[19] S.W. Schaffer, J. Azuma, M. Mozaffari, Role of antioxidant activity of taurine in diabetes, Can. J. Physiol. Pharmacol. 87 (2) (2009), 91-9.

[20] V. Vitvitsky, S.K. Garg, R. Banerjee, Taurine biosynthesis by neurons and astrocytes, J. Biol. Chem. 286 (37) (2011), 32002-10.

[21] H. Ripps, W. Shen, Review: Taurine: A “very essential” amino acid, Molecular Vision 18 (2012), 2673-86.

[22] C.D. Puerta, F.J. Arrieta, J.A. Balsa, J.I. Botella-Carretero, I. Zamarrón, C. Vázquez, Taurine and glucose metabolism: a review, Nutr. Hosp. 25 (6) (2010), 910-19.

[23] K. Zeng, H. Xu, M. Mi, K. Chen, J. Zhu, L. Yi et al., Effects of taurine on glial cells apoptosis and taurine transporter expression in retina under diabetic conditions, Neurochem. Res. 35 (10) (2010), 1566-74.

[24] N. Kumari, H. Prentice, J.Y. Wu, Taurine and its neuroprotective role, Adv. Exp. Med. Biol. 775 (2013), 19–27.

[25] M.C. Shivaraj, G. Marcy, G. Low, J.R. Ryu, X. Zhao, F.J. Roasales et al., Taurine induces proliferation of neural stem cells and synapse development in the developing mouse brain, Plos One 7 (8) (2012), 1-12.

[26] J. Menzie, H. Prentice, J.Y. Wu, Neuroprotective mechanisms of taurine against ischemic stroke, Brain Sci. 3 (2) (2013), 877–907.

[27] G. Paxinos, C. Watson, The rat brain in stereotaxic coordinates, seventh ed., Elsevier Academic Press, New York, 2014, 472p.

[28] G. Caletti, D.B. Olguins, E.F. Pedrollo, H.M. Barros, R. Gomez, Antidepressant effect of taurine in diabetic rats, Amino Acids 43 (4) (2012), 1525-33.

[29] T. Gould, D. Dao, C. Kovacsics. The open field test, in: T.D. Gould (ed), Mood and anxiety related phenotypes in mice: characterization using behavioral tests, Neuromethods, Human Press, 2009, p-1-20.

[30] E. Kovesdi, A.B. Gyorgy, S.K.C. Kwon, L. Daniel, D.L. Wingo, A. Kamnaksh, J.B. Long, C.E. Kasper, D.V. Agoston, The effect of enriched environment on the outcome of traumatic brain injury; a behavioral, proteomics, and histological study, Front Neurosci. 5 (42) (2011), 1-12.

[31] J.I. Malone, S. Hanna, S. Saporta, R.F. Mervis, C.R. Park, L. Chong, D.M. Diamond, Hyperglycemia not hypoglycemia alters neuronal dendrites and impairs spatial memory, Pediatr. Diabetes 9 (6) (2008), 531-9.

[32] H. Y. Kim, H. V. Kim, J. H. Yoon, B. R. Kang, S. M. Cho, S, Lee, J. Y. Kim, J. W. Kim, Y. Cho, J. Woo, Y. Kim, Taurine in drinking water recovers learning and memory in the adult APP/PS1 mouse model of Alzheimer's disease, Sci. Rep. 4 (2014), 7467.

[33] G. Caletti, F.B. G. Almeida, G. Agnes, M.S. Nin, H.M. Barros, R. Gomez, Antidepressant dose of taurine increases mRNA expression of GABAA receptor α2 subunit and BDNF in the hippocampus of diabetic rats, Behav. Brain. Res. 283 (2015), 11-15.

[34] M. Nilsson, E. Perfilieva, U. Johansson, O. Orwar, P.S. Eriksson, Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory, J. Neurobiol. 39 (4) (1999), 569-78.

[35] D.G. Diniz, C.A.R. Foro, C.M.D. Rego, D.A. Gloria, F.R.R. de Oliveira, J.M.P. Paes, A.A de Sousa, T.P. Tokuhashi, L.S. Trindade, E.G. Vasconcelos, J.B Torres, C. Cunnigham, V.H. Perry, P.F. Vasconcelos, C.W. Diniz, Environmental impoverishment and aging alter object recognition, spatial learning, and dentate gyrus astrocytes, Neurosci. 32 (3) (2010), 509-19.

[36] F.E Saravia, Y. Revsin, M.C. Gonzalez Deniselle, S.L. Gonzalez, P. Roig, A. Lima et al. Increased astrocyte reactivity in the hippocampus of murine models of type 1 diabetes: the nonobese diabetic (NOD) and streptozotocin-treated mice, Brain Res. 957 (2) (2002), 345-53.

[37] A. Verkhratsky, L. Steardo, V. Parpura, V. Montana, Translational potential of astrocytes in brain disorders, Prog. Neurobiol. (2015), 1-18.

[38] E. Coleman, R. Judd, L. Hoe, J. Denis, P. Posner, Effects of diabetes mellitus on astrocyte GFAP and glutamate transporters in the CNS, Glia 48 (2) (2004), 166-78.

[39] L.L Williamson, A. Chao, S.D. Bilbo, Environmental enrichment alters glial antigen expression and neuroimmune function in the adult rat hippocampus, Brain Behav. Immun. 26 (3) (2012), 500-10.

[40] N. Thiriet, B. Gennequin, V. Lardeux, C. Chauvet, M, Decressac, T. Janet, M. Jaber, M. Solinas, Environmental enrichment does not reduce the rewarding and neurotoxic effects of methamphetamine, Neurotox. Res. 19 (1) (2011), 172–82.

7. Appendices

Figure 1: Diagram demonstrating experimental groups division

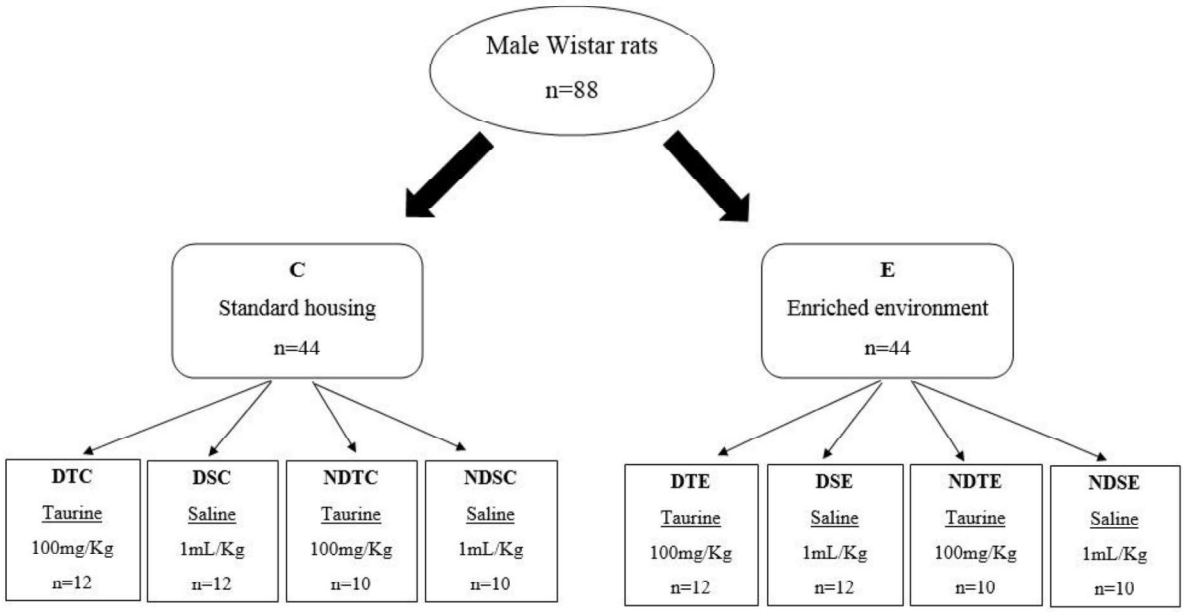


Figure 2: Diagram illustrating experimental design

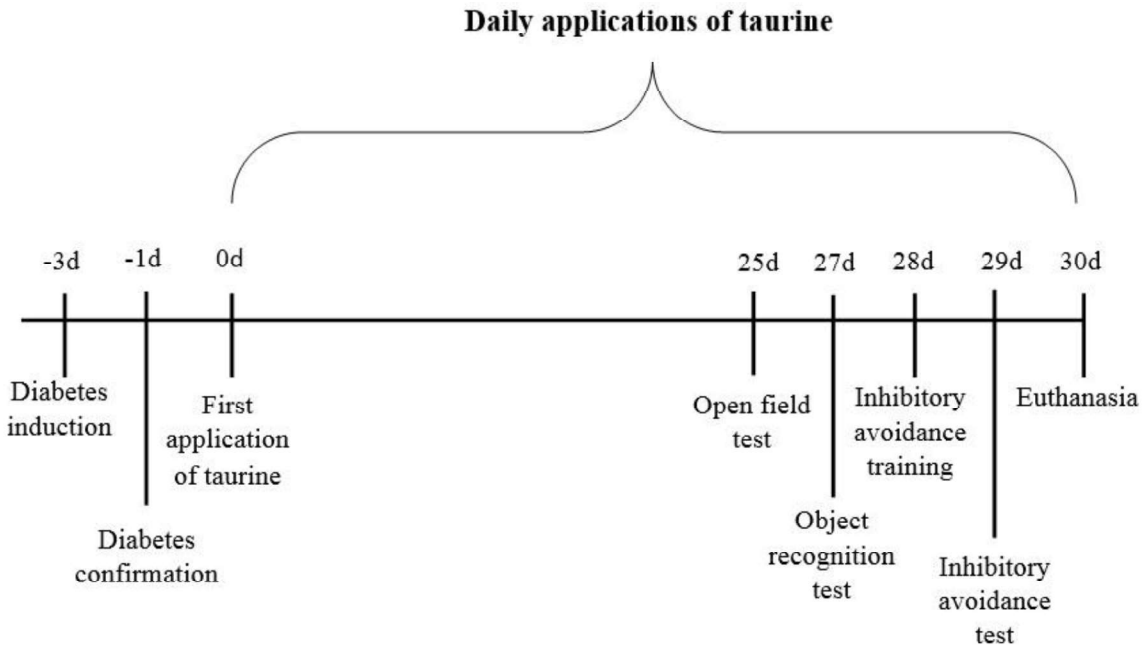


Figure 3: Open field test mean ± standard deviation values for the groups (n=8-10/group):

A: The number of intersections on the periphery of the arena (CROSSINGp): Statistical difference: † $p<0.005$ (DTC vs. DTE, DSC vs. DSE, NDTC vs. NDTE and NDSC vs. NDSE); #p=0.004; *$p<0.001$.

B: Number of times the animal stood on its hind legs (REARING): Statistical difference: † different from the other subgroups with $p<0.02$; *$p<0.001$.

C: Time that the animal spent sniffing (SNIFFING): Statistical difference: † different from NDTC and NDSE, with $p<0.001$; *different from the others of group C, with $p<0.05$; #$p<0.001$;

D: Time spent in the central area of the arena (TIMEc): Statistical difference: †$p<0.001$ (DTC vs. DTE, DSC vs. DSE and NDTC vs. NDTE); *$p<0.001$.

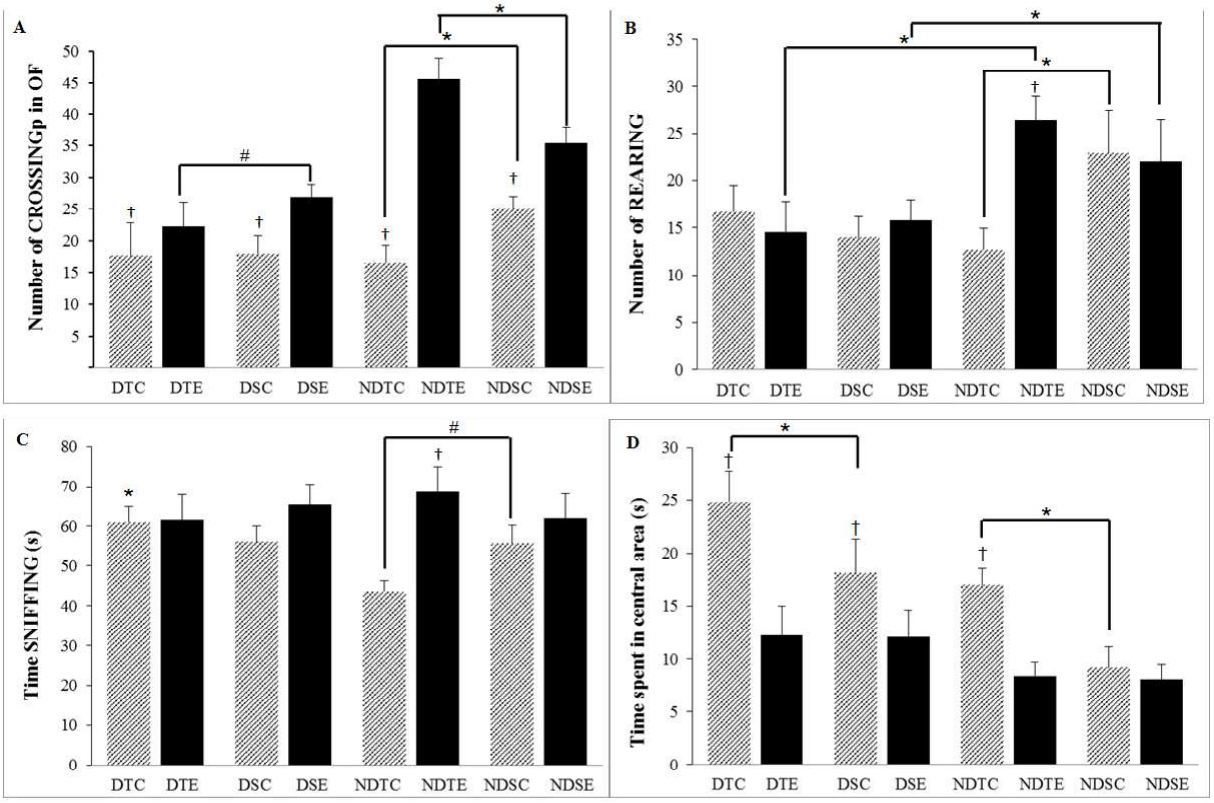


Figure 4: Percentage of preference for the new object in the object recognition test. The values express the mean ± standard deviation for groups (n=8-10/group): statistical difference: *p<0.001; # p=0.005 (DTC vs.DTE, NDTC vs. NDTE and NDSC vs. NDSE).

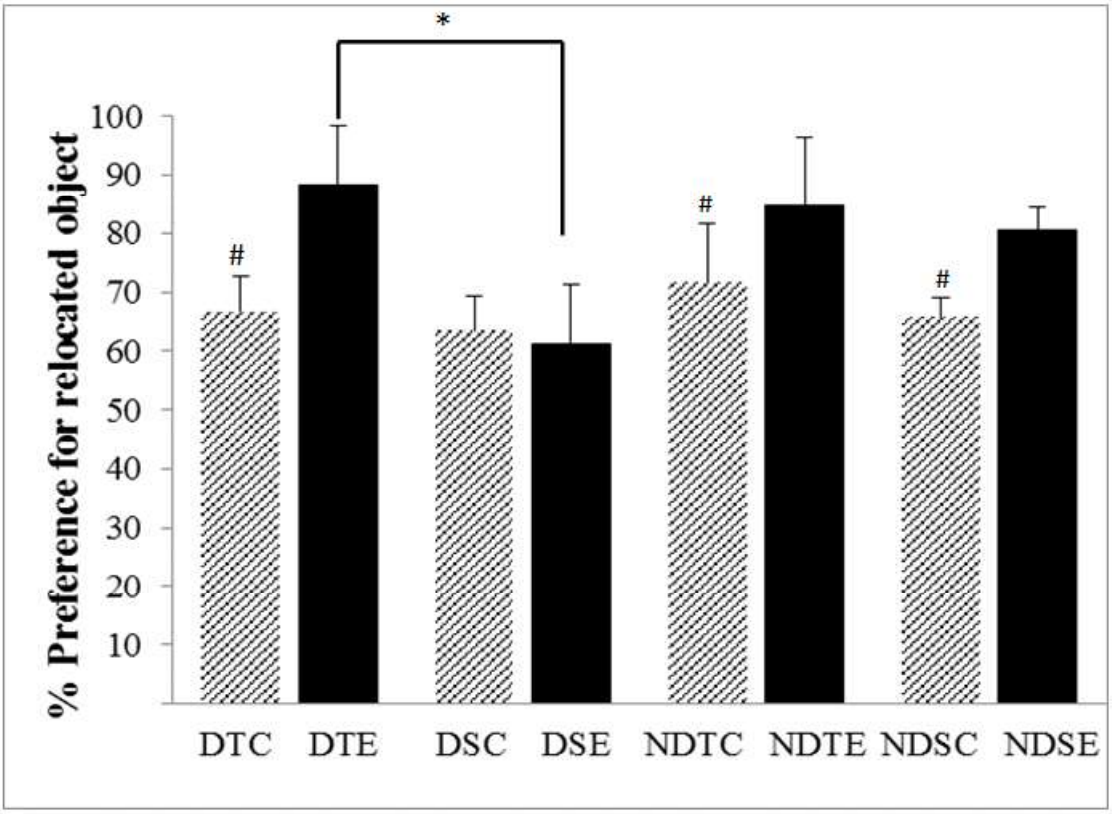


Figure 5: GFAP

A: Optical densitometry of GFAP. Values expressed as means for each group±standard deviation: statistical difference: *p<0.05 relative to other subgroups; #p=0.031;

B: Microphotography dentate gyrus in NDTE group, which showed higher OD values;

C: Photomicrograph of the dentate gyrus in DSC group, which exhibited lower OD values.

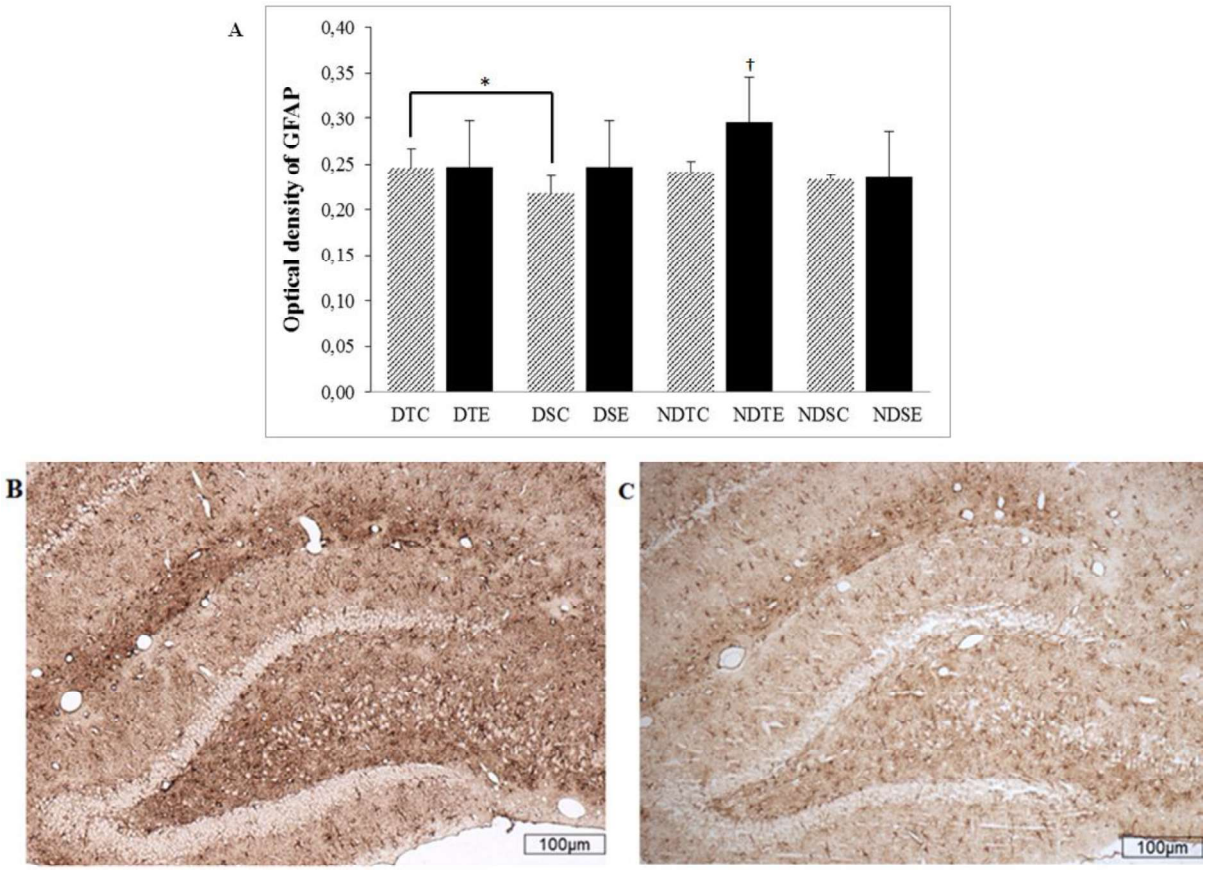
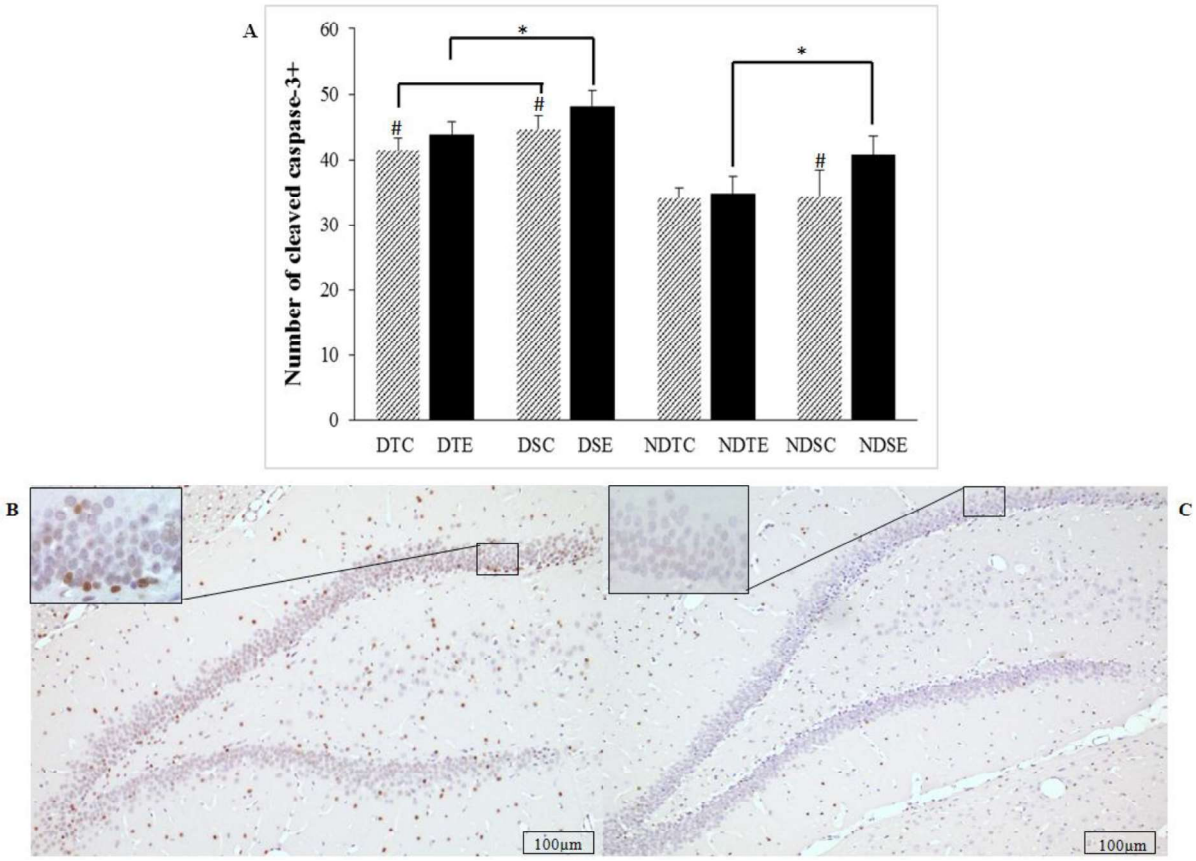


Figure 6: Cleaved caspase-3.

A: Values expressed as means counts of the cells labelled with cleaved caspase-3 ± standard deviation: statistical difference: *p<0.001; #p<0.05 (DTC vs. DTE; DSC vs. DSE and NDSC vs. NDSE).

B: Photomicrograph of DSC, many immunoreactive cells showing caspase-3;

C: Photomicrograph of NDTC, showing few immunoreactive cells.



4 CONSIDERAÇÕES FINAIS

Nossos resultados mostram que de fato, animais diabéticos apresentam déficits de memória e aprendizado em relação a animais não diabéticos, assim como possuem uma maior taxa apoptótica.

Conseguimos demonstrar que a suplementação com taurina apresenta bons efeitos sobre o organismo de animais diabéticos, melhorando seus déficits de memória, aumentando sua atividade exploratória, e parecendo atuar também na diminuição de processos de degeneração astrocitária e apoptose neuronal.

Observamos que o enriquecimento ambiental influencia muito sobre o comportamento e fisiologia dos animais, tanto nos diabéticos como nos não diabéticos. Parece influenciar aumentando a atividade locomotora e curiosidade dos animais. Também, aparenta atuar melhorando mecanismos de aquisição e evocação de memórias, fazendo com que os animais que foram expostos ao AE se sobressaiam positivamente aos outros que não foram expostos ao mesmo ambiente.

No estudo também notamos que em alguns momentos, a taurina e o AE pareciam ter efeitos sinérgicos que estimularam positivamente comportamento animal. Esta sinergia parece proteger contra a morte celular, e ainda, aumentar a DO de GFAP em astrócitos, possivelmente por promover a plasticidade neural e sináptica em decorrência dos muitos estímulos recebidos do ambiente, especialmente em animais não diabéticos.

Diante destes dados, salientamos a importância da continuidade de estudos envolvendo o DM, taurina e enriquecimento ambiental, para confirmar nossos achados e demonstrar se há ainda, efeitos positivos sobre neurogênese e outros processos.

5 ANEXOS

5.1 Normas para publicação da revista Neuroscience Letters

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CEUA –COMISSÃO DE ÉTICA NO USO DE ANIMAIS

PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA E ENSINO

1) PROTOCOLO Nº: 134/13 **Parecer 253/14**

2) DATA DO PARECER: 12/03/14

3) TÍTULO DO PROJETO:

Efeito da taurina no hipocampo de ratos diabéticos.

4) PESQUISADOR RESPONSÁVEL:

Marilda Fernandes

5) RESUMO DO PROJETO:

Estudar em um modelo de diabetes experimental o efeito da administração de taurina sobre a memória de curta e longa duração através de testes comportamentais e a proliferação celular, morte celular e a densidade de células gliais no hipocampo de ratos diabéticos e controles submetidos a ambiente enriquecido ou não.

6) OBJETIVOS DO PROJETO:

- Avaliar a influência da taurina na proliferação celular no giro denteado de animais diabéticos e não diabéticos.
- Verificar se há variação no número de células gliais marcadas com GFAP no giro denteado de animais diabéticos e não diabéticos.
- Verificar se há variação nas células apoptóticas de animais diabéticos e não diabéticos.
- Avaliar o número de neurônios imaturos no giro denteado do hipocampo nos grupos experimentais.
- Verificar se há variação no número de células marcadas com BrdU no GD entre os animais expostos ao ambiente enriquecido e/ou ao diabetes.
- Avaliar o impacto do uso da taurina sobre a memória espacial, de trabalho, de curta e de longa duração) nos diferentes grupos experimentais).
- Verificar nos testes comportamentais se a taurina junto com o ambiente enriquecido potencializou os resultados comportamentais.

7) FINALIDADE DO PROJETO: ☐ Ensino ☒ Pesquisa

8) ITENS METODOLÓGICOS E ÉTICOS DO PROJETO:

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Outra instituição. Qual?

11) CRONOGRAMA DE UTILIZAÇÃO DE ANIMAIS

Data	Espécie	Sexo	Quantidade

12) RECOMENDAÇÃO:

[x] Aprovado

[ ] Com Pendência

REPÚBLICA FEDERATIVA DO BRASIL
MINISTÉRIO DA EDUCAÇÃO

UFCSPA
UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

☐ Não aprovado

Término do projeto 01/12/2017

Comentários gerais sobre o projeto: